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ASIA-PACIFIC FISHERY COMMISSION
FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
REGIONAL OFFICE FOR ASIA AND THE PACIFIC
Bangkok, Thailand

PREPARATION OF THIS DOCUMENT

The publication contains the report of and the technical contributions made to the APFIC Symposium on Fish Utilization in the Asia-Pacific Region held in Beijing, People's Republic of China, from 24 to 24 September 1998, in conjunction with the 26th Session of APFIC. The papers have been edited by D.G. James, Technical Secretary of the Symposium.

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ABSTRACT

The publication contains the report of and the papers presented at the APFIC Symposium on Fish Utilization in the Asia-Pacific Region, held in Beijing, People's Republic of China, 24-26 September 1998. The contributions are divided into four groups. The first group contains papers related to technical approaches to fish utilization in low-cost products, particularly for poor consumers. The second concentrates on processes for full utilization of aquatic resources for food, feed and other purposes, while the third is centered on the safety and quality assurance of fish and fishery products from the region. The final section collects miscellaneous topics of interest to the region.

Distribution:

Participants at the Symposium
Members of the Asia-Pacific Fishery Commission
FAO Fisheries Department
FAO Regional Fishery Officers

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INTRODUCTION

1. The APFIC Symposium on Fish Utilization in the Asia-Pacific Region was held from 24 to 26 September 1998 at the Beijing Continental Grand Hotel, Beijing, People's Republic of China, in conjunction with the Twenty-sixth Session of the Asia-Pacific Fishery Commission (APFIC). The Symposium was attended by 28 participants from APFIC members, two from other FAO members and two from intergovernmental organizations, namely the Southeast Asian Fisheries Development Center (SEAFDEC) and the World Health Organization (WHO). A total of 38 technical contributions, including overview papers by the Session Moderators, was presented for the consideration of the Symposium.

2. At the opening of the Symposium, the Convenor, Mr David James, expressed appreciation, on behalf of the Director-General of FAO, to the Bureau of Fisheries of the People's Republic of China for hosting the Symposium. In view of the rich tradition of fish utilization in China he noted that it was a particularly appropriate place to hold a regional symposium on the topic and that the participants would benefit greatly from the experience. He recorded that the APFIC Working Party on Fish Technology and Marketing, which had proposed the topic for the Symposium, would be abolished after an active life of 25 years. This was in common with all working parties of FAO subsidiary bodies. For this reason, the Symposium participants should consider carefully their recommendations to the Commission on means to provide for continued coordination of activities in the field of fish technology in the Region.

3. On behalf of the Chairman of APFIC, Mr Zhuo You Zhan, Mr Wang Yan Liang, Vice Director-General of the Bureau of Fisheries, delivered an opening address welcoming the participants to China and expressing his best wishes for a successful Symposium.

4. The Symposium was divided into five sessions, each under the direction of a Session Moderator.

SESSION 1: The role of fish in food security and the prospect of maintaining supplies

Moderator: David James

5. The Moderator presented an overview of the pattern of development of world fisheries in relation to production and utilization, noting the predominant position of the Region both in respect to fish catches from the wild and the growing importance of aquaculture. While world fish production was still increasing there were disturbing trends in availability that had a particularly severe impact on the poor. While per capita consumption in the region had risen strongly in recent decades the rate of population growth in the Region was now outstripping production increases. Estimates of a gap between supply and demand of up to 20 million tons by the early part of the next century had been made. A further disturbing trend was the strong demand for low-value fish as feed for the rapidly growing intensive aquaculture sector. These trends place increasing responsibility on fish technologists to provide technical solutions to full utilization of aquatic resources as well as social responsibility to ensure that supplies of safe fish products remain available for the lower socio-economic sector of the community.

6. The Executive Secretary of the Support unit for International Fisheries and Aquaculture Research (SIFAR), Mr Tim Bostock, informed the Symposium of the role of this unit which was established at FAO during March this year. SIFAR would act as a bridge between partner countries and the donor community, helping to identify demand for relevant research initiatives, and assist in the preparation of proposals suitable for donor consideration. Overall, support would be provided for research themes which promote economically efficient development and better management. Research outputs will be used to inform improved national and regional strategic planning, fostering integration of national policy across sectors. Plans to establish an Internet-based electronic forum for

communication of information and discussion on issues related to fisheries and aquatic research called SIFARNET, were explained.

7. Mr Tan Sen Min, Chief of SEAFDEC Marine Fisheries Research Department, presented a short review of the work of SEAFDEC in the field of fish utilization over the last two decades. SEAFDEC has implemented an integrated programme of scientific investigation of the properties of low-value species in the Region, the development of processing technologies using advanced methods and equipment and their transfer directly to the industry. The wide range of products successfully introduced was demonstrated. The approach taken by SEAFDEC of direct interaction with the industry was commended by the Symposium for possible application elsewhere.

SESSION 2: Technological approaches to fish utilization in low-cost products for poor consumers

Moderator: ***Yu Swee Yean***

9. The overview focussed on the more recent technologies that have been developed and tested to use low-value fish in products that could be made available to consumers at relatively low prices. In addition to the well established technologies of fresh fish distribution and traditional processing, the recovery of fish meat by mechanical means is one of the most viable methods of making use of many underutilized species for human consumption. Minced meat recovered from these species can be made into conventional products such as burgers, patties, fingers and pickled products. The minces have also been shown to be suitable as raw material for the traditional Asian fish jelly products, such as fish balls and fish cakes. There is also a renewed interest in fish protein concentrates in the Region as a low-cost product that can find application within the food habits and contribute to food security. More sophisticated products, such as fish hydrolysates which have better functional properties, are also the subject of research in the Region and should find application in the future.

SESSION 3: Requirements and technologies for the full utilization of aquatic resources for food, feed and other purposes

Moderator: ***K Gopakumar***

10. Continuing the search for suitable technologies improvements to fresh fish distribution and products made from whole fish were reviewed and further development of minced fish through the surimi process considered. Suggestions were made for incorporation of hydrolysates included fortified beverages and soup powders. Taking advantage of the rich tradition of fermented products in the region, the use of new species, to substitute for those in short supply, and the development of novel fermented products have both contributed to improved utilization. However, in common with other traditional product technologies there is a need for more detailed attention to the safety aspects of these products.

SESSION 4: Better selection and utilization of bycatch components for direct human consumption particularly as low-cost products

Moderator: ***Attaya Kungsuwan***

11. Participants were informed of the conclusions of an Expert Consultation on Bycatch Utilization in Tropical Fisheries, which had been organized by FAO/DFID (UK) immediately before the Symposium. In addition to representation from the Region, people from Africa and Latin America also contributed to the discussions. While it was apparent that Southeast Asia had had the greatest success in making use of discards and bycatches, the main conclusions of the Consultation were:

- At a world level, discards have been reduced but are still unacceptably high, while bycatches that are landed often bring limited economic returns and their capture may affect the sustainability of the resource;

- In Southeast Asia the previous bycatch component has, in some cases, become the target species as a result of market-driven demand. This has encouraged the introduction of new technologies; and

- In other regions of the world, in particular Central and South America, much bycatch is still wasted.

12. The main recommendations of the Consultation were:

- Better data on bycatches and discards should be collected and for the purpose of statistical reporting discards should be included in catches.

- Discarding and the landing (and utilization) of bycatch that is not sustainable should be prevented by legislation, management and the rewarding of the fisheries sector for compliance.

- Better utilization of sustainable bycatches should be encouraged by the provision of infrastructure for landings, collection and transport systems, research and development and technology transfer, where there are opportunities for transfer from Asia to Latin America.

SESSION 5: Quality assurance and seafood safety

Moderator: Sirilak Suwanrangsi

13. In general the Region has responded positively to the changing requirements of the major importing countries in terms of assurance of the safety of fish and fishery products, although there are serious concerns about safety issues surrounding some aspects of aquaculture in the Region. All exporting countries now have in place the capacity for fish inspection but the extent to which the new approach to HACCP applied by trained staff varies. Control of the safety of fish on domestic markets is deficient in all countries, often as a result of diffuse responsibilities for food control. There is a need for a study of how fish inspection and control, both for the domestic sector and export, fits into the overall pattern of food control in the Region. It is also apparent that the fisheries sector of the Region, both at government and industry levels, is not well integrated into the FAO/WHO Codex Alimentarius system. The Codex Alimentarius is a joint food standards programme implemented by FAO/WHO and is designed to protect the consumers at the same time as promoting trade in food. Its guidelines, codes of practice and recommendations provide the baselines for decision on food safety related issues in international trade as recognized by the SPS/TBT (sanitary and phytosanitary/technical barriers to trade) agreements of WTO. Participation from the Region in the Asian Regional Committee and the Fish and Fish Products Committee of Codex has been weak. As a result, the concerns of the Region and the special considerations of tropical fisheries are not reflected in the standards that are being drawn up. Despite the above and the need for a sustained approach to training, the Region has responded well to the challenge.

CONCLUSIONS AND RECOMMENDATIONS

14. The limits to production from capture fisheries imply that increases in supply can only result from using these resources more wisely and stimulating the availability of fish from aquaculture systems. Increased attention to post-harvest fish technology will be necessary both to convert low-value resources, which are available on a sustainable basis, to products for direct human consumption. Resources which cannot be marketed directly and other waste will be available for feed. There are

also emerging food safety issues associated with aquaculture systems (and some inland fisheries) to which technological solutions must be found.

15. There is a particular need to support the introduction of risk analysis to the aquaculture industry and in the production of traditional products, which are now being widely traded. This should be done on a systematic scientific basis, together with the introduction of HACCP, as a means of effective safety assurance. In the Region at large, there is a definite requirement to expand national quality and safety assurance systems to cover fish and fishery products for the domestic market as well as export. In most cases this will require restructuring of the food safety system. Studies on food safety and quality assurance systems should be encouraged.

16. The development of products from low-value resources was the subject of a recent very useful international research project in which a number of Regional institutes participated. However, this was still a pressing need. Many of the investigations conducted in the Region have been technology driven. The success of SEAFDEC places emphasis on the potential for a market driven approach. The requirements for technology transfer between countries will increase in future.

17. While fish quality and safety assurance in the Region has improved to meet the demands of the major importing countries there is still much to be done, particularly with training at the industry level. The failure of APFIC members to participate in the Codex Alimentarius process has prevented the particular needs of the Region from being reflected in food standards, guidelines, and codes of practice.

18. The benefits of closer regional collaboration have been demonstrated and could be strengthened through cooperation in information exchange and training.

SUGGESTED ACTIONS BY THE COMMISSION

19. In view of the accelerated change from hunting to fish farming, and the emerging food and public safety issues involved, the Symposium concluded that priority attention should be given to this topic and recommends to the Commission that an *ad hoc* Working Group of Experts in Food Safety be established to coordinate the programme as given in the Annex. This Working Group should report on progress to the Twenty-seventh Session of the Commission.

20. The Commission is requested to consider and to endorse the proposed programme on emerging food safety issues in the APFIC Region for implementation.

Programme Proposal: Emerging Food Safety Issues in the APFIC Region

Objective: To establish an *ad hoc* Working Group of Experts in Food Safety, tasked with coordinating a regional programme to address emerging food safety issues associated with fish production in the APFIC region.

Justification:

Aquaculture is currently one of the fastest growing food production systems in the world with production levels increasing at an average rate of 9.6% per year over the past decade. Aquaculture contributes significantly both to the economies and food security of many producing countries. The APFIC region in particular has the longest tradition in aquaculture, producing around 90% of global volume and 82% of value.

While fish from the open ocean are generally considered safe, products from coastal and inland aquaculture are increasingly associated with major food safety concerns. Such concerns are having major impact on the health of millions of people in the region while others threaten the vital international trade in fish products. This programme aims to encourage an integrated approach within the region which will:

- Quantify current and potential health hazards and risks associated with fish products; and
- Develop sustainable management strategies aimed at mitigation.

Data derived from this programme will be used in the process of risk analysis, which is the basis for the development of food standards that both provide adequate health protection and facilitate intraregional and international trade in food.

Participating countries: Interested APFIC members.

Activities: Three main areas of major interest to the APFIC region are initially proposed:

I. Parasitic infestation:

A large number of fish, particularly freshwater species, can serve as a source of human parasitic infestation. In particular, infestation by the fish-borne trematodes of prime concern for human health (including *Clonorchis, Opisthorchis* and *Paragonimus*) is recognized as a major burden to public health throughout the world. WHO has recently estimated that the number of people infected by trematodes is 40 million, with over 10% of the world population being at risk. With regard specifically to the APFIC region, a major concern is that trematodes are endemic for many member countries and related impacts on public health are highly significant.

In support of the objectives of the regional programme, it is proposed to carry out the following activities:

- **Determine the sources and relative impacts of infection.** In particular, to establish the relative importance of aquaculture *vis-a-vis* freshwater capture fisheries as a source of infection. Epidemiological studies would provide quantitative information in support of this activity.

- **Pond management systems.** Working in participation with producers, to identify, test and put into practice, cost effective means of controlling infection through improved management systems. These are likely to be based upon good farming and aquaculture practices.

- **Thermal resistance of parasites**. To carry out resistance trials on fish infested with parasites to determine survival rates under differing processing conditions. The information derived from this work will be used to assist processors in assessing hazards and risks both for local and export markets.

- **Public health policy**. The findings of the above activities will be used to assist partner countries in developing control strategies for prevention. At national level, the results will be used to inform such countries on assessment of risk. At the international level, results would be channeled into the Codex's Codes of Practice relating to products from aquaculture.

II. Disease-causing bacteria (pathogens):

Fish products in particular from aquaculture may be contaminated with pathogenic bacteria such as *Salmonella* and *Vibrio*. These are usually considered as a sign of poor standards of hygiene during handling and processsing and are associated with food borne illnesses. However, under particular circumstances, they may survive in warm water climates and become part of the natural environment. It is of great importance to minimize or eliminate their presence in fish products. Studies are required to distinguish between unavoidable contaminants from the environment and pathogens of human origin that may occur in products as a result of poor hygiene during post-harvest handling and processing and which can be avoided.

In support of the objectives of the regional programme, it is proposed to carry out the following activities:

- **Assess the role of different Salmonella serotypes (in particular *Salmonella weltevreden*) in fish products:** In order to better understand and control contamination of fish products, in particular aquaculture products, research activities should:

    a) determine and monitor national incidence, prevalence and distribution in humans and in farmed fish;
    b) develop outbreak surveillance programmes;
    c) conduct transmission studies to understand environmental source and contamination patterns;
    d) identify and evaluate control measures.

- **Understand the presence and importance of pathogenic and non-pathogenic Vibrios in fish products:** Not all strains of Vibrio cholerae are capable of causing cholera in humans. Since some strains can be part of the natural environment in tropical waters, there is a need to better understand and differentiate between the pathogenic and non-pathogenic strains of these microorganisms by:

    a) Study of the ecology of pathogenic and non-pathogenic strains in aquaculture environment;
    b) Better understanding the differences in pathogenic and non-pathogenic strains in terms of their survival capabilities; and
    c) Identification and evaluation of appropriate control and management measures.

The information derived from this work will be used to assist processors in assessing hazards and risks related to pathogenic micro-organisms of concern in the products of the region both for local and export markets. It will form the necessary basis for the application of Risk Analysis to fish production, enabling producers, regulatory agencies and exporters to develop effective preventive and control measures, which will promote the supply of safe fish products from the region.

III. Emerging safety hazards in aquaculture products:

Certain types of integrated fish farming systems, where livestock consume feed containing antibiotics, may pose a risk of antimicrobial resistance or unexpected residues in fish. Furthermore, emerging new pathogens of concern associated with intensive livestock production may become potential hazards in integrated fish production systems where such intensive livestock production is part of the system. The health implications of this type of artisanal production, combined with antimicrobial use, are poorly understood and more information is needed before a proper assessment can be made.

In support of the objectives of the regional programme, it is proposed to carry out the following activities:

- **Assess the impact of the use of livestock wastes in integrated aquaculture on:**

    a) antibiotic resistant bacteria in farmed fish;
    b) antibiotic resistant bacteria in the environment;

- **Study and evaluate the potential risk** posed by emerging food borne pathogens in the livestock sector to aquaculture products. The data derived from these studies will assist in the identification and prevention of potential emerging hazards in farmed products

Time Frame: The *ad hoc* Working Group of Experts will report to the 27th Session of APFIC.

Inputs:

- FAO through the Fish Utilization and Marketing Service (FIIU) will provide the coordination.
- WHO will provide technical inputs.
- APFIC will provide funding for the meetings of the *ad hoc* Working Group.
- National institutions will be expected to contribute technical expertise through national research programmes.

Outputs:

- Mitigation of public health impacts, reducing the burden of fish borne illness.
- Improved safety of fish supply, both for domestic consumption and export.
- Improved management strategies for the production of safe products from aquaculture introduced.
- Disseminated results of the programme through the Internet, particularly FAO/SIFARNET.

Financial Resources:

FAO (through HQ and RAP), together with WHO, will cover the costs of coordination of the programme.

The participating countries will be expected to commit resources to national research programmes and to their participation in this collaborative activity. The Support Unit for International Fisheries and Aquatic Research (SIFAR) will assist, if and when possible, in finding supporting donors.

APPENDIX A

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TECHNOLOGICAL APPROACHES TO UTILIZING BYCATCH IN LOW-COST PRODUCTS FOR HUMAN CONSUMPTION

by

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ABSTRACT

The paper reviews and evaluates technological approaches to the increased utilization of bycatches, particularly in the Asian region. It notes that a significant reduction has been achieved in wastage in the region by using bycatch species in a variety of conventional and novel products. These include: fish minces, fish jelly products, fish protein concentrates, fish hydrolysates and a range of fermented products.

It concludes by emphasizing the need for a market-driven approach to product development and the requirement to stimulate the search for novel raw materials as components of the products necessary to fill the emerging gap between supply and demand for fish.

INTRODUCTION

Bycatch is defined as the catch of non-target fish, whether kept for market or discarded at sea (Alverson *et al.*, 1994). Discarding is a natural consequence of the very nature of fishing. The direct reasons for discarding are:

biological - there is a mixture of species available in the fishing grounds;
technological - it is difficult to target specific fish for capture;
economic- the accidental capture of fish of no value to the fishermen; and
legal- in some fisheries, fishermen are limited as to the total quantities of fish of certain species that they can land (FAO 1997a).

The remainder is the 'landed catch' or 'retained catch' (i.e. that which is brought ashore). This can be further sub-divided into 'target catch' and 'incidental catch'. A particular species can move from one category to another, depending on size, market demand, season or other criteria. In the context of international fisheries statistics, it should be noted that 'target' and 'incidental' catches are recorded, while discards and other fishery-induced mortalities, which may be substantial, are generally not recorded (FAO, 1997b).

Available data for 1994-1995, suggest that a significant reduction in discards has occurred between the mid-80's and the mid-90's as a result of:

decline in the levels of fishing ;
time/area closures;
new or more selective harvest and utilization technologies;
greater utilization for human consumption and feed for aquaculture and livestock ;
enforcement of prohibition on discarding by some countries; and
a more progressive attitude of fisheries managers, user groups and society to the need to resolve problems resulting from discarding.

The magnitude of the decline has not been quantified, but indications are that the annual discard figure is now of the order of 20 million tons (FAO, 1997c)

Whilst elimination of discards altogether would be an unrealistic goal, the use of discards as a potential source of food will continue to play a role in the reduction of wastage. According to Alverson *et al.* (1994), bycatch from shrimp fisheries represent the single most important sector of the bycatch and discards from world fishing activities. It has been estimated that 11.2 million tons per year are generated world wide and of this 85 percent is discarded. Of particular concern is shrimp trawling in tropical waters, which produces high discard ratios (bycatch fish often outweigh shrimp by five and sometimes ten to one) and contributes a large volume of bycatch and discards. The location of many of these fisheries in waters adjacent to countries with low incomes and food deficits makes it imperative to utilize the discards for human food. Bycatches from shrimp and finfish trawlers are prevalent in South and Southeast Asia. In Southeast Asia, shrimp fisheries are the main contributors to bycatch and discards. The quantum of bycatch and discards for the Southeast Asian region was estimated at 3.4 million tons (Chee, 1997). Between 290 000 to 310 000 tons were discarded from 1992-1994 (Chee, 1997).

Before coastal mariculture was intensified, most bycatch was discarded at sea and only a small portion brought ashore. With the development of chilling systems for on-board storage and the greater demand for feed from aquaculture, much more of the bycatch is landed. Other portions of the bycatch are reduced to fishmeal. In addition, the processing of fish and fish-based products has created ready markets for bycatch, although the utilization of bycatch is still influenced by the location of the fishing grounds and ports, the socio-economic status of fishing communities and the available infrastructure. In poorly-developed areas, there may be no alternative but to discard the bycatch (Chee, 1997).

The inherent problems of bycatch are their extreme heterogeneity of composition, bony structure, dark flesh, small size, unattractive appearance and texture, strong flavour and the possible presence of toxic species. Many of the species are difficult to process by conventional techniques, and if processed, face poor marketability. Previous attempts at technology led utilization programmes have generally not led to long-term solutions especially with regards to economic viability. However, recent trends seem to indicate that, driven by increasing human population, gradual depletion of fish supplies from conventional sources, and the growth of aquaculture, increasing amounts of bycatch are now being used either directly as human food, in aquaculture or as animal feed (FAO, 1997b). The trend in recent years has, therefore, been towards greater utilization with previously-discarded fish species entering the food chain as 'incidental' catch.

This review focuses on the more-recent technologies that have been developed to utilize bycatch in low-cost products for human consumption in Asia, in particular, Southeast Asia.

MINCED MEAT RECOVERY

Due to the great variety and small sizes of fish caught as bycatch, rendering standardized processing and marketing difficult, recovery of meat by mechanical methods is perhaps one of the more viable means of utilizing many underutilized species for human consumption (Flick *et al.*, 1990). For this purpose, a wide range of deboners are available. A common type is the belt and drum model (Wood and King, 1982; Agarwal *et al.*, 1986), where the headed and gutted fish pass between a counter-rotating belt and a perforated drum. Another machine used is the stamp-type deboner, in which the fish is compressed against a perforated steel plate (Ghadi *et al.*, 1976; Suwanrangsi, 1987). The feasibility of deboning several low-value species and using the mince for product development has been reviewed by Grantham (1981), Gopakumar (1987) and Burt *et al.* (1992).

The minced meat obtained after deboning is more unstable than intact fish muscle, (Grantham, 1981; Babbitt, 1986) and must therefore be processed immediately or frozen. Depending on the type of fish,

minces can be stored frozen for up to 8-9 months without appreciable quality deterioration (Revankar *et al.*, 1981; Agarwal *et al.*, 1986). Ghadi and Lewis (1977) found that tripolyphosphate and sodium chloride helped maintain mince quality during frozen storage. Ghosh, Ghadi And Lewis (1977) reported that mechanical deboning did not affect oil emulsification and protein extractability of minces from a variety of low-value fish, including anchovies, croaker, eel, shark and sole. Washing and addition of benzoate-phosphate extended the shelf life of mechanically-deboned bycatch mince in Thailand (Jantawat and Yamprayoon, 1990) for up to 13 days at 0°C.

Chakrabarti (1993) reported using dense media with specific gravities of 1.217-1.220 to efficiently separate meat and bone from small fish, small shrimps (Chakrabarti, 1989) and small crabs (Chakrabarti, 1988). The recovered meat from small fish contained considerably less bone than meat obtained using a conventional deboner. Propionic acid helped to control mould growth and prolonged shelf life for up to 3 to 4 months at ambient temperatures (34 ± 6°C). Processing costs depend mainly on the cost of energy for boiling and the successful recycling of media. However, this method remains largely untested. A code of practice for minced fish production has been provided by FAO (1983).

APPLICATION OF FISH MINCE

Conventional Products

Conventional products that can be manufactured from fish mince include sausages, pastes, patties, balls, wafers, loaves, burgers, fingers, fritters and pickled products. Processes for development of these products have been summarized by Setty (1987), Regier and Raizin (1988) and Gopakumar (1987). Highly-acceptable cutlets from minces of threadfin bream, lizard fish, jew fish and other low-cost fish were prepared by Joseph *et al.* (1984) at the Central Institute of Fisheries Technology, Cochin, India. The development of fish cake has been discussed by Rao (1981). Durairaj and Pichiah (1985) and Venkatanarasimha and Chidambaram (1987) have developed quick-salted fish cakes from sea bream and ribbon fish. Basu *et al.* (1987) developed intermediate-moisture fish cakes from fish mince by adding different humectants. Sankar *et al.* (1992) prepared semi-dried fish cakes from dhoma at 7 percent and 10 percent salt levels. Shelf lives at ambient temperatures were 18 and 21 days respectively. Ramachandran and Solanki (1990) and Ramanchandran and Sankar (1997) studied the sorption behaviour of semi-dried dhoma (*Otolithus ruben*) at different relative humidities, in order to predict the shelf life of the product at different water activities.

Development of salted and dried minces from threadfin bream and Indian oil sardine has been reported by Sudhakaran and Sudhakara (1985). Preparation of fish fingers from croaker (*Sciaenid spp.*) and pink perch (*Nemipterus spp.*) consisted of extruding the mince in the presence of additives, dipping the 6-cm long pieces in batter, rolling in a breading mix, followed by frozen storage of the packaged products (Reddy *et al.*, 1990). Minced fish can be stabilized for room temperature storage after mixing with tapioca starch, soya protein and salt, followed by dehydration at 71-82°C (Venugopal and Shahidi, 1995). A process has been described for preparing restructured sardine meat involving washing the mince, mixing it with alginate, and then dialyzing in a calcium chloride solution (Nakayama *et al.*, 1988). Processing of smoked, dried and powdered sardine into instant soups has been described by Oh *et al.* (1988).

Etoh (1986) has used trash fish from shrimp bycatch in Bangladesh and developed nutritious fish burgers and fish fingers, which were favourably accepted. In Bangladesh also, shrimp bycatch was used to produce fish cakes, fish balls and highly-appreciated breaded fish burgers. Minced fish was handformed and spices such as dill and pepper added to the breading. The fishburgers were sold frozen to retailers who kept them in insulated boxes to be sold the same day for immediate preparation and consumption. The product retailed at 5 Taka for a 2-piece packet in Dhaka and Chittagong (Roessink, 1989). Mince from bycatch and/or trash fish normally sold off at very low prices can be formed into portions of various shapes and sizes, and battered and breaded to produce innovative value-added seafood products. Present market

trends reflect a rapidly-growing market for ready-to-eat convenience products and sophisticated equipment is not necessary for the production of battered and breaded fish products (Roessink, 1989). Ihm *et al.* (1992) developed precooked frozen burgers using sardine meat. Separated sardine meat was chopped and mixed with 14 percent emulsion curd, 8 percent bread crumbs, 3 percent soya protein, 1.5 percent salt, 2 percent sugar, 0.4 percent bicarbonate, 0.2 percent polyphosphate and spices. The seasoned sardine meat was fried in oil at 165°C for 3 min. Yu and Siah (1996; 1997) used trevally and unicorn leatherjacket for battered and breaded burgers from surimi developed from the two species. Feedback from food caterers was favourable.

Convenience food were processed using bycatch in the Philippines (Marfori *et al.*, 1991). Fishballs, noodles and sausages with acceptable sensory qualities and good shelf lives were developed from twelve low-value species, which could be used to supplement the raw material shortage for these products. Ravishankar *et al.* (1993a; 1993b) developed sausages from the mince of Indian oil sardine (*Sardinella longiceps*). Washing the mince six times with water improved meat characteristics and sausage quality (Ravishankar *et al.*, 1993a). Addition of 0.5 percent sodium bicarbonate to the washwater further improved elasticity, gel strength and waterholding capacity of the sausages, (Ravishankar *et al.*, 1993b) by more effective removal of fat, pigments and water-soluble proteins. Fish noodles were also developed from lizard fish in Thailand (Phithakpol *et al.*, 1986). The protein content of the noodles was increased by about 28 percent by incorporation of 25 percent and 30 percent fish into the formulation.

Fish satay is another bycatch product developed from lizard fish (*Saurida spp.*) in Thailand (Pruthiarenun, 1986; Suwanrangsi, 1986; Suwanrangsi, 1988). The fish was deboned, mixed with salt, sugar, flour and sesame seeds, spread into sheets, dried for 4-5 hrs, and then fried. This cheap high-protein snack can be kept at ambient temperatures for at least 5 months, and is marketed locally and exported. Chasanah *et al.* (1986) in Indonesia found that, if properly handled, shrimp bycatch can be processed into fish satay. Yellow goatfish (*Upeneus sulphureus*), a trawl bycatch normally used for fertilizer or animal feed in Malaysia, can be successfully converted to satay fish (Atan and Mohamad, 1986; Bakar, 1987), now extensively sold in the market. A satay-like product has been prepared from Bombay duck (*Harpodon nehereus*), by alternatively pressing the fillets and drying, resulting in a product with 88.5 percent protein and a 6- month shelf life at ambient temperatures. (Doke *et al.*, 1996).

Sophanodora (*pers. comm.* 1997) developed fish satay from *S. leptolepis*, a low-value fish in Southern Thailand. The butterfly fillet was baked or fried. The baked product used raw fish at 40 percent moisture content, which was pressed between drum rollers and dipped in seasoning before baking at 150°C for 80 min. To produce the fried product, the dehydrated and pressed fillets were deep fried in oil at 220°C for 45 seconds, then dipped in seasoning and baked at 150°C for 25 min. The mixture design method of Earle and Anderson (1985) was used to develop the formulations for the seasonings. 83 percent and 89 percent of consumers accepted the baked and fried satay respectively. Products were packed in high-density polyethylene and polypropylene. There were no significant differences in chemical, microbiological or sensory characteristics at up to 5 weeks for baked satay and 8 weeks for fried satay.

Small fishes such as ponyfish (*Leiognathus spp.*), jack (*Caranx spp.*) and sardines (*Sardinella spp.*) have been used for fish paste (Saisithi, 1987; Choorit *et al.*, 1991). Fish biscuits were successfully developed from *Decapterus russelli* (Yu and Kaur, 1992). Two types of fish mince (water-heated and oven-dried) and three levels of fish (8 percent, 16 percent and 24 percent) were used in the formulations. The most-acceptable formulation was obtained using 16 percent of water-heated fish mince. The product had a long shelf life at ambient temperatures (28°C ± 4°C) and large-scale sensory evaluation trials showed good response.

Fish Jelly Products

Traditional fish jelly products, such as fishballs and fishcakes, have been part of the diet of the countries in Asia for decades. They are usually produced in cottage industries using fresh fish. However, with the advent of refrigeration and the increasing demand for these products, many large factories are now producing surimi, the raw material for fish jelly products. Surimi is the minced, washed fish meat to which cryoprotectants are added before freezing. The price varies with fish supply. To date, much of the demand for fish jelly products is still met by small and medium-scale industries, who depend on the day's catch or use frozen-thawed whole fish.

Work directed towards the use of bycatch species for fish jelly products in Southeast Asia, has been undertaken largely by the Marine Fisheries Research Department (MFRD) in Singapore (Poon *et al.*, 1981; Tan *et al.* 1981, 1982 and 1987), and the Department of Fisheries, Thailand (Saisithi, 1981; Suwanrangsi and Kiatkungwalkrai, 1983 and Pruthiarenun, 1985).

In initiating the project on the utilization of bycatch as raw material for the production of fish jelly products in Southeast Asia, the MFRD introduced several basic technological concepts:

the use of a mechanical deboner- this is essential for bulk processing the small sizes of fish, often bony and difficult to head and gut;

leaching of mince meat twice with 4-5 times its volume of iced water (w/w), initially with 0.2 percent salt, followed by 0.3 percent salt. This confers several advantages;

i. eliminates components that interfere with gel formation and makes it possible to utilize not only a wider range of fish species but also raw materials that are not fresh. Cheap and abundant fish resources can now be processed into good-quality fish jelly products (Poon *et al.*, 1981),
ii. whitens the product by removal of blood pigments and kidney tissues,
iii. removes the fishy odour by leaching out the fat components and odoriferous materials, taste can then be adjusted to suit local preferences,

double-step heating, at 40°C for 20-40 min, followed by cooking at 90-95°C for 20 min, determining the optimum conditions (temperature and time) for 'setting' if potential gel strength of the raw material is to be realized. This is especially important because the proteins of tropical fish behave rather differently from those of temperate origin. Traditionally, the fishballs are soaked in tap water (28-30°C) for 2-3 hr before boiling or frying. This time can be reduced to 20-40 min if a temperature of 40°C is used.

Pruthiarenun *et al.* (1985) and Pruthiarenun (1986) sought to produce fishballs from the large quantities of bycatch in Thailand (Pruthiarenun, 1985), generally, used for fishmeal, to supplement supplies required for fishball manufacture. Threadfin bream (*Nemipteridae*), sole (*Synodontidae*), flat-head, (*Platycephalidae*), ponyfish (*Leiognathidae)*, and goatfish (*Mullidae*) were used. Substitution using minced bycatch for fishballs, at a maximum rate of 50 percent and 75 percent for pelagic and demersal species was possible.

In Malaysia, fishballs and fishcakes have been produced from yellow goatfish (*U. sulphureus*) long-finned silver biddy (*Pentaplion longimahus*) and large-scaled branded gunter (*Eutherapa therapa*) (Atan and Mohamad, 1986). Yu (1993) successfully substituted *Decapterus russelli* up to a level of 60 percent in fish cake formulations. Yu and Senapi (1996) have used *Leiognathus equulus*, an abundant low-value specie in Malaysia, for fishball processing. Although its use is limited by its weak gel strength, *L. equulus* can be incorporated at up to 15 percent in the formulation without affecting product quality. Acceptable fishballs were also developed from low-value fish in Bangladesh (Etoh, 1986), the Philippines (Marfori *et al.*, 1991) and from shark meat in Indonesia (Nasran *et al.*, 1986). Irianto *et al.* (1995) used a 4 step washing treatment and various spices to reduce and mask undesirable odour and flavour due to urea and

ammonia in fishcakes produced from Cawtail ray (*Tryygon sephen*). Results showed that a good product can be produced by the addition of 5 percent tapioca flour and 0.4 percent sodium tripolyphosphate. Gopakumar *et al.* (1992) and Muraleedharan and Gopakumar (1997) studied the feasibility of using trawl bycatch in India. Species used include *Saurida tumbil, Johnius dussumieri, Sphyraena spp., Trichiurus lepturus, Scomberoides lysan, Megalaspis cordyla* and *U. vittatus*. Generally, all species were suitable for surimi. Fatty fish yielded darker-coloured surimi with softer gel characteristics. Jasmine *et al.* (1995) at the Fisheries College and Research Institute, Tuticorin, India, used *N. bleekeri,* a trawl bycatch for fish mince. Cryoprotectants sorbitol (4 percent w/w) and ascorbic acid (0.1 percent w/w) effectively prevented protein denaturation during storage at -20°C for 6 months and resulted in products with better gel strength and sensory qualities.

Of the many bycatch species available in Thailand, threadfin bream is in most demand for fish jelly products, i.e. *N. japonicus* and *N. hexoden* (Suwanrangsi, 1988). In response to shortages and price surges of Alaskan pollock (*Theragra chalcogramma)*, the traditional raw material for surimi and fish jelly products manufacture, the Japanese surimi industry has successfully diversified into the use of tropical fish for raw material (Kano, 1992). One of the advantages in using tropical fish species is the stability of their proteins, especially the myofibrillar proteins responsible for gel formation. Alaskan pollock very rapidly loses its gel-forming ability and cannot be used for surimi after 3 months at -25°C (Okada, 1996). If the temperature rises above 15°C during surimi processing, Alaskan pollock gels became fragile and poor, but that of threadfin bream remains strong. Itoyori or threadfin bream surimi is a commercial success, and has been growing in terms of quantity and value. Thai itoyori is regarded in Japan as amongst the highest in quality.

There are now 14 surimi factories in Thailand with a total production of 60,000 tons per year with 90 percent exported to Japan and South Korea. These factories produce surimi from threadfin bream, big-eye snapper and croaker. Some factories also produce lizard fish surimi but these are generally of lower quality, as the gel-forming ability of lizard fish decreases sharply during storage (Yasui *et al.*, 1987; Whitehead, 1995) and it must be processed quickly. Ng *et al.* (1996) found that the formaldehyde level of lizard fish must be less than 15 parts per million to produce best-quality surimi. A two-time leaching in 0.2 percent sodium pyrophosphate enhanced gel-forming ability of lizard fish.

Many processors have also started to use chilled-leached meat from threadfin bream and big-eye snapper for fishball manufacture. 2-3 tons of chilled-leached meat is produced by a Singaporean manufacturer for daily distribution to fishball processors (Tan, 1997). Fishballs produced from chilled-leached meat have better gel strength than those produced from frozen surimi. Chilled-leached meat however, does not have a long shelf and must be processed quickly.

Bycatch is now a supplementary, and in some cases, has become the main source of raw material for the fishball processing industry. Species widely used for frozen surimi include threadfin bream (*Nemipterus spp.*), big-eye snapper (*Priacanthus spp.*), barracuda (*Sphypaena spp.*) and croaker (*Sciaena spp.*), all of which are found in bycatches from the shrimp trawlers in Southeast Asia (Putro, 1989).

Fish Protein Concentrates

Fish protein concentrate (FPC) was one of the earlier products developed from low-cost fish protein for human consumption. There are two types of FPC. Type A is colourless and odourless, with less than 1 percent fat and is produced by solvent extraction. It has poor functionality, may contain residual solvents and suffers from high preparation costs (Finch, 1977). FPC type B is prepared by drying and grinding fish mince, and therefore has a fishy odour due to the presence of higher amounts of lipids. The product has seen some success as a dietary supplement in several countries (Tagle *et al.*, 1977).

Moorjani (1977) developed odourless fish protein concentrate, partially-deodorized and defatted fish and soluble fish proteins from low-value fish. Although insoluble in water, the FPC contained 80 percent protein and could be incorporated as a food supplement. Treatment with ammonia solubilized

the proteins which could then be used for beverage-like preparations. Chen (1981) prepared FPC from three underutilized species in China: filefish (*Navodon modestus*), anglerfish (*Lophiomus litulon*) and undersized hairtail (*Trichiurus haumela*) by solvent extraction. The FPC was effective in treating infant malnutrition. Chaaya *et al.* (1982) reported development of protein concentrate tablets from low-cost fish meat. The authors reported health benefits as a result of administering the tablets. Espejo-Hermes *et al.* (1981) and Orejana (1983) have developed FPC (Type B) from low-cost fish, using appropriate technology, suitable for small-scale industry. The FPC was successfully incorporated into two local products and could be made into tablets and capsules (Orejana *et al.*, 1985), using a locally-fabricated manual tablet machine and capsule filler.

Yamprayoon and Kiatkungwalkrai (1983) improved the sandy texture and water absorbance of FPC type B prepared from trash fish. Three kinds of FPC were produced from dried-ground-whole fish, dried-ground-smoked-whole fish and dried-ground-smoked-headed fish. All the FPC's fulfilled the minimum protein requirement of 60 percent and were found to be acceptable when incorporated into Thai recipes. Pruthiarenun (1986) used sorted bycatch to produce affordable FPC Type B, with high protein content. The approximate yield was 1kg FPC from 46 kg of bycatch. Pruthiarenun and Srisansanee (1987) and Pruthiarenun (1990) showed that sardine protein powder and sardine FPC (type B) were stable for up to a year's storage at ambient temperatures in low-density polyethylene. Fish powder incorporated into nine supplementary food formulations for pre-school and school children were highly acceptable (Charoenphol *et al.*, 1988). Pruthiarenun (1990) improved the quality of the FPC Type B produced by Yamprayoon and Kiatkungwalkrai (1983) by separation of bone before cooking and recombination of the meat and bone after dehydration. The product had better texture, odour, taste, appearance and improved rehydration characteristics.

Fish powder from whole bycatch marine fish was used to produce fish crispies (Charoenphol *et al.*, 1992; Charoenphol and Suanpan, 1992). A high-protein (20 percent), high-calcium (821mg. 100 g^{-1}) snack was developed. They suggested that the product could be an alternative source of protein and calcium for consumers allergic to dairy products. Doke *et al.* (1992) spray-dried *Scoliodon laticandus* with 0.1 percent butyl hydroxyanisole and obtained a colourless, odourless and stable fish powder, compared to the conventional brown fish powder produced by air drying.

Fish Protein Hydrolysates

Development of FPC represented one of the first concerted efforts to increase the use and the value of underutilized fish by converting it into a more-readily acceptable form. However, the FPC produced by these technologies were deficient in some functional properties and their cost of production was high. Recognizing this problem, subsequent investigations have aimed at improving functional properties of FPC by modifying the parameters of extraction and by employing enzymes, acid or alkali, to hydrolyse the proteins.

Venugopal and Lewis (1981) isolated odourless proteins with high-organoleptic ratings from croaker (*J dussumieri*), anchovy (*Trissocles spp.*) and bombay duck (*H. nehereus*). Whole fish was cooked in 0.3 percent orthophosphoric acid, the separated meat was pressed to remove moisture and odoriferous compounds and then hydrolysed using enzyme, acid or alkali. The protein extract was further deodorized by steaming for one hour and then drying. Ghadi *et al.* (1987) used deboned meat from dhoma for hydrolysis with papain at 55°C for 2 hr to obtain a product containing 90 percent protein. Ghosh *et al.* (1992) hydrolysed trash fish proteins using *Bacillus megaterium* cells immobilized in a wood matrix for repeated usage. Mahesh *et al.* (1993) produced functional fish protein hydrolysate from *N. japonicus* using papain for partial hydrolysis, followed by drying. The hydrolysate exhibited better functional and sensory properties compared to conventionally-prepared FPC. Anchovy (*Engraulis japonicus*) was partially and exhaustively hydrolysed using a proteolytic enzyme from *Bacillus subtilis* (Wang *et al.* 1996);. The nonbitter partial hydrolysate (31.86 percent degree of hydrolysis) can be used as a seafood flavouring. A similar product produced by exhaustive hydrolysis (68.66 percent degree of hydrolysis) was high in protein

content and can be used as a protein supplement. The authors estimated that the process can be utilized for the large reserves of *E. japonicus*, an underutilized fatty fish found in the East Sea of China, the Yellow Sea and parts of the Western Pacific, where the anchovy catch can reach upto 500 000 tons a year. Proteins from *Liza subviridis* were hydrolysed using Alcalase 2.5 L (Yu and Ahmad, 1997). The hydrolysate has good water-absorption properties and emulsifying capacity increased with degree of hydrolysis. The occurrence of bitterness (due to a high hydrophobic amino acid content) in fish protein hydrolysates limits its use in human food. Chakrabarti (1995) used ethyl alcohol to remove bitter components from low-value fish hydrolysates. The alcohol was subsequently removed by vacuum drying at 50°C. Wang *et al.* (1996) used active carbon to absorb bitter peptides and masking with β-cyclodextrin. The end product was free from bitterness.

Fermented Products

Fermentation of fish has been used extensively in Southeast Asia as well as Korea, Japan and China, for the preparation of flavoured products, since it offers a simple and low-cost method of preservation. Fermentation products from fish can be classified into two types: fish and salt formulations (high-salt enzyme hydrolysed) e.g. fish sauce, 'nam-pla' in Thailand, and fish, salt and carbohydrate mixtures (low-salt lactic acid bacteria LAB fermented) e.g. 'burong isda' from the Philippines (Owens and Mendoza, 1985).

Velayudham *et al.* (1987) produced fish sauce from silver belly, salted at a ratio of 1:4 at 50°C. Subasinghe *et al.* (1990) used *Amblygaster sirm* for fish sauce as a means of preserving seasonal gluts of small pelagic species which constitute up to 50 percent of total fish landings in Sri Lanka. Caranx, sardine and horse mackerel were found to be suitable for the preparation of 'nam-pla', a Thai fish sauce (Rattagool, P. 1997, *pers. comm.*). Protein solubilization and therefore fermentation rate was faster using minced instead of whole fish. Addition of *Pediococcus halophilus* and 2 percent glucose to 22 percent-salted *Stolephorus commersonii* sauce hastened fermentation time by 20 percent. The sauce produced had acceptable colour and flavour.

Fish sauce production can be take up to 12 months from salting to maturation. Much research has been carried out to hasten the fermentation process by (a) increasing the temperature (Mabesa *et al.*, 1990; Mabesa and Babaan, 1993) and (b) using enzymes (Mabesa *et al.*, 1983; Bigueras *et al.*, 1986; Thongthai and Srisutipruti, 1990; Kumalaningsih, 1990; Mheen, 1993; Putro, 1993). Using *D. macrosoma*, Mabesa *et al.*, (1990) used artificial agitation and/or aeration in addition to heating at 45-50°C for 4 hr a day for 10 days, after an ageing period of about a month. Fish sauce with quality comparable to tra ɔnally manufactured sauce could be obtained in about 2 months or less under certain conditions. In a ion, a 10 percent increase in protein and total yield was achieved. Thongthai and Siriwongpairat (1990) found that fish sauce produced outdoors had a better aroma than that produced in indoors vats. This was associated with a higher temperature, higher salinity and a more rapid penetration of salt into the fish tissues and a lower brine pH. Fermentation time was also shorter. A rapid process utilizing proteases sourced from plants, animals and microbes has been applied industrially in Thailand (Thongthai and Srisutipruti, 1990).

LAB fermentation of fish-carbohydrate mixtures in the presence of small amounts of salt (6-10 percent) provides possibilities for developing a number of products from underutilized fish. Lactic-acid fermented products can be produced in a shorter time (and hence more cheaply) than fish/salt products which depend primarily on autolytic processes. Their lower-salt contents also permit them to be consumed as a main course, rather than as a condiment as in high-salt fish sauces and pastes.

Abraham *et al.*, (1995) used *Leiognathus spp.* for lactic fermentation by *Lactobacillus plantarum.* They found that addition of 4 percent (w/w) glucose or sucrose accelerated fermentation rate. Olympia and Salazar (1997, *pers. comm.*) used *S. longiceps, D.kurroides, Auxis thazard* and *T. haumela* for 'burong isda', a lactic-acid fermented fish-rice product in the Philippines, usually produced from freshwater species and only available in inland areas (Olympia *et al.*, 1995). All species resulted in acceptable products.

Except for *T. haumela,* all the marine species used were histidine-containing ones. However, the production of histamine during fermentation was found to be below the safety limit of 20 mg per 100g. Further tests demonstrated the probability of an *in situ* inhibition of the histamine-producing bacteria by the LAB present. The study also showed that 'burong isda' can be packed in natural casings instead of the usual bulky bottles. They can then be stored chilled or frozen, thus improving marketability. LAB-fermented products could also be produced from bullet tuna (*A. rochii*), mackerel (*Rastrelliger spp*) moonfish (*Mene maculata*), common slipmouth (*L. equulus*), big-eye scad (*C. crumenopthalmus*) and goatfish (*Upeneus spp*) (Olympia, M. 1997. *pers. comm.*). Although these products were judged to be different to the traditional 'bagoong', a fish paste consumed in the Philippines, most panelists agreed that they would be acceptable as an accompaniment to rice. 'Somfag', a Thai-fermented fish, usually produced from freshwater species, was successfully prepared using *Sphyraena abstusata* (Chanthachum and Kimhamanon, 1997, *pers. comm.*). A 60 hr fermentation period resulted in the most-acceptable product, with a pink colour, firm and elastic texture and acceptable odour and flavour. 'Pla-ra' a Thai salt-fish-carbohydrate mixture, could also be produced from anchovy (*S. commersonii*) at a salt concentration of 20 percent and a carbohydrate level of 10 percent, yielding a product with good organoleptic quality (Rattagool, P., 1997, *pers. comm.*)

THE FUTURE

It is obvious that many technological solutions are available for the production of bycatch products for human consumption. Although earlier attempts at technology-led initiatives have generally not provided long-term solutions, particularly with regards to economic viability, recent trends do indicate that increasing amounts of bycatch are now being used as human food, instead of as animal feed.

Apart from solutions offered through fisheries management, such as gear-based selectivity and regulatory-based controls (Alverson *et al.*, 1994), technological approaches can be one of the solutions through which bycatch can be effectively utilized. However, finding a technological solution may not be sufficient in itself. Firstly, although the raw material may be low cost, any form of processing will inevitably add to the cost of production and may not add sufficient value to cover these costs. Any processing involved therefore must contribute minimum added costs. Secondly, since food habits are among the most difficult of all to change in any community, one of the requirements that precedes value-added product development is to know more about the factors that influence consumer acceptance and their relative importance in different societies and under different living conditions i.e. the social, cultural and economic variables that influence consumer attitudes, preferences and consumption. Such knowledge is necessary to design successful approaches to fish promotion, product development and marketing.

This market-driven approach will reflect the needs of the target groups and if programmes and projects are to be increasingly market-driven, there should be greater emphasis on a systems approach, identifying key constraints in the system and subsequently planning research and development activities around these constraints. Technological solutions therefore should be part and parcel of an overall strategy to improve bycatch utilization:

1. evaluate resources and markets;
2. identify and develop products acceptable to potential markets;
3. promote products;
4. monitor handling and processing with respect to product safety and quality; and
5. ensure that adequate standards and specifications are met.

This is all the more imperative as demand for protein escalates. Much of the future increase in demand will come from developing countries, particularly those of Asia (James, 1988). Fish resources in the Asian region are in short supply and as traditional food fish become increasingly under pressure, more non-traditional species in the bycatch will be taken into the food chain. Ultimately, this would result in

more food available for human consumption, reduction of fish prices to the consumer, reduction of pressure on existing fish stocks, reduction of wastes entering the environment and increased incomes to industry.

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UTILIZATION OF BYCATCHES AND LOW-VALUE FISH IN INDIA

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ABSTRACT

India has a coastline of 8 041 km with an annual marine fish landing of 2.97 million tonnes (1997). A total of 238 128 fishing craft are exploiting the marine resources of India and they land a large quantity of bycatch and low-value fishes. The percentage of bycatch varies from 70 to 80 depending upon the seasons. In recent times a sizable amount of low value fish and bycatch are brought ashore by traditional fishing crafts and medium sized fishing vessels due to the economics of fishing operations. In addition market for these fish is expanding. Bycatches are generally used for curing and manufacture of fish meal but those of better quality are also used for surimi production and export as frozen fish.

Bycatch is an excellent raw material for production of minced fish which can be exported and also converted into various value added products like hydrolysates, fish soup powder, fish flakes, moulded products etc. This paper describes in detail the methods of production of several value added products for the economic utilization of low-value fish, particularly the bycatches.

BACKGROUND

India has a coastline of 8041 km with an Exclusive Economic Zone of 2.02 million km^2. The estimated marine potential resource of the country is 3.9 million tonnes (m t). The current level of exploitation (1997) is 2.97 m t. The annual fish production of the country is 5.35 m t with 2.38 m t coming from the inland sector.

A total of 238 128 fishing craft are operational in the Indian waters. Of these 46 918 are mechanised boats, 31 726 motorized traditional craft and 191 207 traditional craft (Anon 1996). The country exported 378,199 mt of marine products earning foreign exchange of around US $ 1 billion (1997). In the field of aquafarming India ranks second in the world and in total fish production sixth in the world. Various figures concerning fisheries of India are given in Tables 1-7.

INTRODUCTION

Bycatches and low value fish that are discarded at sea are subjects of global concern. The bycatches from shrimp trawlers have been an issue of concern and investigation in many tropical countries like India for more than 40 years. This issue has been highlighted in several U.N. resolutions and conferences particularly the International Conference on Sustainable Contribution to Food Security 1995, Kyoto Declaration and notably the U.N. Resolution (AIC.2149.1 50. Rev. 1), which emphasized the vital role of providing sustainable food supplies and livelihoods for present and future generations. This resolution while underlying the significance of the role of bycatches and discards as human food states that this issue **"Warrants serious attention by the international community and a continued and effective response is necessary to ensure the long term and sustainable development of fisheries".** But there are many imponderables on these bycatches or discards about their utilization as they are normally low-value fish. Some of them are listed below:

How economical is it for the vessel owners to preserve and bring their catch ashore?

The method to transport them to deeper interior markets to get better prices.

Improved methods of utilization to convert them to high value processed products.

Certainly discards are a threat to environments and habitats. Often this creates migration of some species. Although many attempts are made to reduce their quantities in fishing nets by exclusion devices they have met stiff resistance from fishers as these devices often reduce the amount of quality fish by around 10-15 percent. The problem is still open and needs global attention.

Table 1. Total fish production in India.

(in '000 tonnes)

Year	Marine	Inland	TOTAL
1950-51	53.4	21.8	75.2
1960-61	88.0	28.0	116.0
1970-71	108.6	67.0	175.6
1980-81	155.5	88.7	244.2
1990-91	230.0	153.6	383.6
1991-92	234.7	171.0	415.7
1992-93	257.6	178.9	436.5
1993-94	264.9	199.5	464.4
1994-95	269.2	209.7	478.9
1995-96	270.7	224.2	494.9
1996-97	296.7	238.1	534.8
1997-98 (Targeted)	292.5	243.8	536.3

Table 2. Investments in various Five Year Plans.

(Rs. in millions)

Plans	Total Plan Outlay	Outlay for Agri. & Allied Sectors	Outlay for Fisheries	% of Fisheries Outlay to	
				Total Outlay	Outlay for Agri. & Allied
I Plan	19 600	2 940	51.3	0.26	1.74
II Plan	46 000	5 290	122.6	0.27	2.32
III Plan	75 000	10 680	282.7	0.38	2.65
IV Plan	159 020	27 280	826.8	0.52	3.03
V Plan	393 220	43 020	1 512.4	0.38	3.52
VI Plan	975 000	66 090	3 711.4	0.38	5.62
VII Plan	1 800 000	105 240	5 465.4	0.30	5.19
VIII Plan	4 341 000	224 670	12 328.2	0.28	5.49
1997-98		15 350*	3 000.0*		

- Tentative allocation for central sector schemes only.

Table 3. Indian fishery resources at glance.

1. Length of the coast Line (Kms.):	8041
2. Exclusive Economic Zone (million km2.):	2.02
3. Continental Shelf Area ('000 km2.):	506
4. Length of Rivers, Canals (km.):	171334
5. Brackishwater Area ('000 ha.):	1422
6. Reservoirs Area ('000 ha.):	2050
7. Tanks & Ponds ('000 ha.):	2855
8. Beels, Oxbow & Derelict Waters ('000 ha.):	788
9. Marine Fish Potential (million tonnes):	3.90
10. Inland Fish Potential (Million Tonnes):	4.50
11. Fish Production (Million Tonnes) 1996-97:	
MARINE:	2.97
INLAND:	2.38
TOTAL:	5.35

Table 4. Market/item wise fish export from India during 1996-1997.

Market Wise			Item Wise		
Japan	Q : V :	64 656 18 860.4	Frozen Shrimp	Q : V :	105 429 27 017.9
European Union	Q : V :	71 236 7 906.9	Frozen Fish	Q : V :	172 627 6 329.5
South East Asia	Q : V :	197 405 8 792.3	Frozen Squid	Q : V :	40 924 2 904.5
U.S.A.	Q : V :	29 792 4 360.5	Frozen Cuttlefish	Q : V :	31 778 2 723.7
Others	Q : V :	15 110 1 293.5	Others	Q : V :	27 441 2 238.0
TOTAL	Q (Quantity- MT): V (Value - Rs. Million) :	378 199 4 121.36			

Table 5. Potential Resources Available, Level of Exploitation and the Potential Available for Exploitation Depth-wise within the Indian EEZ.

(in million tonnes)

Depth Range (m)	**Total**	**0-50**	**50-200**	**200-500**	**Oceanic**
Demersal	1.28	0.625	0.028	-	1.933
Neretic Pelagic	1.00	0.742	-	-	1.742
Oceanic Pelagic	-	-	-	0.246	0.246
Total **(%)**	**2.28** **(58)**	**1.367** **(35)**	**0.028** **(0.7)**	**0.246** **(6.3)**	**3.921**
Present Level of Exploitation*	2.08	0.63	Negligible	Negligible	2.71
Available for exploitation	0.20	0.737	0.028	0.246	1.211

* *1995-96*

Table 6. Estimated group-wise fishery potential of the Indian EEZ

(in '000 tonnes)

GROUPS	Up to 50 m	Beyond 50 m	Total
Elasmobranchs	65	103	168
Eels	7	-	7
Catfish	60	63	123
Oil Sardine	191	-	191
Other sardines	96	-	96
White bait	91	-	91
Other clupeids	157	14	171
Bombay duck	104	-	104
Lizard fish	27	21	48
Perches	114	145	239
Sciaenids	120	22	142
Ribbon fish	95	216	311
Carangids	143	304	447
Silver bellies	82	4	86
Pomfrets	42	12	54
Mackerel	162	62	224
Seer fish	42	-	42
Tunnies	37	242	279
Flat fish	38	-	38
Penaeid prawns	178	-	178
Non-Penaeid prawns	54	-	54
Cephalopods	50	21	71
Priacanthus spp.	-	55	55
Black ruff	-	9	9
Indian drift fish	-	7	7
Deep sea prawns	-	3	3
Deep sea lobster	-	5	5
Oceanic tuna	-	209	209
Bill fishes	-	4	4
Others	254	189	443
Total	**2.210**	**1 690**	**3 900**

Table 7. Fishing crafts in Maritime States/UTs during 1994-95.

State/UTs	Traditional Craft	Motorized Traditional Craft	Mechanized Boats	Total
GUJARAT	8370	4283	8365	21018
MAHARASHTRA	9702	286	7930	17918
KARNATAKA	11952	1189	3655	16796
KERALA	27873	12913	4206	44992
TAMIL NADU	26737	5340	8230	40307
ANDHRA PRADESH	54000	3269	8911	66180
ORISSA	7796	2453	1665	11914
WEST BENGAL	4091	270	1880	6241
LAKSHADWEEP	780	298	443	1521
A & N ISLANDS	1180	160	230	1570
PONDICHERRY	5900	365	553	6818
GOA	1100	900	850	2850
TOTAL	**159481**	**31726**	**46918**	**238125**

THE INDIAN CONTEXT

From traditional and motorized traditional crafts there are no discards now because all catches are consumed fresh locally. In many states like Kerala demand for fresh fish has become great because of large scale export of all good species by processing factories. Fishery operations have become highly competitive in view of the large number of craft operating in inshore waters, which become non-remunerative except in peak seasons. Many vessel owners find it difficult to break-even in view of the high labour charge and cost of maintenance of vessels. An impact of these problems forced the vessel owners to bring fish that were formerly discarded to the land and sell at available rates.

Another factor which contributed to the reduction of the discards is the recent export potential of these low priced fish. Fishes like ribbon fish, cat fish, sciaenids, jelly fish and threadfin bream, which were once considered discards, are now processed and exported to South-East Asian countries. The export of frozen fish from India showed a steady increase from 12.6 percent (1985-86) to 45.64 percent (1996-97). In 1996-97 India exported 173 005 m t of frozen fin fish valued at 6 369.2 million Indian Rupees. The south east Asian Countries offered the market for these low value fish like ribbon fish, threadfin bream, croakers etc. with a unit value realization around 0.8 US S (MPEDA 1998). South East Asian countries shared the major portion of fresh export from India (70.48 percent) underlying the significance of the vast export potential of these low-value fish.

Table 8. Growth of export of miscellaneous bycatches from trawls *(1996-97)*.

	Quantity (m. tons)	**Value (Million Rupees)**
Dried Products		
Jelly Fish	88	8.2
Octopus	32	0.7
Snail meat	532	40.4
Sea dragon/horse	9	1.9
Dried Fish	5918	156
Frozen Octopus	2952	133.5 (+402% growth)
Frozen Fish	173005	6392.2

QUALITY OF BYCATCHES AND DISCARDS

In a multispecies fishery, the concept of bycatch is often a misnomer. Even though shrimp trawlers target their operations on shrimp, substantial quantity of fin fish do occur in the catches. As per price realized at landing centres, these species are categorized into four groups:

Table 9. Quantity of bycatch -fish landed by shrimp trawlers in India*.

Category	Price range (per kg in Rs.)	Quantity (mt)	In relation to total production by trawlers. (%)
(i)	07 – 10	176 000	16
(ii)	10 – 20	561 000	50
(iii)	20 – 50	21 800	19
(iv)	> 50	169 000	15
TOTAL	-	**1 124 000**	**100**

* *Data : Courtesy, Director, Central Marine Fisheries Research Institute, Cochin, Figures average annual production for period 1992-96.*

Bycatch is handled well and processed quickly by local communities. In states like Kerala, Karnataka and Gujarat markets are available and much is used now by surimi plants. In states like Kerala about 70 percent is consumed as fresh fish. Gujarat is another good example where bycatch utilization is virtually 100 percent (Bostock, 1987).

In the Bay of Bengal, the situation is different. There is large variation in fleet size ranging from few days to 30 days of endurance at sea. Bycatch losses have been assessed by the Bay of Bengal Programme and according to their report (Bostock and Ryder 1995) bycatch losses are a function of the time spent at sea and type of vessel, the larger is the vessel, the greater is the discards. The estimated quantity of the discards are 100-300,000 m t from the east coast fleet (Visakapatanam and north to the Sandheads bank), and it was shown that great majority of this figure is derived from large multiday vessels of over 16 m, especially freezer trawlers over 20 m.

Table 10. Bycatch discards from India's East Coast Trawlers (Visakapatham).

Cate-gory	Type of Vessel	Gross tonnage of the vessel	Number of vessels	Voyage time(appro-ximate days)	Annual discards (tonnes)
1.	Double rig trawler of 20 m Length (freezer)	150	150	30 – 50	40-60,000
2.	Double rig trawler of 16-19 meters (mainly ice)	40 – 50	70	21	21-32,000
3.	"Sona" stern trawlers	20-25	70	15	14,000
4.	Mechanized fishing boats (10-11 m)	7-16	320	1-7	18,000
5.	Mechanized boats (Andhra Pradesh)	OAL 10-15	8911 *	1-7	NIL (Total utilization)

Data : Source 1-4: Gordon (1990) 5 (Ministry of Agriculture, Govt. of India).*

SPECIES AND SIZE RANGE

Category I. Shark, lizard fish, jelly fish, octopus, anchovies, threadfin bream, skates etc.

Category II. Sciaenids, catfishes, carangid, chirocentrus, barracuda, ribbon fishes, solefish, scombroids, clupeids, polynemids, tunas etc.

Category III. Flat fishes, perches, tunas, eels, pomfrets (small size), cods, etc.

Category IV. Pomfrets (big size), perches, seers.

Table 11. Protein content of some low-value species.

Common name	Scientific name	Moisture	Protein	Fat	Ash
Anchovy #	*Stolephorus spp.*	79.40	15.10	1.30	2.60
Barracuda	*Sphyraena barracuda*	76.50	21.28	0.64	1.51
Bombay duck *	*Harpodon nehereus*	88.50	7.50	2.00	1.50
Caranx	*Caranx sp.*	76.40	17.60	1.17	2.83
Catfish *	*Arius dussumieri*	75.00	18.00	1.4	1.50
Eel *	*Muraenesox spp.*	83.00	13.50	0.75	1.50
Japanese threadfin bream#	*Nemipterus japonicus*	74.90	18.10	0.76	3.12
Jew fish *	*Sciaena miles*	78.60	17.80	1.36	1.73
Mackerel	*Rastrelliger kanagurta*	71.19	21.21	7.51	1.33
Malabar sole	*Cynoglossus semifasciatus*	77.00	17.50	2.70	3.10
Perch *	*Epinephelus chlorostigma*	75.00	17.00	2.50	1.75
Reef filament threadfin bream	*Nemipterus mesoprion*	76.70	19.80	0.44	0.37
Ribbon fish	*Trichiurus savala*	74.46	22.66	0.42	1.96
Rock cod (juvenile)	*Epinephelus sp.*	67.40	24.30	0.30	2.60
Silver belly	*Leiognathus bindus*	74.70	18.70	0.88	1.97
Snappers #	*Lutjanus spp.*	73.00	20.80	1.40	1.87
Sole *	*Psettodes erumei*	76.50	15.50	1.25	1.75
Spotted butter-fish	*Scatophagus argus*	75.98	18.85	3.77	1.40
Carangids	*Caranx sp.*	77.09	20.97	0.38	1.56
White bait	*Anchoviella sp.*	77.98	18.50	0.46	2.06
Whiting	*Sillago sp.*	76.32	21.90	0.25	1.53

 # From east cost (Lat. 17° 40' E, Long. 83° 20' N)

** From Saurashtra Coast (Lat. 20° 55' E, Long. 70° 20' N)*

Other fishes from South west coast (Lat. 9° 57' E, Long 76° 15' N)

LANDING, PREPROCESS HANDLING AND TRANSPORT

Currently, both the traditional and mechanized sectors do not preserve these bycatches. Normally in vessels of above 18 m OAL, having longer endurance at sea, quality fish are sorted out and stored in the chilled stores and the rest discarded. During the last phases of fishing, depending upon storage capacity, the rest of the miscellaneous fish are also kept. Smaller vessels of OAL 15 m and below usually go for one to three days of fishing. Only shrimps are kept in ice and trash fish is not preserved. In fact, they are brought as such. Most of these species are used for either drying or manufacture of fish meal. Spoilage rates can be around 30 percent or

above. This is one reason that fish meal produced in India is not of the quality suitable for shrimp feed. This type of fish meal is chiefly used as poultry feed.

Some of the problems associated with bycatch handling on board and on shore are the following :

1. Limited ice storage capacity in fishing vessel.

2. Labour associated with sorting these species. The fishing crew seldom get time to do this work on board the vessel.

3. Low market price at landing places where the fish is usually auctioned. As middlemen involved work in groups during auction, price realisation is very poor.

4. Contamination of shrimp by low-quality fish is not now appreciated by shrimp processors due to implementation of HACCP.

PROCESSING AND UTILIZATION OF BYCATCHES

Traditional

Salting and curing

This is still the most popular method of processing bycatches. In fact about 60 percent of bycatches are utilized by this method. The fish is heavily salted in the ratio fish to ice 1:1, 1:0.5 etc. depending on the quantity of fish and salt available. In peak seasons, when fish landings are heavy, less salt is used as salt becomes expensive. Big fish are gutted and split open and salted. Fish like sardines, shark etc. are normally kept immersed in saturated brine solution and then sun-dried. Nowadays consumers generally prefer low-salted fish. Price of fish used for drying varies from Rs.1 to 5/kg. and the market price of dried fish varies from Rs.10 to 20 per kg.

Fish meal

In places like Bombay, Veraval, Visakapatnam and Mangalore, modern fish meal plants are operational and convert bycatches by wet-reduction into quality fish meal.

But there is a traditional fish meal industry which is run very profitably. The marginally spoiled as well as lean fresh fish are dried in the open beach and then pulverized in a mill without further processing. Usually sardines, anchovies and other trash fish are used in this process. Quality of this type of fish meal is poor and sand content can be very high, sometimes up to 7 percent.

Improved methods of utilization:

Although bycatches are rated as low value fish, they are high value fish in terms of nutritional quality. They contain a high amount of valuable proteins ranging from 13 to 21 g/100 g wet fish (Gopakumar, 1997). Fish plays a major role in human protein nutrition by supplying over one third of the total animal protein intake, Thailand (41.1 percent of total animal proteins); Bangladesh (46.7 percent); Japan (47.0 percent); Philippines (51.5 percent); Sri Lanka (51.5 percent); Indonesia (53.5 percent) and Korea DPR (65.2 percent) (Laureti, 1996). Most of these fresh fish are also a rich source of n_3 polyunsaturated fatty acids (Gopakumar 1997). Because of the ready availability, low-cost and nutritional quality it is very important that better utilization of these valuable bycatches be given global attention. Some of the products which can be manufactured commercially and also on a small-scale are described below:

Minced fish

Most species of bycatches can be converted to minced fish by the use of deboning machine of the type Bader 694. Yield of deboned meat varies from 35 to 50% based on type of fish used (Table 12). It is used for the preparation of several value added products like surimi, kamaboko, sausages etc. The separation process is based on physical squeezing of the split fish between a perforated drum and rubber belt. One of the disadvantages of the minced fish is that it often contains small bones, scales and skin as contaminants. Minced fish is unstable and cannot be kept unprocessed for long and can also be contaminated during production if handling practices are not sound. Minced meat is also highly susceptible to microbial, enzymatic and oxidative reactions. Despite many inherent limitations, minced fish has become the most accepted way of utilizing bycatches of low-value fish and pelagics.

Product from minced fish

Block-frozen fish mince.

Fish mince can be pressed into 500g and one kg. blocks and then quick frozen at –40°C. The blocks are very susceptible to freeze dehydration. Hence, it is imperative that they have be glazed with water by dipping in ice-cold water after freezing. When ideally packed in cartons it is an excellent consumer product since a house-wife can prepare a variety of products out of these block-frozen mince. In India block frozen fish mince is a well accepted consumer product and the integrated Fisheries Project at Cochin has established a good market for this product.

Texturized or Moulded products :

Fish mince is an excellent raw material for several reformed products like fish balls, fish burgers, fish fingers, etc. The mince is washed two to three times with water and mixed with polyphosphate (sodium salt) 0.1 percent by weight, sodium chloride (0.1 percent w/w) and starch (0.1 to 0.2 percent w/w). The whole meat is tumbled or macerated. The dough is then shaped to blocks or desired shapes. The blocks are then frozen at –40°C and kept stored at –30°C. They can be cut to different shapes in the form of fingers, cakes etc, battered and breaded and fried in vegetable oil. Seasoning and spices can be added to impart desired flavour and taste. Fish fingers made out of mince may not have the properties of fingers prepared from whole fish fillet, but fingers prepared from minced meat are comparable for most sensory properties like taste and flavour. However, being much cheaper, they offer better market prospects. This is also one of the best-ways to use bycatches as fish fingers are a high value item in western markets. By improving the processing and texturization the product quality can be considerably improved. In fact this is an area of research in food science which needs prioritization.

Table 12. Yield of mince and waste from different species of fish.

Fish	Average	Average length cm	Head + Viscera %	Mince %	Waste after mincing %	Total waste * %
Barracuda *(Sphyraena sp.)*	90.9	22.5	25.4	43.0	30.1	55.5
Thirian *(Decapterus russeli)*	35.5	15.0	37.7	43.3	23.1	53.8
Cat fish *(Tachysurus sp.)*	91.8	20.4	48.0	30.1	21.6	69.6
Goat fish *(Upeneus taeniopterus)*	56.2	15.7	33.3	44.4	20.0	53.3
Caranx compressus	181	25.4	36.0	40.2	23.0	59.0
Caranx malabaricus	105	19.2	47.6	24.3	28.2	75.8
Horse mackerel *(Megalapsis cordyla)*	110	23.2	32.9	29.5	37.5	70.4
Jew fish *(Johnius dissumeiri)*	65.5	17.4	37.3	30.8	32.7	70.0
Pallikkora *(Ottolithes argenteus)*	75.2	18.7	29.0	39.1	27.4	56.4
Ribbon fish *(Trichiurus savala)*	365	61.3	26.4	48.9	25.2	51.6
Pentaprion longimanus	25.5	13.2	32.4	56.8	10.7	43.1
Vala *(Chirocentrus sp.)*	243	43	19.2	54.8	26.0	45.2
Leatherskin *(Scomberoides lyssan)*	340	36.3	20.6	39.7	38.2	58.8
Scad *(Alepes mate)*	57.6	17.4	33.3	29.6	35.8	69.1
Threadfin bream *(Nemipterus japonicus)*	73.6	19.3	30.1	32.9	36.8	66.9
Lizard fish *(Saurida tumbil)*	211.6	30	14.9	58.2	26.7	41.6

* *Includes head + viscera and waste after mincing.*

Fish protein hydrolysates

Protein hydrolysates have an important role in protein fortified foods and beverages, due to their high solubility and digestibility. The technology of production of protein hydrolysates has become cheap and all machinery is available commercially. One great advantage of this product is that all species bycatches and low-value fish can be utilized for the production of hydrolysates compared to many other products which can only be produced from selected fish species. A variety of methods of production techniques are available. The most common among them are listed below :

Acid hydrolysis

The whole fish is cleaned well to be free of slime and adhering dirt. They are then comminuted in a mechanical meat mincer. The minced fish meal is then cooked well with 2 to 6 N acid containing water and maintained at about 90-100°C for 12-24 hours to get a completely soluble finished product. The major disadvantage of this process is that the final finished product, protein hydrolysate, will be acidic and has to be neutralized by alkalies to bring the pH to 7. This step invariably introduces large quantities of salt in the hydrolysates. Apart from this, another major disadvantage is the loss of some acid labile amino acids. This results in the loss of nutritive value.

Enzymatic hydrolysis

In the industrial processes of production of protein hydrolysis a number of enzymes are used. The enzymatic production of protein hydrolysates is perhaps the most convenient and cheapest technique. The process is fast and gives hydrolysates without much loss of essential amino acids. However, a suitable enzyme has to be selected for this process. The choice enzyme depends on factors like stability, cost etc. The important commercially available proteolytic enzymes are papain, pepsin, bromolein, ficin and trypsin. Most protein hydrolysates are highly bitter in taste. Hence, flavouring agents like cocoa are usually used during their fortification in food preparations to mask the bitter taste.

Among the important proteolytic enzymes listed above, the widely used ones are bromolein and papain. The industrial methods of production of protein hydrolysates using bromolein and papain are given below:

Hydrolysis with bromolein

1. Preparation of enzyme

Commercially available enzyme is dissolved in 100 parts of citrate buffer of pH 6. It is then centrifuged and the supernatant is taken. This is the stock enzyme solution for hydrolysis. This is stable, if kept at 3 to 5°C, for a week.

2. Preparation of water fat emulsion :

Ten parts of hydrogenated fat, antioxidant (BHA or BHT, 0.0%) and 0.15 part of sorbitan monostearate (emulsifier) are mixed together and heated to 65°C for about 10 to 15 minutes. To this, water containing 0.35 part sorbitan monostearate is added (90 parts) and the whole mixture is homogenised in a Waring blender and then kept overnight. Next day the soluble oil – water emulsion is separated from the excess fat and taken separately.

3. Hydrolysis

Take 100 parts minced fish meat, 20 parts water and 80 parts oil water emulsion and homogenized in a blender for 5 minutes. To the resulting pasty mass add the enzyme stock solution (six parts by weight) and transfer the whole mass into a reaction vessel maintained at 57°C. The hydrolysis is continued for 15 minutes with continuous stirring. After the reaction is over the mixture is heated to 80°C for 12 minutes to deactivate the enzyme. The whole mass is again homogenised in a blender and then rapidly cooked to 5°C. The resulting fish hydrolysate is dried in a spray drier to get a fine powder.

Hydrolysis with papain

Enzyme stock solution

About 25 g of the enzyme is taken in 100 ml distilled water and centrifuged. The clear filtrate is taken.

Hydrolysis

Comminuted fish meat is cooked with water (1:1 w/v) and this process results in the sterilisation of the mixture also. The pH of the mixture is adjusted with acids to 6.5. The mixture is transferred to a reaction vessel maintained at 55°C and the enzyme is added to this mixture (1:30, enzyme nitrogen to protein nitrogen). The whole mixture is stirred vigorously and the hydrolysis is continued for half an hour until it is completed. The hydrolysate is filtered, concentrated and dried under vacuum to get a fine highly hygroscopic powder.

This hydrolysate can be incorporated in a variety of food preparations like soups, beverages etc. to enhance their protein content and thereby enhance nutritive value.

Table 13. Yield of hydrolysate in sampled marine fishes.

Sl. No.	Common name of fish	Species name	Yield of hydrolysate (g/100 g)
1.	Lizard Fish	*Saurida tumbil*	13.3
2.	Large spined flat head	*Platycephalus macracanthus*	11.0
3.	Ribbon fish	*Trichiurus sp.*	9.9
4.	Barracuda	*Sphyraena sp.*	11.9
5.	Jew fish	*Johnius sp.*	9.9
6.	Threadfin bream	*Nemipterus japonicus*	12.0
7.	Cat fish	*Taehysurus sp.*	10.9
8.	Anchovies	*Thrissocles sp.*	9.7
9.	Sole	*Cynoglossus sp.*	8.6

Enzyme nitrogen : Substrate nitrogen concentrations 1:3, pH 6.5, temperature 55°C

Protein hydrolysate incorporated beverages

Protein hydrolysates are bitter in taste and as such unpalatable. One of the best ways to make the hydrolysates tasty is to add malt, cocoa, sugar and fat to the hydrolysates and the fortification is spray dried to make it a fine powder which is highly hygroscopic and soluble in water. The formula developed at Central Institute of Fisheries Technology, Cochin is given below :

Recipe	% composition by weight
Fish protein hydrolysate	30
Malt	20
Sugar	20
Milk Powder	10
Fat	05
Cocoa	05

This product is highly acceptable to consumers with acceptability in respect of taste, flavour and odour was 90 percent. The Protein Efficiency Ratio of the product is 2.2 compared to casein (3.7) at 10% level of protein.

Surimi

Surimi is a washed refined fish mince, a wet frozen concentrate of myofilbrillar proteins of fish muscle (Lanier, 1986). The process of production and machineries of surimi plants are well documented (Gopakumar K., 1997).

Fish mince is the raw material for production of surimi. Freshness of the raw-material essentially determines the quality of surimi. The fish mince is thoroughly, washed to be free of water soluble proteins, pigments and lipids, leaving a protein concentrate, chiefly myofibrillar proteins, having excellent tensile strength and elasticity. To improve the stability and functional properties, additives like polyphosphate, sodium chloride, starch etc. are added to washed fish mince. The flow diagram for production of surimi is given below :

Raw fish → Washing (5.10°C) → Heading and gutting →
Washing → Minced Fish → Dewatering 5-10°C →
Washing (5-10°C) → Dewatering → Straining and debydration →
Polyphosphate,
Sugar, Salt, Stand → Blending → Stuffing -40°C, plate freezer →
Freezing → Packing → -30oC Storage

Figure 1. Flow diagram of Surimi Production.

Surimi from bycatches :

Surimi is usually prepared from Alaska pollack *(Theragra chalcograma)*. The reduced availability of this fish led the users now to turn their attention to non-traditional fish resources. Properties of surimi prepared from five important species are given below :

The capacity of gel-formation and water retention were seen quite good for all and the surimi prepared from bycatches were of high quality, especially lizard fish and threadfin bream both of which are now used for surimi production in India.

Table 14. Properties of surimi from five species of bycatches.

Properties	Barracuda	Thread fin-bream	Ribbon Fish	Kalava	Lizard fish
Fish Mince colour	Pale Brown	Light Pink	Dull white	Light Pink	Dull White
Yield of mince	41 ± 8.2	38 ± 2.5	34 ± 5.2	35 ± 5.3	45 ± 7.2
Yield of surimi	25 ± 7.8	24.4 ± 2.3	22 ± 4.1	26.9 ± 3.2	30.0 ± 4.5
Water retention of surimi on cooking	86.2 ± 8.3	94.49 ± 0.55	91.8 ± 1	82.5 ± .25	91.8 ± 1.3
Gel Strength g/cm	685 ± 19.7	746 ± 18.2	725 ± 39.2	630 ± 39.2	730 ± 55.1

Mean Value ± SD (n=5) Ref. Muraleedharan, et al (1997)

PRODUCT FROM WHOLE FISH

Fish Pickles

Pickle is a delicacy in many Asian countries. In India pickles made from mango and lime are very popular and consumed by all sectors. Fish pickles are also very popular and a variety of methods are available in India from traditional process to industrial methods. India today exports large quantities of fish pickles to the Middle East and other parts of the world to cater to the needs of people of Indian origin. Pickles are exported in sealed glass or plastic bottles with a net content of around 500 g. During 1996-97 India exported pickles valued Rs. 1.8 million and this market is steadily expanding (Anon 1998). Low-value fish like threadfin bream, scianeids, mackerel, perches etc. are ideal raw materials for the preparation of fish pickles.

Recipe :

Fish :	1 Kg
(dressed and cut into small pieces)	
Mustard seed:	10 g.
Green chilly *(cut into pieces)*:	50 g.
Garlic *(peeled)*:	80 g.
Ginger *(peeled and chipped)*:	80 g.
Chilly powder:	35 g.
Turmeric powder:	2 g.
Gingelly oil:	200 g.
Vinegar:	400 ml.
Salt:	100 g.
Sugar:	10 g.

Preparation:

Fillet the fish and remove the skin. Cut them into small pieces. Small sized species like anchoviella can be used as such after washing and cleaning. Mix with salt in the ratio 1:1 (w/w) and keep for 2-3 hours. Fry the fish in a minimum quantity of vegetable oil of choice like refined peanut oil, olive oil, palm oil, cotton seed oil etc.

All other ingredients, except turmeric and chilly powder, are gently fried in the same oil, in the frying pan used for frying the fish. Turmeric and chilly powder are then added followed by the fried fish. Mix well and add sufficient quantity of boiled and cooled water just to cover the materials. If saltiness is not sufficient more salt should be added. Cool the contents and pack in airtight glass bottles. Acid resistant pilfer proof caps are used to seal the bottle.

Aging: Aging of pickle is very important. Aging for 2 to 3 months add new flavour and impart the traditional taste to fish pickles. In commercial process, pickles are prepared in large quantities and kept packed in large ceramic vats. They are then repacked into consumer packs based on market demands.

Shelf life	***:***	***One year under tropical conditions***
Storage	***:***	***Ambient temperatures***

Fish cutlet or cakes

This is a product, which can be prepared from whole fish or fish mince. Fish cutlet is a highly acceptable consumer product both for urban & rural people. They can be flash fried and kept stored upto 6 months.

Recipe

	Wt. in gm.
Minced meat (raw):	1000
Cooked, peeled potatoes:	300
Peeled chopped onions:	150
Common salt:	30
Ginger pieces:	15
Green chillies chopped:	10
Pepper powder:	2
Clove powder:	2
Turmeric powder:	2
Refined vegetable oil:	100 ml.

Preparation

Cook the fish and separate the meat. Cook the potatoes and peel and mash them. Add the cooked fish mince, salt and turmeric powder and other ingredients and then soft fry in a frying pan using vegetable oil. This may be added to the potato-fish mix and mix well. If spicy cutlets are to be prepared add spice mix at this stage and mix well. Mould 40 g. mix into round shape of around 2 cm thickness. They are then dipped in batter and rolled over bread-crumbs. The battered and breaded fish cutlets are flash fried in vegetable oil maintained at 160°-170°C for five seconds. They are then packed in consumer packets and kept stored under –20°C. Cutlets are to be deep fried prior to consuming.

Storage life : 6 months at –20°C

Fish soup powder

White flesh of many low value fish like threadfin bream, sciaenids, perches etc. can be used to prepare instant fish soup powder. This product has a high consumer acceptability and is now produced in India by several manufactures. Incidentally it is a high value item for the urban population. A common recipe is given below:

Recipe

	Parts by weight
Picked fish meat:	1500
Chopped onions:	1500
Hydrogenated vegetable oil:	200
Common salt:	340
Coriander powder:	35
Cassava starch:	500
Milk powder:	200
Sucrose/glucose:	45
Pepper powder:	36
Garlic:	10
Ascorbic acid:	3
Carboxymethyl cellulose:	6
Monosodium glutamate:	20

Preparation of Soup :

Take 15gm in a vessel. Add sufficient water to make a fine paste. Add 150ml water. Boil for two to three minutes. Serve hot.

Fresh fish
↓
Cook in boiling water --- remove bones and scales
↓
Cooked fish meat
↓
Add fried onions (brown)
↓
Grind to a fine paste
|
| All other ingredients
| (except milk powder)
↓
Grind for five minutes
↓
Spread to a thin layer
|
70°C
↓
Dry under vacuum
↓
Pulverise
↓
Sieve
↓
Mix with milk powder
↓
Pack

Packing :*Laminated pouches*
Shelf Life: *One year at ambient temperatures*

Figure 2. Preparation of fish soup powder.

Fish flakes :

Cooked fish meat or cooked fish mince is used for preparation of flakes. Flakes are to be fried in oil prior to use.

Recipe

Ingredients	Components by weight
Cooked fish meat	2
Corn starch	1
Cassava starch	2
Common Salt	0.05
Water	3.5
Total	8.85

How to use :

The flakes are to be fried in vegetable oil kept heated around 160-170°C. They swell to two to three times its initial volume. They are very crisp and delicious to eat. If dried at high temperature above 60°C. they lose their capacity to swell and become hard on frying. Spices can be added in the final stage of processing to make it appealing to the consumers.

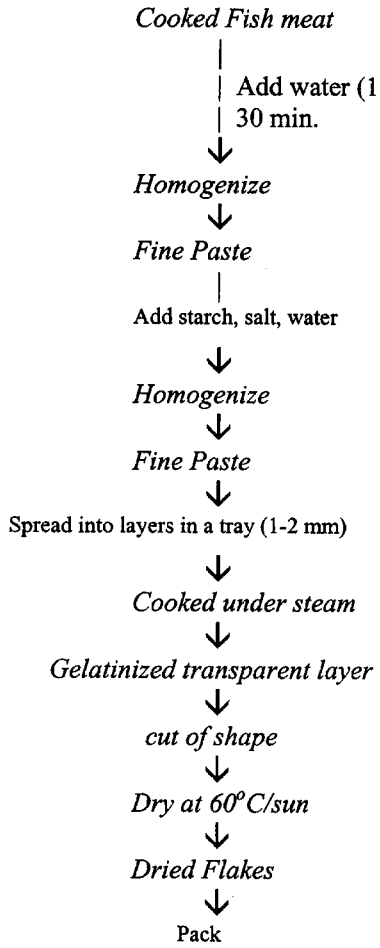

Figure 3. Preparation of Fish flakes.

Fish sauces :

Fermented fishery products are very popular in South east Asian countries. Among them fish sauce is most popular. Fish sauces are a delicacy in countries like Thailand, Vietnam, Philippines, Hongkong, China etc. They are called by different names. Some of the common fish sauces are listed below :

China	Yu-lu
Vietnam	Nuoc-mam
Malaysia	Budu
Thailand	Nam pla
Philippines	Patis
Japan	Shottu suru

Fish sauce is a heavily salted liquid product having salt content varying from 20 to 30 percent depending upon the method and source of preparation. Sauce manufacturing is highly modernized and the whole processing of packing and filling are automated.

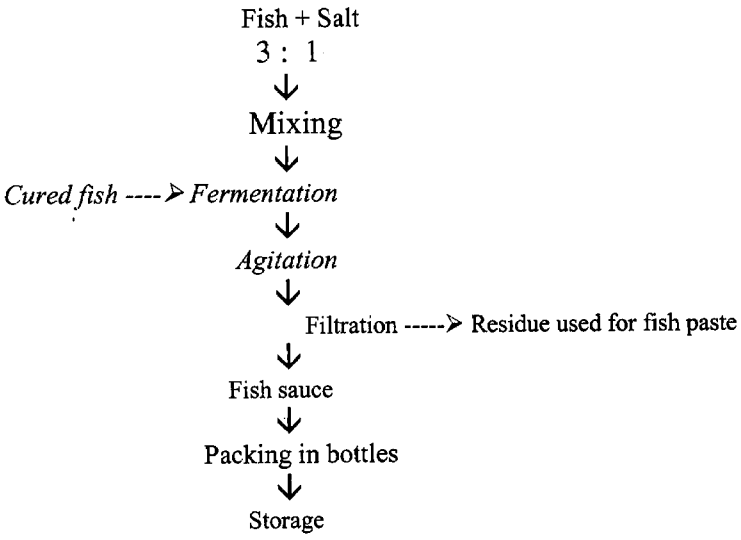

Figure 4. Preparation of sauce preparation.

Fish sauces have a developed market and they are also exported to Europe and America. The technology is very simple. It is one of the best ways of using low-value bycatches.

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UTILIZATION OF TRAWL BYCATCH IN SOUTHEAST ASIA

by

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ABSTRACT

The paper describes the successful implementation of the SEAFDEC/MFRD programme to increase the utilization of bycatch and low-value species in a number of SE Asian countries. Technological advances have contributed lizardfish and small pelagic species as raw material for the dramatic growth of the surimi industry. New products, such as satay fish and battered and breaded surimi chips have been developed and new raw materials sourced for traditional products. An important element of the programme has been the transfer to industry of the technology developed.

INTRODUCTION

A major portion of the catch by fish and shrimp trawlers operating in the Southeast Asian region comprises bycatch fish species which are discarded at sea or used for production of fish meal. Depending on the fishing area and season, the bycatch can comprise from 40% to 60-70% of the total catch. As in many parts of the world the problem of under-utilization of the bycatch and discard of vast quantities at sea is an important area of concern for the Southeast Asian countries. In the late 1970's a survey team of technical experts from Japan recommended that the MFRD look into ways to better utilise the bycatch for direct human consumption. In the early 80's the MFRD conducted research into the species composition of the trawl bycatch and to assess the suitability of the major component species for the production of surimi and surimi products, based on the Japanese technology for surimi production. The MFRD then promoted the concept of surimi to the fish processing industry in Southeast Asia, on the bycatch species as raw materials. This was done through training and lecture-demonstration courses directed at the fish processors from the SEAFDEC Member Countries.

Growth of the surimi industry

The trawl catch in the Southeast Asian region comprises of over 100 species which are sorted on board into high value species like snappers, groupers, pomfrets etc. The larger sized individuals of species like threadfin snappers, big-eye snappers, croakers are also sorted out separately, but a significant proportion of the catch is either iced as mixed species or discarded as bycatch. With the use of threadfin snappers, big-eye snappers and croakers for the production of surimi, the fishing industry is now able to identify a specific market use for these species and as a consequence, better sorting of the bycatch to select these species for the surimi industry resulted in reduction of the bycatch discarded or brought back for fish meal.

Presently, the surimi industry in Thailand, exports more than 60,000 tons of surimi annually, mainly to Japan. These species used comprise mainly the threadfin snappers, big-eye snappers, and croakers. Singapore now imports about 8,600 tonnes of surimi per year mainly from Thailand for its fishball-fish cake industry. Malaysia has about 6 factories each with production capacity of 5 tons/day.

As part of its transmigration policy, Indonesia has developed a fisheries complex in the Aru Islands in the Iran Jaya area, with a fleet of about 200 trawlers. Part of the operations include a 40 ton/day capacity surimi factory to utilise the lower value fish species from the trawl bycatch, as well as a fish meal plant. There is also potential growth for the surimi industry in other ASEAN countries like Vietnam and Myanmar.

RECENT TRENDS IN THE SURIMI INDUSTRY

The present surimi industry in the region is targeted at the production of high grade surimi for export to Japan. However as the domestic fish processing industry sees the benefit of using surimi as a raw material, there is a growing demand for lower grade, lower priced surimi for the local and regional markets. This is also due to the increase in demand and prices for the surimi species like threadfin snappers and big-eye snappers. A factory in East Malaysia produces surimi from mixed species for export to S. Korea. Several manufacturers from Singapore have also moved to the nearby Indonesian Rhio Islands to produce surimi from mixed species from the shrimp bycatch, for the Singapore market.

The use of mixed fish species however can give rise to inconsistent quality in terms of gel-forming ability but this can be controlled to a certain extent through proper sorting. With the increase in use of surimi by the local industry there is therefore a potential to develop this concept to produce lower grade surimi for domestic use. Local fish ball processors in Malaysia and Thailand are now producing leached meat for their own use.

Through this concept, small end-product manufacturers can obtain lower grade surimi from these manufacturers and need not depend an overseas import of surimi, or process their own raw material. At the community or village level, one such factory could serve to utilise the bycatch from the trawlers and produce leached meat for several small end-product manufacturers as part of an integrated fisheries management system. The MFRD will be initiating this system as a case study in Cat Bai, Vietnam as part of the SEAFDEC Integrated Coastal Fisheries Management Project in the Cat Bai and Cat Hai region.

One of the most abundant under-utilised fish species from the trawl bycatch in the region is the lizard fish *(Saurida spp.)*. These fishes are not consumed directly and fetch a relatively low price. Presently the smaller individuals are salted and dried as a traditional product and the remainder is used as minced fish for making lower grade fish jelly products (fried fish cakes etc.). In Japan, the lizard fish is considered as a good raw material for surimi, but in this region, lizard fish has very low gel-forming ability. The MFRD in collaboration with the Fishery Technology Development Institute, Thailand did a study to look into this problem. Lizard fish after death deteriorates very quickly without proper icing, forming formaldehyde as one of its breakdown products. The study indicated that after 6 days in ice the gel-forming ability of lizard fish drops drastically with a corresponding increase in formaldehyde levels. Experiments on polyphosphate leaching resulted in increase in gel-strength but the effect is greatest in fish less than 6 days in ice. With this information several surimi manufacturers have been paying more attention to icing of lizard fish and are now producing lizard fish surimi for export to Japan. Most of the surimi factories in Vietnam are using lizard fish as raw material but can only produce low grade surimi, mainly exported to S. Korea.

During the fishing season, pelagic species such as sardines and round-scads in this region are considered as under-utilised and are often sent to the fish meal factories. In southern Japan, sardine surimi has been used for producing fried tempura fish cakes, chikuwa, and fish noodles (about 20-30 % surimi). In Chile, horse mackerel resources have been used for surimi production and are now exported to Japan for fried tempura, chikuwa production. Several surimi factories in Thailand are also producing sardine surimi for specific markets in Japan.

OTHER PRODUCTS FROM BYCATCH

In terms of product development, the bycatch has been widely used for a wide range of products mainly for local consumption. Malaysia has been very successful in sorting out the goat fish *(Mullidae)* from the bycatch for production of 'satay' fish in the Pangkor Island area. In Thailand, a similar product made from mixed fish, has been very popular. Recently several large fish processing factories in Thailand have also started producing karasaki-ika, a surimi-based product similar to dried cuttlefish/squid slices. Traditional

products like 'kerupok' or fish crackers uses low value fish species like sardines to give the product a better taste.

Recently a low-cost battered-breaded surimi-based 'fish chip' made from lower grade surimi became very popular in Thailand and Malaysia. The fish chip are thin slices of surimi paste mixed with ingredients and flavours to taste, covered with breadcrumbs and frozen. To improve the texture of this product, the MFRD in collaboration with the ASEAN-Canada Fisheries Post-Harvest Technology Project developed a 'cut-and-mixed' fish nugget, using diced cooked fish cakes, mixed with surimi paste or fresh fish mince from lower value fish species (lizard fish) as a binder.

In some of the smaller fishing villages in Japan, the small bycatch species are used for making a product called kawatempura. This is made from very fresh, small, head-gutted, scaled mixed fish, passed through a mincer with small mesh several times to reduce the bone and skin particle size and ground with salt into a sticky paste. Diced vegetables are added for taste and formed into nuggets or cakes and deep fried. This product is quite similar to the Thai fried fish cake with diced vegetables and spices, but in this case the whole fish (including skin and bones) are used.

By-products from tuna processing

Processing of tuna into loins or canned tuna is now a big industry in Thailand and Indonesia. Trimmings from this industry are presently used as pet food, fish meal or discarded. There is a potential to make use of these by-products or the small tuna species presently under-utilised. The MFRD has used technology from Japan, several products like fish sausage, tuna burger, seasoned tuna cubes, tuna stick etc. using these trimmings. The technology has now been transferred to the fish processing industry in the region through training courses manuals etc. A tuna processing factory in Thailand have started commercial production of tuna salami and tuna sausage.

Traditional fish products

When looking at the problem of bycatch utilization it is very important to consider traditional fish products. In Southeast Asia, many of these products are based on bycatch species as raw materials. Small pelagic fish like sardines, scads and croakers are often salted and dried and distributed widely into areas where they serve as an important cheap source of protein. Minced fish meat with low gel-strength is also used in a variety of indigenous products usually as a binder in deep fried fish cakes, rolls, etc. Countries like the Philippines, often indicate that all the bycatch is fully utilised in one way or another in the form of salted, fermented, or dried products. The MFRD has therefore initiated a survey on traditional fish products in the ASEAN, to document the types of products in each country, raw material used, processing methods, production figures, packaging etc. This could perhaps shed some light on how bycatch is used or can be more fully utilised in such products.

CONCLUSION

In looking at the problem of utilization of bycatch in the ASEAN region, MFRD has taken the technology approach, in terms of developing technology to promote greater use of the bycatch species by the industry. Our strategy is then to transfer these technologies directly to the industry, through training courses and manuals in collaboration with the respective department of fisheries and to provide technical assistance to them for commercial application of these technologies.

THE MICROBIOLOGY OF LOW-SALT FERMENTED FISH PRODUCTS

by

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ABSTRACT

A wide range of fermented fish products is produced in South East Asia. The combination of acid production by lactic acid bacteria and addition of salt is used as a low-cost means of fish preservation. Due to the high buffering capacity of fish, a high level of lactic acid bacteria with a capacity to degrade the added carbohydrates is important to obtain the rapid decrease in pH that is required for the production of acceptable and safe products. The salt concentration should be in the range of 3-5% water phase salt (w/w) in order not to inhibit the growth of lactic acid bacteria. *Salmonella, Escherichia coli, Staphylococcus aureus* and *Clostridium botulinum* types A and B are the pathogenic bacteria of particular concern in fermented fish products. Implementation of processing control, and preferably the controlled use lactic acid bacteria starter cultures, is essential to improve the safety of products. The possible use of low-value fish as raw material requires more knowledge of the influence of product composition, e.g. salt concentration, buffering capacity of different fish species and carbohydrate sources on the fermentation process.

INTRODUCTION

Traditionally, the term "fermented fish" covers both enzyme hydrolysed and microbial fermented products (Mackie *et al.*, 1971). However, as suggested by Ozen and Mendoza (1985) only those products involving fermentative growth of microorganisms should be described as fermented. A clear distinction should be made between the microbial fermented products and the hydrolysed products, which are dependent on the activity of indigenous or added enzymes (Table 1). Of course, many products will involve both microbial fermentation and enzymatic activity in particular in the high-salt range of fermented products. This paper will discuss fermented fish products with a salt concentration lower than 8% (w/w). These products have not been studied to the same extent as the high-salt fermented fish and hydrolysed fish. However, health hazards due to a high salt intake limit the use of high-salt products in the daily diet. The production of fermented fish is still carried out at a household level or in small industrial scale with limited or no quality control. However, there is a potential for technological improvement and also for the use of low-value marine fish in processing by increased knowledge of the fermentation process and by the application of lactic acid bacteria (LAB) starter cultures in production.

The raw material used for processing of fermented fish is, with a few exceptions, freshwater fish. This is in contrast to the hydrolysed products, which often are prepared from marine fish species (Kungsuwan, 1998). According to the Fishery Statistical Record of Thailand 1994, issued by the Thai Department of Fisheries (DOF), total freshwater fish production in Thailand was 373,000 tonnes of which 0.44% was used for fish sauce, 0.05% for fish paste and 5.26 % for hydrolysed/fermented fish. *Plaa-ra* is by far the most common product in Thailand. *Plaa-ra* (pla = fish and ra = strong smell) contains from 8-25% NaCl and is today the only hydrolysed/fermented fish product, which is exported and play a role in the national economy (Kungsuwan, A., 1998).

The main preservation factors in fermented fish products are NaCl (reduced water activity) and acid (pH reduction). Others include spices (garlic) and possibly also inhibitory substances (bacteriocins) produced

by LAB. There have been a number of reviews dealing with the characteristics of fermented fish products in South East and East Asia (Adams *et al.*, 1985; Ozen and Mendoza, 1985; Lee *et al.*, 1993). In this paper, a brief overview is given of different types of fermented fish products (Table 2). The role of LAB in the fermentation process is discussed, focusing in particular on products from Thailand, such as *som-fak* and *plaa-som*. The main focus is the influence of preservation factors and product composition on the growth of LAB and thereby on the quality of products. Furthermore, the bacteriological risks associated with fermented fish products are listed and methods to reduce these risks suggested.

INFLUENCE OF PRESERVATION FACTORS AND PRODUCT COMPOSITION ON LAB GROWTH

Indigenous starter cultures

Fermented fish products are produced by spontaneous fermentation or with the use of indigenous starter cultures (Table 2). These are not pure cultures as most often used in the West, but mixed cultures of moulds, yeast and bacteria growing on rice. The starters serve as a source of a variety of enzymes, including amylolytic enzymes, which degrade the starch and thereby provide fermentable carbohydrates for the growth of LAB in the subsequent fermentation process (Lotong, 1998). Japanese *koji* is steamed rice overgrown with *Aspergillus oryzae*, whereas *angkak* used in the Philippines, Thailand and Cambodia is steamed rice overgrown with the red mould *Monascus purpureus*. *Look-pang*, a starter used for the Thai *plaa-chao*, is in the form of dry powder as small flattened cakes. Rice flour makes up the greater part of the starter, but typically it also contains one or more vegetable components (garlic, ginger, pepper).

A lower final pH than in spontaneously fermented products is in general obtained, when indigenous starters, such as *angkak*, are used in the preparation of fermented fish (Sakai *et al.*, 1983a). High amounts of glucose are produced from rice starch by amylolytic enzymes present in the starter and a rapid fermentation by LAB is therefore obtained. The microflora is composed of both LAB and yeasts, where the latter originate from the starter culture. Depending on the species, yeasts are likely to be positively involved in flavour formation as has been found for fish and soy sauce (Mongkolwai *et al.*, 1997). In *som-fak*, which is prepared without indigenous starter culture, yeast are considered as spoilage organisms (Saisithi *et al.*, 1986).

Salt levels

Salting is the most important preservation factor in hydrolysed products, such as fish sauce and fish paste. In contrast, a rapid acid fermentation by LAB is essential for the effective inhibition of spoilage and pathogenic bacteria in the fermented products. However, the level of salting will also affect the growth and selection of micro-organisms during fermentation. In many products an initial salting of the fish is performed. This is done to cause an immediate inhibition of the natural spoilage flora on the fish before acid production and also to improve the texture of the final product.

Water phase salt levels above 7% (w/w) were inhibitory to the LAB fermentation of Thai *plaa-som* (Table 3) (Paludan-Müller *et al.*, 1998b). The product contained an initial high level of the yeast *Zygosaccharomyces rouxii* (approx. 10^6 cfu/g) originating from the palm syrup used in the preparation. *Z. rouxii* was isolated throughout fermentation irrespective of the NaCl concentration (Table 3). *Tetragenococcus* sp. and *Lactobacillus* sp. dominated the microflora (5x108 cfu/g) in *plaa-som* with 5.8 or 7.1% salt (w/w). However, only in *plaa-som* with 5.8% salt (w/w) did pH decrease to below 4.5 within five days. The growth of *Lactobacillus* sp. was inhibited and the microflora dominated by Gram-positive, catalase positive cocci (*Staphylococcus* sp.) in *plaa-som* with salt concentrations above 9% (w/w) (Table 3). Thus, the concentration of water phase salt must be kept lower than 7% (w/w) in this type of product, in order to proliferate fermentation by LAB. Higher salt concentrations will increase the risk of growth of salt-tolerant, potentially pathogenic bacteria, e.g. *S. aureus*. The investigated product contained 10-13% palm syrup (w/w), which was composed of sucrose, fructose and glucose. This high content of mono- and di-saccharides will increase the rate of fermentation by LAB. Thus, an inhibitory effect of salt may be found at lower salt

concentrations in other types of products, which have more complex carbohydrates (e.g. rice starch) as the only carbohydrate source. *Staphylococcus* spp. have previously been isolated from fermented fish products with water phase salt > 5% (w/w) (Tanasupawat *et al.*, 1991 and 1992). In a study of the Filipino product *balao balao*, 3 % salt was found to be optimal for a rapid decrease in pH as compared to 6, 9 and 12 % (Arroyo *et al.* 1978). Investigations of the effect of salt on the growth of *Lactobacillus* sp. isolated from fermented fish products revealed that most strains tolerated up to 6.5% NaCl (Østergaard *et al.*, 1998b). However, 6 % NaCl inhibited the growth of the Gram-negative spoilage flora in *som-fak* prepared from Danish marine fish, but LAB was also inhibited and the fermentation failed (Paludan-Müller, unpublished results). In conclusion, a water phase salt concentration in the range of 3-5% (w/w) is optimal in order to ensure the growth of LAB and thereby a rapid fermentation of the fish products.

Organic acids

The antimicrobial effects of organic acids are due both to the depression of pH below the growth range of spoilage and pathogenic bacteria and to metabolic inhibition by the undissociated acid molecules (Rowan *et al.*, 1998). Lactic and citric acids have low pKa-values of 3.1 and microbial growth is only inhibited at low pH values. Acetic acid has a pKa value of 4.75 and is therefore a better inhibitor in a higher pH range (Eklund, 1989). At pH 5, approximately 35% of acetic acid and only 6% of lactic acid will be in the undissociated form (Silliker, 1980). It is suggested, that the growth of LAB producing mixtures of lactic and acetic acid in the beginning of food fermentation is important for the inhibition of competing spoilage and pathogenic bacteria and to obtain a rapid decrease in pH (Adams and Hall, 1988). However, since a selection of homofermentative LAB is found in most fermented fish products studied, mixtures of organic acids may be important initially, but lactic acid is produced in the highest quantity and is responsible for the maintenance of a low pH. Lactic acid is also the desired end-product of fermentation, considering the organoleptic quality of products.

High amounts of acid (1.8-2.5%) corresponding to a pH-value of 4.5 are formed in *som-fak* (Table 4). *Som-fak* is a minced product and therefore requiring more acid than whole fish to reduce pH. Orillo and Pederson (1968) found a decrease in the buffer capacity among the fish portion, the fish-rice portion and the rice portion in *burong dalag (burong isda* prepared from mudfish). The large amount of garlic in *som-fak* (4-8 %) may also contribute to a higher acid production by LAB (section 3.5).

Buffering capacity of fish

The major buffering constituents in fish muscle have been identified as histidine-related compounds (histidine, carnosine and anserine) and inorganic phosphate (Okuma and Abe, 1992). In general, the buffering capacity of white muscle was higher than that of red muscle. Muscle (red and white) of Pacific blue marlin, a high-speed pelagic swimmer, had two times higher buffering capacity than muscle of the less active rainbow trout (Abe *et al.*, 1985). This could explain the failed fermentation, when small pelagic marine fish species were used as fish raw material in the preparation of *som-fak* (Østergaard *et al.*, 1998a). It is suggested that the main function of increasing the amount of carbohydrate (rice, millet) in fermented fish products is to reduce the buffering capacity of the fish muscle (Owens and Mendoza, 1985). This is supported by studies on *som-fak*, where it was found that, an increase of the percentage of rice in *som-fak* from 5 to 20% caused a slight extra decrease in pH already from day 1, but not a corresponding increase in acid content (Saisithi *et al.*, 1986).

Carbohydrate sources

Fish contains very little carbohydrate (< 0.5%) and other ingredients must be added as substrates for fermentation. Rice is most often used and the capacity of LAB to ferment rice starch is thought to be necessary for the reduction of pH in products prepared without indigenous starter cultures.

LAB with amylolytic activity have been isolated from white *burong isda* (Olympia *et al.*, 1992). However, the role of these strains in the fermentation process was not determined. Starch-fermenting Lc. *lactis*

lactis and *Lb. para. paracasei* were isolated from raw materials (rice and fish) and before start of fermentation of *som-fak* (Paludan-Müller *et al.*, 1998a). A mixed starter culture of these strains was unable to decrease pH in laboratory prepared *som-fak* despite a high level of growth (> 108 cfu/g). Similarly, a starch fermenting strain of *Lc. lactis lactis*, isolated from Thai *plaa-som*, was unable to decrease pH in a fish-salt-rice mixture (Østergaard *et al.*, 1998b). The results indicate that the degradation of rice starch by LAB is not sufficient to obtain a decrease in pH in *som-fak*. Other micro-organisms, such as *Bacillus* sp., could be responsible for an initial degradation of starch to lower carbohydrates, which would then serve as substrates for LAB. However, the LAB completely dominate the microflora after the first day of fermentation, where they grow rapidly from 10^6 to 10^8 cfu/g (Østergaard *et al.*, 1998a). Instead, investigations of the fermenting capacity of LAB isolated from raw materials and during fermentation of *som-fak*, suggest that garlic is acting as substrate for fermentation. (Paludan-Müller *et al.*, 1998a).

Garlic is added to *som-fak* and many other fermented fish products as a flavouring agent, often in high levels (2-6%). Garlic has been found to stimulate the growth of a *Lb. plantarum* starter culture and accelerate the fermentation process of meat sausage (Nes and Skjelvåle, 1982). The active growth stimulating component in garlic has been identified as manganese (Zaika and Kissinger, 1984). However, garlic may be more directly involved in fermentation by supplying LAB with a carbohydrate substrate. Garlic contains approximately 30% of fructo-oligosaccharides, mainly in the form of inulin (Van Loo *et al.*, 1995). *Lb. plantarum* has recently been used as starter culture for the fermentation of blanched garlic with lactic acid as the major end-product (de Castro *et al.*, 1998).

In *som-fak*, an increase in garlic fermenting *Lb. plantarum* strains was found reaching an estimated level of 108 cfu/g after 5 days of fermentation (Paludan-Müller *et al.*, 1998). These strains were able to decrease pH to below 4.5 in culture media with garlic as the only carbohydrate source. Inoculation of a mixed culture composed of garlic or garlic/starch fermenting strains into *som-fak* prepared in the laboratory caused a decrease in pH similar to *som-fak* prepared at a factory. It has also been found that increasing the content of garlic from 2.5 to 5% in Korean *gajami sikhae* resulted in a more rapid decrease in pH and a lower final pH (Souane *et al.*, 1990). Not all fermented fish products contain garlic and the capacity of LAB to ferment rice starch is an important criterion for the selection of starter cultures in those products, e.g. *burong isda* and *balao balao*.

Antibacterial factors

Garlic is also an antibacterial agent, in particular against Gram-negative bacteria, due to allicin (Feldberg *et al.*, 1988; Beuchat, 1994). A growth inhibitory effect of garlic has been found against different types of industrial and food spoilage yeasts (Conner and Beuchat, 1984) and garlic is also found to inhibit food pathogens, such as *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Listeria monocytogenes* (Kumar and Berwal, 1998). Souane *et al.* (1990) found that garlic was more inhibitory against Gram-negative species, *Bacillus* sp. and moulds than against LAB isolated from the Korean product *gajami sikhae*. In contrast, the viability of the parasite *Cysterticus cellulosae* in *nham*, a Thai fermented pork sausage, was found to be prolonged, when 3% garlic was used as compared to lower levels of garlic (Keittivuti *et al.*, 1986). The effect was explained by a slower decrease in pH due to inhibition of LAB by garlic. A starter culture of *Lb. plantarum*, used for sausage preparation, was inhibited by 1% garlic in the culture media, although large inocula (>106 cfu/g) were able to overcome the inhibitory effect (Karaioannoglou *et al.*, 1977). Consequently, the tolerance towards the anti-microbial compounds in garlic is variable among LAB strains. This should be considered, in addition to the capacity of strains to ferment garlic, when criteria for selection of LAB strains for use as starter cultures in fermented products containing garlic are established. If the right LAB strains are selected, garlic may play a dual role by inhibiting Gram-negative bacteria and yeasts and providing the less sensitive LAB with a carbohydrate source for fermentation.

Antibacterial properties of LAB isolated from Thai fermented fish products have been found against *L. monocytogenes, V. cholerae* and *V. parahaemolyticus* (Østergaard *et al.*, 1998b). The activity against *L monocytogenes* was attributed to bacteriocin production, whereas the Gram-negative bacteria were inhibited

by lactic acid. Bacteriocin production by LAB is of minor importance for the inhibition of most pathogenic bacteria, as compared to the inhibitory effect of acid production. However, the capacity of LAB strains to produce bacteriocins could be a factor involved in the selection of LAB strains during fermentation.

SAFETY OF FERMENTED FISH PRODUCTS

Fermented fish products are mostly produced in a household/small factory scale with limited process control to ensure product quality and safety. The microbiological safety is mainly dependent on a successful fermentation. The procedures used for processing of fermented fish do not include steps, such as cooking or pasteurisation, that destroys pathogens and products are often stored at ambient temperature until consumption. Furthermore, some products, e.g. *som-fak* are consumed without prior heating. This, in combination with a low salt concentration, means that very high microbiological risks are associated with those types of products. The risk of intake of parasites is also high when products are not cooked before being eaten. Biotoxins have mainly been studied in marine environments, but the association with freshwater fish can not be excluded, and are therefore a risk in all products. The risk of biotoxins should in particular be investigated if the use of new, low-value marine fish is intended for production of fermented fish. None of the indigenous mould-yeast starter cultures has been described as toxic (Rowan *et al.*, 1998). However, the *koji* mould has been shown to produce mycotoxins during severe stress conditions, although there is little evidence that they are produced in commercial production processes.

This paper focuses on hazards associated with pathogenic bacteria and is thus not further concerned with hazards of non-bacterial origin such as parasites, viruses, biotoxins or mycotoxins.

Pathogenic bacteria

There are two broad groups of pathogenic bacteria of public health significance - those naturally present on the raw materials, referred to as indigenous bacteria, and those introduced from the human animal reservoir, the non-indigenous bacteria (Table 5). The types of indigenous bacteria reflect both the microflora naturally associated with the ingredient and the microflora of the general environment. The presence of non-indigenous bacteria reflects contamination levels, hygiene of handlers, sanitary conditions etc. Most pathogenic bacteria are neutrophilic, that is growing within a pH range of 5-9. Thus, the critical point in processing is the initial time of fermentation, where pH has not decreased to below 5. The most acid tolerant pathogenic bacteria of concern are proteolytic *Clostridium botulinum, Salmonella, Escherichia coli* and *Staphyloccus aureus* (Table 5).

Freshwater aquaculture fish is most often used for preparation of fermented fish including *som-fak.* Pathogenic bacteria associated with farmed fish will include all the indigenous pathogens found in coastal and brackish water systems as well as non-indigenous pathogenic bacteria from disposal of sewage and land run-off (Reilly *et al.*, 1997). Foodborne diseases caused by the indigenous, pathogenic species within the *Vibrionaeceae (Vibrio* spp., *P. shigelloides* and *A. hydrophila*) have mostly been associated with raw shellfish or re-contaminated cooked seafood. The species are not very acid tolerant (Table 5) and are not considered as a hazard in properly fermented fish. However, high numbers of *V. cholerae* on the fish raw material or in the water used for processing can pose a risk, when the fermentation process is not under control. *L. monocytogenes* is ubiquitous in the general environment, but *Listeria* spp. are more common than *L. monocytogenes* in the tropics (Reilly *et al.*, 1997). *L. innocua* was the most frequent species isolated in a study on the incidence of *Listeria* spp. in tropical fish (Jeyasekaran *et al.*, 1996).

The prevalence of different types of *C. botulinum* in the environment depends on the geographical location. The psychrotrophic, non-proteolytic types E, B and F are prevalent in temperate environments, whereas the mesophilic, proteolytic types, A, B and F are more frequently isolated in the tropics as well as the types C and D which are non-pathogenic to man (Dodds, 1993). Human botulism is almost always of type A, B, or E. In surveys of freshwater and marine fish from Indonesian waters, 13-17% positive samples were

found, of which type C spores predominated. In Thailand, less than 1% of 16,000 fish from the Gulf of Thailand were positive, and only type D and E spores were detected (Dodds, 1993). The salt tolerance of *C. botulinum* type E is low (3-5%) and the minimum pH for growth is 5 (Table 5). However, type E toxin is very stable at high salt concentrations and at low pH values (Huss and Rye Petersen, 1980). Pre-formed toxin present in the raw materials used in processing will be preserved during fermentation and strict control with raw materials is therefore essential to prevent botulism. The acid and salt tolerant types A and B have been shown to produce toxins in media at pH lower than 4.6 (Tanaka, 1982). Thus, growth and toxin production by these types is probably a higher risk in fermented fish from tropical environments, than the risks associated with type E.

In a survey on the incidence of Salmonella in 5.219 fishery products from Thailand, only 1.74% samples were contaminated (Keerativiriyaporn, unpublished results). *Salmonella weltevreden*, a serovar not commonly associated with salmonellosis, accounted for 45% of the positive samples. *S. weltevreden* is specificially associated with Asia and has been identified as the principal serovar in brackish water shrimp ponds irrespective of contamination levels (Reilly and Twiddy, 1992).

E.coli is normally used as an indicator organism for faecal contamination, but there is evidence that *E.coli* exists as part of the natural microflora in warm tropical waters (Hazen, 1988). Outbreaks caused by *E. coli* serotype O157:H7 have involved acidic foods such as fermented sausages and yoghurt (Rowan *et al.*, 1998). It has been found, that growth can occur at a pH value of 4.0 and survival even at lower pH values (Conner and Kotrola, 1995). The sustained acid tolerance is not induced by adaptation, but by stationary phase or starvation (Arnold and Kasper, 1995). This so-called acid-tolerance-response has also been described for *L. monocytogenes* (Davis *et al.*, 1996) and *S. typhimurium* (Baik *et al.*, 1996).

S. aureus is ubiquitous in the environment with the animal/human nose, throat and skin as the main reservoirs (ICMSF, 1996). Fish raw material may be contaminated with *S. aureus* via infected food handlers or from the environment. *S. aureus* is able to grow at low water activities (minimum aw 0.90), the main reason for its implication in food poisonings caused by consumption of dry or semi-dry fermented meat sausages (Johnson, 1991). However, the organism is a poor competitor with LAB in fermented meats. Growth and toxin production will be effectively inhibited if lactic acid starter cultures form acid rapidly from the carbohydrate added to the meat (Smith and Palumbo, 1983). High numbers of staphylococci have been isolated from fermented fish with NaCl concentrations > 8% (w/w), when growth of most LAB was inhibited or very slow (Tanasupawat, 1991 and 1992; Paludan-Müller *et al.*, 1998b). Thus, the results indicate, that higher salt concentrations, which are inhibitory to LAB growth, will increase the risk associated with *S. aureus*.

Rice will be naturally contaminated by *Bacillus* sp., but high numbers of *Bacillus cereus* are not suspected in LAB fermented fish products, because the pathogen does not grow at pH values below 5-5.5 (Table 4.2). However, toxin pre-formed in the boiled and cooled rice is a risk, since the toxin will be stable during fermentation. It has been shown, that diarrhoeal enterotoxin is produced before the cells reach a level of 10^7 cfu/g, and it is therefore suggested that levels as low as 10^3-10^4 *B. cereus* cells/g food should be a concern (Granum *et al.*, 1993).

Inoculation studies

Aryanta *et al.* (1991) investigated the survival of *S. typhimurium, E. coli, V. parahaemolyticus, S. aureus, B. cereus* and *C. perfringens* in a fermented fish sausage with a high initial inoculum (>10^7 cfu/g) of *Pd. acidilactici*. The pathogenic bacteria were inoculated individually to give an initial population of 10^3 cfu/g and the sausage was incubated at 30°C. pH decreased to 4.5-4.7 in 48 hours. No growth of *V. parahaemolyticus* and *Cl. perfringens* was observed. *B. cereus* was not detected after 24 hours, whereas the other pathogenic strains showed limited growth during the first 12 hours and were present (10^2-10^4 cfu/g) after 48 hours.

S. typhimurium, E. coli, Cl. sporogenes and *S. aureus* were not able to survive when inoculated individually into a fish-salt-carbohydrate mixture composed of haddock mince, 20% cassava pre-fermented by a *Lb. plantarum* starter culture, 1% NaCl and 5% glucose (Twiddy *et al.*, 1987). pH decreased rapidly to below 4.5 in two days.

A study has been done on the Thai fermented pork sausage, *nham*, which has a composition almost similar to *som-fak*. Minced pork is used instead of minced fish, and glucose or sucrose is added in addition to boiled rice in order to ensure a rapid fermentation (Wiriyacharee, 1992). A mixed starter culture composed of *Lb. plantarum* and *Pd. pentosaceus* was added to *nham* in a level of 10^6 to $5x10^6$ cfu/g (Petchsing and Woodburn, 1990). *S. aureus* (10^3 cfu/g) and *E. coli* (10^4 cfu/g) was inoculated individually and the *nham* was stored anaerobically at 30°C. *Lb. plantarum* persisted throughout fermentation (10^8 cfu/g), whereas *Pd. pentosaceus* gradually decreased during the first 24 hours of fermentation. The growth of the two pathogens was inhibited, but only after 36 and 96 hours of fermentation, respectively. Not more than 1.9% lactic acid corresponding to a pH of 4.6 was formed after 96 hours. The fermentation time of *nham*, prepared without starter cultures, is normally 3-5 days with a final production of approximately 1% lactic acid and a pH of 4.3-4.6. Thus, the results indicate that *E. coli* and other enteric pathogens such as *Salmonella* will be able to survive and grow in *nham*.

The inoculation studies indicate that initial levels of $>10^7$ cfu/g of aciduric LAB starter cultures (e.g. *Lb. plantarum, Pediococcus* spp.) resulting in a rapid decrease in pH to 4.5 will inhibit the growth of most pathogenic bacteria. A high capacity of the starter culture to ferment the available substrate is essential. The LAB microflora in spontaneous fish fermentation is initially a mixed flora composed of both hetero- and homo-fermentative species (Paludan-Müller *et al.*, 1998a; Olympia *et al.*, 1992; Solidum; 1979). In *som-fak*, a mixed LAB flora of $4x10^6$ cfu/g was able to produce 2.5% lactic acid (w/w) in only 2 days (Østergaard *et al.*, 1998a). It is likely, that a "houseflora" of LAB adapted to the fermentation of garlic/starch has been established in the factory environment. An initial heterogeneous LAB microflora may also be necessary for the right development of flavour.

Safety record for 'fermented' fish products

Only 1.6% of factors that contributed to the occurrence of outbreaks of food-borne disease in the United States from 1977-1982 (total 766 outbreaks) was attributed to improper fermentation (Bryan, 1988). This is in accordance with the microbiological safety generally associated with fermented foods produced under controlled conditions, e.g. dairy products and meat sausages. However, when factors contributing to outbreaks caused by foods prepared in homes were identified, improper fermentation were responsible for 4.6% of outbreaks (Bryan, 1988). This reflects the higher risks associated with fermented foods produced by an uncontrolled, spontaneous process dependent on naturally occurring micro-organisms. The latter is the case with most South East Asian fermented fish products.

Examination of 165 outbreaks of type E botulism caused by fish products showed that fermented fish products accounted for 68% of the outbreaks (Huss, 1994). However, the products were all high salt products, thus rather enzymatically hydrolysed than fermented. The outbreaks were all associated with fish products from temperate waters, and especially home-made Japanese hydrolysed fish (Nakano and Kodama, 1970) and native Alaskan hydrolysed fish heads, fish eggs, seal, beaver or whale have caused human botulism (Shaffer, 1990).

Outbreaks of type E botulism has been associated with traditional salted fish in Cairo (Weber *et al.*, 1993), New York (Telzak *et al.*, 1989) and New Jersey (CDC, 1992). Common for all three cases was that the fish was uneviscerated, pickled in brine, purchased as whole fish and consumed raw. The salt level in the fish from New York was above 18% in the aqueous phase, but *C. botulinum* isolates obtained from remains of the fish did not grow in a salt concentration > 3.5%. It is suggested, that either toxin production occurred before salting or that the pathogen grows and produce toxins in the protective environment of the viscera. In Hawaii, a botulism outbreak caused by type B was associated with consumption of grilled, fresh fish (CDC, 1991). Also in this case the fish was ungutted and the patients had eaten the intestines. The fish might have been held at

high temperatures on the market before it was purchased, allowing *C. botulinum* to grow and produce toxin in the intestines. From these cases it can be concluded, that evisceration is essential to reduce the risk of botulism.

The fermentation process of *som-fak* produced in small industrial scale is today without any kind of quality control. This means that all the indigenous and non-indigenous pathogens mentioned above should be considered as potential hazards, when products are not thoroughly cooked prior to consumption. If products are cooked immediately before consumption, those hazards are eliminated.

REDUCTION OF BACTERIOLOGICAL RISKS

A high quality of raw materials is essential. Fresh fish with a low level of pathogenic bacteria and no pre-formed toxin (*C. botulinum*) must be used. The correct salt concentration (< 5-6% (w/w) water phase salt) is important to ensure a rapid growth of LAB and a decrease in pH to below 4.5 in 2-3 days. To ensure the safety of fermented fish products, implementation of quality control of the processing and distribution is required, preferably based on the Hazard Analysis Critical Control Point system (HACCP). Growth of spoilage/pathogenic bacteria and contamination can be prevented by control of factory hygiene, sanitation and water quality. Quality assurance has already been implemented for some fish sauce producers and such control systems should be extended to all producers of fermented fish. In Thailand, a Quality Control and Training Center (QCTC) for soybean fermentation has been successfully established for small and medium size soy sauce producers, with the aim of improving quality and manufacturing techniques, including introduction of an improved microbial inoculum (Mongkolwai *et al.*, 1997). Such a concept could improve transfer of technological advances made in more developed economies to traditional technologies in small-scale productions, which do not have the economic and scientific qualifications for such developments.

A more consistent and safe production can be the result if LAB starter cultures are used, securing a rapid growth of LAB to 10^8 cfu/g and a reduction of pH to below 4.5. LAB starter cultures should be selected based on several criteria, including a high capacity to ferment the available carbohydrates in the products and the ability to produce the desirable sensory changes. The advantage of using mixed starter cultures with a capacity to ferment complex carbohydrates, such as inulin in garlic and starch in rice, could be a more controlled and faster fermentation, because the carbohydrate substrates specifically (more or less) supports the growth of the added culture. Investigation of the preparation of starter cultures as freeze-dried cultures or as starters resembling the indigenous starter cakes, like the Thai look-pang, is necessary, since pure culture inocula may not be applicable in developing countries.

CONCLUSION

The major role of LAB in fermented fish products is to produce organic acids and reduce pH in order to inhibit spoilage and pathogenic bacteria and thereby preserve the products. In addition desirable flavours, or at least no undesirable sensory changes, are formed during fermentation. A high acid production and low final pH (< 4.5) require a rapid growth of LAB with a capacity to ferment the available carbohydrate sources. Inulin, the main carbohydrate component in garlic, is found to act as substrate for fermentation in the Thai product *som-fak*. Further investigations of the fermenting properties of LAB strains isolated from spontaneous, successful fermentation is important for the establishment of criteria for the selection of starter cultures. The production of organic acids during fish fermentation remains to be studied. The succession of homo-fermentative LAB strains during most fermentation suggests that lactic acid is the major acid produced. However, mixtures of organic acids could be an important factor to secure an initial dominance of the microflora by LAB. Fish, and in particular marine, pelagic species, has a high buffering capacity and high amounts of acid are therefore needed to obtain a sufficient decrease in pH. The water phase salt concentration must be in the range of 3-5% (w/w) in order to ensure a rapid growth of LAB. At higher salt concentrations, there is a risk for growth of the salt tolerant pathogens *S. aureus* or *C. botulinum* types A and B. Growth of the enteric pathogens, *Salmonella* and *E. coli*, is also a risk in fermented fish products, when a rapid fermentation by LAB is not under control. To ensure the safety of fermented fish products, implementation of quality

control of the processing and distribution is required preferably based on the HACCP principles. An initial high level of LAB (>10^7 cfu/g) resulting in a rapid decrease in pH (< 4.5 in two to three days) is essential. The controlled use of lactic acid bacteria starter cultures would therefore greatly reduce the bacteriological risks associated with fermented fish products.

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Table 1. Categories of enzyme hydrolysed/bacterially fermented fish products.

Category	Salt % (w/w)	Carbohydrate	Indigenous starter culture
Hydrolysed	> 15-20 (high)	-	-
Hydrolysed/ fermented	8-15 (high)	+	-
Fermented	< 8 (low)	+	-
	< 8 (low)	+	+

Table 2. South East and East Asian low salt fermented fish products[1].

Country	Name	Ingredients	Indigenous starter	References
Thailand	*Som-fak*	minced fish, salt, rice and garlic	-	Østergaard *et al.*, 1998; Saisithi *et al.*, 1986
	Plaa-som	fish, salt, palm syrup, (roasted rice)	-	Paludan-Müller *et al.*, 1998
	Plaa-chao	fish, salt, rice	*look-pang*	NRCT, 1981-1982
	Plaa-paeng-daeng	marine fish, salt, rice	*Angkak*	
Cambodia	*Mam-chao*	fish, salt, syrup, rice	*angkak*	Westenberg, 1951
	Mam-ca-loc	fish, salt, ginger, sugar, pineapple, roasted rice, *mak-hak*	-	
The Philippines	*Burong isda*	fish, salt, rice	*+/- angkak*	Sakai *et al.*, 1983a & b
	Burong bangus	fish, salt, rice	-	Olympia *et al.*, 1992
	Burong dalag	fish, salt, rice	*angkak*	Orillo and Pederson, 1968
	Balao balao	shrimps, salt, rice	-	Solidum, 1979; Arroyo *et al.*, 1978
Korea	*Gajami sikhae*	fish, salt, millet, garlic, ginger, pepper	-	Souane *et al.*, 1990
Japan	*I-sushi*	fish, salt, rice	*koji*	Tanikawa, 1971
	Saba-narezushi	fish, salt, rice	-	Fujii *et al.*, 1992

[1] *The table is not comprehensive. The listed products are mostly products, where the fermentation process and microbiology have been investigated.*

Table 3. The effect of NaCl concentration on chemical and microbiological changes during fermentation of *plaa-som.*

Salt (% w/w)	Microflora	cfu/g	pH, day 5
10,5	*Zygosaccharomyces rouxii*	10^6	6,0
	Staphylococcus sp.	10^7	
	Zygosaccharomyces rouxii	10^6	
9,1	*Staphylococcus* sp.	10^9	5,8
	Tetragenococcus sp.	10^9	
	Zygosaccharomyces rouxii	10^6	
7,1	*Tetragenococcus* sp.	$5x10^8$	4,8
	Lactobacillus sp.	$5x10^8$	
	Zygosaccharomyces rouxii	10^6	
5,8	*Tetragenococcus* sp.	$5x10^8$	4,4
	Lactobacillus sp.	$5x10^8$	

Table 4. Chemical and microbiological characteristics of *som-fak.*

Salt % (w/w)	% garlic	pH < 4.5 (days)	% acid[a]	LAB counts (cfu/g)[b]	References
2.9	6	2	2.5	$5x10^8$	Østergaard *et al.*, 1998a
3.5	4	2	2.0	10^7	Saisithi *et al.*, 1986
4.0	8	3	2.6	$2x10^9$	Chanthachum, unpubl.
4.2	4	2	1.8	10^9	Østergaard *et al.*, 1998a

[a] *total titratable acid calculated as lactic acid when pH < 4.5.*

[b] *when pH < 4.5.*

Table 5. Limits for growth of pathogenic microorganisms*.

Pathogenic bacter	pH minimum	NaCl (%) maximum
Indigenous		
Fish/tropical waters		
Aeromonas	4.5	4-5
Cl. botulinum proteolytic type A,B,F	4.0-4.6	10
Cl. botulinum non-proteolytic type B,E,F	5	3-5
Cl. perfringens	5.5-5.8	5-8
Listeria monocytogenes	4.6	10
Plesiomonas	5	9
Vibrio cholerae	5	< 8
Vibrio parahaemolyticus	4.8	10
Vibrio vulnificus	5	5
Indigenous rice/spices		
Bacillus cereus	5	7.5
Human/animal reservoir		
Escherichia coli	4.4	6-8
Salmonella	3.8	4-5
Staphylococcus aureus	4	10-15

* *Data are from ICMSF (1996), Huss (1994) and Notermans and Batt (1998).*

TRADITIONAL FERMENTED FISH PRODUCTS IN INDONESIA

by

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ABSTRACT

Fermented fish products have an important role in stimulating appetite by providing unique aromas and flavours. Many kinds of fermented fish products can be found in various parts of Indonesia. Raw materials include whole fish, comminuted fish and viscera from marine and fresh water fishes. The products are peda, jambal roti, kecap ikan, terasi, ikan tukai, bekasang, bekasam, naniura, picungan, and cincaluk.

INTRODUCTION

Traditional processing makes a significant contribution to fish preservation in Indonesia as about 50 percent of the total marine catch is processed and traditional methods account for more than 40 percent of that.

One of the traditional fish processing methods is fermentation and many kinds of fermented fish products can be found in parts of Indonesia. Those products are able to provide specific unique characteristics. In fermented processing, there is a transformation of organic substances into simpler compounds either by the action of micro-organisms or by the action of enzymes from the fish (Beddows, 1985). At the same time, the fish odour of the starting material is changed to the flavour and aromas of certain foods (Bukholder *et al.*, 1968). Enzymes are most significant in changing the texture and producing some of the flavour but micro-organisms aid in the development of aroma and flavour (Beddows, 1985).

Consumption of fermented fish products in Indonesia is mainly to introduce specific flavours which encourage the appetite. The many fermented fish products available in Indonesia provide many different flavours which meet various consumer appetites. Unfortunately, most of the fermented products are only available locally. Thus, a fermented product in one province will be very difficult to find in other provinces.

This paper mainly addresses fermented fish products found in Indonesia. Due to lack of scientific information about most of the products, the paper emphasizes processing technologies, which are traditionally employed by processors.

PRODUCT TYPES

Based on the information which has been compiled, Indonesian fermented fish can be divided in several ways, such as in terms of raw materials, fermentation types and final product forms.

a. Raw Materials

1. fresh water fish: bekasam
2. marine fish:
   2.1. whole gutted fish: peda, jambal roti, ikan tukai, cincaluk, picungan, and naniura
   2.2. pounded fish/shrimp: terasi
   2.3. visceral: bekasang

b. Types of Fermentation

1. fish and salt fermentation: peda, jambal roti, ikan tukai, bekasang, terasi, and kecap ikan
2. fish, carbohydrate and salt fermentation: bekasam, cincaluk, naniura, and picungan

c. Final Product Forms

1. dried fish: peda, jambal roti, and ikan tukai
2. moist fish: bekasam, naniura, and picungan
3. lumped pounded fish/shrimp: terasi
4. liquid/semi-liquid: kecap ikan, bekasang, and cincaluk.

FERMENTED FISH PRODUCTS

Peda

Peda is mainly found in Java. This product is processed using mackerel (*Rastrelliger negletus*) as raw material. Fresh water fish processed into peda did not result in products with similar quality (Sukarsa, 1979). Nutritional quality of peda can be seen in Table 1.

Table 1. Nutritional quality of Peda.

Parameter	Amount in 100 g sample
Moisture content (g)	46
Energy (Calorie)	156
Protein content (g)	28
Fat content (g)	4
Ca (mg)	174
P (mg)	316
Fe (mg)	3.1
Vitamin A (IU)	110
Vitamin B1 (mg)	trace
Vitamin C (mg)	trace

Source: Soedarmo and Sediaoetama (1977)

The basic method of peda processing is a salting process with two steps. The first salting step takes several weeks to develop its characteristic flavour and texture, and this is followed by a maturation phase. Salting is carried out using a 1:3 salt to fish ratio. Fish and salt are arranged in layers alternately in a salting tank. By the end of the process, the fish are soaked in saturated brine pickle with coarse salt remaining at the bottom and the top covering the fish. This salting process normally takes 3 days. Maturation is performed by mixing the fish and salt thoroughly. The amount of salt used is a one third of fish weight. During maturation, the fish are kept in bamboo baskets, the process taking around 1-2 weeks (Rahayu, 1992). Better products can be obtained by using eviscerated fish (Hanafiah, 1987) and without salt addition in the second fermentation (Hanafiah, 1987; Irianto, 1990; Irianto and Brooks, 1994).

Investigations conducted by Nur and Sjahri (1979) indicate that fish viscera and vacuum encourage the fermentation process. Use of a starter made from matured peda has a similar effect to fish viscera. Sjachri and Nur (1979) improved pedah quality by introducing antifungals and antioxidants during processing.

Microbiological studies on peda conducted by Hanafiah (1987) revealed Gram-positive cocci predominated and some were identified as lactic acid bacteria. Isolation of halo-tolerant bacteria from peda by Suwandi (1988) concluded that bacterial growth in peda was characterized as Gram positive cocci, which were non-motile, aerobes or facultative aerobes, catalase positive, non-indole producers and oxidase negative. They can utilize citrate as the only carbon source, ferment glucose and show proteolytic activity. Some of them are able to reduce nitrate. These bacteria can be classified as mesophiles and require a pH of 6-8. They show variations in salt tolerance and can be divided on the basis of salt tolerance as weak, moderate and halotolerant bacteria. Idawati (1996) showed that lactic acid bacteria isolated from peda were homo-fermentative and hetero-fermentative *Lactobacillus* sp., *Leuconostoc* sp., and *Streptococcus* sp..

Jambal Roti

Jambal roti is processed from marine catfish (*Arius thalassinus*). Production centres in Java are Pekalongan, Cilacap, Cirebon and Pangandaran. Basically, processing methods applied in all production places are the same, but each location has their own specific ways to process jambal roti.

In the processing method employed by processors in Cirebon, the fish are first beheaded and eviscerated. They are then washed and soaked in fresh water for 24 hours. After draining, the fish are arranged in a basin. Each layer of fish is sprinkled with salt and the bellies are also filled with salt. Total amount of salt used for salting is 30-35% of fish weigh and the salting process takes around 24 hours. After salting, the fish are freed from excessive salt and washed. Clean fish are soaked in fresh water for 15-20 minutes and subsequently split into butterfly form. The split fish is sun dried for 3-5 days (Burhanuddin *et al.*, 1987).

Fish which has been iced usually produces a worse quality product than fish without icing. Nuraniekmah (1996) investigated a new method for processing jambal roti from iced fish by soaking the fish in warmer water to bring the temperature back to normal before processing. The higher the water temperature, the shorter the soaking period will be. Soaking the fish at 40°C needs 60 minutes to reach normal temperature.

Erwan (1992) developed a modified method of jambal roti processing by soaking the fish in 30% coconut sugar solution before salting. He also found that 20% of fish weight was enough for salting. Proximate composition, salt content and pH of jambal roti noted by Nuraniekmah (1996) can be seen in Table 2.

Table 2. Proximate composition, salt content and pH of jambal roti.

Parameter	
Moisture content (%)	49.27 - 49.68
Protein content (%)	54.17 - 61.86
Fat content (%)	0.69 - 1.19
Ash content (%)	34.93 - 38.80
Salt content (%)	7.38 - 8.53
pH	6.57 - 6.91

Source: Nuraniekmah (1996)

Terasi

Terasi is consumed in small quantities as a flavour. The product is not only for local consumption, but is also exported, mainly to the Netherlands and Suriname, in powder form. Terasi is usually made from planktonic shrimp *"rebon"* (*Atyas* sp. or *Mycis* sp.) (Budhyatni *et al.,* 1982). Terasi processors can be found in Java and Sumatera islands. According to Yunizal (1998) terasi can be processed in two ways, i.e. (1) with salt only and (2) with salt and other ingredients.

In the processing of terasi with salt only, firstly rebon is washed, drained and dried, until half dried. During drying, impurities, such as small fish, mussel shells and coral, are removed. After that, semi dried rebon is sifted to separate sand and other undesirable materials. The rebon is then left overnight at ambient temperature and pounded the next day. During the first pounding, salt is added (around half of the total salt required during processing). Total amount of salt used in terasi processing is 2-5% of rebon weight which should be added as solution. Pounded rebon is sun dried and subsequently kept in a container at ambient temperature for 2-3 days. The stored rebon is then pounded for a second time, while the remaining salt is added. After that, the pounded rebon is sun dried and kept at ambient temperature for 2-3 days until soft. It is then ground by many passes through a meat grinder until fine. Fine rebon is formed in cubes or cylinders of 1 kg weight and subsequently fermented for a week or more at ambient temperature (Yunizal, 1998). Proximate composition and important mineral contents of this type of terasi can be seen in Table 3.

Table 3. Proximate composition, energy, and mineral contents of terasi.

Parameter	Amount in 100 g sample
Moisture content (g)	40
Energy (Calories)	174
Protein content (g)	30
Fat content (g)	3.5
Ca (mg)	100
P (mg)	250
Fe (mg)	3.1

Source: Soedarmo and Sediaoetama (1977)

Processing method of terasi with salt and other ingredients is similar to the processing method of terasi with salt only. Specifically, in the second pounding, the salt solution is mixed with coconut sugar and tamarind prior to be added to rebon. For 10 kg fresh rebon, 200g and 250g respectively of tamarind and coconut sugar are used. The additional ingredients accelerate the fermentation process (Yunizal, 1998).

Budhyatni *et al.* (1982) investigated micro-organisms in terasi powder during ambient storage. Results showed that the number of *Lactobacillus* sp was constant during storage. Pathogenic bacteria: *Staphylococcus* sp., *Bacillus* sp., and *Proteus* sp. were present, but *Salmonella* sp., *Clostridium* sp., *Vibrio* sp. and *E. coli* were absent.

Kecap Ikan

Kecap ikan (fish sauce) is not really popular in Indonesia due to strong competition from soy sauce, but is well recognized in West Kalimantan Province (Mulyokusumo, 1974). In East Java, kecap ikan is processed from oil sardine (*Sardinella lemuru*) (Putro, 1993).

Kecap ikan is traditionally produced by fermentation using high salt concentrations. Generally fermentation takes a long time to complete (Suparno and Silowati, 1982).

In the processing of kecap ikan described by Putro (1993), fish are firstly washed and minced. Minced fish are mixed with salt (25-30% of fish weight) and allowed to ferment for 10-12 months. The fermented mixture is then filtered and finally brown sugar and spices are added. Results of chemical analysis of commercial kecap ikan can be seen in Table 4.

Table 4. Chemical composition of kecap ikan.

Parameter	Kecap Ikan A	Kecap Ikan B
Moisture content (%)	66.67	76.89
Ash content (%)	23.5	21.95
Salt content (%)	21.16	11.60
Protein content (%)	10.17	10.51
Fat content (%)	0.50	0.70
Carbohydrate content (%)	1.50	0.30
Energy (calories/g)	5.41	5.24

Source: Poernomo et al. (1984)

Idawati (1996) produced kecap ikan from the brine waste of salting peda. The brine waste was fermented for a month. Identification of lactic acid bacteria isolated from that kecap ikan revealed that bacteria involved in the fermentation of kecap ikan were homo fermentative *Lactobacillus* sp, *Pediococcus* sp and *Streptococcus* sp.

Kumalaningsih (1986) studied the improvement of fish sauce quality by introducing bromeline and papain to prepare fish hydrolysate as well as the use of *Brevibacterium linens* and *Micrococcus* sp. According to the formation of amino-N, the incorporation of *Brevibacterium linens* strain C and *Micrococcus* were considered to be promising.

Ikan Tukai

Ikan tukai is a traditional fermented fish product which is only found in West Sumatera. Ikan tukai is also called *lauak tukai* or *ikan sambal lado*, and is mostly processed from barracuda (*Sphyranea* sp.).

The traditional processing method of ikan tukai is unique. Barracuda is washed and soaked in 20% brine for around two hours. After draining, the fish are dried for a day. Dried fish are then wrapped with taro leaves. The wrapped fish are kept underground for two days to allow fermentation and then sun dried until dry. Chemical characteristics of commercial ikan tukai can be seen in Table 5.

Table 5. Results of chemical analyses of ikan tukai.

Analyses	
Moisture content (%)	51.01
Salt content (%)	5.05
pH	6.93
TVN (mg/100g)	113.22
VRS (meq/g)	40.97

Source: Effendi (1995)

Effendi (1995) processed ikan tukai using a modified method, in which fermentation and drying were carried out in vacuum incubator and oven respectively. His microbiological studies concluded that bacteria

having a significant role in the fermentation process of ikan tukai were *Micrococcus* sp., *Pediococcus* sp., *Lactobacillus* sp, *Pseudomonas* sp and *Staphylococcus* sp.

Bekasang

Bekasang is a traditional product in North Sulawesi and the Moluccas and is processed from the viscera of skipjack (*Katsuwonus pelamis*), which is waste from *cakalang fufu* (smoked skipjack) processing in North Sulawesi and *cakalang asar* (smoked skipjack) processing in the Moluccas.

Processing methods of bekasang employed by processors in Manado-North Sulawesi are as follows. Skipjack viscera obtained from cakalang fufu processors is washed and mixed with salt at a ratio of 2.5:1. The mixture of viscera and salt is kept for a week to allow fermentation. After fermentation ceases, the fermented viscera are boiled for 2 hours and filtered using gauze. The filtrate is bottled and ready to sell (Wudianto *et al.*, 1996).

Subroto *et al.* (1984) introduced a mincing treatment to viscera before mixing with salt, this resulted in better quality bekasang. Setiabudi *et al.* (1985) investigated salt levels in bekasang processing by varying salt addition from 10 to 20%. Conclusions from the results were that the higher salt addition, the better bekasang quality obtained. Comparison of protein content and pH of experimental bekasang with commercial bekasang can be seen Table 6.

Table 6. Protein content and pH of experimental and commercial bekasang.

Samples of Bekasang	Protein content %)	pH
Experimental bekasang:		
- from minced viscera	37.95	5.65
- from whole viscera	36.52	5.72
Commercial bekasang	22.37	5.77

Source: Subroto et al. (1984)

Bekasam

Bekasam is a fermented fish product processed from fresh water fish which can be found in South Sumatera and Central Kalimantan. Bekasam is served by mixing with chilli and sugar. In bekasam processing, inspite of the salt addition, carbohydrate sources are also incorporated to stimulate the growth of lactic acid bacteria by decomposing into simpler compounds. Carbohydrate sources used are cooked rice, roasted rice and sticky rice Murtini (1992).

Processing of bekasam from common carp (*Cyprinus carpio* Linn) was described by Murtini (1992). Firstly, the fish are beheaded, descaled and eviscerated. They are then cut into butterfly forms and washed. Washed fish are eventually soaked in 16% brine solution for 48 hours prevented from floating by weights. The fish are then drained and cooked rice and sticky rice, up to 50% and 25% of fish weight respectively, are added. Finally the mixture of fish and rice is sealed in plastic jars and allowed to ferment for a week or more. Proximate composition and salt content of common carp bekasam are shown in Table 7.

Table 7. Proximate composition and salt content of common carp bekasam.

Parameter	
Moisture content (%)	66.95
Ash content (%)	5. 76
Protein content (%)	4.81
Fat content (%)	14.95
Salt content (%)	5.72

Source: Murtini (1992)

In order to improve bekasam quality, Murtini *et al.* (1997) added the liquids of cabbage and Chinese leaf pickles as lactic acid bacteria sources into spotted gouramy bekasam. Sensory evaluation indicated that the best bekasam was produced by the addition of Chinese leaf pickle. All bekasam in this study was organo leptically still acceptable after 8-weeks storage.

A similar product called *Naniura* is found in Riau and North Sumatera provinces. In naniura processing, the fish are firstly soaked in lemon juice or 25% acetic acid solution for three hours. After that, ground boiled rice is added and the fish is then packed and allowed to ferment.

Another similar product to bekasam is *Picungan* which can be found in West Java. This product is processed using marine fish. Picungan seeds are used as a carbohydrate source. The seeds are cut into small pieces before mixing with fish and salt.

Cincaluk

Cincaluk is a traditional fermented fish product from Riau Province. This product is usually processed from rebon.

There is no prescribed method to process cincaluk. In a method employed by processors in Bengkalis, fresh shrimp are mixed with boiled rice and salt in a pan. For 1 kg shrimp, the rice added is around 200-300g, while salt is approximately 300g. The pan is then sealed with the lid to avoid air entry and kept for 4 days until liquid release. After that the mixture is put into bottles and eventually sealed tightly. Proximate composition and other chemical properties of cincaluk processed using this method can be seen in Table 8.

Another method to process cincaluk is by mixing shrimp with tapioca flour, salt and sugar at the ratio of 20:1:1:1. In its processing, shrimp are descaled and then washed. Tapioca flour is dissolved in water, gelatinized and allowed to cool. Shrimp are then mixed thoroughly with salt, sugar and gelatinized tapioca flour. The mixture is filled to washed bottles and sealed firmly and fermented at ambient temperature for 1-2 weeks.

Table 8. Proximate composition and other chemical properties of cincaluk.

Parameter	
Moisture content (%)	69.76
Ash content (%)	12.43
Protein content (%)	16.23
Fat content (%)	1.57
Salt content (%)	10.11
Lactic acid content (%)	2.34
pH	4.82

CONCLUSIONS

Indonesian fermented fish products vary in form, raw material and fermentation type. Most of them have not been studied in detail, thus scientific information relating to those products is difficult to find. More studies identify lactic acid bacteria involved in the fermentation which suggests improved product quality can be achieved by using selected lactic acid bacteria.

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MARKET POTENTIAL OF PROCESSED TASMANIAN JACK MACKEREL (*TRACHURUS DECLIVIS*) FOR HUMAN CONSUMPTION

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ABSTRACT

In response to declining world fish stocks, a study of the market potential for developing a product for human consumption using the underutilized species, Tasmanian jack mackerel (*Trachurus declivis*), was undertaken.

A review of seafood consumption, the changing racial composition in Australia and the importance of market intelligence was performed, along with a market survey to determine current consumption of similar products.

Results of the survey indicate that products such as smoked fish, satay fish and fish pates are being consumed and that a further study of the financial and economic benefits and constraints of setting up full-scale commercial production would be worthwhile.

INTRODUCTION

The consumption of seafood and fish products has generally increased. In developed countries, due to a variety of reasons, but in no small part to increased awareness of health benefits, greater disposable incomes, greater promotion of seafood and new product innovation. By the same token, fisheries within the developed world have reached the limits of their capacity. World demand for seafood products is increasing but currently demand exceeds supply (Homer *et. al* 1994). The only likely improvement will come from better fisheries management, increased use of underutilized species and aquaculture.

No longer is the value of Australia's fin fisheries able to be increased by increasing just the volume of catch as was possible in the age of technological advancement like that of the seventies. Instead, one step that should be taken is to enhance the value of landed catch by further processing. With the status of most Australian fin fisheries at full capacity, it makes sense for manufacturers to broaden their production into value adding particularly of those species recognised as underutilized or currently used for purposes other than for human consumption.

In Australia the domestic market consists mainly of low-value finfish supplied by small owner operators and importers. Very little value adding to products occurs on a domestic basis (National Seafood Study, 1993). Underutilized species or those that are processed adding further value, have the ability to dramatically increase the value of the Australian catch.

Historically, Australians typically consumed white boneless fish fillets cooked by grilling or frying, but Australian society is changing, larger percentages of Asians and Europeans are migrating to Australia bringing with them different tastes and traditional foodstuffs. The influence on the diets of Westerners that this has had is yet to be accurately determined but coupled with increased awareness of health issues, and the benefit of

consuming fish, the shift in the type and amount of seafood demanded could be partly accredited to these reasons.

LITERATURE REVIEW

POTENTIAL OF JACK MACKEREL

Jack mackerel are available in large quantities off the South East Coast of Tasmania. As they appear in large schools, they are ideally caught by purse seine. Because of the ease of capture, they are of interest to fishermen. The current annual catch is approximately 20,000t based mainly at Triabunna on the East Coast of Tasmania. The fishing season at Triabunna lasts from October to May. Smaller fisheries also exist in South Australia, Victoria and New South Wales.

The species is well studied and understood with figures of the maximum sustainable yield in Australia estimated to be at 30 000 to 50 000 t a year.

Currently, less than 1% of the total catch of jack mackerel is processed for human consumption. Mainly the catch is processed for fishmeal and frozen bait. Jack mackerel is ideally suited to further processing due to the flesh composition. As it is not a true mackerel but belongs to the jacks or trevally family, it is related to many species currently used for human consumption. Jack mackerel is a popular fish, eaten particularly in Japan and Korea.

Jack mackerel shows high potential as a species for post harvest processing, particularly in areas such as smoke preserving. As some of the components of smoke are fat and water soluble, fatty fish smoke better than lean ones. Jack mackerel shows seasonally high fat contents and has been reported by Hookway (1994) to produce a good quality smoked product.

Smoking is the oldest method for preservation of protein food stuffs but due to changes in taste preferences, smoking is now more concerned with presenting alternative and distinctive tastes to the consumer (Kow, 1995). Colour is shown to be the most important factor, since vision is the first sense consumers use in evaluation of the products, this is followed by aroma, flavour and texture. One benefit to be gained from smoking jack mackerel is disguising the darker flesh that has been shown to be disagreeable to consumers

The main interest in value adding in Australia is to improve the value of low value products like jack mackerel. The majority of the Australian catch is low volume, high value species like prawns or rock lobster which are exported either whole or live. This is because the value of the species is high enough without the requirement or desire for further processing. Value adding for fishermen is synonymous with profit adding when dealing with what is commonly referred to as low grade or value fish. The main impediment to value adding in Australia is its cost. Weighing up the cost to benefit is the most important consideration when deciding whether to start processing seafood. There are instances where deciding to implement the required technology needed to process seafood will give little return for the money out laid, but there are instances, when transforming a low value product into a gourmet item pays off (ASTEC, 1989).

INDICATIONS OF CHANGING DEMOGRAPHICS

In 1947, 97.4 % of the population was born in Australia, New Zealand or the United Kingdom and Ireland. The abandonment of the Australian government's " White Australia" policy saw a large decrease in the proportion of anglo-saxon arrivals. Data from the latest national census (1991) indicates the numbers of Asian immigrants has greatly increased. In 1992, 13.0 % of settler arrivals were from the United Kingdom, 18.3 % from Asia and 26.3 % from Europe. Overall, in the last decade, migration from south east Asian countries like

Malaysia and Singapore has risen by 131%, migration from north east Asian countries has risen by 267 % and migration from southern Asian countries such as India and Pakistan has risen by 97 % (ABS 1994).

Australia's population growth was 1.02% in 1993, the lowest since 1976. This growth of 178,900 comprised of 143,800 from natural increases and 35,100 from overseas migration (ABS 1994). The population is projected to rise to 19.0 million in the year 2000 and up to 21.0 million sometime between 2009 and 2011.

The demand for seafood is influenced by income and its distribution, the population size, tastes and eating habits of the population as well as the price of seafood and its substitutes. The relationship between demand of seafood and its price is negative. The substitutes for seafood are usually recognised to be chicken and pork products, but in the case of smoked or pate seafood they may be products such as vegetable pates, preserved meats and other similar delicatessen lines. Increases in population size also display positive effects on the demand for seafood. ABARE (1992) states that Australia displays a positive increase in seafood demand with increases in income. Due to the decreased economic growth in this country, this is likely to slow the demand for certain luxury items of seafood. Attitudes to health and the ageing society in Australia will have a positive effect on the amount of seafood purchased and consumed in home (ABARE 1992)

Australia, though having a large exclusive economic zone, has relatively unproductive waters and little production of processed fish products such as smoked fish and smoked fish pates. For this reason the majority of Australian seafood products are sourced by imports. The total value of Australian seafood imports rose by 12% to $ 666 million dollars in 1994. These imports were mainly sourced through Thailand (25%), New Zealand (18%) and America (7.2 %) (ABARE 1995). As most Australians are currently aware there is much talk about the balance between the value of Australia's imports and exports. Seafood imports could be decreased by domestic production of many of the products demanded and currently sourced offshore.

MARKETING INTELLIGENCE

Understanding consumer attitudes is paramount in seafood marketing. The first step in producing a successful new seafood product, or any new product for that matter, is to understand the needs and desires of the consumers as well as the size and location of this demand (Wood 1987). Usually this information is obtained through market analysis. There are many different methods in use to analyse a market but probably the simplest to understand and most useful is the standard consumer survey. A survey allows information to be gathered on a specific market, the present and future needs and how to best meet these needs (Barker et al 1994).

Many previous studies have discussed the matter of promotion of seafood products, and find is sadly lacking. The Senate Standing Committee on Trade and Commerce (1992) found that "The industry as a whole must engage increased national promotion of products and educate its consumers in its use before benefit from increased domestic seafood consumption can be obtained". The main objective of promotion is to increase awareness of products and hence demand. Activities like trade fairs, retail promotions and packaging and labelling are good methods of seafood promotion (Wood 1987).

General market characteristics which provide good opportunities for promotion include the existence of a favourable demand trend, the presence of potential consumers and a situation where quantity demanded of a product is very responsive to price change (Battaglene 1990).

The eating habits of this generation are very different to those of previous ones. Many different environmental factors interact to influence a consumer's purchasing decision. Healthy low fat meals are in demand while at the same time the average Australian is working longer and harder, not to mention the decrease in "stay at home mothers". Hence it leaves little time for leisure without having to spend more than absolutely necessary on domestic chores like meal preparation. Convenience is the buzzword of the nineties in the seafood industry. Obtaining this type of information before introducing new food products is vital to produce a product that not only obtains initial consumer interest but a continuing interest that results in repeat sales (Wood 1987).

METHODS

A stratified survey was performed in Melbourne between July 30th and August 6th 1996. A sample questionnaire shown is in Figure 1. Four areas in Melbourne were chosen because of historical ethnic settlement in the areas. To enable selection of the survey areas Australian Bureau of Statistics information was obtained. The statistical racial breakdown by labour force as zoned by the ABS, included information on the population in the main statistical regions of Melbourne, the racial breakdown by region, country of birth and the birth place of Mother by birth place of Father.

SURVEY SHEET FOR MARKET ANALYSIS OF JACK MACKEREL POTENTIAL

AREA NAME
SURVEY NO#

		YES	NO
QUESTION 1a	DO YOU EAT SMOKED FISH?	.....	.....
QUESTION 1b	DO YOU EAT SATAY FISH	.....	.....
QUESTION 1c	DO YOU EAT FISH PATES	.....	.....

QUESTION 2 HOW OFTEN DO YOU CONSUME THESE PRODUCTS?
tick the correct box **1** **2** **3** **4**

QUESTION 3 DO YOU PREFER TO PURCHASE IMPORTED OR AUSTRALIAN PRODUCED PRODUCTS OF THIS TYPE YES NO

QUESTION 4 WHAT, IN ONE WORD, IS THE MAIN FACTOR THAT INFLUENCES THIS CHOICE TO PURCHASE IMPORTED OR AUSTRALIAN PRODUCED PRODUCTS

QUESTION 5 ARE YOU INTERESTED IN TRYING A NEW AUSTRALIAN PRODUCED YES NO

AGE GROUP: <18 18-30 30-40 40+

MAIN LANGUAGE SPOKEN AT HOME OR PARENTS BIRTHPLACE

Figure 1. Sample questionnaire. For question 2. 1= once a week or more 2= once a month or more, 3= at least once a year and 4= rarely or special occasions.

The surveys were analysed by proportion of respondents and their responses to each question. The answers to the questions hence needed to be put into a code to be useable. Responses to question 4 needed to be categorized. Because this question was open ended, there was a large variety of answers. Seven categories were chosen these being; price differential between imported and domestically produced products, quality differential, perception of healthiness, perception of freshness/safety, advertising programs particularly "Buy Australia" campaign, habit and taste. All responses were able to fit into these categories. The coded responses were then analysed by ethnicity and product type. Further proportional analysis would have been performed if results from an evoked response set showed that other factors such as age were significant. To determine the significant factors affecting the purchasing behaviour of consumers an evoked response set analysis was performed to enable a logit model to be run. This model is in the form of:

$$PF = a_0\ a_1EX\ \ a_2P\ \ a_3Q\ \ a_4HLTH\ a_5F\ \ a_6CMP\ a_7H\ a_8T\ a_9A\ a_{10}LA\ N$$

Where PF = preference, EX = experience, P = price, Q = quality, HLTH = healthiness, F = freshness, CMP = campaigns, H = habit, T = taste, A = age, and LAN = language spoken.

To be able to perform an evoked response set analysis the data needed to be put into binary code. For this purpose a positive answer was assigned a value of 1 and a negative answer assigned a value of 0 (Table 1). Because some of the questions had more than two possible answers it was necessary to decide what level was relevant and what would be discarded. To perform a logit analysis a measurable variable is necessary, therefore each age group was averaged, and the respondents assigned the median age. This data was analysed using the statistical program Shazam 7.0, which generates the T ratio, to enable determination of each variable significance. The data was analysed three times by language group, to determine significant factors that influence the purchasing decision of consumers by ethnicity.

The data for English speaking respondents was shown to suffer from multicollinearity, this is due to the models independent variables being strongly correlated. This is overcome by dropping insignificant variables out of the model, as was performed with the data set for English speaking respondents. All variables except price differential and quality perception were dropped. As no multicollinearity was shown in the data sets for Europeans and Asians this was not required.

Table 1. Binary code for each question in the survey.

QUESTION NUMBER	RESPONSE AND CODE ASSIGNED
1a, b & c	yes = 1, no=0
2	once a week or more & once a month or more = 1 once a year at least & rarely or special occasions = 0
3	yes = 1, no = 0
4	because each respondent only chose one factor of significance if that factor was chosen = 1 all other responses for that respondent = 0
5	yes = 1, no = 0
6	each respondent was assigned the mean age for the age group they came under
7	data analysed three times, DATA SET 1; if Asian =1 all others = 0. DATA SET 2; if European = 1 all others = 0 DATA SET 3; if English =1 all others =0

RESULTS AND DISCUSSION

Of the surveys performed, 106 respondents were obtained. Of these respondents, 24% were European, 34% were Asian and 41% were English speaking. Three different product forms that are currently available in retail outlets throughout Australia were investigated. These were smoked fish types, satay fish on a stick products and fish pate type products.

The survey was designed to determine five factors that were considered important in analysing the buying behaviour of consumers. Firstly, the consumption of similar products and the frequency of that consumption, secondly if loyalty to Australian produced products existed and the consumers main consideration

in the purchasing decision. Demographic data that was considered important was also analysed and this included ethnic background or language spoken at home and the age of the respondent.

CONSUMPTION

The results from the survey show that overall processed fish products are currently being consumed and that there may be room for new types of product without excessive competition. Overall, 61.3 % of all respondents questioned ate smoked fish of some kind, 33% ate satay fish and 50% ate fish pates, mousses and terrines. This is a fairly significant result, showing that processed fish products are a popular form of seafood consumption. The perception of these types of products differed between each ethnic category. For example English speaking respondents perceptions of what was considered a smoked fish product differed from that of European respondents. Most English-speaking respondents perceived smoked fish as smoked salmon, cod, or haddock as used in breakfast dishes such as kippers. Europeans referred to smoked mackerel and haddock. Unfortunately the questions in the survey were not specific enough to determine whether consumption of hard smoked products is different from soft smoked products.

The survey did not ask respondents if the products were eaten in home or out of home. A large percentage of the consumption of products such as these may well be consumed in restaurants. The National Seafood Consumption Study (ARDC 1992) found that many respondents though liking fish, did not know many ways to prepare it or disliked the mess involved with it's preparation, therefore pre-packed dishes of this type could appeal to these people. The lifestyles of Australians nowadays are very different to that of previous years. With more women entering the workforce and all of us working longer hours, convenience is a big selling point. Therefore, a heat and eat meal would also appeal to those looking for convenience and ease of preparation.

Hookway (1994) found that ethnic taste testers of a pate prototype utilising jack mackerel, mainly did not differentiate between a commercially available mackerel pate and the jack mackerel prototype. She postulated that a market for a jack mackerel product might exist without trying to replace existing products.

CONSUMPTION FREQUENCY

The frequency of purchase or consumption of these products can be termed as the consumers' experience. A large percentage of respondents purchased similar products at least once a month or more showing that significant consumption of similar products, particularly smoked fish and smoked fish pate are occurring in all ethnic groups. The structure of Australian society has changed quite dramatically in a short period of time, influencing the demand for type and amount of seafood products.

The measurement of preference of Australian products over imported ones was of the utmost importance to this project. Of the English- speaking respondents 51.3% preferred to buy Australian, showing the highest preference followed by Europeans at 40% and Asians at 33.3%. This data was used to determine if an Australian produced product had. The number of respondents that preferred to buy Australian over imported goods show that an Australian produced product containing jack mackerel may not need to compete with already established products but fit neatly into its own market niche. Packaging and labelling may therefore become a major factor in the success of a new product. Consumers could choose the product solely on the fact that it is Australian produced. Fish that is sold fresh or frozen without any identifying marks makes brand promotion and loyalty very difficult. (Bose 1996 pers comm.). In this case loyalty is likely to be concentrated through the retailer rather than the final consumer.

Actual sample products that have been trialed previously have obtained a good reception in Victoria, NSW and Tasmania despite the perception of the eating properties being poor (Hookway 1994). Overall 93.4 % of all respondents to the surveys performed in this investigation were interested in trying a new Australian produced fish pate, satay or smoked fish product.

Generally, consumers know very little about the fish they buy. The purchase is made of species that are familiar to them. Promotion of a more diverse range of fish could cause greater consumption of less known species. There is very little fish caught which cannot find a market somewhere or which cannot be considered as suitable for further processing. I am convinced that there is a market for virtually all seafoods. (Townsend, 1984)

PURCHASING DECISION

To enable analysis of the factors influencing the purchasing decision by consumers a logit analysis was performed on the survey results. The outcome has been determination of the principal influences concerned with consumer's decision making process where food products are concerned. These results are important for determining the correct strategies for marketing new products on the market. Large percentages of new products introduced fail within the first twelve months and this is particularly true in respect to food products in a country where choice is virtually unlimited. The factors of significance were found to be taste of product by Asians, while Europeans were influenced by advertising campaigns such as "Buy Australia". Quality was found to be the most important influence by English-speaking respondents. Price, of course should have shown significant influence in all ethnic groups, and during analysis the usually negative relationship between demand and price was displayed. It is interesting to note that in none of the ethnic groups was price the determinative factor in the purchasing decision. The interesting results to this analysis give an insight into marketing techniques for different demographic market segments. It is important that marketing is directed towards the consumer most likely to be interested in the product and that it informs them that this product is capable of fulfilling their needs. The best way of doing this is to understand what drives that person's behaviour and their decision on whether to purchase or not. It is very important that consumer preferences are known so that the correct marketing strategies may be employed.

CONCLUSIONS AND RECOMMENDATIONS

The proportion of respondents that consumed smoked fish and fish pates were significant, over 50% in both cases. Of these respondents, a high percentage consumed them more than once a month. The result for satay fish was hardly significant, but encouraging none the less. Other ready-made meal type products produced from jack mackerel could be investigated. A large number of the respondents preferred to purchase Australian produced goods offering an avenue for promotion. As well the main reasons for the consumers purchasing decisions are relatively easily met.

When this project was first undertaken, part of the aim was to find if the reason why speciality type seafood products produced in Australia were not doing well was because of language barriers in packaging or habit and loyalty to imported brands. It is encouraging to see that this is not the case. The main considerations are quality, taste and promotion, which need to be focused on when marketing. Taste is probably the most difficult to deal with, as each ethnic group has their own preferences. During the surveys, it became quite clear that Asians do not think that Australian products are spicy enough. Making the product spicy may not appeal to the majority of Caucasians.

One of the main impediments to the marketing of these products may be the consumers association of the name jack mackerel with a fish not possessing good eating properties. If at all possible it may be advantageous to market under another commonly used name, such as cowanyoung as the jack mackerel is known in NSW or herring scad as it is known in New Zealand.

The main limitation of this study was the small numbers of respondents in the surveys performed. Additional information would have greatly enhanced the analysis of this project, such as income data.

It is recommended that value added products be developed and study be made to investigate the market potential of these products.

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MAXIMIZING UTILIZATION OF LOW-VALUE FISH FOR A BETTER FUTURE - USING BLACK TILAPIA AS THE MODEL

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ABSTRACT

The estimation for the cost of production of breaded tilapia and hydrolysate from the head were carried out to determine the feasibility of commercialising the products. This was in view of maximizing the utilization of the resources. It was found that the costs of producing breaded tilapia fillets was Rm 13.72 and that of hydrolysate was Rm 1.81[1]. Based on the comparison of prices of competing products in the same category of products, it was concluded that the production of both the commodities were very viable.

INTRODUCTION

Fish harvested from marine resources are not as controllable as fish from aquaculture activities. Black tilapia is one of the many fishes which are produced from these activities. Its production volume is constrained by its pattern of consumption and utilization i.e. mainly in fresh or unprocessed form. Its marketability in some Asia-Pacific countries such as Malaysia is also limited by the less popular characteristic muddy odour and flavour. This characteristic is mainly due to the presence of geosomin and 2-methylisoborneol (Yurkowski and Tabachek,1974, 1980; Kuusi and Suihko, 1983). Off-flavour in fish is a world-wide problem in cultured fish (Lovell et al., 1978). One of the possible avenues for increasing its market value and share is by developing new value-added products and by utilizing the by-products of the industry. Studies carried out at the faculty has proven that the battered and breaded tilapia fillets are marketable (Jamilah and Wong, 1996; Jamilah and Siti Aini, 1997); however, the market price as well as the commercialisation of the product has not been tested. Presently, there are many breaded fish products sold in the market - filleted and surimi based, in particular. These products are non-tilapia, non-freshwater/brackishwater fish based. They are potential and direct competitors to breaded tilapia. Thus, the pricing of breaded tilapia must take into account the competitors' pricing i.e. the price of breaded tilapia should be at par or lower than that of the competitors' prices. The costing of hydrolysate which is the by-product of breaded tilapia will also be considered. At the time of this study, no production of hydrolysate from local companies could be registered. Therefore, the objective of this paper is to estimate the cost of production of breaded tilapia and hydrolysate to determine the feasibility of commercialising these products.

[1] At the time of writing, the exchange rate was approx. Rm 4.1 to US$ 1.00

METHODOLOGY

Live black tilapia (*Oreochromis mossambicus*) was purchased from a fish farmer nearby the university. They were brought alive to the processing laboratory and killed immediately with a sudden blow on the head. Filleting was then done manually and the fillet was first washed in ice-slush and rinsed in cold water to remove blood and slime. Weight of fillets and heads obtained were recorded to calculate the yield. They were then pooled and placed in the refrigerator prior to the battering-breading and hydrolysis process. The battering and breading of the fillets for the production of breaded product was according to Jamilah and Siti Aini (1997). The fish heads were washed, homogenised and hydrolysed according to Yanez et al. (1976) and Mackie (1982). The yield of the hydrolysate was determined by measuring the volume of hydrolysate obtained after centrifugation versus the original volume.

Each processing step was timed to gather information on the time required to complete the job. Information on cost of equipment was sourced from recent actual purchasing of the equipment or was sourced from the local suppliers. Information on insurance and others were sourced from agents. Detailed information on oil and breading material pick-up were obtained from Jamilah and Wong (1996). The pricing of competitive products as surveyed in several chain supermarkets and hypermarkets within 50 km radius of the university campus. The costing for the production of breaded tilapia and hydrolysate was based on the capacity identified for medium scale industry (capital investment of Rm100K to 200K).

RESULTS AND DISCUSSION

Like all other manufacturing activities, the manufacturing cost must be known to measure inventory values (and so the cost of goods sold) and profitability (revenues minus expenses, which include cost of goods sold).

Breaded tilapia:

Three cost elements in the production of breaded tilapia are identified. They are the direct material, direct labour and factory overheads. The direct materials (Table 1) are black tilapia, breading material and frying oil. The average yield of black tilapia fillet is 38%, the breading material and frying oil pick-up are 3 and 2% respectively. Cumulatively, the yield for every kg of black tilapia is 43% or 430 g of breaded tilapia.

Based on the statistics derived in Table 1, the amount of black tilapia required to produce 1 kg of breaded fillet can be computed. Table 2 shows that for every 2.63 kg of black tilapia, 1 kg of fillet will be produced. At the time of study, the average cost of black tilapia was Rm3.20 per kg. Thus, the total main material cost to produce 1 kg of breaded fillet is Rm8.42. However, this cost can be lower since the ex-farm price is at Rm2.40 - 2.80 when purchasing is done at bigger quantities. As cooking oil and breading material are considered as part of the direct material costs, they are then added together to determine the total direct material cost. Therefore, the total estimated direct material costs to produce 1 kg of breaded tilapia is approximately Rm8.70.

Table 1. Yield calculation for breaded tilapia and hydrolysate.

Yield from 1 kg of live tilapia:

1 Kg of live tillapia can produce	380 gms of fillet	38%
Breading pick-up	30 gms	3%
Frying oil pick-up	20 gms	2%
Yield	430 gms	43%

Based on yield statistics above raw materials used can be compute follows:

Fillet:

1 Kilogram of live tilapia X 38 % = **380 gms**

Breading material required to produced 1 Kg of fillet:

1,000 gms X 30 gms = **79 gms**
380 gms

Cooking oil required to produced for every 1 Kg of fillet:

1,000 gms X 20 gms = **53 gms**
380 gms

HYDROLYSATE:

Raw material is derived from live tilapia fish head as follows:
1 kg of live tilapia is equivalent to 180 gms (18%) of fish head.

Table 2. Estimated production cost of 1 kg of Breaded Tilapia.

Manufacturing cost elements	Estimated Quantity	Estimated x cost per kg	Total
Raw materials:			
	Kg	Rm	Rm
Live Tilapia	2.63	3.2	8.416
Breading	0.079	2	0.158
Cooking oil	0.053	2.4	0.1272
			8.7
Direct labour:			
	Estimated hours	Estimated cost per hour	
Labour hours used	0.67	5	3.35
Manufacturing overhead:			
Estimated overhead			1.65
Estimated cost of 1 kg of breaded tilapia			13.7

It requires 0.67 hr of direct labour to produce 1 kg of breaded tilapia. Currently, the average market rate for labour is Rm5.00 per hr. Hence, the average cost of direct labour to produce 1 kg of breaded tilapia is Rm3.35 (Table 2).

Finally, the firm has to incur the factory overhead which consists of costs that are indirectly associated with the manufacture of the finished product. They are manufacturing costs that cannot be identified as either direct material or direct labour. Table 3 details the manufacturing costs that should be

captured under the heading of factory overheads - utilities, packaging material, supervision, indirect labour, factory insurance, and depreciation of fixed assets (i.e. depreciation of factory buildings and machinery). Table 5 shows a detail listing of fixed assets required to produce breaded tilapia. Based on a one yr projected production of 36,400 kg of Rm1.67.

Table 3. Estimated production cost of 1 L of Hydrolysate.

Manufacturing cost elements	Estimated quantity Kg	X	Estimated cost per Kg Rm	Total Rm
Raw material:				
Tilapia head	1		0	0
Direct labour:				
Labour hours used	0		0	0
Manufacturing overhead:				
Expected overhead per litre				1.81
Estimated cost of 1 L of hydrolysate				1.81

To sum up, the estimated total manufacturing cost of 1 kg of breaded tilapia is approximately Rm13.72 (Rm8.70 + 3.35 + 1.67). The cost derived is only the manufacturing cost which exclude the administrative and selling expenses. To make profit, the firm's projected/planned selling price should be greater than the projected manufacturing costs, administrative and selling expenses. This estimated cost seems to be competitive since the present closest competing product is a breaded marine product which is selling at Rm4.30 - Rm4.90 per 250 g pack. This approximates to Rm17.20 per kg based on the average selling product price of Rm4.60 per 250 g. However, surimi-based products are cheaper, i.e. selling at an average price of Rm4.90 per 500 g.

Hydrolysate

The costing of the hydrolysate which is produced from the fish heads (the industrial waste) can contribute positively to the firm's profit. This would mean that the raw material cost for the production of the hydrolysate is nil given the firm's main objective is to produce breaded tilapia and the production of hydrolysate is secondary i.e. it will be produced when the firm decides to produce breaded tilapia. On the other hand, if in the beginning it was decided that the firm's main objective is to produce both products than it would be fair to include the fish head as a direct cost in the production of hydrolysate. Thus, it is logical to assume that there is no direct material cost required to produce the hydrolysate (Table 3).

It requires 95,732 kg of live tilapia to produce the projected 36,400 kg of breaded tilapia. The amount of fish heads that can be collected is 17,232 kg. The estimate is based on the average yield weight of fish heads which is 18% (Table 1). The starting volume of hydrolysis was 34,464 L due to the adjustment made to obtain approximately 8% protein content in the starting material. The yield of the hydrolysate is 80% after the removal of the sediments which is equivalent to 27,571 L (Table 4).

Table 4. Computation for factory overhead, administration and selling expenses.

	Factory Overhead			Admin. & Selling Expense
	Total	**Breaded Tilapia**	**Hydro-lysate**	
Activity Index		Kilogram	Litres	
Amount of production for one year (Note 1)		36,400	27,571	
Costs	**Rm**	**Rm**	**Rm**	**Rm**
Variable costs:				
Utilities (allotted based on 8:2)	17,000	13,600	3,400	
Packaging-plastic bags (Rm0.3x91,000 pieces)	27,300			27,300
Packaging – aluminum laminate of 1 kg (Rm1 x 17,232 pieces)	17,232			17,232
	61,532	13,600	3,400	44,532
Variable cost per kilogram		0.374		
Variable cost per litre			0.123	
Fixed costs:				
Supervision (1 x Rm1,400 per month x 12) allotted based on 8:2	16,800	16,800	4,800	2,400
Indirect labour (2 x Rm 800 x 12)	19,200		19,200	
Insurance (allotted based on floor space) Note 2)	7,180	6,424	460	294
Depreciation (see Table 3)	48,460	23,780	22,200	2,480
Clerk (Rm 1,000 x 12 months)				12,000
Utilities				1,000
	91,640	47,004	46,660	18,174
Absorption rate for fixed cost based on per kilogram of raw material used:		95,732	17,232	
		Rm0.491	Rm2.70	
Estimated predetermined manufacturing overhead rate to produce 1 unit of product:				
Fixed overhead	0.374		0.123	
Variable overhead	1.291 (Note 3)		1.688 (Note 4)	
	1.665		1.81	

Another interesting feature in the production of the hydrolysate is that it requires a negligible effort from labour. This is indirect labour and not a direct labour. Efforts from labour is only used for the transfering of fish heads into the hydrolysis vessel, monitoring of the hydrolysis and dispensing of hydrolysate into the packaging material which requires minimal effort. Thus, like the raw material which carries no cost to the firm, there is also no direct labour cost (Table 3) to produce the hydrolysate.

The only manufacturing cost required in the production of hydrolysate would be the factory overhead. These would include packaging material, supervising, indirect labour, depreciation of machinery - hydrolysis vessel, centrifuge, spray dryer, factory building (Table 5), and insurance (Table 4). To sum up, the cost of producing 1 L of hydrolysate is approximately Rm1.81. Imported fish and cuttlefish hydrolysates from China and Thailand are priced at an average price of Rm3.90 per L.

Table 5 Types, Number of Units and Cost of fixed assets required in the production of breaded tilapia and hydrolysate and the estimated depreciation cost per year.

	Depreciation					Amount of depreciation allotted per year		
	Number of unit	Cost (Rm) per unit	Total (Rm)	Rate applied	Depre-ciation per year	BREADED TILAPIA	HYDRO-LYSATE	OFFICE
Breading machine	1	50,000	50,000	0.1	5,000	5,000		
20 litre batch fryer	3	15,000	45,000	0.1	4,500	4,500		
20 kg washing machine	1	10,000	10,000	0.1	1,000	1,000		
Blast freezer (20 kg)	1	50,000	50,000	0.1	5,000	5,000		
Walk-in chiller (10 x 10 sq. ft)	1	8,000	8,000	0.1	800	800		
Ice making machine	1	9,600	9,600	0.1	960	960		
Walk-in freezer (270 sq. ft (15'x18'x10')	1	30,000	30,000	0.1	3,000	3,000		
Hydrolysis vessel	1	80,000	80,000	0.1	8,000		8,000	
Centrifuge	1	50,000	50,0000	0.1	5,000		5,000	
Spray dryer (50 kg input)	1	80,000	80,000	0.1	8,000		8,000	
3 units of 3HP air-cond. at Rm 5,000 per unit	1	15,000	15,000	0.1	1,500	1,000		500
Building (3,500 sq. ft @ Rm60 per sq. ft) See Note 1 (allotted based on the following ratio-21:10:4)	1	210,000	210,000	0.02	4,200	2,520	1,200	480
Land (10,000 sq ft x Rm 20 per sq ft)	1	200,000	200,000	0	0	0	0	0
Personal computer	1	5,000	5,000	0.2	1,000			1,000
Office furniture (table, chairs and filing cabinet)	1, 3 and 1		5,000	0.1	500	0	0	5,000
TOTAL			847,600		48,460	23,780	22,200	2,480

Note 1: Building space will be utilized as follows:

Breaded Tilapia	3131 sq ft	(89.5%)
Hydrolysate	225 sq ft	(6.4%)
Administrative	144 sq ft	(4.1%)
Total	3500 sq ft	(100.0

SUMMARY

The estimated cost of manufacturing breaded tilapia and hydrolysate from tilapia heads are Rm13.72 and Rm1.81 respectively. Based on the prices of similar groups of products for breaded tilapia and hydrolysate selling price, it is apparently viable to commercialized and fully utilized black tilapia. Although the production of breaded tilapia alone is economical, the production of hydrolysate from the waste of the industry is certainly more profitable.

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A STUDY ON THE FISH PRICE AND CONSUMPTION OF FISH IN THE NINE CITIES OF FUJIAN PROVINCE OF CHINA IN 1997

by

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ABSTRACT

This paper reports changes in the price of the main fish species in nine cities of Fujian province of China in 1997. The characteristics of fish consumption in these regions have been studied. The results show that there were significant differences among the nine cities on the role of fish in household food supply. The consumption habits of the people appear to be more important than fish price. Advice and promotion of consumption will enhance the socio-economic role of fish in household food security.

Fujian province on the southeast coast of China has a mild climate, abundant rainfall, many crisscrossing rivers, 3053 km. of coastline on the mainland, 12 500 km2 of fishing grounds and 0.36 million ha. of inland waters containing productive resources of aquatic animals and plants. These unique conditions are favourable for the development of Fujian fisheries. With convenient communication links, Fujian is in an excellent position to develop its international trade in aquatic products.

There are nine administrative cities in Fujian province (Fig1): Fuzhou, Xiamen, Putian, Quanzhou, Zhangzhou, Longyan, Sanming, Nanping and Ningde. Longyan, Sanming and Nanping are situated inland, the other six are in the coastal area. Xiamen is a special economic zone.

In 1997 the population of Fujian was about 32 million. The annual output of Fujian aquatic products is 4.29 million tons, about 12% of the total national production and in third place in China. The figure for per caput consumption in Fujian is 133 kg/yr, the highest in China. The value of fish production is second to that of agriculture of the province, it includes 2.0 million tons (46%) from marine fishing and 2.29 million tons (54%) from aquaculture. Mariculture output is 1.88 million tons, and the freshwater fishery produces 0.41 million tons.

In a market economy fish supply and consumption are reflected by differences in fish price. In this study, a comparison between the prices of the main fish (hairtail, silver carp and tilapia) and pork, chicken and egg in 1997 have been made. The characteristics of fish consumption in these nine cities have been studied with the hope of enhancing the socio-economic role of fish in the household food supply.

The monthly average prices in Fujian province from Jan. to Dec. 1997 of the main fish species: hairtail, silver carp and tilapia (hairtail is from marine capture and silver carp and tilapia are from aquaculture) and other competing foods are shown in Fig 2. The monthly average prices of fish from the nine cities in 1997 are almost the same, and so are those of pork, chicken and egg. The highest price is for pork, about Y19/kg, the second for hairtail and chicken, both are about, Y14/kg, and the cheapest is tilapia, silver carp and egg, all below Y10/kg. It is evident that the food supply in the province is sufficient. However, it is notable that the price of hairtail, a marine product, is higher than that of the freshwater fish and that fish is not the most important animal food in the province.

Because the annual average prices of fish from all the nine cities cannot reflect the differences and the characteristics of fish price and fish consumption between the cities, it is necessary to know the annual average price of fish in each of the nine cities. Fig 3 indicates that in the coastal cities the price of hairtail is far higher than that of freshwater fish, even higher than the price of chicken. In the inland cities, Longyan,

Sanming and Nanping, the prices of hairtail are lower than that of chicken, close to the prices of silver carp and tilapia. The differences between the prices of freshwater fish in the nine cities are small. From the annual average prices of fish in each of nine cities it shows that the people living in coastal zone prefer to eat pork and marine fish, while the people living in inland zones prefer pork and chicken. Neither marine fish nor freshwater fish rate as a preference for the people from the inland cities.

In Fuzhou, Xiamen, Sanming, and Nanping, there were some different characteristics in the monthly variation of fish price in 1997. The monthly change of the fish price in the coastal cities is larger than that in the inland cities. The prices of hairtail in Fuzhou and Xiamen are higher than that of chicken, and are close to the price of pork. It shows that hairtail and pork are equally preferred in the two cities. On the other hand, the fish prices in Sanming and Nanping, are all lower than the prices of pork and chicken, indicating that fish prices are set by consumer preference. As marine fish consumption in the coastal cities is greater than that in the inland cities fish prices in the inland cities are all lower than those in the coastal cities and change only slightly.

The conclusion is that there are obviously different habits of fish consumption which affect demand more than the fish price. To develop fisheries, we need to encourage people to consume more aquatic products, especially to enhance the socio-economic role of fish in household food. To achieve this aim, it is necessary to run campaigns to encourage people to consume more fish.

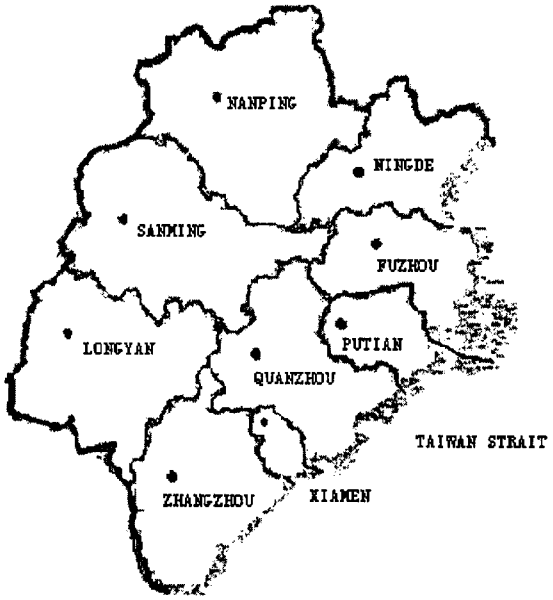
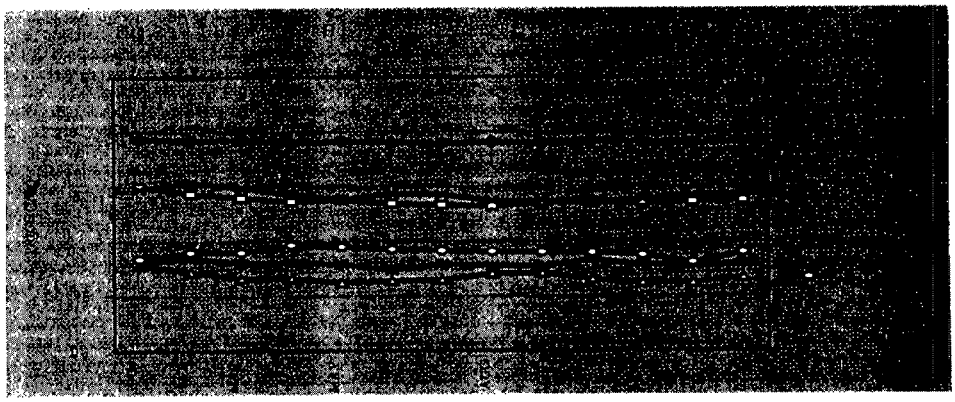
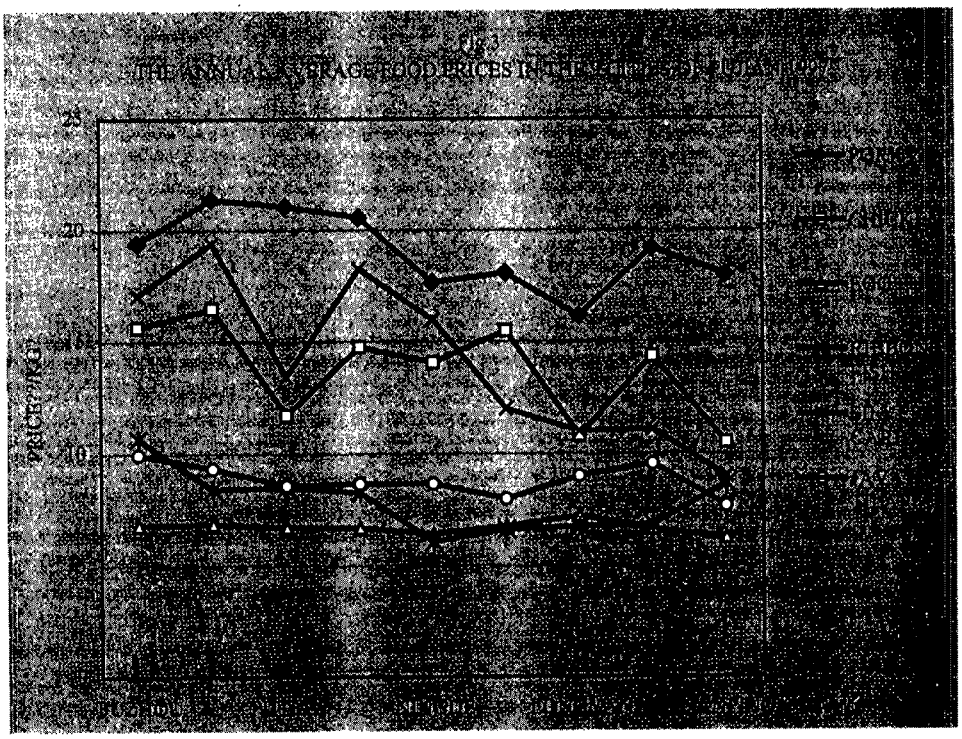


Fig. 1. Fujian administrative area.

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PREPARATION AND STORAGE STUDIES OF SQUILLA PICKLE

by

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ABSTRACT

Stomatopods are landed in considerable quantities in almost all maritime states of India. Total landings 1996 in India were 72,342 tonnes. In states like Karnataka, their landings were as high as 50% of the total crustacean landings. The stomatopod *Oratosquilla nepa* (squilla) forms an important component of the bycatch of the shrimp trawlers. It is also widely distributed in the Indo-Pacific region, extending from Pakistan to Australia. Though squilla is landed in large quantities, no concerted attempt has been made to study the utilization of this resource as a good source of protein for human consumption. Currently the major catch is discarded at sea and those that are landed are converted into fishmeal or manure.

In view of the high nutritive value of squilla as a human food and the fact that pickles are very popular in India and other South East Asian Countries, pickle was prepared out of squilla meat and studies on its keeping quality were carried out to estimate its shelf-life. The yield of the meat from whole squilla is 20%. The paper discusses the biochemical composition of squilla meat and its seasonal changes. The composition of the pickle is also discussed. There was a decrease in the pH value throughout the period of storage. The pH of the pickle was found to stabilize at 3.72 after 6 months of storage. The NaCl content did not show much variation during the storage period but moisture was found to decrease. The total bacterial count showed a gradual reduction during the initial period of storage. The results of the sensory assessment shows that the pickle is acceptable for test marketing on commercial scale.

INTRODUCTION

India, which ranks 7th in world fish production, is on the threshold of a blue revolution. At the same time, it is worth noting that a significant proportion of the total available catch consists of commercially unimportant varieties and hence is underutilized. This has resulted in the industry processing only selected fish, leaving others unutilized.

There is the need for the utilisation of the total fish catch so as to compensate the depletion of the commercially important fish stocks. In view of the nutritional importance of fish as human food, attempts are made to utilize *O. nepa,* which forms a component of the bycatch of shrimp trawls. Different products like pickles, cutlets, fingers and flakes were prepared as an attempt to utilize this unutilized resource.

Pickling is one of the oldest methods of preserving food material. Pickles have been of commercial importance in some developing countries like Korea, where pickles are made out of anchovies, shrimps, squid, oyster and sea urchin. Several workers have studied on the pickles prepared from different fish in India, namely fresh water fish (Chattopadhyay *et al.*, 1985), low cost marine fish (Vijayan *et al.*, 1989), clams (Yellappa and Chandrasekharan 1989), blood clam (Gupta and Basu, 1985), edible oyster (Sugumar *et al.,* 1994) and chunk (Sugumar *et al.*, 1994). Jawahar and Shetty (1994) did a detailed study on the preparation of pickles from crustaceans. Since squilla has not so far been used for the preparation of pickle, a study was conducted on pickles developed out of *O. nepa*, to assess the acceptability and storage characteristics.

MATERIALS AND METHODS

Fresh *Oratosquilla nepa* of varying size groups, without sorting sex-wise, was collected from the Cochin Fisheries Harbour was used for the study. The study was conducted for a period of one year in 1995. Standard methods as described in AOAC (1975) were used to determine the moisture, crude fat, and protein and ash contents of squilla meat. Sodium and potassium were estimated using the flame photometer (Elico model 26 D). Sodium chloride was estimated by the method of AOAC (1975). Carbohydrate was analysed adopting the phenol sulphuric acid method of Du-bois *et al.* (1956). Chitin nitrogen was estimated by the method followed by Garg *et al.* (1977) by digesting the crude fibre obtained after alkali digestion and estimating the nitrogen in the digest by micro-Kjeldahl .

Peeling of squilla is a difficult task and to facilitate a comparatively easy peeling and to get better yield, the fresh squilla has to be iced for 18 hours (Rajeswary ,1996). After keeping squilla in ice for 18 hours, the shell was removed by cutting with a pair of scissors from the abdominal region up to the 5th thoracic region. This was found to be the best method for manual peeling of the meat, because a higher yield was obtained through this method (Rajeswary, 1996). Pickles were prepared using the same ingredients as reported by Rajeswary and Hameed (1998) (Table 1). The meat was fried in vegetable oil for about 4-5 minutes and set apart. Ginger, garlic, chilly and turmeric were fried in the remaining oil by thoroughly mixing them in oil under low flame for few minutes. After removing from the fire these ingredients were mixed with fried meat, vinegar and sufficient quantity of boiled and cooled water to completely cover the ingredients. After mixing together all the ingredients with salt, the pickle was left to mature for 2 days and then packed in clean dry bottles and sealed airtight. While packing care was taken to prevent the exposure of the meat and a layer of oil was always kept covering the pickle at the top. Previously heated and cooled gingerly oil was added if necessary to ensure a protected layer of oil at the top. The product was stored at room temperature and periodically examined for changes in the physical, chemical and microbiological characteristics.

Table 1. Ingredients in the prepartion of pickles from Squilla.

Squilla meat	1000g
Ginger	180g
Garlic	80g
Chilly	50g
Turmeric	5g
Asafoetida	11g
Cummin	25g
Mustard	11g
Fenugreek	5g
Chilly powder	35g
Spice mixture (pepper, cardamom, cinnamon and clove)	11g
Vinegar (3% acetic acid)	450 ml
Water (boiled and cooled)	400 – 600ml
Gingerly oil	250ml

For the determination of the pH, a representative sample of 10 g including the meat, oil, spices, etc. was ground thoroughly to a smooth paste, diluted with 20 ml water and the pH of the resulting solution was measured using a digital pH meter. Total bacterial count was estimated using tryptone glucose beef extract agar. Coagulase positive staphylococci were determined as per FDA (1973) and salmonella was detected by the method of Galton *et al.* (1968). A panel of 8 members using 5-point scale assessed the organoleptic qualities.

RESULTS AND DISCUSSION

The yield of meat from processing of squilla was only 20%. The average proximate composition of *O. nepa* (Table 2) meat has been estimated based on the monthly samples drawn from Cochin Fishing Harbour. The squilla meat has a protein and fat content almost comparable to that of other crustaceans like prawns and crabs. Chitin nitrogen is another significant component, which constitutes about 0.75% of the dry weight. The sodium level in squilla is comparatively higher (compared to teleosts) than other crustaceans. The sodium content of the individual species of salt-water fish ranges from 34-96 mg/100g with an average of 68 mg/100g. The potassium is less than that in teleosts with an average of 88.19 mg/100g. The level of potassium has been found to be 240-400 mg/100g for marine fish. Variation in the amount of sodium and potassium are related to the size of the animal and the fishing season. Sodium is higher for crustaceans and molluscs compared to teleosts and the reverse applies to potassium. Sodium levels are appreciable in shellfish and are considered to be important in food due to their involvement in coronary heart disease, hypertension and osteoporosis (Swaminathan, 1991). According to Sunderrao *et al.* (1992) the mineral composition of shellfish is 164mg/100g and that of potassium 185mg/100g, which is comparatively higher than that of *O. nepa*.

Table 2. Proximate composition # of Squilla.

	Whole (%)	Flesh (%)
Moisture	78.22	82.75
Protein*	44.57	68.31
Fat *	2.86	3.12
Ash*	27.61	11.88
Chitin nitrogen*	1.05	0.75
Carbohydrate*	2.29	2.55
Sodium* (mg/100g)	54.33	98.4
Potassium* (mg/100g)	65.39	88.19

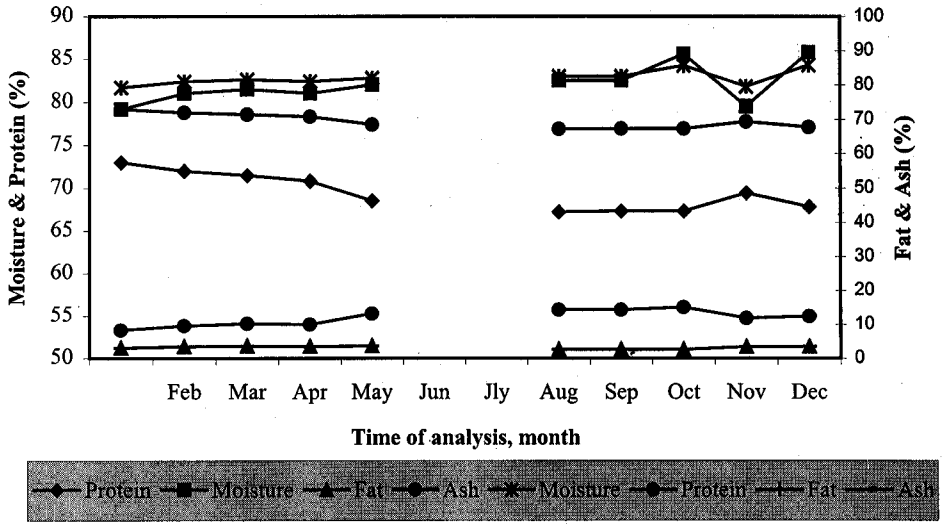
*# Average * on dry weight basis*

Figure 1 shows the seasonal variation in the biochemical composition of squilla meat. The meat contains a higher water content of around 82.75%. According to Mukundan *et al.* (1981), the moisture content of shellfish is comparatively high when compared to that of fish, probably in accordance with the well-known fat-moisture relationship. The moisture content increased gradually up to October and then dropped. A definite co-relation could be established between the fluctuation in the moisture content and the maturity and spawning behaviour in *O. nepa* (Rajeswary, 1996). According to her study the *O. nepa* had spent gonads during August and December which corresponded to the peak level of moisture in the flesh.

There was a decrease in the values of protein during the beginning of the year up to May. By monsoon, the values were found to pick up and from then onwards an increase was observed in the values till the end of the year. An inverse relationship is noticed with respect to moisture and protein. During mature seasons, the protein is found to be highest and during the spent season it is found to be lowest. Hence this dilution of the body fluids has a marked effect on the level of organic constituents in the body. The inverse relationship that the organic constituents hold to water level in molluscan bivalves as observed by Durve and Bal (1962) and Mohammed (1979) agrees with the present findings.

Higher values of fat were noticed during May, November and December and this follows an inverse trend to that of the water content of the meat. This difference in the seasonal trend of fat may be the result of spawned individuals present in the samples taken for fat analysis. It was also observed that in the food and feeding habits of *O. nepa*, the maximum number of gorged stomachs were observed during the month of December. This was the time when the highest fat content was observed in the samples.

Fig 1. Seasonal variation in the proximate composition of squilla, *O. nepa.* (no data for June & July as no sampling possible due to fishing ban)



The ash was found to increase during the beginning of the year and by October had reached around 15%, almost double the value of the beginning of the year. There was a fall in the ash content of the meat during the month of November and by December they again showed an increase. There was not much variation in the carbohydrate content of the meat during the entire period of study. It was also noticed that there was a considerable level of chitin nitrogen in the flesh and this has a role in the formation of the exoskeleton. It was observed that the chitin nitrogen was very low during September and October when juveniles were dominant. According to Garg *et al.* (1977), in general the total nitrogen and fat (ether extractable) contents of squilla are lower and the ash and chitin nitrogen contents are higher than those of *Acetes* spp.

Table 3. Changes in Squilla pickles during storage at room temperature.

Duration, days	pH	NaCl (%)	Moisture (%)
Initial	4.46	3.03	64.34
7	4.29	3.03	63.58
14	4.22	3.55	63.82
21	4.22	3.26	64.88
30	4.20	3.38	65.50
60	3.88	3.55	66.40
90	3.93	3.64	64.13
120	3.78	2.93	68.62
150	3.72	2.89	63.78
180	3.72	2.92	62.99

Table 3 shows the chemical changes in the pickle for a period of 6 months. The pH of the pickle was 4.46, which dropped gradually during storage. Similar decreasing trend in pH during storage of pickles was reported by many authors (Dhanapal *et al.*, 1994; Gupta and Basu, 1985; Chattophadhyay and Battacharya ,1985; Behanan *et al.*, 1992). The decrease in the pH could be due to the uptake of acid by the meat during storage. According to Sugumar *et al.* (1992) low pH inhibits most of the bacterial activity.

NaCl did not show a significant variation throughout the period of study. NaCl content of the freshwater fish pickles such as *E. vacha* was 3.48%, 3.76% for *G. guerus* and 3.53% for *S. canula*. This falls within the range as observed in *O. nepa* pickle. In the case of acetic acid treated clam pickle, NaCl was found to increase with storage whereas in the case of *O. nepa* pickle there was a slight reduction in the NaCl levels by the 6th month of storage. The moisture content in the pickle was found to show decreasing values during storage. Similar observation was reported in clam pickles (Dhanapal *et al.*, 1994).

The result of the microbiological analysis is shown in Table 4. Total bacterial count was found to show a gradual reduction during storage. By the end of the 6th month, the values had increased. *Escherischia coli*, Staphylococci and Salmonella were absent throughout the study. It was also observed that molds could be sighted by the 6th month of storage. The absence of *E. coli*, Staphylococci and Salmonella was in agreement with earlier studies. The absence of these organisms was reported to be due to the inhibitory action of low pH and high salt content in pickles. The reduction in the bacterial population in the initial stages of storage and later on the increase may be due to the multiplication of acid tolerant bacteria. Similar observation was also made by Vijayan *et al.* (1989).

Table 4. Microbiological changes in squilla pickles during storage at room temperature.

Duration, Days	E.coli	Staphylococci	Salmonella	TPC / gm
Initial	-	-	-	1.96×10^3
7	-	-	-	1.92×10^3
14	-	-	-	1.66×10^3
21	-	-	-	1.25×10^3
30	-	-	-	5.98×10^2
60	-	-	-	2.85×10^2
90	-	-	-	1.60×10^2
120	-	-	-	1.21×10^3
150	-	-	-	3.61×10^3
180	-	-	-	4.35×10^2

The sensory attributes like appearance, colour, texture and taste were assessed under organoleptic assessment. It was observed that throughout the period of study, the appearance was rated to be good. The colour was observed to be reddish brown during the initial period of study. At the end of 6 months, the colour had started fading, thus rated as fair by the test panel. In the initial period, the odour was good but at the end of 6 months, it was rated as poor due to the development of off odour. In the beginning, the texture was tough but started getting soft and after 3 months of storage it became soft. The flavour was found to be good. In the opinion of the test panel the flavour improved during storage. This could be due to the aging of the pickle combined with the proper mixing of the ingredients on storage. According to the opinion of the test panel, the pickle had the taste similar to that of prawn pickle.

To conclude, it is found that the squilla pickle can be kept in good condition at tropical temperature for six months. It is also found that since it is a low fat species and the seasonal fluctuations in the fat is negligible and it can be utilized for this purpose throughout the seasons.

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PREPARATION AND PROPERTIES OF FUNCTIONAL PROTEIN CONCENTRATE FROM TUNA (*Euthynnus affinis*)

by

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ABSTRACT

A process for the utilization of tuna (*Euthynnus affinis*) meat in the form of a functional protein concentrate was developed. Mechanically separated mince was washed first with water, then with 2% (w/v) aqueous solution of sodium bicarbonate and finally with water to remove pigments, lipids, colour and odour-bearing compounds. Homogenization of the washed meat in water followed by acidification of the dispersion with acetic acid produced a weak gel which was diluted and spray dried to get a light yellow powder having a faint fishy odour. The spray dried protein powder contained 5% moisture, 1.2% lipid and 89.5% protein and had good functional properties. Packed in 300 gauge polyester/polythene laminate pouch, it did not undergo significant browning for 3 months during storage at ambient temperature.

INTRODUCTION

Tuna is emerging as one of the commercially important fish species in many developing nations. In India tuna is still an underexploited item. The potential yield of coastal tunas from Indian waters is 263 000 tons against a catch of 37 722 tons (James *et al.*, 1993). Major share of the catch is the little tunny (*Euthynnus affinis*) which lacks market demand because of its high black meat content and unappealing taste. The International Workshop on Post harvest Fishery Losses (ICMRD 1988) emphasized the need for developing new products for nutrition enhancement and utilization of resources. In recent years, value addition to tuna and tuna wastes has witnessed significant growth in major markets (Subasinghe, 1996).

One of the value-added fish products of global interest is surimi, a washed myofibrillar protein concentrate of fish mince, having characteristic elastic and gelling properties. In the case of dark meat fish, repeated washings with large quantities of water are required to decolorize and deodorize the mince and to remove the sarcoplasmic protein thereby facilitating gelation of the fibrillar protein. Overdilution of wash systems and low freshness of fish make the mince overhydrated and hence unsuitable for surimi (Gopakumar *et al.*, 1992). In such cases the swelled meat can be converted into alternate protein preparations by appropriate technology. The present work reports a method of converting the washed mince of *Euthynnus affinis* into a free flowing dispersion which is spray dried to a concentrated protein powder.

MATERIALS AND METHODS

Tuna (*Euthynnus affinis*), (length 45-50 cm) was collected from the local fishing harbour immediately on landing. The fish were brought to the laboratory in iced condition. Heading, gutting and filleting were done manually. Mince was prepared using a laboratory model meat bone separator fitted with a drum having 4 mm diameter perforations.

Washing

Washing of fish mince was done first by cold water, followed by 2% (w/v) sodium bicarbonate solution and finally by cold water. The washing conditions were those described by Muraleedharan *et al.* (1997) for surimi preparation. Washing was done with a mince/water ratio 1:3 : In between washings the mince was strained with a close mesh sieve. After the final washing the meat was strained and gently pressed to remove excess water.

Preparation of dispersion

Washed mince was suspended in 3 times its weight of water. Glacial acetic acid was added dropwise with stirring to a final concentration of 1% (v/w). The suspension was allowed to stand for 2 hrs at room temperature for gelation.

Spray drying

The dispersion was homogenized using a mixer (Hobart A-120) and diluted with water to a free flowing consistency. The liquid was then subjected to spray drying at an inlet temperature of 200°C, and outlet temperature of 110°C. The spray dried fish powder was collected and used for further studies.

Proximate composition

Moisture, ash, crude protein and lipid were determined according to AOAC (1990) methods. Pepsin digestibility of protein was determined as described by Ambe and Sohonie (1957).

Oil Emulsification Capacity

The oil emulsifying capacity of the protein powder was determined by the method of Carpenter and Saffle (1964) and was expressed as ml of oil emulsified by 100 mg of protein.

Salt solubility

Fish powder (1g) was blended with 100 ml water or 1M sodium chloride solution for 1 min. in a Hobart (A-120) blender. Aliquots of 25 ml were centrifuged at 5000 x g for 5 min. Protein was estimated in the supernatant.

Density

A preweighed 10 ml graduated cylinder was filled with the powder up to 10 ml mark with gentle tapping. The weight of the powder was noted to determine the density.

Storage stability

The tuna protein concentrate was packed in 300 gauge polyester/polythene laminate pouches in the presence of air and was stored at 10°C or at ambient temperature (25-29°C). The samples were observed for changes in colour and odour at monthly intervals.

RESULTS AND DISCUSSION

Raw tuna had about 40% of its body weight as dark meat. Upon mincing, the dressed fish gave 47% mince yield. The mince was of oily appearance, dark red colour and strong odour. The proximate composition

and other characteristics of the raw mince and washed mince are given in Table 1. After the final wash, the meat swelled up because of protein hydration and resulted in a higher level of moisture in the washed mince. The alkali washing removed most of the lipids and blood pigments and greatly improved the appearance. Some of the soluble protein was invariably lost, but it improved the gelation of the fibrillar fraction as reported by Muraleedharan and Gopakumar (1996). Washing with bicarbonate increased the pH of the meat to 9.2 but the final wash with cold water reduced the alkalinity and lowered the pH to 7.1. Exposing the mince to alkalinity, and followed immediately by bringing back to neutrality also seem to have activated the fibrillar protein to improve its gelling ability (Schmidt, 1981). Washed fish proteins formed a weak gel on the addition of acetic acid (pH 3.9). Removal of the lipids and the low molecular weight compounds attached to the structural proteins during washing enhanced the hydrophilic character of the proteins, causing gelation which was further increased with lowering of pH to 3.9 by the addition of acid (Fretheim *et al.*, 1985). This weak gel after proper dilution and homogenisation, formed a free flowing fluid facilitating easy dehydration in the spray dryer. The yield of the powder was 2.5% on whole fish weight. The characteristics of the spray-dried protein concentrate are given in Table 2.

Dehydrating the gel in mechanical dryers (45°C, 60% RH) or vacuum dryers produced a coarse powder with brown colour and fishy odour. But dehydration by spray drying produced a light yellow coloured powder of low density and faint fishy odour. Since no solvents are employed for the removal of lipids, the protein in free from the harmful effects of solvent residues.

The solubility of the protein in water was about 7.6% but the presence of 1M NaCl enhanced the solubility to about 19%. The high functionality of the powder is also shown by its oil emulsification capacity. The product contains higher protein content (89.5%) compared to the recommended minimum of 75% in type A and 70% in FPC type B defined by FAO (Halliday and Disney, 1974). The Indian Standards specify a minimum protein content of 70% on a dry basis and a maximum fat content of 0.75% on a dry basis in FPC (IS 9808-1981). Total lipid content of the tuna protein concentrate is also lower (1.2%) compared to 5.0% in FPC type B (FAO).

Storage characteristics

The influence of storage conditions on colour and odour of the protein concentrate are given in Table 3. It was observed that the product could remain without any change in colour or odour at 10°C for 3 months. Even at ambient conditions these changes were negligible. The polyester/polythene laminate having good barrier properties could effectively prevent quality deterioration of the spray dried protein concentrate during the storage period, at both conditions of storage.

Table 1. Characteristics and proximate composition of unwashed and washed tuna mince.

	Unwashed mince	**Washed mince**
Colour	Dark red	Brown
Odour	Strong fishy oily	Slight fishy
Moisture %	71.46±2.3	81.91±1.2
Lipid %	6.89±0.5	2.15±0.1
Protein %	23.5±1.9	16.93±0.4
pH	6.7	7.1

Values: Mean±s.d. : (n=5)

Table 2. Characteristics of Tuna Protein Concentrate.

Yield % (on whole fish weight)	2.5
Moisture %	5.49+0.8
Protein %	89.5+1.3
Lipid %	1.2+1.1
Ash %	2.08+0.7
Pepsin digestibility %	91.2 +1.6
Oil emulsification capacity/100 mg powder ml	125+8.2
Solubility in water %	7.6+2.8
Solubility in 1M NaCl %	19.1+4.3
Colour	Pale yellow
Odour	Faint fishy
Density	0.35

Values: Mean±s.d. : (n=5)

Table 3. Storage characteristics of Tuna Protein Concentrate

Storage in weeks	**Storage temp. (°C)**	**Colour**	**Odour**
0	--	Pale yellow	Faint fishy odour
4	10	Pale yellow	-do-
4	ambient	Pale yellow	-do-
8	10	Pale yellow	-do-
8	ambient	Light brown	Slight rancid
12	10	Pale yellow	Faint fishy
12	ambient	Light brown	Slight rancid
16	10	Pale yellow	Slight rancid
16	ambient	Brown	Rancid

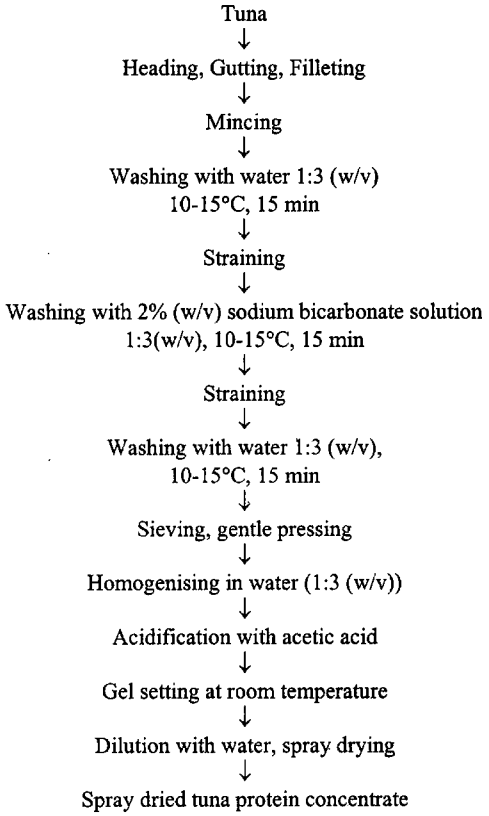

Fig. 1. Flow chart for the preparation of Tuna Protein Concentrate.

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USE OF SOYBEAN FLOUR IN FISH SAUSAGE PROCESSING

by

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ABSTRACT

Soybean flour was made from defatted soybean meal, a by-product of soybean oil processing. The flour processing waste was steamed, pressed, dried and ground. A preliminary experiment was directed to determine the optimum addition of soybean flour by varying the levels from 10 to 50%. An optimum level of 30% in fish sausage processing was suggested. The product was then stored at 7-10°C and evaluated organoleptically, chemically and microbiologically to determine its stability and quality changes. Fish sausage produced with no addition of soybean flour was also observed. Sensory evaluation indicated that the sausage was acceptable until the fourth day of storage.

INTRODUCTION

Defatted soybean meal (DSM), known as 'bungkil kedelai', is a by-product of soybean oil processing after the pressing step. It still contains high protein (approx. 45% db) suitable for human needs. So far, in Indonesia, the DSM is commonly used as one of materials in the production of livestock feed, but only one percent of the DSM (of about 82% DSM produced by soybean oil industries) has been used in the food industries. In other countries, primarily developed countries such as Japan, USA and Europe, the DSM is used in the formulation of various food products, e.g. meat analogues, sausage, bread, cake, noodle and macaroni (Kinsella, 1979 *cited by* Muctadi, 1989). This is due to the characteristic of soybean protein which can improve the functional properties of the products, such as emulsification, water adsorption and ability to provide good texture. In addition, the digestibility and other nutrition values of DSM are high and the price is relatively low.

Several experiments on the use of soy protein in the food processing have been reported (Cross *et al.*, 1975, Seidemen *et al.*, 1977, Andayani, 1981 and Matulis, *et al.*, 1995). However, information on the use of soy protein in sausage processing (especially fish sausage) is still limited. The research was undertaken to study the possibility of increasing the added value of DSM, as soybean flour in the fish sausage processing.

MATERIALS AND METHOD

Materials

Yellowpike congro (*Congresox talabon*) obtained from Muara Angke, Jakarta, was used as raw material in this experiment. Defatted soybean meal ('bungkil kedelai') was obtained from PT Sarpindo Soyabean Industri, Jakarta. Salt, onion, garlic, ginger, pepper, sugar, monosodium glutamate, vegetable oil and tapioca flour were the other ingredients used.

Method

Soybean flour preparation-

- Soybean flour was prepared by using the method of Koswara (1995) as follows :
- Waste of soybean oil processing was washed and steamed at 100°C for 20 minutes.
- After steaming, the product was then pressed and sundried.
- The dried product was ground and finally screened to 100 mesh. The flour was ready to be used in sausage processing.

Fish sausage processing

Processing of fish sausage was conducted using a method described by Purnomo *et al.* (1992) as follows:

- Fish was washed, filleted and minced using a meat separator.
- The minced fish was then added and mixed thoroughly with ingredients. The dough was eventually stuffed into collagen casings.
- The sausage was heated in water at 60oC for 20 min. and followed by 80oC for 40 min.
- The product was then packed in plastic bags and stored in a refrigerator at 7-10oC.

Observation

1. Raw material

Proximate composition was conducted for yellowpike congro (*C. talabon*) and the soybean flour. Fish freshness, i.e. pH and TVB content were also determined. Proximate composition including moisture, protein, fat, ash and carbohydrate content were measured using AOAC (1984) method, as follows:

- Moisture was determined using a direct heating method at 105oC
- Protein content was determined using the Kjeldahl method
- Fat content was determined using Soxhlet extraction
- Ash content was determined by incinerating a sample at 550oC
- pH was measured using a pH meter and TVB content was determined using the microdiffusion method.

2. Fish sausage produced by the addition of some levels of soybean flour

Sensory acceptability tests were carried out to determine the optimum level of soybean flour used in fish sausage processing. Five levels of soybean flour concentration were used, i.e. 10, 20, 30, 40 and 50%. A six-point hedonic scale (1: dislike very much, 6: like very much) was used to evaluate the acceptability of the products with the limit score of 3.5.

3. Fish sausage stored in a refrigerator at 7-10°C

Based on the best result, fish sausage with the optimum level of soybean flour was evaluated its quality changes during chill storage compared to control (fish sausage with no addition of soybean flour). Evaluation was carried out for proximate composition and physical properties at the initial storage and quality parameters during storage including organoleptic, chemical and microbiological quality. Physical properties observed were emulsion stability and hardness of sausage. The emulsion stability of fish sausage was determined using Webb's method (1975) and the hardness was determined using a texture analyzer having a 2 mm/sec speed. Organoleptic tests to evaluate appearance, colour, odour, flavour and texture used a 6-point hedonic scale as mentioned above. Moisture content, pH and TVB represented chemical quality while microbiological quality was represented by the total bacterial count, which was determined by the pour plate method.

RESULTS AND DISCUSSION

Raw Material

Table 1 summarizes the proximate composition of fish and soybean flour as raw material of the fish sausage. It shows that soybean flour has a high protein content (46.87% db). To indicate fish freshness, the fish was also analyzed for its pH and TVB content. The fish used had a pH value of 6.57 and a TVB content of 6.8 mgN%, indicating good quality as implied by Egan (1981) that the limit TVB content of fresh fish is 20 mgN%.

Table 1. Proximate analysis of yellowpike congro (*C. talabon*) and soybean flour as raw material of fish sausage.

Ingredient	*C. talabon*	Soybean flour
Moisture (% wb)	81.02	7.86
Ash (% db)	5.26	7.07
Protein (% db)	91.46	46.87
Fat (% db)	3.16	4.26
Carbohydrate (% db)*	0.12	41.80

* by difference

Effect of soybean flour on the acceptability of fish sausage

Soybean flour was added at 5 concentrations, i.e. 10. 20, 30, 40 and 50%. Based on panellist acceptance, as shown in Table 2, the addition of soybean flour at the level of 40 and 50% produced fish sausage with odour and taste scores which were lower than the acceptance score limit (3.5). Therefore, the maximum soybean flour added in fish sausage processing was 30%. In terms of texture, all of the sausages produced had coarse and porous texture causing low scores. This was due to the characteristic of soybean flour as a protein source with a limited carbohydrate content. To produce fish sausage with a better texture, tapioca flour was added at levels of 5 and 10%. The addition of this flour (tapioca) as a filler increased the water holding capacity and gave better texture. Observation showed that addition of 5% tapioca flour produced a solid and elastic product. The panellist scores increased from slightly dislike to like very much. During cooking, the starch was gelatinized giving a solid dough, then produced better texture and elasticity. Addition of 10% tapioca flour produced sausage with a harder texture and less elasticity decreasing the acceptance score. Further experiments on quality changes of fish sausage used 30% soybean flour and 5% tapioca flour.

Table 2. Average of hedonic scores of fish sausage added by soybean flour.

Soybean flour concentration (%)	Appearance	Colour	Odour	Taste	Texture
10	5.0	4.8	4.2	4.0	3.0
20	5.3	5.0	4.3	4.7	3.4
30	4.8	4.3	3.8	4.2	3.3
40	4.5	4.2	3.2	3.3	3.3
50	4.5	4.0	3.3	3.2	2.8

Quality of fish sausage

Fish sausage quality is shown in Table 3. It was pointed out that the use of soybean flour increased ash, fat, crude fibre and carbohydrate contents of fish sausage. While protein content of fish sausage containing soybean flour was lower than the control. This was probably due to the raw materials used, described in Table 1.

Protein content of soybean flour was much lower than the protein content of yellowpike congro (46.87% compared to 91.46%). Consequently, the addition of higher soybean flour will reduce the fish flesh content in the sausage dough, which directly had impact in decreasing protein content of the sausage.

Fat content of the sausage was in the range of 9.49 - 10.36% (db). It was relatively much lower than fat content of commercial sausage reported by Haq *et al.* (1994). The commercial sausage, particularly beef or chicken sausage had a fat content of 24.15 - 65.66% (db). Currently, most people pay more attention to their diet, especially in relation to dietary fat. Saturated fat (animal fat) is known to cause hypercholesterolemia in humans. Fish sausage, with low fat content and rich in polyunsaturated fatty acids, was found to be hypocholesterolemic. It was therefore considered to be beneficial to the health compared to beef or chicken sausage.

Crude fibre is defined as part of cell wall of plant, which is resistant to acid and base treatments. The table demonstrated that fish sausage with 30% soybean flour contained a crude fibre of 10.91%, much higher than control (1.81%). Indicating that the crude fibre content of this sausage resulted mostly from the soybean flour.

Table 3. Quality of fish sausage.

Parameters	Soybean flour percentage (%)	
	0	30
Moisture (% wb)	77.17	73.80
Ash (% db)	7.04	8.94
Protein (% db)	64.78	55.46
Fat (% db)	9.49	10.36
Carbohydrate (% db)	15.15	24.59
Crude fibre (% db)	1.81	10.91
Emulsion stability (%)	76.25	85.37
Hardness (load/mm)	79.00	65.88

In term of physical properties of fish sausage, the use of soybean flour in fish sausage processing at the level of 30% significantly increased the emulsion stability from 76.25% to 85.37%.

Koswara (1995) stated that soybean protein improved the process and stability of oil/water emulsion. Mattulis *et al.* (1995) also indicated that the cohesiveness in the sausage formulation decreased in proportion to soybean protein concentration.

Hardness, another physical characteristic of fish sausage, decreased with the addition of soybean flour, probably due to the muscle protein in the sausage formulation being substituted with soybean protein. It is known that one of the factors affecting the sausage hardness is type of protein. The protein, which has an important role in influencing product texture, is muscle protein (actine and myosin). According to Wirakartakusumah *et al.* (1992), during cooking, muscle protein denaturated and produced a harder meat texture. Cross (1975) investigated the addition of soybean flour which decreased the hardness of the muscle protein and increased the tenderness of processed minced meat.

Quality changes during chill storage

Chemical quality

Chemical quality changes of fish sausage are presented in Table 4. It is indicated that storage time did not significantly affect the moisture content. On the contrary, TVB content was influenced significantly by

storage time. TVB content increased during chill storage. This was related to degradation of protein or its derivatives during storage, producing volatile bases such as methyl and ethylsulfide, mercaptan, ammonia, amine compounds (histamine, tiramine, piperidine, putresine and cadaverine), indol and skatol (Frazier and Westhoff, 1988).

Similar to TVB content, pH was significantly affected by storage time. At the second day storage, pH increased at the initial storage and then decreased until the sixth day storage. It might be due to the breaking down of other components of the product, primarily carbohydrate, producing acid compounds, e.g. lactic acid (Fardiaz, 1992).

Table 4. Chemical quality changes of fish sausage during chill storage.

Parameter/ Soybean flour percentage	**Storage time (days)**			
	0	**2**	**4**	**6**
Moisture (% wb)				
0%	77.17	77.75	77.93	77.47
30%	73.80	74.13	73.42	74.06
TVB (mgN%)				
0%	12.4	13.2	14.6	28.8
30%	15.6	16.2	17.2	22.8
pH				
0%	6.32	6.46	6.26	5.72
30%	6.68	6.68	6.29	5.41

Microbiological quality

Total microbial load of the fish sausage as one parameter of microbiological quality was observed during chill storage. It was shown in Table 5 that total plate count of the fish sausage increased from $3.9x10^4$ - $6.4x10^4$ colonies/g at the initial storage to $1.4x10^8$ - $1.5x10^8$ colonies/g at the sixth day storage. Connel (1985) stated that the maximum TPC for processed food to be consumed safely was 10^7 - 10^8 colonies/g. The sausage, therefore, has been microbiologically acceptable up to 4 days of storage.

Organoleptic quality

Sensory evaluation results showed that the main factor affecting the panellist's acceptance was flavour and taste. At the sixth day storage, both flavour and taste scores were much lower than the limit acceptance. While for the colour, panellist did not show any rejection up to the same storage time. However, the scores mostly tended to decrease during chill storage.

Table 5. Total plate count of fish sausage during chill storage.

Soybean flour concentration	Storage time (days)			
	0	2	4	6
0%	$6.4x10^4$	$2.2x10^6$	$4.9x10^7$	$1.4x10^8$
30%	$3.9x10^4$	$1.4x10^6$	$4.7x10^7$	$1.5x10^8$

Table 6. Organoleptic quality of fish sausage during chill storage.

Parameter/ Soybean flour concentration (%)	Storage time (days)			
	0	2	4	6
Appearance				
0%	5.6	5.6	5.3	3.3
30%	5.0	5.2	4.9	3.3
Colour				
0%	5.2	5.5	5.1	3.5
30%	5.0	4.8	4.6	4.5
Flavour				
0%	4.7	5.4	3.8	2.0
30%	4.8	4.7	4.5	2.8
Taste				
0%	5.0	5.4	4.1	2.5
30%	4.9	4.8	4.6	2.5
Texture				
0%	5.0	5.5	5.0	3.7
30%	4.9	5.4	4.6	3.3

Changes in both flavour and taste scores were related to degradation process of protein during storage. Deterioration occurred because micro-organisms produced protease enzymes, breaking down the protein. The degradation of protein produced volatile compounds, e.g. indol, skatol, methylamine and ammonia which then causing undesirable flavour and taste. At the sixth day of storage, appearance score showed that the sausage did not meet the panellist's acceptance. Slime on the sausage surface was the main cause of the panellist's rejection. Slime is one of the results of bacterial spoilage in breaking down the products during deterioration process (Buckle *et al.*, 1987).

CONCLUSION

- Up to 30% soybean flour made from defatted soybean meal can be added to fish sausage.
- The shelflife of fish sausage was 4 days at chill storage.
- Addition of soybean flour in fish sausage processing increased carbohydrate, fat and crude fibre content. It also improved the emulsion stability and the tenderness of the sausage. On the other hand, the flour reduced the moisture, ash and protein content of the sausage.

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STUDIES ON THE EXTRACTION OF FISH PROTEIN CONCENTRATE (FPC) BY SOLVENT EXTRACTION

by

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ABSTRACT

Fish protein concentrate (FPC) was made from low-value fish by solvent extraction with isopropanol at special temperature and time. The protein, fat, ash and moisture contents were 92.28%, 0.09%, 3.42% and 4.22% respectively. The quality was far better than that of FPC-A as defined by FAO. The amino acids composition for human nutrition were complete, approaching the model chart, and were easily absorbed. The nutritional quality of the trial-FPC was near to that of cheese, egg and breast milk, and exceeded that of soybean and wheat.

INTRODUCTION

For many years research on the production of FPC has been conducted in various countries and a number of factories have been established. FPC can be made by physical, chemical and microbiological methods. The output of low- value fish from China's rich resources is about 60%~70% of that of the ocean capture fishery. Most is used for producing fishmeal or feed, so their utilization value was low. Experimental solvent extracted FPC was made in order to utilize these resources and satisfy the needs for protein. The technological conditions of producing FPC were refined and the products tested. This demonstrated that trial-FPC was rich in protein and essential amino acids and that its quality reached the standards of FPC-A as defined by FAO. Used to fortify food, FPC could contribute to improving the nutrition of babies and young children and to raising the health level of people.

MATERIALS AND METHODS

Raw Materials

Fresh low-value fish.

Organic Solvent

Isopropanol, alcohol, ethyl acetate (all chemical-pure).

Extracting

The internal organs of fresh low-value fish were removed, and the fish washed and minced by a mincing machine. The extracting solvent was added to the fish meat, followed by dehydration. After de-fatting (including removing of smell and colour) and refining for different temperatures and times, the product was dried, ground and packed.

Analyses

Protein ***Semimicro Kjeldahl.***
Fat ***Soxhlet extraction.***
Moisture ***Drying at 1050C.***
Ash ***Furnace at 5500C.***
Amino Acid ***Automated analysis.***

Yield

$$Y(\%) = \frac{W1}{W2} \times 100$$

Y----yield, W1----weight of FPC from fish meat, W2 ----weight of fish meat.

RESULTS AND DISCUSSION

Extracting Solvent

The effects of dehydrating, defatting, removing smell and colour of fish meat were different with different kinds of extracting solvent, and the yield of FPC was different, too. For better extracting effects, isopropanol (A), alcohol (B), isopropanol and ethyl acetate (C), alcohol and ethyl acetate (D) were used in the experiments for comparison. The results were shown in Table 1.

Table 1. The effects and the yields of dehydrating, defatting, removing smell and colour with four extracting solvents.

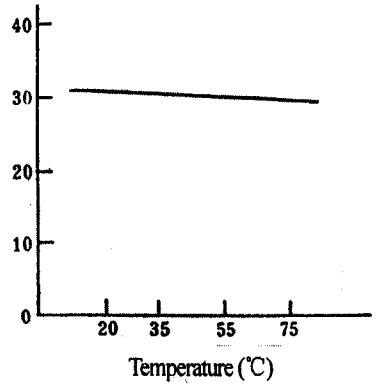
Extracting Solvents	Moisture (%)			Fat (%)			Colour	Smell	Yield (%)
	Raw material	After Dehydra-ting	Raw Mate-rial	Dehydra-ting Stage	Defat-ting Stage	Refining Stage			
A	80.50	29.35	4.47	4.14	0.34	0.12	White	No	17.1
B	80.50	33.73	4.47	4.17	1.45	0.86	Pale Yellow	little	16.3
C	80.50	40.35	4.47	1.85	0.21	0.10	Greyish	No	15.0
D	80.50	36.29	4.47	2.46	0.29	0.13	Grey	No	16.0

The results showed that the effects of extracting with B were not good in the same conditions. Extracting effects with C and D were better. The effects of defatting were better in dehydrating stage with C and D, but the colour of product was not good, and the yield was lower. The abilities of dehydrating and defatting with A were better, and the colour of product was good, and the yield was higher.

Factors Working on Dehydrating and Defatting of Isopropanol

Temperature

The effects of temperature on dehydrating and defatting are shown in Figures 1 and 2.



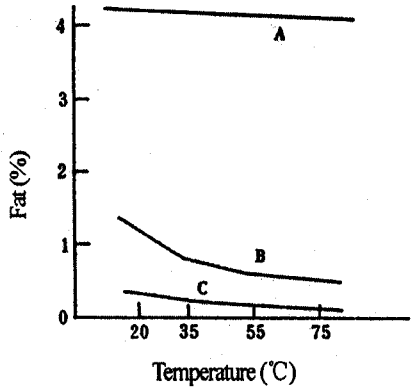


Fig. 1. The effect of temperature on dehydrating

A: Dehydrating stage B: Defatting stage

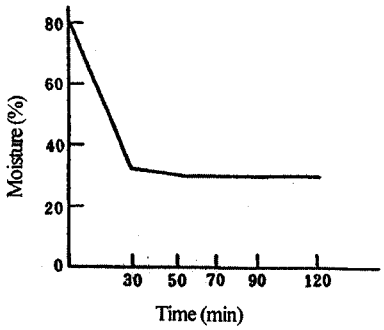
Fig. 2. The effect of temperature on defatting.

C: Refining stage

The effect of temperature on dehydrating was very weak because the dissolving capacity of isopropanol with water was very good in room temperature (Fig.1). The concentration of isopropanol was so reduced, because of being dissolved in a lot of water in dehydrating stage, that its defatting ability was very weak. So room temperature was the best in dehydrating stage. Before defatting and refining, most of the moisture in fish meat had been removed, so the concentration of isopropanol was high, which led to its high defatting ability. The combination of spreading and permeating was speeded and defatting effect was increased because the movement of molecular was quickened with increasing temperature. When the temperature nearly went up to the common boiling point (80□) of isopropanol and water, the defatting ability of isopropanol got to maximum. Therefore, 75□ was the best choice so as to obtain fully defatting and to avoid boiling loss of isopropanol.

Time

The effects of time on dehydrating and defatting were shown in Figures 3 and 4.



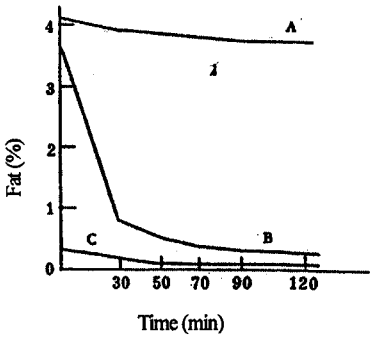


Fig. 3. The effect of time on dehydrating.

A: Dehydrating stage B: Defatting stage

Fig. 4. The effect of time on defatting.

C: Refining stage

The extraction was dependent on time. The moisture and fat in the surface layer of fish meat were extracted immediately. Then those from the internal part diffused to the surface. So the moisture and fat in the fish meat reduced gradually, and the rate of dehydrating and defatting went down afterwards. It was found best to dehydrate for 50 minutes, to defat for 90 minutes and to refine for 50 minutes.

Isopropanol Volume

The effects of isopropanol volume on dehydrating and defatting are shown in Figures 5 and 6.

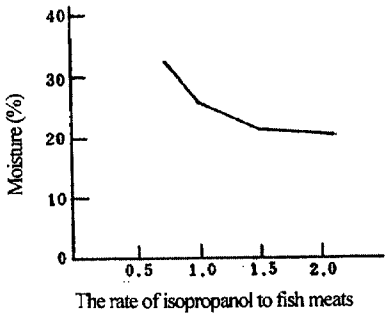


Fig. 5. The effect of isopropanol on volume dehydrating.

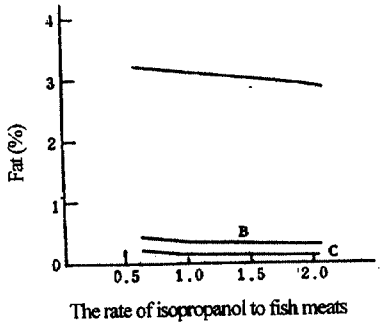


Fig. 6. The effect of isopropanol volume on defatting.

A: Dehydrating stage B: Defatting stage C: Refining stage

The dehydrating and defatting abilities of isopropanol were better when more isopropanol was used at a given temperature and time. Using 1.5 times the weight isopropanol to the weight of fish meat in the dehydrating stage and 1.0 times isopropanol in the defatting and refining stages was found to be best.

Nutritive Value of FPC

Nutritional composition of FPC is shown in Tables 2 and 3.

Table 2. Comparison of the standards of FPC-A (FAO), and nutritional composition of trial FPC and four kinds of food.

Name	Protein (%)	Fat (%)	Moisture (%)	Ash (%)	Calcium (%)	Phospho-rus (%)
FPC-A of FAO	>75	<0.5	<10	<15	-	-
Trial FPC	92.28	0.09	4.22	3.42	0.900	0.563
Egg	11.8	15.0	70.8	-	0.58	0.248
Milk powder	26.2	30.6	2.0	-	1.03	0.883
Milk	3.3	4.2	86.7	-	0.122	0.090
Soybean powder	32.4	28.5	3.7	-	0.272	0.490

The protein of trial FPC was higher and fat, moisture and ash were lower than those of FPC-A as defined by FAO (Table 2). The quality of trial FPC exceeded that of FPC-A. The rates of protein of trial FPC to that of egg, milk powder, and milk were 7.8, 3.5, and 28 respectively. The contents of calcium and phosphorus of trial FPC were higher than those in other foods.

Table 3. Essential amino acid contents of a few proteins and essential amino acid requirements for humans (mg /g protein).

Amino acid	Trial - FPC	Cheese	Egg	Human Milk	Soy-bean	Wheat	Providing need of FAO			
							Baby	Child	Adult	Model-chart
LYS	96	81	70	66	63	20	52	75	22	55
TRP	14	13	17	17	13	11	8.5	4.6	6.5	10
THR	46	43	47	43	38	29	44	44	13	40
VAL	53	74	66	55	52	19	47	41	18	50
MET+CYS	39	31	57	42	13	14	29	34	24	25
ILE	51	66	54	46	53	32	35	37	18	40
PHE+TYR	76	54	93	72	48	48	63	34	25	60
LEU	87	101	86	93	77	74	80	56	25	70
HIS	25	-	22	25	-	-	-	-	-	-

The essential amino acid requirements for humans were met by trial FPC, indicating that the human body can easily absorb it. Its nutritive quality is close to that of cheese, egg and human-milk and better than that of soybean and wheat. Therefore the trial FPC was an excellent protein for people, especially babies and children.

CONCLUSIONS

(1) FPC was extracted with isopropanol at given temperature and time. The contents of protein, fat, ash and moisture were 92.28%, 0.09%, 3.42% and 4.22% respectively. The FPC did not have a bad smell and its quality greatly exceeded that of FPC-A as defined by FAO.

(2) The protein content of the FPC was high, and the essential amino acids balance met human requirements.

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PROCESSING OF FISH FLOUR FROM CROAKER (*Pseudociena amoyensis*)

by

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ABSTRACT

The experiment aimed at studying fish flour processing, particularly to determine the best method to produce fish flour that meets the requirements for a food product. Four methods were tried, (1) without steaming, spray drying; (2) without steaming, drum drying; (3) steaming, spray drying and (4) steaming, drum drying.

The results indicated that the best quality was obtained by the third method which consisted of the following steps: dressing - mincing - washing three times - dewatering - steaming- pressing - blending - spray drying. The proximate composition of fish flour produced was 4.66% moisture, 92.48% (d.b.) protein, 1.62% (d.b.) fat) and 2.77% (d.b.) ash. The fish flour was also physically good with a whiteness value of 85.55% and microbiologically free from *E. coli* and *Salmonella*.

INTRODUCTION

Although several countries have developed fish flour or fish protein concentrate (FPC) commercially and used the products to supplement or fortify foods like bread and noodles, limited attempts have been made in Indonesia. Some studies have been conducted to produce FPC from shark (Astawan, 1990), FPC from the by-products of the tuna industry (Sulistiani, 1994), fish flour from shark (Juwono, 1989) and fish flour from sardine (Nurhayati, 1994).

This research studied the effect of processing method on the quality of fish flour from croaker, *Pseudociena amoyensis*, a demersal fish, often found in North Java waters. This fish contains relatively low fat, approximately 2%, and is commonly marketed fresh, cooked or dry-salted at relatively low prices. It is hoped that the results will provide more information and alternative products to support the fish product diversification programme.

MATERIALS AND METHOD

Croaker or 'gulamah' (*Pseudociena amoyensis*) was used as raw material. The fish was obtained from Indramayu, West Java with the physical size and proximate composition as presented in Table 1.

Fish flour processing was done using the method of Juwono (1989), slightly modified as described in Figure 1. Firstly, the fish was washed, eviscerated and filleted. The fillet was then minced using a meat-bone separator and washed 3 times with cold water (5 - 10°C). After de-watering by centrifuge, the minced fish was steamed for 30 minutes followed by pressing, blending and drying. Drying was either by a spray dryer at 180°C or a drum dryer at 100-110°C and pressure of 4.5-5.0 Bar.

Table 1. Physical size and proximate composition of croaker (*P. amoyensis*).

Parameter	
Physical size	
- Total length (cm)	28.5 ± 2.66
- Width (cm)	7.52 ± 0.83
- Thickness (cm)	3.67 ± 0.43
- Weight (g/fish)	292.75 ± 91.83
Chemical composition	
- Moisture (%)	79.40
- Ash (%)	1.32
- Protein (%)	16.64
- Fat (%)	0.52

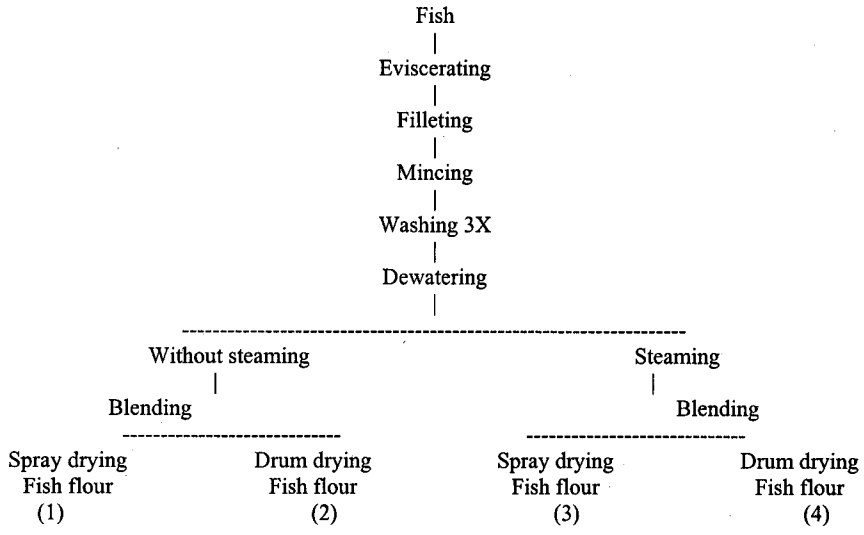

Fig. 1. Schematic diagram of fish flour processing.

Quality of fish flour was assessed by chemical, physical, microbiological and organoleptic analysis. Moisture, crude protein, crude fat and ash contents were determined according to AOAC (1984). Non protein nitrogen (NPN) was determined according to Apriyantono (1989) using the Kjeldahl method. Water solubility was determined using Lembono's method (1989), while water absorption was determined according to Beuchat (1977). Whiteness was measured using a whiteness meter with a green filter and sodium carbonate as standard (with a score of 100). Microbiological analysis was for TPC (Fardiaz, 1987), *E. coli* (Miwa, 1992) and *Salmonella* (Miwa, 1992). Organoleptic assessment was carried out by a descriptive scoring method and data were statistically analyzed using a Complete Randomized Design.

RESULTS AND DISCUSSION

Yields

Processing of fish into fish flour caused a large reduction of the material weight due to losses during preparation (dressing up to washing/after mincing) and moisture losses during pressing and drying. The average

processing yields obtained from the four methods (1-4) were 4.44%, 8.54%, 5.15% and 4.67% respectively. The yields were significantly affected by the processing method ($p>0.01$). Spray drying produced a lower yield of fish flour than drum drying because the preparation for spray drying requires blending finely. Consequently, losses from spray drying were more than for drum drying.

Physical Properties

The effect of processing method on the physical properties of fish flour is shown in Table 2. The results revealed that the processing method significantly affected whiteness and water solubility of fish flour, but no significant effect was shown in water absorption. Spray drying resulted in a whiter fish flour compared to drum drying (method 1 and 3 vs method 2 and 4) probably due to the HTST (high temperature short time) in spray drying reducing oxidative browning. Drum drying in open air accelerated oxidation.

Table 2. Physical properties of fish flour from croaker.

Parameter	Processing method			
	1	2	3	4
Whiteness (%)	84.25[a]	82.00[b]	85.50[a]	79.75[c]
Water absorption (%)	248.26[a]	171.71[a]	204.76[a]	179.71[a]
Water solubility (%)	15.96[a]	21.51[b]	38.27[c]	15.01[a]

Note : The same letter at the same row indicates not significant ($p>0.01$).

Water absorption is one of the important characteristics of protein. Interaction of protein and water affects hydration, swelling, viscosity and gelation (Hutton and Campbell, 1981). Table 2 shows that the water absorption of fish flour from croaker was in the range of 171.71 - 248.26%, indicating that water absorption was insignificantly affected by processing method. Astawan (1990) found that water absorption of FPC from shark produced by extraction using cold ethanol reached 482%. The lower water absorption of croaker flour might be due to the heating/steaming and drying process causing denaturation and aggregation of protein molecules thus reducing water absorption.

Processing method significantly affected water solubility with the highest water solubility (38.27%) obtained from the third method (combination of steaming and spray drying).

Chemical Composition

Table 3 shows that processing method has a significant effect on the chemical composition, particularly moisture and fat content. Fish flour produced from method 1 and 3 contained lower moisture than those from method 2 and 4. This means that spray drying resulted in a lower moisture than drum drying. According to Kulikov (1971) moisture content of fish flour and FPC type B should be not more than 10%. Therefore, all of the processing methods except method 2, met the requirement.

Fat content for methods 1 and 2 was not significantly different from those from method 3 and 4, meaning that steaming had no significant effect on the fat content. On the contrary, drying method greatly influenced the fat content, indicated by significant difference in fat contents between methods (1 and 3) and methods (2 and 4). The fat contents of fish flour from drum drying were lower than spray drying. It is suspected that some of the fat in the drum drying process separated from the solid material because of the heating.

Table 3. Chemical composition of fish flour from croaker.

Parameter	Processing method			
	1	2	3	4
Moisture (%)	4.51[a]	13.01[b]	4.66[a]	8.08[c]
Ash (% db)	3.50[a]	3.13[a]	2.77[a]	2.12[a]
Protein (% db)	92.13[a]	93.25[a]	92.48[a]	91.36[a]
Fat (% db)	2.15[a]	1.10[b]	1.62[c]	1.04[b]
NPN(% db)	0.15[a]	0.20[a]	0.21[a]	027[a]

Note : The same letter at the same row indicates not significant (p>0.01).

Compared to the standard for fish flour as stated either by Kulikov (1971) or FPC type B (FAO, 1964), the fish flour from croaker met the requirements for fat and protein contents. The requirement of FAO (1964) for FPC type B was 65% protein (minimum) and 3% fat content (maximum), while Kulikov (1971) lists a protein content of more than 70% and a fat content less than 3% for fish flour.

Microbiological quality

Microbial load of fish flour was in the range of 1.5×10^2 - 3.3×10^4 as described in Table 4. The load of TPC was not significantly affected by the process method. *E. coli* which is often associated with faecal contamination was not found in the fish flour. *Salmonella* was also absent, implying that products were safe as foodstuffs or for fortification.

Table 4. Microbiological quality of fish flour from croaker.

Parameter	Processing method			
	1	2	3	4
TPC (cfu/g)	1×10^{4a}	$2.1\ 10^{3a}$	3.3×10^{4a}	1.5×10^{2a}
E. coli	negative	negative	negative	negative
Salmonella	negative	negative	negative	negative

Note : The same letter at the same row indicates not significant (p>0.01).

Organoleptic quality

Organoleptic quality of the fish flour is presented in Table 5. Processing method significantly affected appearance, colour and acceptance of the fish flour, but no significant effect was shown on both odour and texture. Spray drying resulted in improved organoleptic quality compared to drum drying. The highest score of organoleptic parameters was obtained from the third method (steaming and spray drying) with description of the fish flour as follows: good appearance, fine and homogenous particle, no contamination (dust, insect, etc.), white-creamy, specific odour and dry.

Table 5. Organoleptic score of fish flour from croaker.

Method	Appearance	Colour	Odour	Texture	Acceptance
1	4.55[ac]	4.35[a]	4.45[a]	4.70[a]	4.50[ac]
2	3.90[b]	3.85[bc]	4.40[a]	4.30[a]	3.70[b]
3	4.80[c]	4.60[a]	4.65[a]	4.75[a]	4.70[a]
4	4.15[ab]	3.45[b]	4.60[a]	4.55[a]	4.05[bc]

Note : The same letter at the same column indicates not significant (p>0.01).

CONCLUSION AND SUGGESTION

Of the four processing methods, the best was: dressing - filleting - mincing - washing three times - dewatering - steaming- pressing - blending - spray drying.

Even though the method provided good quality fish flour, the processing yield was relatively low (5.15%) making it economically difficult to produce commercially. Therefore, it is necessary to make efforts to develop alternative methods that are able to give higher yields.

Experiments on the stability of the fish flour during storage should be carried out to determine the storage life of the product.

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Appendix

Organoleptic Score Sheet for Fish Flour

Date : Panellist : Fish :

Please, give the score for the sample of fish flour at the appropriate scale line !

Example :

	A	C		B	
		D			
1	2	3	4	5	

PARAMETERS	DESCRIPTION	SCORE					
		1	2	3	4	5	
APPEARANCE	Very dirty						Very clean
	Many contaminants						No contaminants
	unhomogenous particle size						Homogenous particle size
	Very dull						Very shine
COLOUR	Brown						White
ODOUR	Specific odour /fish flour						Neutral
	Strong rancid odour						No rancid odour
	Strong musty odour						No musty odour
	Strong burnt odour						No burnt odour
	Strong acid odour						No acid odour
	Strong putrid odour						No putrid odour
TEXTURE	Clumpy						Not clumpy
	Wet						Dry
	Coarse						Fine
ACCEPTANCE	Do not like						Like

THE UTILIZATION OF FISH PROTEIN AND OIL FROM ANCHOVY (*Engraulis japonicus*) FOR HUMAN CONSUMPTION

by

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ABSTRACT

The comprehensive utilization of fish protein and oil from anchovy (*Engraulis japonicus*) for human consumption was studied. Proteolytic enzymes were used to hydrolyze anchovy protein and the bitter taste of the hydrolysate was eliminated by active carbon and beta-cyclodextrin. Another non-bitter fish protein hydrolysate also could be prepared by limited hydrolysis with proteolytic enzymes. The final protein product from anchovy can dissolve in water and form a transparent solution, which is rich in peptides and free amino acids with an appropriate essential amino acid ratio. A highly polyunsaturated fatty acids (PUFA) product was prepared from anchovy oil by molecular distillation. The contents of EPA/DHA in oil products with light yellow color from anchovy can reach 70%. The wastes from anchovy after protein and oil recovery were converted to fish meal. Growth and metabolic experiments with young male rats indicated that the efficiency of digestion and utilization of the protein from the anchovy hydrolysate were very close to that of milk protein. The product can be used as a protein supplement for babies and patients, or as raw materials for seafood flavorings.

INTRODUCTION

The underutilized fish anchovy *Engraulis japonicus* is one of the important resources in China for manufacturing fish meal and fish oil. It is rich in fish protein with a well-balanced amino acid composition, and the lipid is rich in highly polyunsaturated fatty acids (PUFA). Because of its rapid spoilage, it is difficult to process for human food.

The trend in the utilization of underutilized fishery resources is toward direct consumption as human food, such as food products and food supplements used in different nutrition programmes (Lalasidis *et al.*; 1978, Mackie, 1982; Yanez *et al.*, 1976). The usual method is hydrolysis where enzymes are used to produce protein rich food for human consumption. Lack of functional properties and a bitter taste have, however, inhibited the use of hydrolysates as human food (Hevia *et al.*, 1977; Lalasidis *et al.*, 1978; Vega *et al.*, 1988).

Fish oil products derived from omega-3 fatty acids have been suggested as beneficial in the prevention and/or treatment of major cardiovascular diseases affecting human health (Gordon *et al.*, 1992). The present experiments were undertaken to convert anchovy protein into a water-soluble protein product suitable for direct addition to food systems, and to prepare anchovy oil as a PUFA concentrate for use in medicine and health foods. The comprehensive utilization process from anchovy is also discussed.

MATERIALS AND METHODS

RAW MATERIALS

Anchovy, caught in the Yellow Sea in February 1990, was supplied by the Qingdao Marine Fisheries Co., China, and was frozen until required. The crude composition of raw material was as follows: 16.70%

protein (N x 6.25), 10.82% fat, 1.40% ash and 69.75% water. The neutral proteolytic enzyme from *Bacillus subtilis* was purchased from Wuxi Enzyme Products Factory, China.

PREPARATION OF PROTEIN HYDROLYSATES

1. Exhaustive hydrolysis for enzymatic anchovy protein hydrolysates (EAPH).

A homogenate was prepared from frozen anchovy by thawing, mincing and suspending in the same volume water. The pH was adjusted to 7.0 with NaOH and kept at this value at 50°C. After that 0.7% (w/w) of neutral proteinase was added. The hydrolysis continued for 12 h. After hydrolysis the enzyme was inactivated by heating the homogenate to 85°C for 10 min and the suspension was centrifuged to remove the insoluble materials. Then the supernatant was treated by absorption with active carbon and beta-cyclodextrin to remove the bitterness (Helbig *et al.*, 1980; Lalasidis *et al.*, 1978). The clear aliquots of hydrolysate were spray dried for analysis and preparation of food supplements.

2. Partial Hydrolysis for EAPH.

The process of partial hydrolysis is similar to that of exhaustive hydrolysis. The anchovy homogenate was hydrolyzed with 0.1% (w/w) neutral proteolytic enzyme at 50°C, pH 7.0 for 1.5 h. The hydrolysate was treated with 1% w/v active carbon. The clear aliquots of hydrolysate were analyzed and spray-dried.

SENSORY TEST ON BITTERNESS

All the taste evaluations were carried out according to the methods of Helbig *et al.*(1980) and Lalasidis *et al.*(1978). The bitterness scores of hydrolysates were graded on a five point scale (0 = no bitterness; 1 = weak aftertaste; 2 = weak bitter taste; 3 = bitter; 4 = strong bitter taste; 5 = extremely bitter) by a panel of 10 trained members.

PREPARATION OF PUFA CONCENTRATES

Refined anchovy oil was prepared from crude fish oil, which was obtained from exhaustive hydrolysis process, by a general oil refining process such as degumming, deacidification, decolorization and deodorization. PUFA concentrates were prepared by urea addition and molecular distillation (Sumerwell, 1957; Ackman *et al.*,1973). Before PUFA concentration fish oil needed to be converted to the ethyl ester by transesterification with a catalyst. In the urea addition method, the amounts of urea used in three experiments were 1.5,2.0,and 2.5 times the of ethyl ester of fish oil. The ester mixture was distilled in two stages on a SIBATA MS-300 molecular still.

BIOLOGICAL EVALUATION OF EAPH

Biological evaluation was performed according to McLaughlan (1980) and Pellett *et al.* (1980). Thirty male Wistar rats weighing 57±1 g were randomly divided into five matched groups. The experimental feeds had the following nitrogen sources: A, nitrogen-free; B, milk powder protein; C, EAPH (from exhaustive hydrolysis); D, wheat protein; E, wheat protein supplemented with EAPH (12% protein in the feed was from EAPH). The feed compositions were as follows: 10±0.12% protein; 10±0.36% fat; 5% mineral mixture; 2% vitamin mixture; 5% nitrogen-free cellulose; and up to 100% corn starch. Feed and water were offered *ad libitum*. During the 4-d nitrogen balance experimental periods, weight gain and feed intake were measured, and urine and feces were collected separately for nitrogen determination. During the 4-week growth experiment, feed intake and weight gain were measured. At the end of the experiments, all rats were put to death, and analyses of fresh organs (liver, kidney, lung and spleen) were performed.

RESULTS

EAPH FROM EXHAUSTIVE HYDROLYSIS

The degree of exhaustive hydrolysis was 68.66%. The hydrolysate prepared from exhaustive hydrolysis had a strong bitter taste (bitterness score 4.5). The hydrolysate can be debittered by absorbing the bitter peptides with active carbon and masking with beta-cyclodextrin. The end product EAPH was free from bitterness (bitterness score 0.5). The yield of soluble nitrogen in EAPH was 89.80%. The spray dried product from the pilot plant production was a fine white powder with a pleasant odor containing 88.2% protein, 6.89% ash, 0.38% fat and 3.82% moisture. The amino acid composition was well balanced, and the content of essential amino acids was higher than that of enzymatic fish protein hydrolysate (EFPH) used as a reference from Yanez *et al.* (1976). EAPH had a high content of free amino acids (62.70% of total amino acids) (Table 1). Synthetic milk-like beverages were prepared with EAPH, and stored at room and refrigerated temperatures for several weeks with no evidence of de-emulsification. As a protein supplement in cake formulations, the product could replace wheat flour with no change in cake volume and texture. Therefore EAPH may be used as a food ingredient or additive.

EAPH FROM PARTIAL HYDROLYSIS

The degree of partial hydrolysis was 31.86%. The hydrolysate prepared by partial hydrolysis had no bitterness (bitterness score 0.5). The yield of soluble nitrogen in EAPH prepared by partial hydrolysis was 41.17%. The spray dried product was a powder with a seafood flavour containing 93.10% protein, 2.28% ash, 0.52% fat and 2.17% moisture. The amino acid composition was also well balanced. The amount of essential amino acids was also higher than that of EFPH (Table 1). It can also be used as a seafood flavoring or a seafood additive.

Table 1. Amino acid composition of EAPH (g/100g protein).

Amino Acids	**Raw anchovy**	**EAPH**			**EFPH**
		a	**b**	**c**	
Asparagine	9.21	8.63	3.41	8.66	13.9
Threonine	4.11	3.73	0.25	3.62	3.7
Serine	3.97	3.30	1.48	3.08	3.5
Glutamic acid	13.62	19.30	8.87	19.03	17.7
Glycine	5.47	4.87	2.31	4.88	3.9
Alanine	6.51	7.57	5.85	7.45	5.7
Cysteine	0.35	0.25	0.54	0.25	0.8
Methionine	3.65	3.18	2.68	3.48	3.4
Valine	6.60	6.58	5.36	6.48	Trace
Isoleucine	3.75	5.58	5.05	4.58	4.4
Leucine	7.51	9.93	8.88	9.80	9.5
Tyrosine	2.94	2.47	2.48	2.50	3.4
Phenylalanine	3.97	3.37	Trace	3.18	3.2
Lysine	7.53	10.05	6.24	10.09	11.8
Histidine	9.15	3.38	2.66	3.20	1.4
Arginine	8.47	6.66	5.76	6.62	6.5
Tryptophan	1.46	1.19	0.88	1.19	1.3
Proline	1.23	Trace	Trace	1.90	Trace
T	99.50	100.00	62.70	99.99	94.1
E/T	38.77	43.61	46.79	42.42	39.6

a: from exhaustive hydrolysis; b: free amino acids from exhaustive hydrolysis; c: from partial hydrolysis; EFPH: enzymatic fish protein hydrolysate; T: total amino acids; E/T: Essential amino acids/total amino acids(%).

HUFA CONCENTRATES

The concentration of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in HUFA concentrates prepared by urea addition method increased with the amount of urea used in the process. In the concentrate obtained by using 2.5 times of urea, the content of EPA and DHA was up to 70.0% (EPA 30.7%, DHA 39.3%). In the molecular distillation method, the content of EPA and DHA in HUFA concentrates fraction obtained in the first stage was 48.1% and in the second stage, up to 70.1% (EPA 15.8%, DHA 54.3%). The analysis of iodine values indicated that the degree of unsaturated fatty acids in HUFA concentrate was high. The colour values showed that the products were yellow and transparent(Table 2).

Table 2. PUFA concentrates from anchovy fish oil.

Fatty acids	Raw Oil	Urea addition			Molecular distillation		
C14:0	7.1	0.8	0.7	0.9	15.5	/	/
C16:0	18.6	/	/	/	31.4	1.5	1.2
C16:1	6.9	8.2	6.3	0.8	12.1	0.7	0.5
C18:0	3.7	/	/	/	3.5	2.3	2.0
C18:1	12.3	14.6	3.8	0.8	12.0	5.9	6.0
C18:2	3.6	10.6	8.1	1.9	3.5	1.6	1.6
C18:3	1.7	4.8	3.8	1.5	1.5	1.6	1.0
C20:1	2.3	0.8	/	/	1.0	4.9	4.4
C20:4	1.1	2.0	3.0	3.2	0.5	1.2	1.6
C20:5	9.5	16.7	26.4	30.7	4.2	9.8	15.8
C22:1	/	/	/	/	0.3	10.6	10.4
C22:6	17.2	18.4	34.6	39.3	3.5	38.3	54.3
Other	16.0	23.3	13.3	20.9	11.0	21.6	1.2
EPA+DHA	26.7	35.1	61.0	70.0	7.7	48.1	70.1
Yield	100	25.2	22.1	19.0	53.3	45.3	20.5
Iodine value	120	160	280	320	86	220	320
Color L value	38.2	69.2	71.3	70.6	85.0	68.7	72.0
A value	8.9	8.6	9.3	9.5	14.1	10.1	9.4
B value	3.2	7.5	7.3	8.1	5.6	6.4	7.9

a, b, c: from process using 1.5,2.0,2.5 times of urea, respectively. A: saturated fatty acid fraction from the first stage; B: concentrated HUFA from the first stage; C: concentrated HUFA fraction from the second stage.

BIOLOGICAL EVALUATION OF EAPH

The nitrogen balance experiments indicated that the biologic value (BV), true digestibility (TD) and net protein utilization (NPU) were significantly higher for the EAPH-based feed (Table 3). Growth experiments showed that the growth rate and protein efficiency ratio (PER) of rats fed with EAPH-based feed was also higher than that of rats fed with milk protein-based feed. Supplement with EAPH gave a significant increase in the PER value of the wheat protein. The results from analysis of fresh organ weights showed no significantly different values among the tested rats. Histological examination of the livers, lungs, adrenals and kidneys revealed no differences between control and experiment groups.

Table 3. Nitrogen balance and growth experiments on rats fed with different feeds.

Nitrogen Balance Experiments	A	B	C	D	E
Feces nitrogen (g)	0.03	0.11	0.08	0.11	0.08
Urine nitrogen (g)	0.06	0.16	0.15	0.23	0.16
Nitrogen intake	Trace	0.66	0.69	046	0.52
Absorbed nitrogen (g)	Trace	0.58	0.64	0.38	0.47
Retained nitrogen (g)	Trace	0.48	0.55	0.21	0.37
True digestibility(TD)	87.88	92.75	82.26	90.38	
Biological value(BV)	82.76	85.94	55.26	78.72	
Net protein utilization(NPU)	72.73	79.71	45.65	71.15	
GrowthExperiments					
Weight gained	-31.32	103.38	106.27	25.46	70.52
Growth rate	-54.04	176.37	184.56	44.13	122.73
Diet intake(g)	347.71	339.65	335.68	386.78	358.62
Protein intake(g)	trace	34.30	33.90	37.40	31.60
Protein efficiency ratio(PER)	3.01	3.13	0.68	2.23	

A: nitrogen-free; B: milk powder protein; C: EAPH(from exhaustive hydrolysis);
D: wheat protein; E: wheat protein supplemented with EAPH.

DISCUSSION

In the exhaustive hydrolysis of anchovy protein, some factors such as different proteolytic enzymes, the hydrolysis temperature, the hydrolysis time and the amounts of enzyme addition should be carefully considered. To compare with specific sulfhydryl group proteolytic enzyme such as papain and bromelin, neutral proteinase No. 1398 has higher hydrolysis efficiency. High yield of soluble nitrogen, well balanced amino acid composition and the high contents of free amino acids. The hydrolysate had a strong bitter taste which was eliminated by using active carbon and beta-cyclodextrin. Bitterness of the fish hydrolysate comes from short peptides with hydrophobic groups such as Leu (Gly)-ASP-Lys. Different sizes of active carbon, the amount of its addition, beta-cyclodextrin addition, the combination of the two substances also affected debittering activities in fish hydrolysate preparation.

The final product had a faint marine taste and odour. In partial hydrolysis, the fish protein was not fully hydrolyzed and little bitter-taste peptides were less produced, but the yield of soluble nitrogen was much lower than that of exhaustive hydrolysis. The exhaustive hydrolysis processes from under utilized fish have been proven practicable in the pilot-plant production and large scale industrial production. In the nutritional evaluation experiments, all feeds had the same composition except for the amino acid composition. EAPH showed the high biological value (BV) as well as the PER value. It had a high nutritive value and was easily digested, adsorbed and utilized and can be used as a protein supplement in a variety of foods, especially cereal.

Anchovy oil contains high contents of HUFA and HUFA concentrates can be prepared by several methods. Among them the urea addition method and molecular distillation method have been used for industrial production in China. The molecular distillation is carried on under a high vacuum condition and by two to five stages, 70% or more of EPA and DHA concentrates can be prepared.

Anchovy fish is rich in the East Sea of China, the Yellow Sea, and other parts of the West Pacific. The resources have not been utilized and developed. The idea of the comprehensive utilization of under utilized fish such as anchovy has been pointed out. The preparation of four products are EAPH for use as a protein supplement; fish oil for animal feed additives and for HUPA production; HUFA concentrates with different contents of EPA and DHA and the residue from EAPH to make fish meal.

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UTILIZATION OF FISH WASTE FOR FISH SILAGE POWDER AND ITS APPLICATION AS FEED FOR CHICKEN AND FISH CULTURE

by

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ABSTRACT

Fish waste (such as bone, viscera, skin and meat) generated from the fish processing industry can be a source of pollution and environmental contamination. On the other hand, this waste contains high amounts of protein, fats and minerals and can be utilized as chicken or fish feed.

Utilization at fish waste and by catch for production of fish silage powder (FSP) and its application for chicken and fish feed were investigated. The processing steps were addition of 3% formic acid (v/w) and incubation at ambient temperature for 7 days; neutralization of pH to about 6 by addition 1% Na_2CO_3 ; mixing with rice bran with ratio 1:1 (w/w); drying and grinding.

The result of proximate analysis (protein, fat, ash, water and carbohydrate) as well as omega 3 fatty acid (EPA and DHA) of whole fish (Lizard fish/*Saunida tumbil*) were 35,73%; 15.88%; 8.00%; 17.07%; 0.66% and 2.75% respectively while for fish silage powder from fish waste were 22.00%; 7.7%; 9.77%; 12.18% and 6.62% for protein, fats, ash, moisture and omega 3 fatty acid respectively.

As feed for chicken, FSP showed no significant difference in weight gains or RFC (Relative Food Conversion).

INTRODUCTION

All fish processing plants generate various kinds of wastes. The specific wastes streams vary with the plant and the products being processed. However, typically fish processing plants produce solid waste, which include such items as bones, shells, skin viscera, head and a proportion of meat. These wastes are a potential source of pollution and contamination of the environment, as they tend to spoil relatively quickly by enzymatic and bacteriological processes and accumulate flies, rodents, and other vermin. On the other hand, they contain high amounts of protein, fats and minerals which could be utilized as nutrient for chicken and fish feed.

The percentage of waste varies from near zero to about 80%. The average amount of waste for all fish is about 30% (Carawan *et al.*, 1979). Preparation and filleting fish generate 30-60% of solid wastes (Anon., 1992).

Other activities that produce waste include unloading fish in fish landing places due to bad handling in fishing vessels. Studies in northern Java indicate that about 20-30% of the fish landed are damaged and unused for human consumption.

Two main products for utilization of fish waste are silage and fish protein hyrolysate (Morrissey, 1988). Many studies have been published on the fish silage involving addition of acid to fish (Gildberg, 1974, Santos, *et al.*, 1974; Kompiang, 1977; Tatterson and Windsor, 1974). There are very few studies on fish silage powder as feed for chickens and fish culture.

MATERIALS AND METHODS

The raw material used for these investigation was solid waste from salt fish processing (viscera, bone, skin and head) and whole fish (lizard fish) a by-catch of low quality.

The raw materials were mixed with 3% formic acid (v/w) and incubated at ambient temperature for about 7 days until 60% of raw material become liquid. The mixture was neutralized to pH (5-6) by addition 1% of Na_2CO_3 and mixed with rice bran at a ratio 1:1 (w/w); dried under the sun and ground. The fish silage powder was then tested as chicken feed for the grower stages (average weight of 600 gram) and the relative food conversion (RFC) was measured. Before feeding to chicken fish silage powder was mixed with commercial feed at a ratio of 75%:25%; 50%:50%; 25%:75% and 0%:100%.

The nutrient composition of fish silage powder was determined (proximate, carbohydrate, pH and omega 3 fatty acids (EPA and DHA).

Feeding trials were also carried out for cultured fish (*Cyprinus carpio*) by the Fish Stock Center of Wanayasa, West Java and the normal growths and spawning rate were observed. All chemical analyses were carried out using the methods of the National Standards of Indonesia (SNI).

RESULTS AND DISCUSSION

Proximate composition, carbohydrate, pH and omega 3 fatty acid are given in Table 1.

Table 1. Proximate composition, pH, Carbohydrate and omega 3 fatty acid of fish silage and FSP of whole fish (Lizard fish) as well as FSP of fish waste.

Parameter	Fish Silage of Fish Lizard	FSP of fish Lizard (Whole)	FSP of Fish Waste
- Protein (%)	17.15	35.73	22.00
- Fats (%)	6.12	15.88	7.70
- Ash (%)	3.28	8.00	9.77
- Moisture (%)	73.32	13.50	12.18
- Carbohydrate (%)	-	17.07	-
- Omega 3 fatty acid (%)	-	3.41	6.62
- EPA	-	0.66	-
- DHA	-	2.75	-
- pH	4.0	5.73	6.00

Fish silage is a liquid product with a moisture content of 72.32%; if it is dried directly, some nutrients will drain out. The silage was therefore mixed with rice bran before drying.

Rice bran also contains protein, fats and crude fiber, according to Sudaryani and Santoso (1995) 10.1%, 4.9% and 15.3%, respectively. The pH of fish silage is quite low (4.0) that is unattractive for chicken and fish, for this reason neutralization pH by addition of 1% Na_2CO_3 was done before mixing with rice bran and drying.

Protein content of FSP of both whole fish and fish waste is quite high (35.73% and 22.00%) considering that the maximum protein and fat content of chicken feed are 20% and 6 % respectively. FSP for feed could thus be mixed with other components such as corn, soybean or flour meal.

The feeding trial of FSP from whole fish to chickens in the grower stage in this study was conducted by mixing FSP with "commercial feed". The chemical analysis of FSP mixed with commercial feed is shown in Table 2.

Table 2. The commercial analysis of FSP mixed with "Commercial Feed".

Parameter	Ratio of FSP to Commercial Feed				
	100%:0%	**75%:25%**	**50%:50%**	**25%:75%**	**0%:100%**
- Protein (%)	35.73	26.74	23.20	21.09	19.72
- Fats (%)	15.88	11.70	10.60	9.34	7.25
- Ash (%)	8.00	7.10	6.17	5.11	4.14
- Carbohydrate (%)	17.07	30.20	36.77	41.34	45.64
- Omega 3 fatty acid (%)					
- EPA	0.66	0.57	0.50	0.35	0.35
- DHA	2.75	2.15	1.69	1.06	0.42

Protein and fat content of feed (FSP) decreased considerably by addition of commercial feed although they are still higher than the maximum level of feed that chickens require.

Omega 3 fatty acids of FSP mixed with commercial feed are higher compared to commercial feed (100%). Table 2 shows that there is correlation between fat content and omega 3 fatty acid content. The higher fats content, the greater omega 3 fatty acid.

The ratio of FSP and commercial feed has a significant effect on the omega 3 fatty acid content of chicken meat as shown in Table 3. The higher the ratio of FSP, the higher the omega 3 fatty acid content of the meat.

Table 3. Correlation between ration of FSP and Commercial feed given to chicken and omega 3 fatty acid content in chicken meat.

No.	Ratio of FSP and Commercial Feed	Omega 3 Fatty acid Content	
		(in feed)	**(in chicken meat)**
1.	75% : 25%	2.72	1.96
2.	50% : 50%	2.19	1.52
3.	25% : 75%	1.41	0.98
4.	0% : 100%	0.77	0.56

The effect of a chicken feeding trial for grower stages using a combination of FSP and commercial feed is shown in Table 4.

Table 4. Weight changes, Amount of feed given and relative feed conversion (RFC) of 25 days feeding trial to the chicken.

No.	Ratio of FSP and Commercial Feed	Initial Weight (average in gr.)	Final weight (average in gr.)	Amount of feed given (gr.)	RFC
1.	75%:25%	615	1,775	1,981	1,70
2.	50%:50%	615	1,880	2,258	1,78
3.	25%:75%	600	1,950	2,104	1,55
4.	0%:100%	610	2,100	2,407	1,61

Statistical analysis shows no significant differences among all treatments at the 0.01 level. The average weight gain is 1,316 gr., where the average amount of feed given is 2,187 gr. and the average relative feed conversion (RFC) is 1.66

Based on data above, FSP could be used as a feed for chicken, especially in the grower stages. Feeding studies have shown that the addition of fish meal to animal rations caused increased growth over and above that produced by nutritionally equal diets that do not contains fish meal (Fredrieck and Thomas, 1985). As the FSP is also made from fish, FSP could be used to substitute fish meal in feed.

A preliminary study on feeding FSP in substitution of fish meal in feed for fresh water fish (*Caprinus carpio*) found that the fish grows normally and spawned faster compared to fish fed with commercial feed (quantitative data is not complete yet, as the feeding trial is still in progress).

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UTILIZATION OF FRESH WATER CATLA (*Catla catla*) FOR PREPARATION OF MYOFIBRILLAR PROTEIN CONCENTRATE

by

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ABSTRACT

Minced fish offers a new area of fish utilization and scope for diversification of international trade. Washing the minced fish to remove the solubles and to increase the frozen storage life opens a new dimension to mince based technology. The global demand for the washed mince or myofibrillar protein (MFP) for diversion to surimi and other use is on the increase and the industry is in the look out for new sources.

Carp culture is gaining importance in recent times and marketing in the fresh condition is going to be a constraint if alternate utilization methods are not developed. Here a study has been made on the feasibility of preparing MFP from a major carp (*Catla catla*). The deboned meat was washed once, twice and three times with water (1:4 fish to water) with stirring for 10 minutes. A major portion of the solubles was lost in the first washing. Based on the round weight, the yield of MFP was 24%. Besides the loss of lipids, considerable loss of salt soluble protein was also noticed. The natural sea weedy or muddy odour associated with the fish was lost to a large extent as a result of washing. The changes in the rheological properties – folding test, gel strength and compressibility are discussed. There is overall reduction in the compressibility and gel strength of gel made from the MFP in comparison to unwashed mince, though the results of folding tests remained almost the same.

INTRODUCTION

Fish is an important food commodity world-wide. The technology of fish mince based products has undergone tremendous advancement in recent years. The era is slowly shifting to the development of fish mince based industry.

Fish itself is a highly perishable commodity. When the fish is comminuted to fish mince, due to the damage of the actual tissue make up a lot more changes could be expected and the same has been established (Anon, 1981). The spoilage of fish during storage is due to the combined effect of autolysis and bacterial action. Mincing accelerates decomposition, aggregation and cross-linking of myofibrillar protein (Laird et al., 1979) with consequent decrease in soluble proteins, water holding capacity and other rheological properties. However, mincing of fish opens a new field of fish utilization that offers scope for diversification of industry and international trade in value added products.

Further development of mince based technology following the commercialization of surimi led to the production of white, odourless and bland flavoured products. In surimi preparation, fish mince is washed in chilled water to remove the solubles which interfere with the storage and rheological characteristics (Lee, 1984). Washing fish mince decreases its stability but increases the storage tolerance (Shimizu and Fujita, 1985) and the tolerance is species dependent. The material obtained as a result of washing is nothing but the concentrated myofibrillar protein. Grinding the same with cryo-protectant makes surimi with better storage and rheological functions and this is used as a raw material for a number of fabricated seafood products.

Because of the high demand for the mince based products the preparation of mince and surimi is extended to more fish species.

The study on the suitability of freshwater species for mince based technology has gained importance due to the reduction in fish landings from marine sources and the importance given by several countries to fresh water fish culture to increase fish production. Freshwater fish, in the coastal states of India, is not that easily accepted primarily for its muddy flavour and the presence of pin bones. Hence, the objective of the present study is to:

1) Standardize the washing schedule for the preparation of surimi from one of the most popular fresh water carp, *Catla catla*.
2) Study the changes in the fish mince as a result of washing.

MATERIALS AND METHODS

Three different lots of pond fresh catla (*Catla catla*) were brought from the freshwater pond in iced condition (1:1) and kept for 24 hours to resolve the rigor. The post rigor fish was used for experiment. The fish was processed to remove head, fins and viscera and washed in ice cold water to remove remaining slime, scales, blood and adhering viscera. The flesh was separated from the processed fish using a Baader 694 deboning machine equipped with a rotating drum with 5-mm hole and operated at medium pressure to eliminate bones and for optimal recovery.

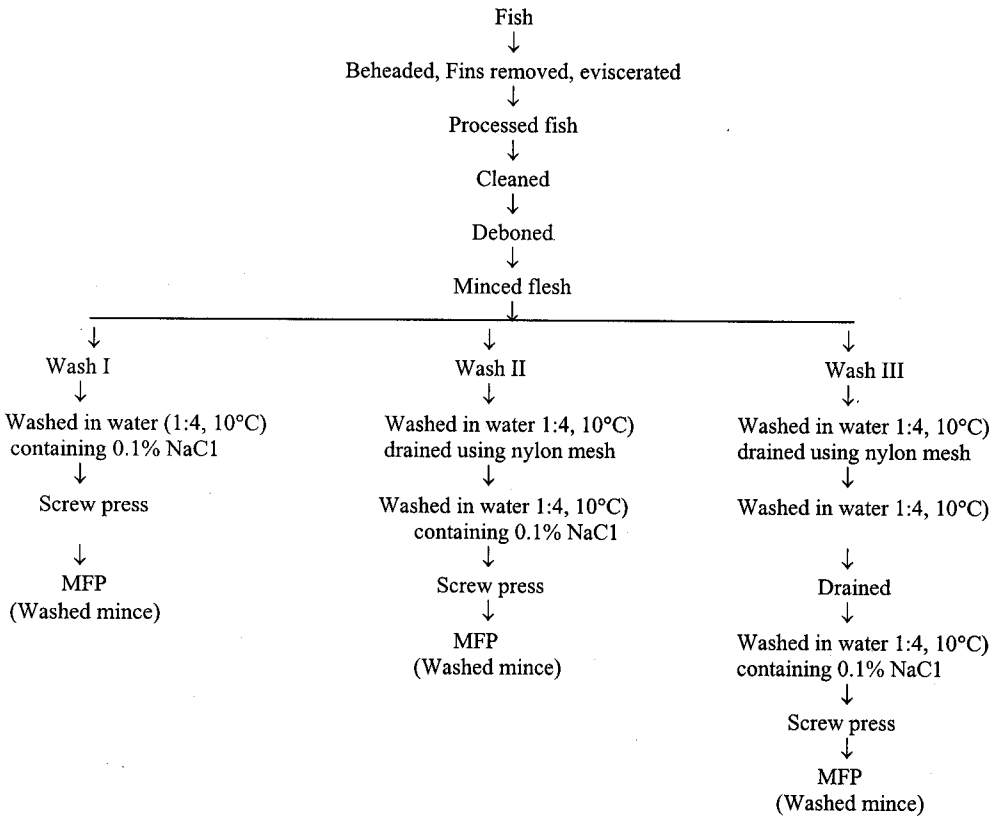

Fig. 1. Washing schedule for the preparation of myofibrillar protein from catla.

Flesh was then washed (Fig.1) in a stainless steel vessel once, twice and three times, in ice cold water (8-12° C) in the ratio of 1 to 4 (W/V). After stirring the mixture for 10 minutes, it was dewatered as much as possible using a laboratory model manual screw press. 0.1% NaCl was used in the last wash to facilitate dewatering. Washing efficiency was calculated by measuring and comparing the total solids and protein recoveries and reductions in the lipid and ash of the minced flesh before and after washing.

Moisture, total nitrogen, lipid and ash were analysed using AOAC (1976) procedures. The salt soluble proteins were extracted (Monterchia et al.., 1997) by homogenizing the meat in 5% NaCl containing 0.02M $NaHCO_3$ for 60 seconds followed by centrifugation at 10000 rpm for 20 minutes at 5°C. The supernatant was taken as salt soluble protein. The water-soluble protein was extracted in the same way using 0.02M $NaHCO_3$ instead of just water. The protein content was estimated by the biuret method (Gornall et al., 1949).

The rheological characteristics were studied by folding test, gel strength and compressibility of the gels prepared. The gel was prepared (Lee, 1984) by grinding the myofibrillar protein with 3% NaCl for 20 minutes using a mortar and pestle maintained at about 15°C. The paste was filled manually into polypropylene tubing of 6.0 cm diameter, taking care to eliminate the trapped air as much as possible. The ends of the tubes were tied and cooked by immersion in a boiling water bath for 30 minutes. The gels thus formed were cooled at once in ice and then kept at 5°C over night and analysed.

For folding test (Lee, 1984) a 3 mm piece (of diameter 37cm) of the gel was cut and folded between thumb and index finger and depending on the breakage they are graded as AA, A, B, C etc.

The gel strength and compressibility were studied using a 'SUN' rheometer using a 2.5 cm long (diameter 37cm) gel (Lee,1984). Gel strength (g x cm) is the product of force and deformation while the force (g) required to compress the sample by 4 mm is taken as compressibility.

RESULTS AND DISCUSSION

Manual processing yielded 61.87% processed fish based on round weight (Table 1). Machine separated minced flesh was 30.71% based upon round fish and 58.81% based processed fish. Gleman and Benjamin (1987) reported 41.4-45.2% mince yields in the case of silver carps. Generally, higher yields of mince are reported for marine fishes (Perigreen, 1979; Joseph and Perigreen, 1983; Muraleedharan, 1996). Yield of fish mince depends on the processing method and the type of equipment used (Crawford, 1972; Chang Lee et al., 1990). The yield in this case was less because of the size of its head, which in the case of fresh water catla accounts for about 25-27% of the total weight of fish.

Table 1. Yield based on round weight from processed Catla before and after mechanical flesh separation.

Batch	% Yield	
	Processed Fish	Minced Fish
1	62.93	30.74
2	62.48	31.28
3	60.21	30.12
Mean	61.87	30.71
SD	1.46	0.57

Washing the flesh to remove the solubles enhanced the over all characteristics of the fish flesh. A fish to water ratio of 1:4, with 10 minutes stirring time, was reported (Lee, 1986) to be adequate and economical. Excessive washing results in the hydration of the mince making water removal difficult. Recoveries of solids protein and ash during washing depend on the amount of water used for washing though the effect is not directly proportional (Pacheco-aguilar, 1989). In this experiment, first exchange yielded 24% myofibrillar protein on round weight basis and the second and third washing reduced the yield by further 3% and 7%, respectively (Table 2).

Table 2. Yield of myofibrillar protein based on round weight and minced fish.

Washing cycle	Yield based on	
	Round fish	Minced fish
1	24.6 (1.55)	71.2 (3.42)
2	21.4 (3.22)	70.3 (3.62)
3	17.0 (2.23)	56.3 (1.05)

Standard Deviations given in parenthesis.

The fresh water carp had a moisture content of 83.3%. The low fat content (0.45%) and the high protein content (16.54%) of the fish may be related to the quality of the fish, its maturity stage and the condition of storage prior to processing. A moisture content of 76% and protein content of 19.1% have been reported in the case of fresh rohu (Gopakumar, 1993). In this experiment the fish was kept in ice for 24 hours to resolve the rigor which could have enhanced its water content. The fresh fish had a salt soluble protein content of 9.7% and about 50% of this is water-soluble protein. The soluble protein content of mince was usually lower than that of fillets indicating denaturation of protein as a result of mincing (De Koning and Mol, 1991).

Table 3. Effect of washing on the composition of *catla* minced meat.

	Unwashed	Washing cycles		
		I	II	III
Moisture (%)	83.3	85.32	86.47	84.60
Solids (%)	16.7	14.68	13.52	15.40
Protein DWB	97.04	95.44	95.23	94.63
Lipids DWB(%)	2.70	2.32	1.48	1.76
Ash DWB (%)	0.60	2.52	2.63	2.62

Washing results in the increase in the water content (Table 3) and a corresponding decrease in solids. On comparing protein content on a moisture-free basis, it is seen that the protein content decreases as washing progresses. Maximum loss of 4% was noticed in the first washing itself. Only 15% of oil is lost in the first wash and up to 45% is lost in the second washing. But this being lean fish, the importance of fat removal is not that important. The initial ash content itself is low in this case; however, the increase shown during washing is as a result of the use of NaCl for final washing to effect efficient dewatering.

About 26% of total soluble protein was lost in the first exchange and water-soluble protein contributes to about 50% of it (Fig. 1). The solubility of myofibrillar protein in water and low ionic strength buffers was reported in the case of cod (Stefensson and Hultin, 1994). This loss of myofibrillar protein on a moisture-free basis was calculated to 42%. But subsequent washes removed the water-soluble fraction and enriched the myofibrillar fraction resulting in the retention of myofibrillar protein. Lin and Park (1996) showed increased loss of protein as a function of washing cycles and further demonstrated that the protein loss decreased when the wash solution contained less than 1% salt in the wash solution.

Table 4.Effect of washing on the organoleptic characteristics of catla minced meat.

	Unwashed	Washing cycles		
		I	II	III
Colour	1	3	5	5
Flavour	1	3	4	5
Texture	1	3	5	5

Numerical scores used in the evaluation.
Colour and appearance: 1- characteristic pale red, 3 - slight loss of pale red, 5 - whitish
Texture: 1 – soft slippery, 3 – firm, 5 – firm chewable
Odour: 1 – muddy, 3 – slight loss of sea weedy, 5 - bland

The acceptability of the fresh water fish was very much limited due to its characteristic strong muddy odour. This is related to the algal flora of the water source. The odour gets diluted and the pale red colour of the meat is also becomes almost white as a result of first washing (Table 4). Though further washing improves the colour, the yield of the mince is considerably reduced.

Table 5. Effect of washing on the rheological properties of catla minced meat.

	Unwashed	Washing cycles		
		I	II	III
Folding test	AA	AA	AA	AA
Gel Strength (gxcm)	211	136	88	136
Compressibilty (g)	100	92	76	92

The rheological properties (Table 5) did not improve to any large extent as a result of washing although the acceptability in terms of its odour and flavour improved to a large extent. The flesh from fresh water carp itself had a very high binding characteristic and had a folding test grade of AA which in not normally seen in the unwashed marine fish flesh. The folding test grade is not affected as a result of washing. The gel strength and the compressibility of the gel, however, got reduced, signifying that the unwashed flesh itself is comparable with that of the washed flesh of marine fish but for the smell associated.

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CONTROL OF SALTING SCHEDULE AND ITS EFFECT ON THE QUALITY AND STORAGE LIFE OF CURED FISH

by

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ABSTRACT

Results of investigation on the effect of salt penetration on the quality and storage stability of salted-dried croaker (Jonius sp.) and salted-dried lizard fish (Saurida sp.) are reported. Dressed fish salted for less than 24 h at a fish/salt ratio 4:1 and dried to 35% moisture did not develop the characteristic cured quality and were understabilized with less than 45 days storage at ambient conditions. With higher durations of salting the dried product became brittle and fibrous and became prone to early onset of rancidity. Salt-induced dehydration of the fish was significant at 12 to 24 h of salting and thereafter it almost levelled off. The study thus reveals that intermediate moisture foods of reasonable storage stabillity could be prepared from croaker and lizard fish by salting at a salt/fish ratio 1:4 for 24 h followed by drying to 35% moisture level. The optimized method reduces processing costs and improves product quality.

INTRODUCTION

In India intermediate moisture foods are still in great demand because of the low production costs and consumer appeal. Storage stabililty of the cured fish is due to the restricted availability of water for microbial growth and biochemical reactions, brought about by drying and facilitated greatly by the addition of sodium chloride which lowers the water activity (aw) to a safer level. It is necessary to optimize the salting process to stabilize the fish for sufficiently long storage life. Salting of fish is thus a process which aims at reaching the saline equilibrium between the muscle and the surrounding salt solution in a specific time. In commercial practice fish are generally salted for days depending upon the weather and most often the market demand (George Joseph *et al.*, 1983, Muraleedharan *et al.*, 1989). This practice makes the product understabilized resulting in strong consumer resistance. The present work aims at optimizing the salting of two underutilized fish viz. lizard fish and croaker. The effect of different durations of salting on the quality of the dried product are presented and discussed.

MATERIALS AND METHODS

Lizard fish (Saurida sp. - average length 23 cm, average weight 145 g) and croaker (Johnius sp. - average length 25 cm, average weight 218 g) were collected from the trawl catches on board a deep sea research vessel. The fish were immediately frozen and stored at –20°C. and brought to the shore laboratory when the vessel touched port (5 days). The fish were thawed in running water, gutted and washed free of slime and blood. Salting was done with curing salt (IS:594 1962) at a ratio 1:4 (salt:fish w/w). The salted fish were kept in PVC containers with lids. Sampling of the salted fish was done at 3, 6, 12, 18, 24, 36 and 48 h for analysis of different quality parameters. Drying of the salted samples was done by spreading on nylon mesh trays and exposing to the sun till the moisture reduced to about 35%. The cured fish were packed in 25x18 cm pouches of 50u polyester polythene, sealed and stored at ambient conditions. (temperature 26-31*C.RH 80-90%). Moisture, salt and protein were analysed according to AOAC (1990) methods. Fat was determined by extraction with petroleum ether using a soxtlet analyser. TVN was estimated by the microdiffusion method of Conway (1962). Reconstitution properties were assessed by the method of Sen *et al.* (1961). Organoleptic evaluation of the desalted (cold water, 1h) and cooked (boiling water, 10 min) samples was conducted by a panel of 6 members.

RESULTS AND DISCUSSION

Proximate composition of the fish were comparable. Croaker: of 71% moisture, protein 19.72 % and 4.2% fat and lizard fish: of 73% moisture, protein 23.39% and 3.9% fat. Thus the body constituents of the fish had similar and comparable influence on the penetration of salt and moisture removal when the dressed fish were kept salted. Fig. 1 and 1A show that salt uptake reached its maximum after 24 h salting with little subsequent increase, while moisture loss increased rapidly to 24 h salting and almost ceased thereafter indicating the formation of a saline equilibrium. Drying of fish salted for different periods took varying times for dehydration (data not given) to reach a moisture level of about 35%, which is the standard specified for salted- dried fish by the Bureau of Indian Standards (IS :8836,1985). Fish with low salt content took more time to dry. However, salting beyond 24 h did not significantly reduce the drying period. Fish salted for longer than 24 h gave dried products of brittle surface with visible salt crystals. Higher salt content might have induced protein denaturation causing decreased water retention ability (Poernomo *et al.*,1992). This leads to rapid moisture diffusion to the fish surface during the early stages of drying facilitating salt crystal formation, and consequent brittleness. Reconstitution ability of the dried fish decreased gradually with increase in salt content (Figs. 2, 2A), indicating again salt-induced protein denaturation and consequent loss of water retention property. Fish salted for longer than 24 h showed a tendency for fragmentation during reconstitution. It appears that the extent of salting can affect disintegration of fish tissues. The salted-dried products were evaluated by a panel for sensory quality. The panel showed preference for fish that was salted for less than 24 h. Fish salted for 48 h obtained the minimum score because of the poor appearance, hard texture and extremely salty taste. The results indicated consumer resistance for high salted product from the point of view of palate and of health considerations. Salting fish for more than 2 days is practised by traditional curers, depending upon the weather and perhaps the market demand. But this practice will affect the appearance and texture of the product in addition to elevating cost of production. From these studies, therefore, it may be concluded that salting time in commercial conditions can be reduced to 24 h for lizard fish and croaker in view of the product quality, and consumer appeal.

Storage characteristics

Under similar conditions of salting (24 hrs.) and drying, cured croaker had 35.5% moisture, 70.12% protein, 4.66% fat and 25.9% sodium chloride while cured lizard fish had 35.15% moisture, 69.85% protein and 26.34% sodium chloride, 3.46% fat expressed on a dry matter basis. Both products showed similar water activities, 0.75 for cured croaker and 0.74 for cured lizard fish. The sample packed in 50 micron polyester/polythene pouches and stored for 60 days at ambient conditions showed variations in colour and odour. Browning and rancid flavours were significant in the high salt (>27%) products, indicating the effect of salt content. However, the stabilizing property of salt against microbial growth was clear from the observation that the TVN formation was greater in the less salted samples (Figs.3, 3A) which became unacceptable within a short span (tables1, 1A). The salt induced hardness was evident in the samples having high concentration of sodium chloride which affected the water retention property of the fibrils causing reduced succulence and harder texture.

The study thus leads to the following findings:

1. Salting for 24 h at a salt /fish ratio 1:4 is optimum to get quality cured products from croaker and lizardfish.
2. Significant dehydration takes place in 12 to 24h of salting and thereafter it almost levels off.
3. Increasing the salt content by prolonged salting of the fish has no added advantage; on the contrary it adversely affects the quality and appearance of the cured product.
4. Fish salted for 24h and dried to 35% moisture could be kept for 3 months at ambient condition without any quality deterioration.

Table 1. Changes in physical characteristics of salted and dried lizard fish during storage.

Sample	Initial	15 days	30days	45days	60days
3 h	Light yellow normal cured odour, soft, firm	Yellow-brown off odour Not acceptable Discarded			
6h	Pale white to yellow soft, firm normal cured odour	Yellow soft, cured odour	Yellow-brown off odour Not acceptable Discarded		
18h	Off white normal cured odour soft, firm	Off white normal cured odour hard	Light yellow Slightly rancid hard	Yellow-brown Rancid, off odour Discarded	--
24h	White, normal cured odour hard	white, fibrous normal cured odour, hard	White-yellow normal cured odour, hard	Yellow slightly rancid hard	Brown, rancid Fungal spots off odour, Discarded
36h	White, normal cured odour soft, firm	white, fibrous hard	white, fibrous rancid, hard	yellow rancid, hard	brown rancid fungus off odour Discarded
48h	white, normal cured odour soft, firm	white, normal cured odour hard	white, fibrous, hard	yellow-brown rancid hard	brown putrid fungus, rancid

Table 1A. Changes in physical characteristics of salted and dried croaker during storage.

Samples	Initial	15 days	30 days	45 days	60 days
3h	Light yellow normal cured odour	yellow-brown off odour Discarded	--	--	--
6h	Light yellow normal cured odour, soft,	yellow slight off odour, soft	yellow fungus, soft off odour Discarded	--	--
18h	White, normal cured odour	dull white normal cured odour, hard	yellow rancid hard	brown off odour fungus Discarded	
24h	white, soft firm, normal odour	dull white normal odour, firm	yellow normal odour, hard	brown S. rancid hard	brown rancid bitter taste fungus
36h	- do -	yellow, normal cured odour	yellow normal odour	yellow rancid fibrous	brown bitter taste off odour
48h	- d0	yellow fibrous hard	yellow S. rancid hard	brown hard rancid	brown rancid off odour fungus

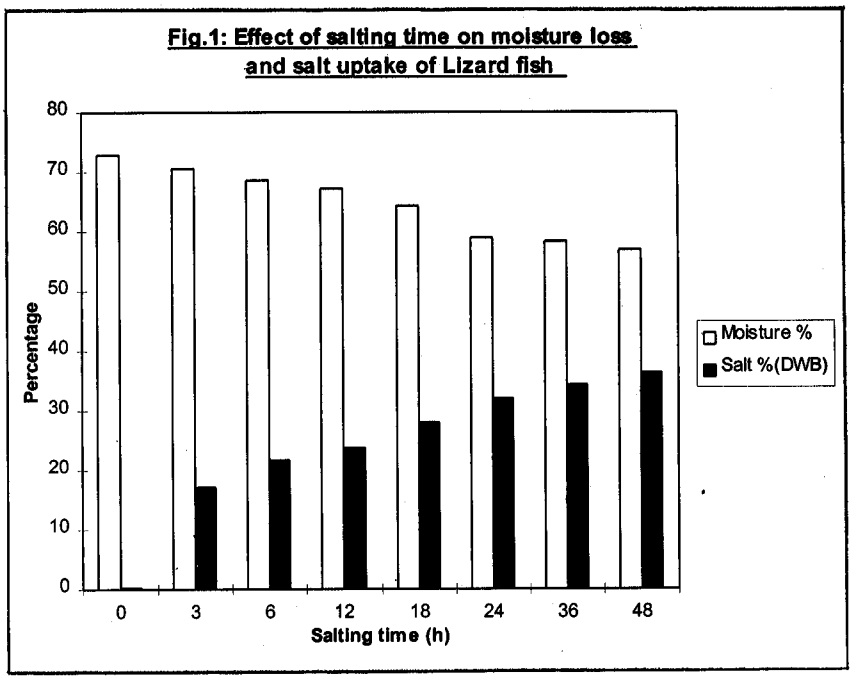


Fig.1: Effect of salting time on moisture loss and salt uptake of Lizard fish
80
70
60
50
40
30
20
10
0
Percentage
0
3
6
12
18
24
36
48
Salting time (h)
Moisture %
Salt %(DWB)

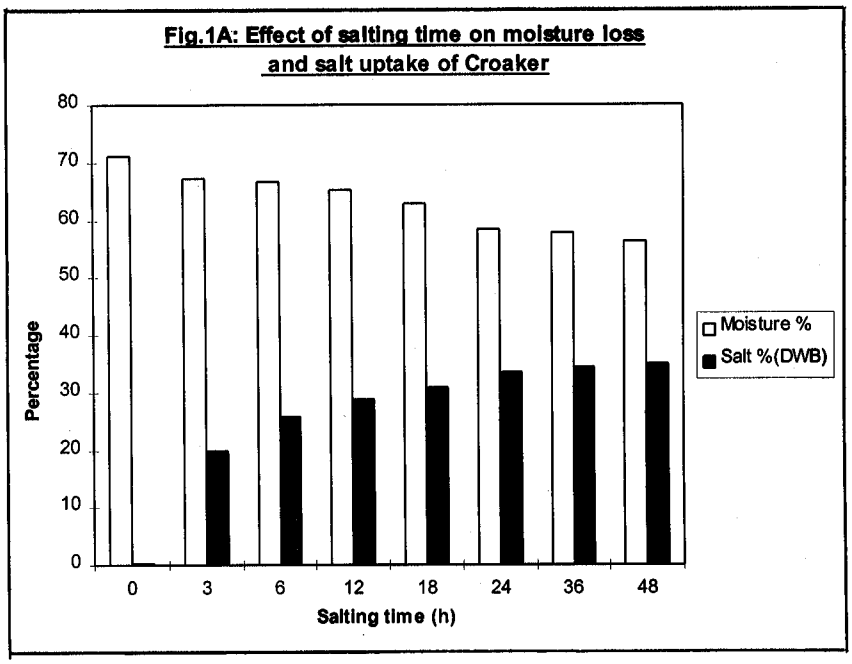


Fig.1A: Effect of salting time on moisture loss and salt uptake of Croaker
80
70
60
50
40
30
20
10
0
Percentage
0
3
6
12
18
24
36
48
Salting time (h)
Moisture %
Salt %(DWB)

Fig.2: Effect of salting time on reconstitution property of cured Lizard fish during storage

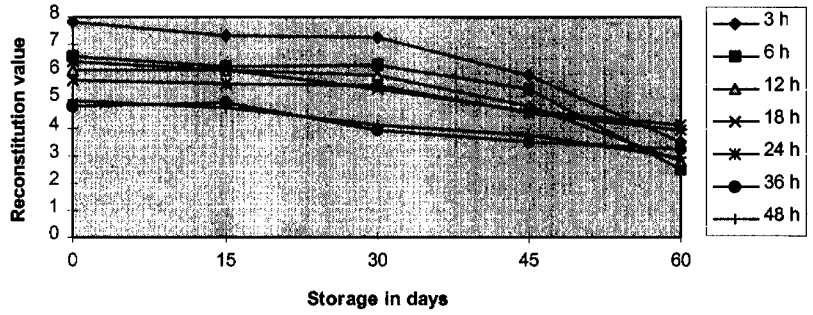


Fig.2A: Effect of salting time on reconstitution property of cured Croaker during storage

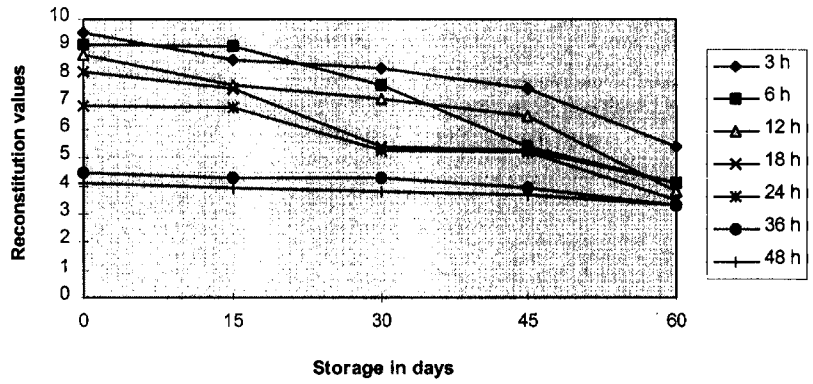


Fig.3: Effect of salting time on TVN formation during storage of cured Lizard fish

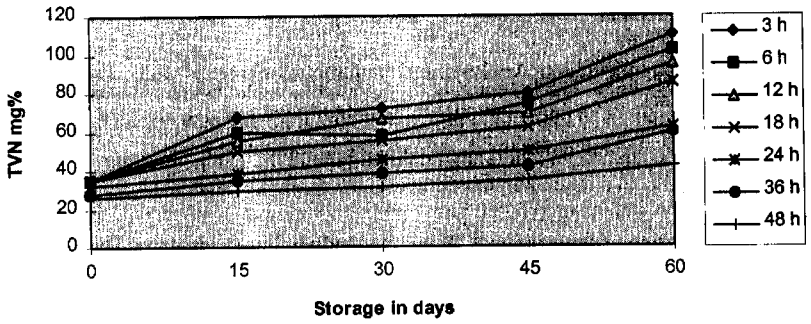
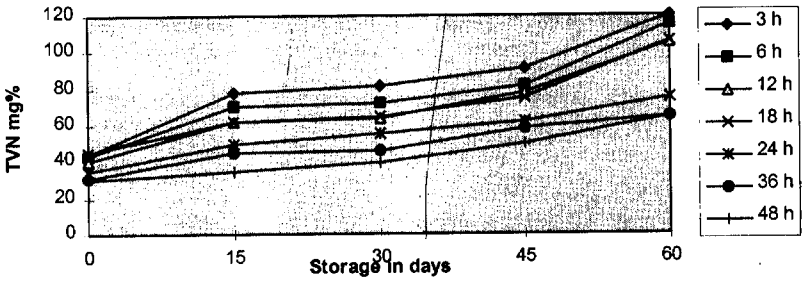


Fig.3A: Effect of salting time on TVN formation during storage of cured Croaker



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CHANGES IN TISSUE PROTEINASE ACTIVITY OF INDIAN MACKEREL UPON CURING IN BRINE AND SALT

by

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ABSTRACT

Autolytic activity in the muscle of Indian mackerel (*Rastrelliger kanagurta*) cured in saturated brine and dry salt, was monitored at pH 3, 4, 9 and 10 along with weight loss, moisture, salt and α-amino nitrogen, at 3, 6, 12, 24 and 48h of curing. The fish lost 14 to 29% of the initial weight after 48h of curing, in brine and dry salt, respectively. Correspondingly the moisture decreased from 76 to 57% in brine cured and 52% in dry salted fish. Salt content in the muscle increased from 1% to 14.82 and 13.32% in the case of brine cured and dry salted mackerel respectively, with slower uptake of salt in the latter because of the lesser surface area in contact. Tissue proteinases were activated initially in all cases when salting commenced, with higher activation in the case of pH 3 and 4. But subsequently increasing salt concentrations suppressed the autolytic activity. Autolytic proteinases in the mackerel muscle were suppressed between 37 to 78% of their original activity in case of the brine cured fish and between 36 to 74% in the dry salted fish, at the pH assayed. Concentration of α-amino nitrogen in the muscle increased initially in both types of curing at 3 h, but decreased later on. Autolytic activity assayed in the presence of salt containing buffers showed that at 10-20% salt level, (the concentrations prevailing in fish after 48h salting), 36-71% of the original tissue proteinase activity was still intact, which can affect cured product quality.

INTRODUCTION

Curing with salt, drying and smoking are traditional preservation methods of fish, dating from prehistoric times, which are still widely practised in tropical countries. Although successful preservation by salting essentially hinges on lowering of water activity, chemical changes such as lipid oxidation (Khuntia *et al.*, 1994), protein denaturation (Tambo *et al.*, 1992, Raghunath *et al.*, 1995) occurring during salting can also affect the product quality. Proteolytic enzyme activity in fish muscle can lead to rapid deterioration of quality in some fish such as chum salmon (Yamashita and Konagaya, 1991) and squids (Stanley and Hultin, 1984). But their activity is also well known to contribute to the process of fish sauce formation where proteolysis proceeds over long periods, albeit extremely slowly due to the high salt concentrations (Yamashita *et al.*, 1991). In lightly salted products proteinases from salt adapted bacteria contribute to the processing of fermented anchovy paste (Cha and Lee, 1989). However, the activity of fish tissue proteinases under salting conditions where fairly high salt concentrations prevail have not been investigated although their presence has been demonstrated in salted squid preserves (Makinodan *et al.*, 1993). Indian Mackerel (*Rastrelliger kanagurta*), a fish which is widely used for salting in India (Gopakumar and Bhattacharyya, 1992), is well known for its strong tissue proteinase activity (Jose and Raghunath, 1998), and hence was chosen for this study.

MATERIALS AND METHODS

Indian mackerel (*Rastrelliger kanagurta*) procured from the local fish market of Cochin, in post-rigor condition was brought immediately to the laboratory. The fish were beheaded, eviscerated and carefully washed to remove all traces of viscera from the abdominal cavity. Dressed fish were divided into three lots.

For curing in brine, each batch of four pre-weighed fish (Average weight of batch 220g) were immersed in 1.5 vols. of saturated brine (with a small amount excess undissolved NaCl). In case of curing with dry salt, the fish were intimately mixed and covered in 4x their weight of salt. Each batch of fish were taken out of the brine/salt after 3,6,12,24 and 48h, drained/wiped free of brine/salt and weighed again. The weighed fish were cut into small pieces, minced and taken for analysis. Moisture was determined as per AOAC method (AOAC, 1975). Salt was estimated as total chlorides with silver nitrate after digestion with nitric acid (Raghuramulu *et al.*, 1983). Free (α-) amino nitrogen in 10% trichloroacetic acid extract of the muscle was determined by the EBC Ninhydrin method (EBC, 1975). Activity of autolytic proteinases in the muscle homogenate (1:4, muscle: water) was determined by incubating 2ml of the homogenate with 4ml of buffer at 50°C for 1h, reaction terminated with 5ml of 11% TCA and the Folin positive material in the supernatant measured as per Herriott (1955). Activity was expressed as μMoles of Tyrosine released / g muscle / min. Citrate phosphate and Tris -HCl buffers (0.3M) were used at pH 3 and 4 and 9 and 10 respectively. Assays were carried out at pH 3,4, 9 and 10 which are known to contain most of the autolytic activity in mackerel muscle (Jose and Raghunath, 1998). Additionally, autolytic activity of unsalted muscle was also assayed with buffers containing 10, 20 and 30% salt as detailed before.

Commercial solar salt was used for curing purposes and for assaying of autolytic activity in presence of salt containing buffers. All other chemicals used were of AR or equivalent grade.

RESULTS

The loss in weight of mackerel, cured in brine and dry salted conditions over 48h of salting are shown in Tables 1A and 1B. Both the rate and extent of loss were higher in dry than in wet salted (brine cured) samples reaching 30% in dry and 14% in wet salted fish. After 24h, the loss in weight was marginal, indicating establishment of near equilibrium conditions. Concomitantly, the moisture content in the samples decreased quickly in the first 12h, but more slowly later on (Table 2). The dry salted samples reached a lower moisture content than wet salted ones, but difference between the moisture contents was small as the saturated brine contained some undissolved salt to compensate for dilution. Changes in the salt content of the mackerel (Table 2) during curing were similar in both forms of curing, with a marginally higher rate of salt penetration in the first 6h followed by a nearly uniform rate of increase till the end of salting period. Salt penetration was higher in the case of brine cured fish owing to the better contact between fish and curing media.

Tissue proteinase activity (TPA) in mackerel muscle because of the changing composition over time due to moisture egress and salt ingress, have been presented on a moisture and salt free basis (Tables 3 and 4). The TPA in brine cured mackerel ultimately decreased to levels lower than the original, but were initially activated to different extents(Table 3). TPA at pH 3 and 4 were activated to a maximum of nearly threefold after just 3h of salting, and decreased to 72 and 59% respectively after 24h of salting. This was followed by a slight increase to 76 and 78% of the original activity at pH 3 and 4 respectively, at 48h. But the activities remained suppressed below original levels of activity. TPA at pH 9 and 10 increased after 3h of curing as in the acid pH range, albeit to a lesser extent, but decreased steadily later and retained just 50 and 37% of their original activity after 48h curing.

The fate of TPA in the case of dry salted mackerel was largely similar, but extent of activation were much higher than in wet salted mackerel (Table 4). The activation in case of pH 3 and 4 was much higher than in alkaline pH (9 and 10). The activated levels of autolysis decreased steadily with progress of salting, but showed a slight increase of about 1 and 15% at pH 3 and 4 from 24h to 48h, as was earlier observed in case of brine cured mackerel. Decrease in autolytic activity at pH 9 and 10 was quite similar to that at pH 3 4, and retained just 48 and 36% of original activity respectively after 48h of curing.

The effects of autolysis in the tissue should be discernible by the presence of the products viz., free amino acids and peptides. The ninhydrin positive free amino nitrogen (FAN) in the cured fish are shown in Table 5. The concentration of FAN increased initially at 3 h of curing, with higher increase in dry salted fish.

This perhaps is analogous to the increase in autolytic activity (Table 4). Later, in case of wet salted fish, FAN increased marginally at 12h, but this decreased to levels lower than the initial by 48h. The reduction of TPA in the tissue as salt concentration increased, coupled with diffusion of FAN compounds out of the tissue in to the curing brine could be the reason for the decrease in FAN. However, in dry salted mackerel, FAN decreased steadily after 3h, along with decreasing TPA (Table 4), but slightly increased between 24-48h, reaching nearly original levels at 48h. The different behaviour of the dry salted fish is perhaps due to the lower diffusion rates of FAN compounds out of the tissue in the absence of a diffusion facilitating liquid brine.

As the TPA assays were done at low salt concentrations to enable full expression of autolytic activity, the question remains whether the autolytic proteinases were indeed active at the high salt concentrations actually prevailing in the salted fish. This was tested by assay of TPA in unsalted fish muscle, in buffers containing high salt levels. The results are shown in Table 6. Autolysis in the presence of salt was strongly inhibited, the extent of inhibition increasing with salt concentration. Between 10-20% salt level; concentrations which prevailed in the fish after 48h of salting, 36-71% of the original autolytic activity remained at various pH. Thus, at salt concentrations encountered in salted fish, autolytic activity is expressible, even if at a reduced level.

DISCUSSION

High salt concentrations denature muscle proteins like myosin during salting of fish (Iizuka *et al.* 1995), which is attributed to cross-linking (Oka *et al.* 1994). Both *in-vitro* and *in-vivo* digestibility of mackerel subjected to curing and drying are known to be affected (Raghunath *et al.*, 1995) which further affirms the denaturation of muscle proteins. Tissue proteinases may also be affected likewise, but are shown not to be completely inactivated, since activity of cathepsins D, B and L-like proteinases have been demonstrated in 'ika-shiokara', a Japanese salted squid preserve (Makinodan *et al.* 1993). The activation or increase in autolytic activity observed initially in our experiment can perhaps be attributed to denaturation of membrane structures releasing the proteinases bound in tissue lysosomes. Proteinases in the acid and alkaline pH range, however, seem to be at slightly different locations in the cells, as they were activated to different levels and showed slightly different patterns of inactivation. In addition they have also been observed to behave differently when autolytic activity was fractionated into sarcoplasmic and myofibrillar fractions (Jose and Raghunath, 1998). The increasing concentration of salt in the muscle, eventually does suppress autolytic activity, which is in agreement with similar observations in "shottsuru" fish sauce at 5M NaCl concentrations (Yamashita *et al.* 1991). But, as seen from this experiment, salt concentrations in cured mackerel in the absence of drying reach only about 2.5M level on as is basis, and as demonstrated, TPA is only partially suppressed at such salt concentrations. Hence, tissue proteinases can still be active in cured mackerel. Cured fish have a tendency to suffer from physical damage (fragmentation) as observed by Wood *et al.* (1986), but whether tissue autolysis has any role in this, is not known. *Rastrelliger kanagurta* cured even in low strength brine (21%) and dried, is sufficient to produce salted fish with a reasonable shelf life (Poernomo *et al.*, 1992). As autolysis in mackerel is slowed down but not completely stopped by salting, tissue proteolytic activity can affect cured product quality. Further investigations are needed to find out if cured mackerel product quality can be improved by inhibition of autolytic activity using inhibitors other than salt.

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Table 1. Weight changes during curing of Mackerel (*Rastrelliger kanagurta).*

Time of Curing, h	Initial Weight, g	Final weight, g	Loss in weight,%
A. Brine Curing			
3	227.48	208.92	8.16
6	220.17	196.88	10.58
12	245.48	215.81	12.09
24	207.13	172.1	16.91
48	213.19	182.48	14.41
B. Dry salting			
3	203.19	184.82	9.04
6	232.56	203.5	12.50
12	222.15	182.13	18.02
24	218.09	163.51	25.03
48	216.41	151.67	29.92

Table 2. Changes in moisture and salt content of mackerel during curing (as is basis).

Time of Curing, h	Moisture, g / 100 g		Salt concentration, g / 100 g	
	Brine cured	Dry salted	Brine cured	Dry salted
0	75.94	75.94	1.04	1.04
3	71.14	70.91	3.69	1.82
6	66.13	69.41	5.61	3.54
12	65.71	62.16	7.94	6.99
24	60.26	60.45	10.95	10.23
48	56.91	52.38	14.82	13.32

Table 3. Autolytic activity in brine cured mackerel muscle during curing on moisture and salt free basis (values in parenthesis indicate activity as % of activity at zero time).

Time of Curing, h	pH 3	pH 4	pH 9	pH 10
	(micromoles Tyrosine / g muscle / minute)			
0	0.1565(100)	0.1130(100)	0.1624(100)	0.1791(100)
3	0.5204(332)	0.3482(308)	0.2763(170)	0.3181(178)
6	0.2518(161)	0.1562(138)	0.1349(83)	0.1541(86)
12	0.2568(164)	0.2409(213)	0.1771(109)	0.2340(131)
24	0.1138(73)	0.0674(60)	0.0904(56)	0.0836(47)
48	0.1189(76)	0.0877(78)	0.0811(50)	0.0668(37)

Table 4. Autolytic activity in dry salted mackerel during curing on moisture and salt free basis (values in parenthesis indicate activity as % of activity at zero time).

Time of curing, h	pH 3	pH 4	pH 9	pH 10
	(micromoles Tyrosine / g muscle / minute)			
0	0.1565(100)	0.1130(100)	0.1624(100)	0.1791(100)
3	0.6807(435)	0.4555(403)	0.3613(222)	0.4160(232)
6	0.3239(207)	0.2001(177)	0.1734(107)	0.1982(111)
12	0.2031(130)	0.1905(169)	0.1400(86)	0.1851(103)
24	0.1119(71)	0.0662(59)	0.0888(55)	0.0823(46)
48	0.1135(72)	0.0838(74)	0.0775(48)	0.0638(36)

Table 5. Changes in alpha amino nitrogen content during curing of mackerel (g/100 g of salt free dry matter).

Time of Curing, h	Brine cured	Dry salted
0	6.53	6.53
3	9.81	15.23
6	6.00	9.16
12	7.95	6.12
24	5.57	5.82
48	2.71	6.23

Table 6. Autolytic activity in presence of salt containing buffers, (μMoles Tyrosine/g muscle/min, as is basis).

Salt concentration (%)	**pH of assay**			
	3.0	4.0	9.0	10.0
Control	0.0382	0.0311	0.0416	0.0402
10.0	0.0174	0.0123	0.0209	0.0297
20.0	0.0000	0.0112	0.0297	0.0223
30.0	0.0032	0.0000	0.0179	0.0061

PROCESSING AND FROZEN STORAGE CHARACTERISTICS OF RAY FILLETS

by

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ABSTRACT

Rays, belonging to the class Elasmobranchii, constitute a major fishery in many states in India like Tamil Nadu, Gujarat, Andhra Pradesh, Kerala and Maharashtra. The estimated landings are 21,700 tonnes per annum. Even though the meat of rays is nutritious and free from bones and spines, there is little demand for fresh meat due to the presence of a high urea content. The landings are mainly used for salt curing which fetches only very low prices for the producers.

Urea nitrogen constituted the major component (50.8%) of the non-protein nitrogen of the meat. An attempt has been made to standardize the processing steps to reduce the urea levels in the meat before freezing by using different simple techniques like dipping the fillets in stagnant chilled water, dipping in chilled running water and dipping in stirred chilled running water. It was found that meat dipped in stirred running water for two hours reduced the urea level of the meat by 62%. The yield of the lateral fin fillets and caudal fin fillets vary with the size of the ray. The drip loss during frozen storage is found to be more in the case of samples frozen stored after the treatment for urea removal by the method of stirring in running water. The samples treated in stagnant chilled water had the lowest drip loss. The total nitrogen was higher in samples treated in stagnant chilled water and lowest in the samples treated in stirred running water. The overall acceptability was high in the case of samples treated with stirred running water and frozen stored.

INTRODUCTION

Elasmobranchs constitute a major fishery in India. The total landings of elasmobranchs and the contribution of rays (Table 1) indicate a potential for utilisation. Gujarat has the maximum landings of elasmobranchs among the states in India with average landings during the last 12 years of 14,300 tonnes. Tamil Nadu has an average landing of 13,000 tonnes, Maharashtra 10,000 tonnes and Kerala 5,100 tonnes (Anon., 1995; Anon., 1996; Anon., 1997). In Tamil Nadu, elasmobranchs accounted for 6.9% of the total marine fish landings. Central Marine Fisheries Research Institute (CMFRI) has estimated the potential yield in Tamil Nadu as 20,000 tonnes using a Maximum Contribution Approach (Anon., 1987). In Andhra Pradesh, the potential yield is expected to be 12,000 tonnes (Anon., 1987a). Gujarat and Maharashtra have a substantial elasmobranch resource. The important genera of rays found in India are *Dasyatis, Gymmura, Himantura, Urogymnus, Aetobatus, Aetomylaeus, Rhinoptera, Manta, Mobula and Nercine* (Anon., 1987).

Rays make up 67.7% of elasmobranch landings in Tamil Nadu. In Kerala, rays contribute 33.9% and in Karnataka 20.6% of the total elasmobranch landings. The contribution of rays to the total elasmobranch landings in Maharastra is 15.1%, Gujarat 20%, and Andhra Pradesh 30%. Substantial quantities of rays are landed as bycatch from shrimp trawls in Gujarat (Badonia *et al.*, 1990). Among elasmobranchs, sharks are mostly preferred by most people as technologies are already evolved for their processing (Joseph and Solanki, 1985) and the studies on the removal of urea, which contributes to undesirable odour, have been carried out by Kandoran *et al.* (1965); Solanki and Venkataraman (1978). Production of semi-dried fish products from shark, an intermediate moisture product, also increased its utilization for human consumption (Ramachandran and Solanki, 1991).

Table 1. Total Elasmobranch landings and contribution of Ray in India during 1985-96.

Year	Total Elasmobranch landing	Contribution of Ray (%)
1985	52154	29.9
1986	51898	29.9
1987	56585	39.3
1988	56847	31.8
1989	49979	39.1
1990	49820	48.0
1991	50420	34.5
1992	63132	25.3
1993	66423	28.3
1994	58097	31.1
1995	69274	30.3
1996	58634	37.1

However, no such concentrated efforts have been made to utilize the major families of rays belonging to the super order *Batoidimorpha* of subclass *Elasmobranchii* for human consumption, though they are believed to be similar to sharks in composition and are landed in substantial quantities. Hence, the objectives of the present study are:

1. To develop a simple method for the easy removal of urea from ray fillets so as to improve commercial acceptability.
2. To prepare fillets from ray and elucidate the frozen storage characteristics.

MATERIALS AND METHODS

Fresh rays belonging to the species *Himantura uarnak* (Forsskal, 1775), popularly called honeycomb sting ray, was used for the study. The rays are classified into different size groups for the purpose of filleting as, 4.00-4.99 kg, 5.00-5.99 kg, 6.00-6.99 kg, 7.00-7.99 kg, 8.00-8.99 kg, 9.00-9.99kg, 10.00-10.99 kg, 11.00-11.99 kg, 12.00-12.99 kg, 13.00-13.99 kg, 14.00-14.99 kg, and 15.00-15.99 kg. The yield of skin-on lateral fins and skinless lateral fins and caudal fins obtained from these size groups were then calculated. For the purpose of studies on urea removal and frozen storage characteristics, fillets of uniform sizes (average length 22 cm, average breath (measured at the central point) 18.5 cm and thickness (measured at the central point) 3.81 cm) fillets were prepared. The fillets were treated by three different methods - immersion in chilled stagnant water (20:1 v/w water to fillets), in chilled running water with a flow rate of 2 litres/minutes and in chilled running water with stirring having a rate of flow of 2 litres/ minutes and a stirring speed of 2000 RPM /minutes, approximately. A micro tissue homogenizer was used to get the stirring effect.

The treated samples were surface dried using filter paper and taken for analysis. Moisture, salt, total nitrogen (TN) and non-protein nitrogen (NPN) were analysed by AOAC methods (1975). About 10g muscle was homogenized in chilled distilled water (20V) and centrifuged to collect the water-soluble nitrogen (WSN). The salt-soluble nitrogen was estimated by the methods of King and Poulter (1985) using pre-cooled buffers. Nitrogen was estimated by the micro Kjeldahl method. The trimethylamine (TMA), total volatile nitrogen (TVN) and urea nitrogen were estimated by the diffusion method of Conway (1947). Urea was calculated by multiplying urea nitrogen with 2.14. The total bacterial count was estimated using tryptone-glucose agar as per the standard method and incubated at 37°C.

A test panel of 8 members evaluated the physical and olfactory characteristics of the fillets treated by different methods. Similarly, the same test panel assessed the physical and olfactory characteristics of the

frozen stored products during a period of six months. The scale used for scoring the physical and olfactory characteristics is given in Table 2. The average of the scores given by the test panel members is reported.

Table 2. Proximate biochemical composition of Ray.

Moisture	78.16±1.7
Protein (TN-NPN)	17.16±1.4
Fat g %	0.31±0.1
Ash g %	1.09 ±0.1
NPN g %	1.10±0.06
Urea N g %	0.54
WSN (% of TN)	36.40
SSN (% of TN)	48.38
Salt g %	0.506

RESULTS AND DISCUSSION

The ray was processed into lateral fin fillets and caudal fin fillets as these two were the only fleshy parts of the body, which could be used for human consumption. The yield of fillets from lateral fins varied from 35% to 60% by weight depending on the size of the ray while that from caudal fin ranged from 0.75 to 1.75%. Fig. 1 shows the yield of fillets from different sized rays and it can be seen that the larger the animal the lesser the yield of fillets. More than 50% yield was obtained from animals of up to 8 kg. Above this, the increase in weight of the rays the yield dropped to about 35%. This may be due to the calcification of the cartilaginous skeleton, especially of the central part of the body separating the two lateral fins. It was also noticed that in bigger size rays the central cartilaginous skeletal support is heavy and occupies a greater volume, probably to adapt to its swimming nature.

The composition of the ray used for the experiment is given in Table 2. The composition compares well with fish and other elasmobranchs except that it contains large amounts of NPN, especially urea. Ray has about 28% NPN and urea nitrogen contributes to about 47% of NPN. Considering the other nitrogenous fractions, salt soluble nitrogen contributes about 49% while the water-soluble fraction to about 36% of TN. The composition was in agreement with the earlier report (Kandoran *et al.*, 1965) and compares well with the composition of sharks (Solanki and Venkataraman, 1978; Ramachandran and Solanki, 1990).

The results of experiments conducted to remove the urea from the meat to improve its consumer acceptability are given in Figures 2 and 3. It can be seen from Fig. 2. that stirring fillets in ice chilled running water for 2h removes as much as 65% of the urea. Immersing in ice chilled running water for 1h removed about 46% urea, which is comparable to 2h treatment in either running water alone (46%) or in stagnant water (41%). The stirring in running water treatment accounts for about 29% loss in the total nitrogen (Fig. 3). A 45% loss in the NPN and 32% loss in the water-soluble nitrogen were also seen. The SSN content was found to be 1.94 g/100g muscle. Thus the method also effectively removes NPN, especially urea, and makes the fillets comparable to other fish products. Kandoran *et al.* (1965) reported that salting, desalting followed by resalting removes as much as 98% urea in both sharks and rays. Similarly, Solanki and Venkataraman (1978) reported that by simply keeping the shark fillets in ice for 7 days removes up to 25% urea. However, in both the cases the fish is exposed for a longer time at higher temperature, which affects the actual quality of the meat.

Table 3. Organoleptic evaluation of Ray fillets during processing.

Duration	Types of treatment		
	Urea removal in stagnant water	In running water	In running water with stirring
1 h			
Colour and appearance	4.6	4.7	4.8
Texture	4.8	4.6	4.5
Odour	1.8	2.8	3.6
Total	11.2	12.1	12.9
Overall acceptability	good	very good	excellent
2 h			
Colour and appearance	4.6	4.8	4.8
Texture	4.6	4.4	4.2
Odour	2.8	3.6	3.8
Total	12.0	12.8	12.8
Overall acceptability	very good	excellent	excellent

The organoleptic scoring showed (Table 3) improvements in the colour and appearance, texture, odour and overall acceptability as a result of washing by different methods. The overall acceptability was in greater for fillets subjected to urea removal in running water with stirring and this correlates well with the urea content in treated fillets (Fig. 2). Hence, the method described here is mild and can be used effectively by the industry.

The moisture content of the fillets treated for urea removal, increases as a result of the treatment. About 7% increase was noticed in stirring in running water (Fig. 4) compared to about 5% in the other two methods. There is a decrease in the moisture content of the fillet treated by the earlier method during frozen storage up to 120 days (Fig. 4) and this may be due to the loss of water holding capacity of the meat as a result of excessive uptake of water. But in the other cases the moisture remained at the initial level up to about 4 months. This finding is supported by the loss in weight during frozen storage (Fig. 5). The fillets kept stirring in ice chilled running water for 2h recorded about 12 to 14 % loss of weight while in the other cases only 4 to 6 % weight loss was noticed through out the frozen storage period.

The samples treated in stagnant and running water lost about 7% TN (Fig. 6) by the end of 180 days while only 3.9% loss was noticed in the samples stirred in running water. The low initial TN value in the case of the latter experiment could be the reason. The loss of SSN contributed more to this, as the loss of WSN was almost negligible during frozen storage (Figures 7 and 8). A major portion of the WSN and SSN were lost in the initial urea removal stage. The SSN and WSN showed a slight increase during initial storage, which could be due to the excessive moisture loss noticed in the respective samples. The NPN value did not show any change during frozen storage.

Organoleptic evaluation showed (Table 4) considerable changes in acceptability of ray fillets subjected to different treatments of urea removal and subsequent storage at -18°C. The fillets treated in stagnant water showed good acceptability up to 120 days, while the fillets treated by the other methods were in good condition even after 180 days of frozen storage. The major problem noticed was the ammoniacal odour, which affected the overall acceptability of the fillet treated in stagnant water.

Table 4. Organoleptic evaluation of Ray fillets during frozen storage at –18°C.

Duration of Storage (days)	Types of treatment		
	Urea removal in stagnant water	In running water	In running water with stirring
0 day			
Colour and appearance	4.6	4.8	4.8
Texture	4.4	4.4	4.2
Odour	3.0	3.8	4.0
Total	12.0	13.0	13.0
Overall acceptability	very good	excellent	excellent
30th day			
Colour and appearance	4.5	4.6	4.8
Texture	4.2	4.6	4.2
Odour	3.0	3.8	4.1
Total	11.7	13.0	13.1
Overall acceptability	very good	excellent	excellent
60th day			
Colour and appearance	4.4	4.6	4.6
Texture	4.2	4.0	4.2
Odour	3.1	3.9	4.0
Total	11.7	12.5	12.8
Overall acceptability	very good	excellent	excellent
120th day			
Colour and appearance	4.0	4.4	4.6
Texture	3.8	3.6	3.5
Odour	3.2	3.9	4.1
Total	11.0	11.9	12.2
Overall acceptability	good	very good	very good
180th day			
Colour and appearance	3.8	4.0	4.1
Texture	3.0	3.0	3.0
Odour	3.0	3.8	3.8
Total	9.8	10.8	10.9
Overall acceptability	satisfactory	good	good

Numerical scores were given based on the following scales:

Colour and general appearance: 5 = characteristic, 4 = slightly dull, 3 = dull, 2 = dull with slight discoloration, 1 = discoloured.

Texture: 5 = firm, 4 = slight loss of characteristic texture, 3 = loss of characteristic texture, 2 = slightly soft, 1 = very soft.

Odour: 5 = complete loss of characteristic urea smell, 4 = considerable reduction in urea smell, 3 = Reduction in urea smell, 2 = slight reduction in urea smell, 0 = characteristic urea smell.

The average total bacterial count of the fillets before treatment was 2.08×10^4. Upon treatment for urea removal, the bacterial count was reduced by one log cycle in the fillets treated with running water. The treatment involving stirring reduced the count further. There was no reduction in the bacterial count in the fillets treated with stagnant water compared to untreated fillets. The microbiological analysis (Table 5) showed a similar pattern in the fillets during frozen storage except that the microbial content of the fillets treated in stagnant water was slightly higher than that of the other fillets. There was a slight increase in the count during the initial period of storage up to 60 days then decreased.

Table 5. Changes in total bacterial count in Ray fillets during frozen storage.

	Stagnant water	**Running water**	**Stirring in running water**
0 day	2.41×10^4	2.86×10^3	1.69×10^3
30 days	5.66×10^4	1.56×10^4	9.73×10^3
60 days	2.87×10^4	1.56×10^3	4.30×10^3
120 days	8.80×10^2	3.06×10^2	2.25×10^2
180 days	2.57×10^2	2.21×10^2	160×10^2

It can be summarized that the utilization of rays can be improved by effectively removing the urea by the method stated above and the treated fillets were in acceptable condition after treatment and also after frozen storage up to six months.

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EFFECT OF INDIAN GOOSEBERRY (*Phynanthus emblica*) ON FISH OIL ANTIOXIDATION

by

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ABSTRACT

Fish oils are becoming important due to their nutritional implementations. Development of rancidity in fish oils is very fast and leads to reduction of the qualities of fish oils as well as fish. Prevention of development of rancidity in fish oils by the fruit of Indian Gooseberry (*Phynanthus emblica*) extracts was studied in detail. The ethanolic, methanolic and water extracts of Indian gooseberry were applied to fish oils and the activity of the different extracts was determined by measuring peroxide value (PV), free fatty acid value (FFA) and fatty acid composition (FAC) on eight occasions over forty four days of storage at room temperature (30°C). In the second experiment, active components of the ethanol extract were separated by hexane, carbon tetrachloride, chloroform, ethyl acetate and water, and the activity of these fractions were also measured by applying to fish oils.

In the first experiment, the ethanolic extract treatment recorded the highest activity in prevention of formation of peroxides, free fatty acids, and the conversion of fatty acids than the methanolic and water extracts. The amount of saturated and monounsaturated fatty acids increased whereas the polyunsaturated fatty acids decreased during the storage. The water extract treatment showed the lowest while the methanolic extract was intermediate. Results from the second experiment showed that the antioxidant activity of ethyl acetate fraction was higher than the other solvent fractions used in the study and this indicated that the active compounds might have a medium polarity. The study suggests the possibility of using Indian gooseberry to prevent rancidity of fish oils in industrial uses.

INTRODUCTION

The importance of antioxidants to prevent rancidity in fat and oils is increasing. Polyunsaturated fatty acids in fish oils tend to oxidize and hydrolyse due to their high unsaturation (Stansby, 1967; Khayat and Schwall, 1983). Most synthetic antioxidants cause some undesirable side effects and therefore there is an increasing demand for natural antioxidants (Haigh, 1986).

A number of plants have been recognized as natural antioxidants for a long time. Rosemary *(Rosemerinus officinalis L)* and sage (*Salvia officinalis*) are two with strong antioxidant efficiency. The antioxidant properties of the fruit of Indian gooseberry (*Phynanthus emblica*) was reported very recently (Edirisinghe *et al.*. 1996). This highly acidic, vitamin C rich, bitter tasting fruit has been used in traditional medicine for many purposes. In previous studies, the active components were extracted using two organic solvents, methanol and carbon tetrachloride, and the reported activity was high (Edirisinghe *et al.*. 1996). Methanol and carbon tetrachloride are poisonous and therefore the extracted product was suitable only for laboratory studies. The extraction of active components in non-poisonous solvents, i.e. ethanol and water, may

give opportunities for Indian gooseberry as an antioxidant in the food industry. This study was carried out to evaluate the activity of crude ethanol and water extracts of Indian gooseberry and to assess the activity of purified extracts in preventing oxidation in fish oils. The purification process may lead to identification of the active compounds present in this fruit.

EXPERIMENTAL

Materials

The dried fruit of Indian gooseberry was obtained from local market in Colombo, Sri Lanka. The fish oil of sudaya (*Sardinella albella*) was extracted by steam rendering (Edirisinghe *et al.*, 1998). The extracted fish oil was dried over anhydrous sodium sulphate for 24hrs. Analytical grade solvents and chemicals were obtained from Sigma Chemicals Co. Ltd, UK.

Experiment –1 Evaluation of anti-oxidant activity of Indian gooseberry using different solvent systems.

Extraction of antioxidants from Indian gooseberry

Three samples of 5 g of dried, powdered Indian gooseberry were extracted with 150ml of ethanol, methanol and water, respectively by Soxhlet extractor for 4hrs. After the extraction, the solvents were removed by rotavapor at 38°C. The products obtained were dried in an oven (40°C) for 24hrs and stored in a freezer (-18°C) until use.

Assess of activity

Each of the three products as extracted above was (weight : 1g) dissolved in three sets of 1ml of ethanol and added to the fish oil separately at concentrations of 2000 ppm and the reference samples were treated with 200ppm BHT. Treated samples were stored at room temperature (30°C). The quality of the fish oil was measured by peroxide value (AOAC 1980), free fatty acid value (AOAC 1980), and fatty acid composition (by gas chromatography) over a 44 day storage period. The quality of the samples was compared with the control.

Experiment –2. Purification of crude Indian gooseberry extract and the examination of preservative action of purified fractions

Crude ethanol extracts of Indian gooseberry were obtained by Soxhlet extraction as described in experiment -1. The dried ethanol extract (10 g) was dissolved in 100 ml of warm water (45 - 50°C). This aqueous solution was extracted with 100 ml of hexane, carbon tetrachloride, chloroform and ethyl acetate in order to increase the polarity. The final residue remained in the water. The solvents were removed using a rotavapor at 38°C. The procedure is as follows:

Dried Indian gooseberry

↓ Soxhlet extraction with ethanol

↓

10g of crude extract (dried)

↓ dissolved in 100ml of water (slightly hot ~ 50°C)

↓

aqueous solution

↓ extraction with 100ml of hexane

↓

mixture → separation of hexane phase, removal → residue 1
of solvent using rotavapor

↓

residue in aqueous phase

↓ extraction with 100ml of carbon tetrachloride

↓

mixture → separation of carbon tetrachloride phase, removal → residue 2

↓ of solvent using rotavapor

residue in aqueous phase

↓ extraction with 100ml of chloroform

↓

mixture → separation of chloroform phase, removal → residue 3

↓ of solvent using rotavapor

residue in aqueous phase

↓ extraction with 100ml of ethyl acetate

↓

mixture → removal of ethyl acetate phase, removal → residue 4

↓ of solvents using rotavapor

↓

residue in water → removal of water using rotavapor → residue 5

The five extracts were dried at 40°C for 4 hrs and at ambient temperature (30°C) for 24 hrs. The final residues were mixed with fish oil in 1000ppm concentrations using ethanol and stored in screw capped vials ambient temperature (30°C). The efficiency of the treatments were determined by measuring PV, FFA and FAC as experiment -1 over a 16 days storage period, and compared with the treatment with 200 ppm BHT.

Determination of fatty acid composition:

The fatty acid methyl esters, for fatty acid analysis, were prepared (AOCS 1989 and FF Method 1991) and these methyl esters were separated by packed column gas chromatography on a Shimadzu GC-14A gas chromatograph using GP 10% SP 2330 on chromasorb WAW (100-120 mesh) packed glass column (2.1 m * 3.2 mm). The temperature programming used was 20 min. at 175°C, then up to 190°C at a rate of 1°C/min, 10 min at 190°C, then up to 240° at a rate of 3°C/min and 10 min of holding time at 240°C. Helium was used as the carrier gas at a flow rate of 35 ml/min at 175°C with a flame ionization detector (FID). The temperature of the detector and injector was maintained at 270°C. The peaks were identified by comparing retention times of the methyl esters in standard mixtures from Larodane Fine Chemicals AB, Sweden and Neu-Chek pack, U.S.A. The weight of individual fatty acids was calculated as mg by using a C 17:0 internal standard.

Four replicate analysis were carried out to determine PV and FFA and the results were statistically analyzed ($p < 0.05$). Fatty acid analysis were carried out in duplicate and the results were given as mean values

RESULTS AND DISCUSION

Experiment –1.

The quality of the fish oil samples which were treated with the three different extracts and BHT were found to be different from each other and from the blank sample.

Peroxide and free fatty acid values

Variation of peroxides and free fatty acids in these treatments were shown in Tables 1 and 2.

It was observed that there were significant differences in peroxide values between the blank sample and the other four treatments. When comparing the three treatments, the ethanol extract was found to have very low PV up to the 30th day and after that period this value rapidly increased. The treatment with methanolic extract showed a low peroxide value up to the 24th day and the water extract showed the lower level up to nearly 16 days. The peroxide value of the fish oil sample treated with 200 ppm BHT maintained a lower level than the blank but higher than all the other three treatments of Indian gooseberry extracts up to the 16th day. The values of final peroxide levels showed that two observations were nearly equal and very high.

Table 1. Variation of peroxides in fish oil during storage with different solvent extracts of Indian gooseberry. (mean ± S.D, n= 4, $p < 0.05$)

Peroxide Value (P.V) m.eq/kg)	**Storage Time / Days**							
Sample No.	**1**	**4**	**9**	**16**	**24**	**30**	**37**	**44**
1	10.2 ±0.2	17.8^{b} ±3.8	66.1^{b} ±5.4	165.7^{b} ±9.7	500.4^{b} ±13.9	661.8^{ab} ±54.0	705.7^{c} ±27.3	778.7^{a} ±46.5
2	10.2 ±0.2	1.0^{c} ±0.0	24.5^{b} ±1.2	54.4^{d} ±7.9	108.0^{c} ±9.7	143.1^{c} ±10.7	834.2^{ab} ±63.4	841.1^{a} ±52.9
3	10.2 ±0.2	15.3^{b} ±0.0	25.2^{b} ±1.5	67.0^{cd} ±4.8	126.2^{c} ±17.7	678.1^{ab} ±48.8	775.8^{bc} ±49.6	833.1^{a} ±39.8
4	10.2 ±0.2	16.5^{b} ±0.7	43.3^{b} ±1.5	85.7^{c} ±8.5	536.8^{b} ±25.7	560.6^{b} ±163.5	856.7^{a} ±47.9	822.0^{a} ±30.9
5	10.2 ±0.2	34.3^{a} ±2.5	487.9^{a} ±12.9	543.7^{a} ±23.2	660.2^{a} ±36.8	763.9^{a} ±118.3	748.8^{c} ±32.7	682.6^{a} ±32.1

1 – Sample treated with BHT (200ppm); 2 – Sample treated with Indian gooseberry extracted in EtOH (2000ppm)
3 – Sample treated with Indian gooseberry extracted in MeOH (2000ppm)
4 – Sample treated with Indian gooseberry extracted in Water (2000ppm) 5 – Control

Table 2. Variation of free fatty acids in fish oil during storage with different solvent extracts of Indian gooseberry. (mean ± S.D, n= 4, $p < 0.05$)

Free fatty acid value (FFA) (%)	Storage Time / Days							
Sample No	**1**	**4**	**9**	**16**	**24**	**30**	**37**	**44**
1	3.5 ±0.3	4.0^{ab} ±0.0	5.1^{b} ±0.2	5.2^{b} ±0.4	5.8^{b} ±0.3	7.2^{b} ±1.1	8.6^{b} ±0.5	10.3^{b} ±0.3
2	3.5 ±0.3	3.6^{b} ±0.5	5.5^{c} ±0.3	4.4^{c} ±0.3	5.6^{b} ±0.2	5.0^{b} ±0.2	5.5^{d} ±0.7	8.0^{d} ±0.4
3	3.5 ±0.3	4.1^{ab} ±0.3	5.3^{b} ±0.2	4.5^{c} ±0.2	5.7^{b} ±0.3	5.6^{b} ±0.5	7.6^{c} ±0.3	9.6^{c} ±0.3
4	3.5 ±0.3	4.1^{ab} ±0.1	5.5^{ab} ±0.3	4.7^{bc} ±0.5	5.8^{b} ±0.1	7.0^{b} ±0.3	8.6^{b} ±0.2	10.3^{bc} ±0.5
5	3.5 ±0.3	4.5^{a} ±0.4	5.8^{a} ±0.3	6.7^{a} ±0.5	11.4^{a} ±0.1	11.4^{b} ±1.9	12.6^{a} ±0.7	14.4^{a} ±0.5

The percentage of FFA in the blank sample was high from the beginning but the values from other treatments were considerably lower than the blank sample. The lowest value in the oil sample treated with ethanol extract varied from 3.5 to 8% during storage. The treatments with methanolic and water extracts showed lower free fatty acid values. There is no significant difference between free fatty acid values of treatments of BHT and water extracts during storage. This indicated that the activity of the water extract in preventing the formation of FFA's was comparable with BHT at this concentration. This results showed that the ethanol extract has the highest ability to prevent formation of peroxides and free fatty acids, but methanolic and water extracts also showed considerable activity.

Fatty acid composition

The fatty acid composition results showed the stability of individual fatty acids during the experiment. The stability of the saturated fatty acids (SFAs) were very high during the storage (Fig. 1). During storage the amounts of SFAs increased at varying rates in all five treatments. The highest rate was recorded in the blank sample and the lowest in the sample treated with ethanol extract. The SFAs of the other three treatments varied between these two treatments.

The variation of MUFAs was observed with respect to palmitoleic acid, oleic acid and with minor quantities of myristoleic acid, eicosenoeic acid, eruc acid and nervoneic acid. The variation of fatty acids of five treatments was different from each other (Fig. 2). During the study MUFAs increased at different rates but similar to the changes observed in SFAs. The ethanol treatment of Indian gooseberry showed the lowest rate of increase while the control sample showed the highest rate of increase of monounsaturated fatty acids.

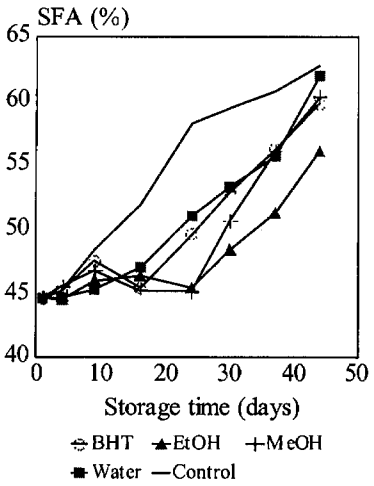

Fig. 1. Variation of saturated fatty acids of fish oils treated with different solvent extracts of Indian gooseberry during storage at 30°C.

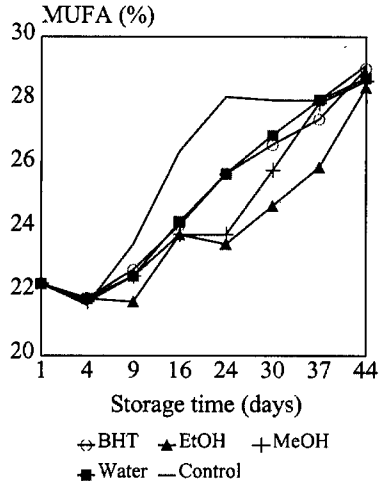

Fig. 2. Variation of monounsaturated fatty acids of fish oils treated with different solvent extracts of Indian gooseberry during storage at 30°C.

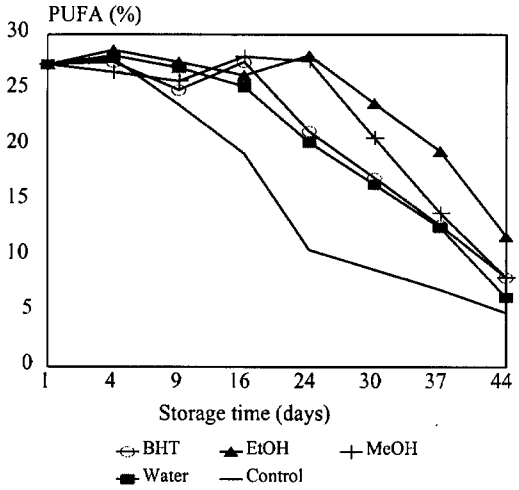

Fig. 3. Variation of polyunsaturated fatty acids of fish oils treated with different solvent extracts of Indian gooseberry during storage at 30°C.

The changes in the polyunsaturated fatty acids (PUFAs) of these five treatments were totally different from the variation of saturated and monounsaturated fatty acids during storage (Fig. 3). The amounts of PUFAs in the five samples were found to decrease at different rates during storage. The amount of PUFAs remained high up to the 24th day and then decreased slowly in the ethanol and sharply in the methanolic treatment. PUFA's decreased sharply in the control from the beginning of storage.

The ethanol extract of Indian gooseberry showed the highest ability of preventing the formation of peroxides, free fatty acids and showed the highest stability of fatty acids. The methanolic and water extracts also showed considerable activity against oxidation and hydrolysis. The different types of solvents were used to extract antioxidant components from different sources and their activity was different from each other. Wu *et al.* (1994) reported that the antioxidant activity of ethanol and methanol extracts of wild rice (*Z. aquatica L.*) were higher than the activity of an ethyl acetate extract. The antioxidant activity of 2000 ppm methanolic extract of ajowan seed (*Carum coptimum*) was a little lower than the activity of 200 ppm of BHT and this was measured by measuring peroxide values of stored soybean oil (Mehta *et al.*, 1994). Results from the current study showed that the activity of methanolic extract was higher than the BHT at the same concentration. The activity of these crude extracts depends on the other methanol soluble materials which do not have antioxidant activity. Chang *et al.* (1977) used seven solvents with different polarities (except ethanol) to extract antioxidant compounds of rosemary and sage and concluded that the methanolic extract had the highest activity. Both Chang et al (1977) and Wu et al (1994) concluded that high polar solvents extract active compounds more efficiently. The activity of the extracts depends on the polarity of the active compounds present in the material, and solvents with similar polarity to the compounds extract them. Other compounds with similar polarity, but no antioxidant activity may be extracted with the active ones and change the overall performance. This can be minimized by purification after extraction.

Experiment –2. Activity of purified compounds from Indian gooseberry

Variations of peroxides and free fatty acids in the treatments are shown in Figs. 4 and 5. The significantly lower PV ($P < 0.05$) for the ethyl acetate fraction than for the other treatments indicated that the antioxidant components were extracted more by ethyl acetate than other solvents. The variation of free fatty acids was very similar to the variation of peroxides. The highest and the lowest values of FFA were recorded in the control and the ethyl acetate extracts, respectively. The other solvent extracts also recorded lower PV's than the control and therefore showed some antioxidant activity. Among the Indian gooseberry treatments, the hexane extract showed the lowest effect on preventing formation of peroxides and free fatty acids. According to the present results the highest antioxidant activity was recorded in the ethyl acetate fraction (1000ppm) and this activity was higher than the crude extract as well as 200ppm BHT. There is no significant difference, in both PV and FFA, between the ethyl acetate extract treatment and the BHT treatment.

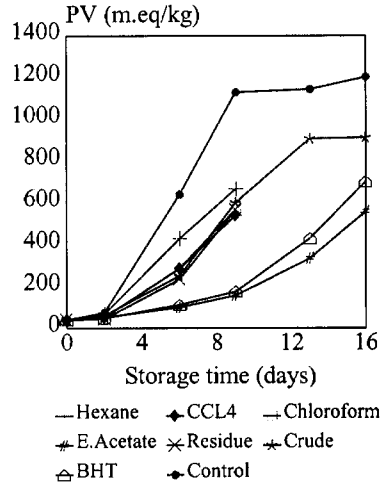

Fig. 4. Changes in the levels of peroxides in fish oils during storage with purified Indian gooseberry.

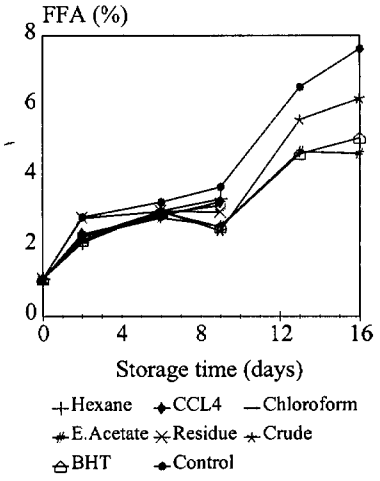

Fig. 5. Changes in the levels of free fatty acids in fish oils during storage with purified Indian gooseberry .

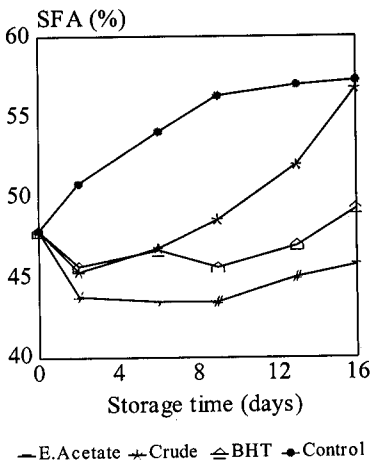

Fig. 6. Changes in the levels of saturated fatty acids in fish oils during storage with purified Indian gooseberry.

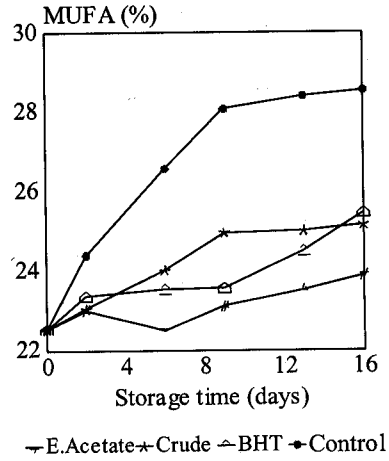

Fig. 7. Changes in the levels of monounsaturated fatty acids in fish oils during storage with purified Indian gooseberry.

The variation of fatty acids of stored fish lipids with antioxidant treatments were plotted in Figs. 6, 7 and -8. During storage, the amount of saturated and monounsaturated fatty acids increased and the amount of polyunsaturated fatty acids decreased. When treated with the antioxidant, the increasing rate of SFAs and MUFAs, and the decreasing rate of PUFAs was low and remained stable. The ethyl acetate treatment gave the highest stability of the fatty acids of fish lipids. The crude Indian gooseberry treatment also showed some activity, but lower than the treatment of BHT at this particular concentration.

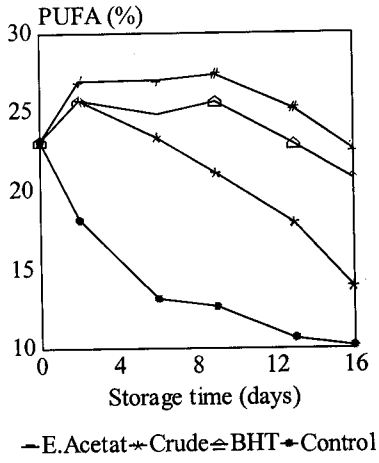

Fig. 8. Changes in the levels of polyunsaturated fatty acids in fish oils during storage with purified Indian gooseberry.

All these quality parameters indicate that the antioxidant components of Indian gooseberry can be easily separated by ethyl acetate and this indicated that this active compounds might have a medium polarity (relative polarity of ethyl acetate = 0.58).

CONCLUSIONS

The fruit of Indian gooseberry is reported to have considerable antioxidant activity in preservation of fish oils. The active components of this fruit can be extracted by ethanol and water and the activity of these two solvent extracts showed the possibility of using Indian gooseberry extracts as an antioxidant on a large scale. The antioxidant components extracted to these non-toxic solvents can be used without removing solvent or even without purification of the obtained product. The active components of Indian gooseberry can be easily separated and purified by ethyl acetate and the active compounds might have a medium polarity. The study suggests the possibility of using Indian gooseberry for industrial uses.

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FATTY ACID COMPOSITION OF SOME SMALL PELAGIC FISHES IN SRI LANKA

by

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ABSTRACT

Consumption of fish lipids, more precisely omega-3 polyunsaturated fatty acids has prophylactic effects minimizing the development of a number of chronic degenerative diseases. In this study, fatty acid profiles and the content of fatty acids of forty small pelagic fish species in Sri Lanka were studied by gas chromatography.

The more abundant fatty acids were myristic acid (C 14:0), palmitic acid (C 16:0), palmitoleic acid (C 16:1), stearic acid (C 18:0), oleic acid (C 18:1), eicosapentaenoic acid (C 20:5 n-3, EPA) and docosahexaenoic acid (C 22:6 n-3, DHA), but the most predominant fatty acid was palmitic acid which contributed to 20-39% of the total fatty acids. Saturated fatty acids were the largest group in the total fatty acid profile while content of mono-unsaturated fatty acids was much lower. Poly-unsaturated fatty acid content was intermediate.

In most species studied, the total amount of omega-3 PUFA contributed nearly 90% of the total PUFAs. The most important omega-3 PUFAs, namely EPA and DHA contributed to 85% of the total omega-3 polyunsaturated fatty acids. The total omega-3 PUFA contents of thirty six fish, out of forty species, were higher than 20% of total fatty acids and indicating that the lipids of small pelagic fish species can provide greater health benefit.

INTRODUCTION

Fish are highly nutritive and the omega-3 polyunsaturated fatty acids (PUFA), which are high in fish lipids, are reported to have number of health benefits (Kinsella, 1988. These fatty acids, specially eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), are reported to have the ability to decrease levels of cholesterol, triacyleglycerols, very low-density lipoproteins (VLDL) and low density lipoproteins (LDL) in blood. The omega-3 PUFAs also play a major role in controlling the excess production of prostaglandins, which are known as powerful metabolic and physiological regulators (Kinsella, 1986, 1988; Ackman 1988. Therefore, consumption of fish, more precisely fish lipids, provides a number of prophylactic effects by minimizing the development of a number of chronic degenerative diseases.

The best sources for omega-3 PUFAs are fish oils. The fatty acid composition of fish from the Bay of Bengal (Yusuf *et al.*, 1993), Icelandic fish (Sigurgisladothir, and Palmadothir, 1993), Australia (Gibson, 1983) were reported. In addition, fatty acid profiles of different fish species were reported by Ackman (1974), Kaitaranta (1980), Ackman and McLeod (1988), and Low *et al.* (1994. Most of the early work about the Sri Lankan fish were limited to the proximate composition (Lantz and Gunasekara, 1957; Peiris and Grero, 1972, 1973; De Silva and Rangoda, 1979. Jayasinghe (1994) reported the fatty acid composition of ten marine fish species and three prawn species. The present study was carried out to find the fatty acid profiles and to evaluate the health benefits of n-3 PUFA from forty small pelagic fish species commonly available in Sri Lanka.

MATERIALS AND METHODS

Forty fish species from ten different families were purchased from Chilaw and Negombo fish landing sites on the North-West Coast of Sri Lanka. These fish samples were packed in ice and immediately transferred to the laboratory of National Aquatic Resources Research and Development Agency, Colombo and stored in a freezer (-18°C) until use.

A sample of four fish from each species was taken for analysis and length (cm) and weight (g) were measured (Table 1). The amount of lipids present in the whole fish was extracted and determined by a modified Bligh and Dyer method (Hans and Olley, 1963). The fatty acid methyl esters (FAME) of these lipids were prepared by base hydrolysis followed by trans-esterification (AOCS, 1992. The methyl esters were separated by gas chromatography on a Shimadzu GC-14A gas chromatograph using GP 10% SP 2330 on Chromasorb WAW (100-120 mesh) packed glass column (2.1 m * 3.2 mm. The temperature programming used was as follows: 20 min. at 175°C, then increasing up to 190°C at a rate of 1°C/min, 10 min at 190°C, then increasing up to 240° at a rate of 3°C/min and 10 min. of holding time at 240°C. Helium was used as the carrier gas at a flow rate of 35 ml/min at 175°C with a hydrogen flame ionization detector (FID). The temperature of the detector and injector was maintained at 270°C. The peaks obtained from the methyl esters in fish oil samples were identified by comparing with retention times of methyl esters in a standard lipid mixture from Larodane Fine Chemicals AB, Sweden; Inhouse Fame, Denmark; and Neu-Chek pack, U.S.A. The absolute amount of fatty acids were determined using heptadecaenoic acid (C:17) as an internal standard.

RESULTS AND DISCUSSION

There were differences in composition of fatty acids among the species studied. The more abundant fatty acids in fish were myristic acid (C 14:0), palmitic acid (C 16:0), palmitoleic acid (C 16:1), stearic acid (C 18:0), oleic acid (C 18:1), eicosapentaenoic acid (C 20:5 n-3, EPA) and docosahexaenoic acid (C 22:6 n-3, DHA). The most predominant was palmitic acid which contributed to 25-30% of total fatty acids (Table 2). Of the three groups, i.e. saturated, monounsaturated and polyunsaturated fatty acids, the n-3 family of the polyunsaturated fatty acids is the most important to human health.

The absolute amount of fatty acids in fish depends on the percentage of individual fatty acids as well as the total lipid content of fish. Most of these species contained very low amount of fat (< 4%) and therefore the amount of fatty acids present is also very low. The absolute amount of individual fatty acids (mg/100g fish) are shown in Table -3.

Saturated fatty acids:

Saturated fatty acids (SFAs) contributed to the major part of the fatty acid profile and range from 39.1 (Largescaled terapon) to 51.4% (Straked spinefoot) of the total fatty acids. This SFA group consisted of myristic acid (C 14:0), pentadecanoic acid (C 15:0), palmitic acid (C 16:0), margaric acid (C 17:0), stearic acid (C 18:0) and lignoceric acid (C 24:0. Palmitic acid contributed the largest proportion (>20%) of the total fatty acids. Streaked spinefoot recorded the highest percentage of C 16:0 acid, 38.8%, and white spotted spinefoot the lowest percentage (20.7%. Myristic acid and stearic acid also contributed a considerable proportion but the C 15:0 acid and C 17:0 acid were comparatively low (Table 2). Lignoceric acid (C 24:0) has been found in a few species. The highest amount of saturated fatty acids was in hilsa shad (5844.5mg/100g fish) while palmitic acid contributed 3345 mg/100g fish. Pathamadiya (*Cynoglossus. sp*) had the lowest saturated fatty acids; 180.2 mg/100g fish (Table 3).

Monounsaturated fatty acids:

Monounsaturated fatty acids (MUFAs) were mainly palmitoleic (C 16:1) and oleic acid (C 18:1) with minor quantities of myristoleic (C 14:1), eicosenoeic (C 20:1), erucateic (C 22:1) and nervoneic acid (C 24:1. The highest percentage of MUFAs was recorded in long-finned herring (31.9%) including 22.6% of C 18:1 acid. The lowest percentage of MUFAs was recorded in white sardine (14.4%) with equal amounts

(6%) of C 16:1 and C 18:1. Hilsa shad and white sardine had the highest (3033.4 mg/100g fish) and the lowest (75.6 mg/100g fish) monounsaturated fatty acids respectively (Table 3).

Polyunsaturated fatty acids

The polyunsaturated fatty acids were the second largest group, slightly less than the monoenes in only five of the forty fish species studied. The percentage of polyunsaturated fatty acids (PUFAs) ranged from 14.3% (silver sillago) to 37.3% (brushtooth lizardfish). Among the species studied, 20 out of 40 contained more than 30% PUFAs and 12 out of the remaining 20 contained more than 25% PUFAs of total fatty acids (Table 2). The highest and the lowest amount of polyunsaturated fatty acids were recorded in hilsa shad (3310.7mg/100g fish) and pathamadiya (116.5mg/100g fish) respectively (Table 3). The PUFAs mainly consisted of omega 3 (n-3) polyunsaturated fatty acids, contributing to nearly 85% of the total PUFAs.

n - 3 Polyunsaturated fatty acids

This group of fatty acids in fish oils is reported to have a therapeutic effect on a number of pathophysiological and heart diseases. Most species contained high levels, around 30%, of n -3 PUFAs but contained low levels of other positional isomers. Among the species studied, the highest percentage of n-3 PUFAs were present in buccaneer anchovy (*Stolephorus punctifer*, 36.2%, 218.7mg/100g fish), brushtooth lizardfish (*Saurida undosquamis*, 34.6%, 361.4mg/100g fish), yellow stripe scad (*Selaroides leptolepis*, 34.4%, 579mg/100g fish), dorab-wolf herring (*Chirocentrus dorab*, 33.9%, 517.9mg/100gfish), big-eye barracuda (*Sphyraena jello*, 33.6%, 204.3mg/100 fish), Indian scad (*Decapterus ruselli*, 32.4%, 376mg/100g fish) and spotted sardinella (*Amblygaster sirm*, 32.3%, 920.6mg/100g fish. The lowest percentage of n-3 PUFAs, 7.1%, was reported in silver sillago (*Sillago sihama*, 144.3mg/100g fish. Streaked spinefoot and small scaled terapon also contained lower levels of, 12.5% (685.9mg/100g fish) and 13.7% (416.7mg/1000g fish), n-3 PUFAs (Table 2 and 3) respectively.

EPA and DHA content

The n-3 PUFAs mainly consisted of C 18:4 n-3, C 20:4 n-3, eicosapentaenoic acid (EPA, C 20:5 n-3), docosapentaenoic acid (C 22:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3. But in most species the two most biologically active n-3 PUFAs, EPA and DHA, contributed to nearly 85% of the total n-3 PUFAs. The highest percentage of EPA, 13.9%, was in hilsa shad (*Tenualosa ilisha*) and the lowest value, 1.9%, in whitespotted spinefoot (*Siganus canaliculatus*). Generally, DHA content was greater than EPA except in very few species such as hilsa shad, silver sillago and otomebora mullet. The highest (24.6%) and the lowest (2.41%) values of DHA were recorded for brushtooth lizardfish and silver sillago respectively. Ackman and Burgher (1964) regarded DHA and EPA as interchangeable and this suggestion was supported by von Schacky and Weber (1985) who found that DHA could be retroconverted *in vivo* to EPA. Armstrong *et al.* (1991) considered the combined EPA and DHA levels, rather than simply EPA alone, when assessing the nutritional quality of lipids.

The high proportion of omega-3 PUFAs and low proportion of SFAs and MUFAs acids indicate the good quality of the lipids with respect to health benefits. The omega-3 PUFAs, known to provide a number of health benefits, originate in unicellular phytoplankton or in some areas, in seaweeds (Ackman, 1979). When considering these quality parameters, buccaneer anchovy (36.2% n-3 PUFAs, 15.4% MUFAs, 41.1% SAFs), brushtooth lizad fish (34.6% n-3 PUFAs, 17% MUFAs, 39.2 SFAs) and yellowstripe scad (34.4% n-3 PUFAs, 17.2% MUFAs, 40.8% SAFs) provides the highest health benefits. In addition to these species, spotted sardinella, double-lined fusilier, blacktip sardinella, dorab-wolf herring, Indian scad and big-eye barracuda were found to consist of 32.1 - 33.9% n-3 PUFAs, 16.2 - 19.1% MUFAs and 40.6 - 43.8% SFAs and therefore also provide good health benefits. Non of these seven fish species recorded fat contents of more than 3% and most of these are very small fish in size. Ackman (1979) showed that the SFAs, MUFAs and PUFAs of capelin, mackerel and Atlantic herring was in the range of 22.6 - 30.3%, 43.9 - 61 % and 16.4 - 25.8% respectively. In Ackman's study the percentage of MUFAs was higher and SFAs are lower compared to the results from the present study. The percentage of saturated, monounsaturated and n-3 polyunsaturated fatty acids of dorab-wolf herring in the present study is very similar to the results recorded by Jayasinghe (1994).

In most species of the current study, the total n-3 PUFA content (thirty two out of forty species) were higher than 25% of total fatty acids. Among the ω3 PUFAs, EPA and DHA contribute a very high proportion (nearly 85%) to the total ω3 PUFAs. The content of these two acids in seafoods or fish oils varies immensely between species of fish and marine mammals (Ackman, 1982; Kinsella, 1987) and largely determines the efficacy and dose required for treatment. According to the results of the present study, DHA is the major fatty acid of this n-3 PUFA group, recording very high percentages in brushtooth lizard fish, dorabwolf herring and in big-eye barracuda. A number of research workers have concluded that DHA is the main n-3 PUFAs in marine fish (Gibson, 1983; Armstrong *et al.*, 1991; Yusuf *et al.*, 1993; Reena *et al.*, 1996). The n-3 PUFA contents of Australian fish were in the range of 9.6 - 48.2% (Gibson, 1983), and the upper limit of this range is higher than in the present study. According to the report by Armstrong *et al.* (1991), the SFAs and n-3 PUFA contents of some fish in temperate Australian waters were in the range of 7.3 - 33.6% and 31.1 - 42.9% respectively, and are lower than those found during the present study. Yusuf *et al.* (1993) reported that the n-3 content of marine fish in the Bay of Bengal varied from 8.9 - 35% and DHA contributed the highest proportion to the total n-3 PUFAs. Reena *et al.* (1996) report ed the fatty acid composition of 32 fish from Indian waters. According to their results the total percentages of SFAs, MUFAs and PUFAs of Decapterus russelli were 31.75, 16.45 and 47.48% respectively. The size of fish they studied was smaller than in the present study and the period of harvesting was different. They also pointed out that the variation of PUFAs in their samples to be in the range of 20.9 - 57.9%, which is higher than the results in the present study. The results of the present study generally show a lower EPA content than DHA, but in few cases such as hilsa shad (family: Clupeidae) and otomebora mullet, EPA content is higher. Low *et al.* (1994) reported that longtail shad (family: Clupeidae) had higher EPA than DHA. The Icelandic fish, capelin (*Mallotus villosus*), shows the same pattern. i.e. EPA > DHA. Herbivores and phytoplankton feeders tend to have more EPA than DHA (Sigurgisladottir and Palmadottir, 1993. Some of the fish species studied were moderate sources of these fatty acids, but a few species, such as silver sillago, long-finned herring and white spotted spinefoot, contained low proportions of n-3 PUFAs (7.1 - 16%) with high proportions of SFAs (45.0 - 46.6%) and MUFAs (29.5 - 32%) and therefore are poor sources of the beneficial fatty acids.

The total lipid content of the fish analyzed in this study varied between 0.69 - 14 6%. Most fish which contained high n-3 PUFAs were poor sources of lipids, i.e. there was an inverse relationship between the n-3 fatty acids and total lipid content (figure -4.44). This type of relationship was also reported in fatty acids of Icelandic fishes by Sigurgisladottir and Palmadottir (1993).

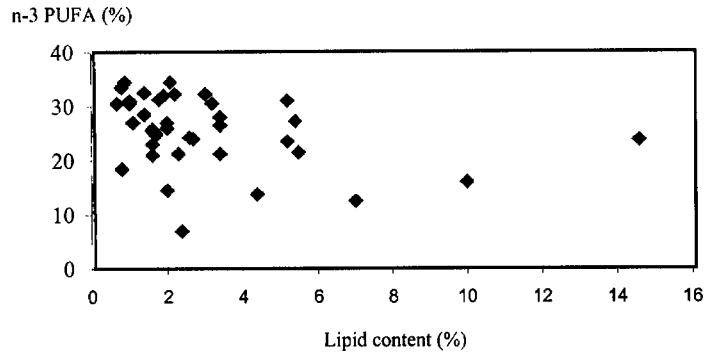

Fig. 1. The relationship between lipid content (%) and n-3 polyunsaturated fatty acids (%).

CONCLUSIONS

The saturated fatty acids dominated the fatty acid profile of fish lipids. Most small pelagics contained around 30% of omega-3 polyunsaturated fatty acids in total fatty acids. Buccaneer anchovy, brushtooth lizardfish, and yellowstripe scad showed higher nutritional values where as silver sillago, thryssa and small scaled terapon showed the lowest nutritional value with respect to fatty acids. The two most important n-3 PUFAs, i.e. EPA and DHA, contributed to nearly 85% of the total n-3 polyunsaturated fatty acids. The total n-3 PUFA content in thirty six fish species out of forty, were higher than 20% and this indicated that the lipids of small pelagic fish provide greater nutritional value as well as higher health benefits.

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Table 1. List of Species studied.

No.	Date	English name	Family	Scientific name	Common name	Weight (g)	Length (Total /cm)	Oil (%)
1	96/11/06	--	*Cynoglossidae*	*Cynoglossus. sp*	Pathamadiya	--	--	0.6
2	96/09/23	Baelama anchovy	*Engraulidae*	*Thryssa baelama*	Lagga	12.2	11.3	2.3
3	96/09/23	Bhestripe herring	*Clupeidae*	*Herklotsichthys quadrimaculatus*	Koramburuwa	23.2	11.2	5.5
4	96/09/12	Big eye barracuda	*Sphyraenidae*	*Sphyraena forsteri*	Theliya	125.2	25.7	0.8
5	96/08/22	Blacktip sardinella	*Clupeidae*	*Sardinella melanura*	Salaya	22.0	13.1	1.9
6	96/09/23	Brushtooth lizardfish	*Synodontidae*	*Saurida undosquamis*	Bolanda	32.1	15.0	0.9
7	96/09/23	Buccaneer anchovy	*Engraulidae*	*Stolephorus punctifer*	Halmassa	20.1	26.5	1.6
8	96/09/12	Commerson's anchovy	*Engraulidae*	*Stolephorus commersonii*	Halmassa	2.8	6.7	1.6
9	96/09/12	Common ponyfish	*Leiognothidae*	*Leiognathus equulus*	Karalla	14.0	7.4	2.0
10	96/09/12	Dorab-wolf herring	*Chirocentridae*	*Chirocentrus dorab*	Katuwalla	61.0	24.5	2.0
11	96/08/22	Double-lined fusilier	*Caesionaidae*	*Pterocaesio digramma*	Angaya - N	55.1	14.0	2.2
12	96/09/12	Fin-stripe goatfish	*Mullidae*	*Upeneus taeniopterus*	Nagari	46.0	22.0	1.6
13	96/11/06	Flying fish	*Exocoetidae*	*Exocoetus volitans*	Piyamassa	--	--	2.7
14	96/09/12	Gold band fusilier	*Caesionaidae*	*Pterocaesio chrysozona*	Angaya -R-Y	47.9	13.1	3.4
15	96/10/10	Hilsa shad	*Clupeidae*	*Tenualosa ilisha*	Katukoiiya	43.0	38.7	14.6
16	95/08/10	Indian anchovy	*Engraulidae*	*Stolephorus indicus*	Handalla	16.1	11.8	1.0
17	96/10/10	Indian mackerel	*Scombroidae*	*Rastrelliger kanagurta*	Kumbalawa	78.4	16.8	1.7
18	96/09/23	Indian pillona	*Clupeidae*	*Pillona ditchela*	Udassa	--	--	1.7
19	96/08/22	Indian scad	*Carangidae*	*Decapterus russelli*	Linna	43.0	28.4	1.4
20	97/02/06	Largehead hairtail ribon	*Trichiuridae*	*Trichiurus lepturus*	Sawalaya	510.0	66.1	5.2
21	96/09/12	Largescaled terapon	*Teraponidae*	*Terapon theraps*	Keeliya	45.0	21.0	3.4
22	96/10/10	Long-finned herring	*Clupeidae*	*Opisthopterus tardoore*	Tottawa	10.0	10.5	10.0
23	96/09/12	Malabar thryssa	*Engraulidae*	*Thryssa malabarika*	Lagga	18.1	11.5	1.6
24	95/08/18	Moonfish	*Menidae*	*Mene maculata*	Hunupenna	40.0	11.5	1.0
25	96/07/11	Otomebora mullet	*Mugilidae*	*Liza melinoptera*	Godaya	70.2	17.0	2.6
26	96/09/12	Pickhandle barracuda	*Sphyraenidae*	*Sphyraena jello*	Theliya	58.1	19.0	1.1
27	96/09/12	Pugnose ponyfish	*Leiognothidae*	*Secutor insidator*	Karalla	7.6	6.8	5.4
28	96/09/23	Rainbow sardine	*Clupeidae*	*Dussumieria acuta*	Thonda Huralla	32.5	13.7	3.2
29	96/09/12	Shortnose ponyfish	*Leiognothidae*	*Leiognathus brevirostris*	Karalla	7.2	6.6	3.4
30	96/08/12	Silver sillago	*Sillaginidae*	*Sillago sihama*	Kalanda	38.8	16.0	2.4
31	96/09/12	Small scaled terapon	*Teraponidae*	*Terapon puta*	Keeliya	32.1	12.0	4.4
32	96/09/12	Smithurst's ponyfish	*Leiognothidae*	*Leiognathus smithursti*	Karalla	18.2	9.5	1.4
33	96/08/22	Spotted sardinella	*Clupeidae*	*Amblygaster sirm*	Hurulla	68.3	17.7	3.0
34	96/08/08	Streaked spinefoot	*Siganidae*	*Siganus javus*	Orawa	82.1	23.4	7.0
35	96/09/12	Toothpony	*Leiognothidae*	*Gassa minita*	Karalla	17.3	10.4	1.8
36	96/07/17	Whipfin silver-biddy	*Gerredae*	*Gerres filamentosus*	Thirali	28.1	16.0	2.0
37	96/08/22	White sardine	*Clupeidae*	*Escualosa thoracata*	Wella Sudaya	5.3	20.8	0.7
38	96/08/22	White sardinella	*Clupeidae*	*Sardinella albella*	Sudaya	18.4	10.2	5.2
39	96/08/22	White spotted sprinfoot	*Siganidae*	*Siganus canaliculatus*	Orawa	146.7	29.6	0.8
40	96/07/25	Yellowstripe scad	*Carangidae*	*Selaroides leptolepis*	Suraparawa	17.0	10.4	2.1

Table 2. Fatty acid composition of small pelagics.

Small pelagic	Percentage fatty acids																								
	14:0	15:0	16:0	17:0	18:0	24:0	**SFA**	14:1	16:1	18:1	20:1	22:1	24:1 n-9	**MUFA**	16:2 n-4	18:2 n-6	18:4 n-3	20:2 n-6	20:4 n-3	20:5 n-3	22:4	22:5 n-3	22:6 n-3	**PUFA**	**n-3 PUFA**
Baelama anchovy	7.4	1.0	29.3	2.0	8.2		**47.9**		7.3	11.7	0.6		0.7	**20.2**	0.6	1.2	1.0			7.9		1.4	13.0	**25.2**	**23.4**
Bhestripe herring	6.6		32.7	2.1	8.3		**49.7**	1.1	6.5	11.5			1.2	**20.4**	0.8	0.8			0.4	7.5		1.8	11.9	**23.3**	**21.6**
Big eye barracuda	6.8	0.8	23.5	1.0	8.4		**40.6**		7.4	10.0			1.7	**19.1**		1.1	1.0			7.6	0.5	2.3	22.7	**35.2**	**33.6**
Blacktip sardinella	8.6	0.9	25.4	1.7	6.0		**42.7**		7.7	6.9	0.7		1.9	**17.1**	1.2	0.9	1.1		0.4	10.0	0.6	1.9	18.7	**34.7**	**32.1**
Brushtooth lizardfish	2.9	0.7	22.2	3.3	10.2		**39.2**	0.6	4.0	9.9	0.7		1.7	**17.0**	1.2		0.3			7.4	1.4	2.3	24.6	**37.3**	**34.6**
Buccaneer anchovy	6.5	0.4	24.6	1.4	8.3		**41.1**	0.9	3.9	8.7	0.5		1.5	**15.4**		0.6	1.5			10.9		2.2	21.6	**36.8**	**36.2**
Commerson's anchovy	6.8	1.3	28.0	0.9	8.2		**45.3**		9.1	7.2	0.4		0.9	**17.7**	0.9	1.3	2.2		0.2	7.6		1.2	16.8	**30.3**	**28.1**
Common ponyfish	5.5	1.7	24.9	2.2	9.6	0.3	**44.2**	0.2	9.0	7.6	1.3		1.8	**19.8**	1.6	0.5	1.3		0.3	9.2	1.5	2.3	13.9	**30.5**	**26.9**
Dorab-wolf herring	6.4	0.7	26.4	1.2	9.1		**43.8**		6.2	8.7			1.3	**16.2**		1.2	1.1			7.7	0.6	1.5	23.7	**35.7**	**33.9**
Double-lined fusilier	6.3	1.0	25.6	1.6	7.0		**41.5**		6.2	9.1			2.4	**17.7**	1.3	0.8	1.4		0.6	9.0	0.8	1.3	20.0	**35.2**	**32.3**
Fin-stripe goatfish	3.7		29.2	1.4	9.7		**44.0**	0.6	5.9	16.3	0.8		0.7	**24.2**	1.3	1.3	1.0			7.7		1.6	12.8	**25.6**	**23.1**
Flying fish	4.5	1.2	24.4	2.0	8.0		**40.0**	0.3	5.5	17.1	2.1		1.6	**26.6**	0.8	1.4			0.5	5.4	1.6	2.7	15.4	**27.8**	**24.0**
Gold band fusilier	7.6	0.7	27.3	0.9	8.6		**45.1**		7.2	12.3	0.7		1.9	**22.2**	0.5	0.9	1.1		0.5	7.0	0.5	2.5	15.3	**28.4**	**26.5**
Hilsa shad	12.6	0.5	26.5		6.7		**46.2**		14.6	8.4	0.6		0.5	**24.0**	1.0	0.5	1.5	0.3	0.3	13.9	0.7	1.4	6.7	**26.2**	**23.8**
Indian anchovy	5.4	0.9	26.3	1.3	7.3		**41.1**	0.3	6.1	9.3		0.3	1.9	**17.7**	0.2	0.9	1.1		0.4	10.0	1.7	1.4	18.0	**33.8**	**30.9**
Indian mackerel	10.9	0.2	24.1	1.3	8.0		**44.6**	0.6	14.2	6.1	1.4		0.7	**23.1**	1.6	0.5				10.7		1.6	12.7	**27.0**	**25.0**
Indian pillona	6.2		28.7	1.8	9.7		**46.3**	0.9	6.1	13.7	0.9		1.1	**22.7**	1.0	0.8	0.9		0.3	8.1		1.8	13.8	**26.6**	**24.8**
Indian scad	4.5	0.9	24.8	1.5	9.7		**41.3**		4.8	11.6	0.5		1.1	**18.0**	0.2	1.3	1.4		0.3	9.9	0.4	2.2	18.6	**34.3**	**32.4**
Largehead hairtail ribbon fish	6.6	0.7	28.7	1.6	9.4		**47.0**		8.1	14.2			1.0	**23.3**					0.4	5.5	0.4	2.4	15.2	**24.0**	**23.6**
Largescaled terapon	5.4	0.6	24.0	1.5	7.7		**39.1**		8.8	18.2	1.4		1.5	**30.0**	1.1	1.7			0.3	5.1	1.1	2.7	13.1	**25.2**	**21.3**
Long-finned herring	5.3	0.6	32.4	1.1	7.3		**46.6**		8.0	22.6	0.8		0.6	**31.9**	0.6	1.1			0.4	4.9	0.6	2.4	8.3	**18.3**	**16.0**
Malabar thryssa	5.0	1.1	29.2	1.7	10.8		**47.7**	0.1	6.3	11.8			1.5	**19.8**	1.7	1.7	0.1	0.5	0.3	7.1	0.5	1.3	12.2	**25.4**	**21.0**
Moonfish	6.0	0.5	23.9	1.1	8.0		**39.5**		6.4	12.5		0.3	2.2	**21.4**		0.8	0.2		0.6	8.8	1.1	2.8	18.3	**32.7**	**30.6**
Otomebora mullet	10.5	1.9	22.2	0.9	7.5		**43.0**		13.5	6.0			0.8	**20.3**	3.6	1.4	2.4	0.3	0.7	11.9	1.1	2.1	7.1	**30.6**	**24.2**
Pathamadiya	5.2	1.0	21.9	3.0	12.7		**43.8**	0.2	6.6	10.9	3.0		1.4	**22.1**	2.0				0.7	8.4	2.5	4.4	10.3	**28.3**	**23.8**
Pickhandle barracuda	4.7	0.5	27.3	1.5	8.4	0.1	**42.5**		6.7	15.1	0.7		1.4	**23.8**	1.0	1.2	0.7		0.1	6.1	0.4	1.6	18.5	**29.5**	**27.0**
Pugnose ponyfish	6.4	0.7	28.2	2.0	8.2		**45.5**		8.5	8.9	0.4		1.4	**19.2**	1.1	1.1	0.7		0.4	9.4	0.6	1.7	15.1	**30.2**	**27.3**
Rainbow sardine	6.0	0.9	27.7	2.7	9.0		**46.2**	0.1	6.3	7.9			1.5	**15.9**	0.8	1.0	1.7		0.2	8.9	0.3	1.2	18.4	**32.6**	**30.4**
Shortnose ponyfish	6.2	1.5	27.1	2.0	8.4	0.2	**45.4**		9.2	7.7	0.5		1.4	**18.8**	1.5	0.8	1.2		0.4	9.5	0.8	2.2	14.0	**30.3**	**27.3**
Silver sillago	3.5	1.0	28.9	2.0	9.6		**45.0**		10.8	14.9	2.7	0.6	0.4	**29.5**	0.9	1.6				2.6	4.7	2.1	2.4	**14.3**	**7.1**
Small scaled terapon	4.8		28.9	1.7	8.3		**43.7**	0.2	10.0	17.5	1.6		0.7	**30.0**	1.2	1.2			0.2	6.9	0.9	1.8	4.9	**17.0**	**13.7**
Smithurst's ponyfish	3.9	1.1	25.7	2.0	10.5		**43.1**		5.9	12.4	0.2		1.6	**20.2**		0.6	0.6		0.2	6.2	0.3	2.5	19.0	**29.3**	**28.5**
Spotted sardinella	6.3	1.0	25.6	1.6	7.0		**41.5**		6.2	9.1			2.4	**17.7**	1.3	0.8	1.4		0.6	9.0	0.8	1.3	20.0	**35.2**	**32.3**
Streaked spinefoot	5.4	0.6	38.8	0.7	5.9		**51.4**		9.9	12.8	0.2		0.8	**23.6**	0.7	2.4			0.3	3.8	0.6	4.5	3.8	**16.5**	**12.5**
Toothpony	3.8	0.6	25.0	2.1	10.0		**41.5**	0.7	5.0	10.9			1.2	**17.9**	2.1	1.0				7.3	0.2	2.3	21.7	**34.6**	**31.3**
Whipfin silver-biddy	4.4	1.9	27.5	1.8	10.7		**46.2**	0.3	6.7	15.4	3.3		1.6	**27.2**	0.5	1.5		0.3	0.2	4.0	1.7	3.0	7.4	**18.4**	**14.5**
White sardine	6.1	0.7	26.2	1.9	9.0		**43.9**		6.0	6.0	0.8		1.6	**14.4**		0.3	1.2		0.4	9.0	0.8	2.3	17.5	**31.5**	**30.4**
White sardinella	10.1	1.0	24.1	1.4	6.4		**43.0**		9.2	6.5			1.5	**17.2**	1.3	1.0	2.4		0.4	10.9	0.8	1.7	15.5	**34.0**	**30.9**
White spotted sprinfoot	4.9	1.9	20.7	2.7	11.5		**41.7**	0.4	4.3	8.8	1.4		6.8	**21.7**	1.4	0.9		0.3	0.5	1.9	1.6	3.8	12.1	**22.5**	**18.4**
Yellowstripe scad	3.9	0.6	26.0	1.5	8.7		**40.8**		4.6	11.4	0.3		1.0	**17.2**		0.6	0.9		0.2	11.3	0.7	2.9	19.1	**35.7**	**34.4**

Table 3. Amount of fatty acids present in small pelagics.

(mean ± S.D, n = 4)

Name of fish	Amount of fatty acids (mg/100g fish)													
	14:0	15:0	16:0	17:0	18:0	24:0	SFA	14:1	16:1	18:1	20:1	22:1	24:1 n-9	MUFA
Baelama anchovy	126.5	16.4 ±1.4	504.1 ±31.2	34.6 ±2.8	141.6 ±11.5		823.3		124.5 ±5.6	200.9 ±7.2	10.6		11.8 ±1.2	347.7
Bhestripe herring	299.1 +6.8		1473.5 ±53.2	94.3 ±8.0	373.3		2240.2	50.7 ± 4.3	294.1 ±6.8	519.6 ±15.2			54.3 ±2.1	918.6
Big eye barracuda	41.5 ±2.8	5.1 ±0.7	143.2 ±5.9	5.8 ±0.7	51.2 ±6.1		246.8		44.9 ±5.0	60.9 ±1.9			10.4	116.2
Blacktip sardinella	126.9 ±5.6	13.6 ±1.0	373.0 ±7.6	25.7 ±2.5	87.5 ±7.6		626.7		112.6 ±6.1	100.9 ±4.5	10.0		27.9 ±0.5	251.4
Brushtooth lizardfish	30.5 +1.8	7.1 +0.5	231.4	34.6 ±4.1	106.2 ±6.8		409.8	6.5 ±0.3	41.5 ±6.1	103.6 ±6.7	7.6		18.2 ±0.8	177.4
Buccaneer anchovy	39.0 ±4.2	2.4 ±0.3	148.8 ±14.2	8.2 ±0.8	50.0		248.4	5.3 +0.1	23.3 ±0.8	52.4 ±4.1	2.9		9.2	93.0
Commerson's anchovy	86.7 ±7.8	16.7 ±0.8	356.1	11.2 ±0.8	104.7 ±14.2		575.5		116.2 ±7.1	91.6 ±5.2	5.3		11.4 ±0.5	224.5
Common ponyfish	61.1 ±6.8	18.8 ±0.8	275.7 ±15.2	24.6	106.3 +14.3	2.7	486.4	2.1 ±0.2	99.3 ±4.6	84.1 ±4.7	14.0		19.4 ±1.8	219.0
Dorab-wolf herring	97.1 ±4.8	10.6 ±1.4	403.5 ±20.1	19.0 ±2.4	138.7 ±17.6		668.9		94 5 ±3.9	132.7 +3.5			20.0 ±1.0	247.2
Double-lined fusilier	113.5 ±5.2	17.2 ±1.6	459.8 ±18.2	29.6 ±3.0	125.7		745.8		111.5 ±4.5				42.3 ±2.1	317.3
Fin-stripe goatfish	54.6 ±3.5		432.1	20.2 ±1.6	143.0 ±4.8		649.9	8.7 +0.6	86.8	240.4 ±6.8	11.1		10.4	357.4
Flying fish	99.4 ±6.8	25.9 ±1.3	544.3 ±31.2	43.7 ±5.4	177.5 ±8.0		890.8	7.6 ±0.3	122.7 ±4.8	380.0 ±11.2	47.0 ±0.2		36.1 ±3.5	593.3
Gold band fusilier	228.3 ±9.4	20.7	815.9 ±25.3	26.7 +2.1	255.4 +17.6		1347.0		216.0 ±8.6	366.9 ±14.0	20.6 ±2.1		58.2 ±3.1	661.7
Hilsa shad	1597.5 ±54.9	58.8 ±8.1	3345.1 ±92.4		843.1 ±45.2		5844.5		1843.8 ±57.3	1060.4 +20.5	71.2 ±1.2		57.9 ±3.4	3033.4
Indian anchovy	48.2 ±5.6	7.7 ±0.8	234.6 ±11.5	11.6 ±0.8	65.3 ±4.8		367.3	2.4 ±0.2	54.1 ±4.6	83.0 ±7.1		2.3	16.7 ±1.6	158.4
Indian mackerel	139.8 ±8.7	3.1 ±0.4	307.2 ±23.4	16 8 ±1.2	102.0 ±7.3		568.9	8.0 ±0.5	181.6 ±8.6	77.8 ±5.7	18.3		9.6	295.2
Indian pillona	84.8 ±5.8		390.0 ±21.8	23.8 ±2.4	131.2 ±11.5		629.8	12.8 ±1.4	82.3 ±6.1	186.5 ±10.2	12.8		14.7 ±0.8	309.1
Indian scad	51.7 ±7.1	9.9 ±0.6	288.0 +17.6	17.6 +1.5	112.4 ±7.6		479.5		55.9 ±2.8	135.1 ±8.2	6.1		12.3	209.3
Largehead hairtail ribbon fish	215.7	24.4	946.5	53.4	309.2		1549.1		266.8 ±32.0	468.0 +24.8			32.9 ±2.1	767.7
Largescaled terapon	115.2 ±7.2	13.0 ±1.1	512.1 ±36.4	31.2 ±2.1	163.6		835.1		188.1 ±5.8	389.9 ±19.4	30.4 ±2.1		32.7 ±2.0	641.0
Long-finned herring	489.3 ±31.2	51.0 ±4.9	2987.9 ±68.2	100 8 ±6.4	671.2 ±23.1		4300.1		735.9 ±16.5	2085.0 ±24.0	72.6 ±4.0		55.7 ±3.8	2949.3
Malabar thryssa	52.6 ±.2	11.4 ±0.7	308.9 ±14.2	17.8 ±1.5	113.8 ±8.0		504.4	1.4	66.5 ±3.9	125.0 ±5.8			16.3 ±1.3	209.3
Moonfish	47.9 ±1.3	3.8 ±0.2	190.6 +5.9	8.5 ±14.2	63.8 ±3.8		314.5		50.8 ±4.8	99.9 ±3.5		2.1	17.5 ±1.5	170.4
Otomebora mullet	211.8 ±2.4	39.1 ±2.3	448.4 ±24.8	18.2 ±1.1	151.4 ±10.2		868.9		273.2 +20.0	120.4 ±4.9			17.1 ±2.1	410.6
Pathamadiya	21.3 ±3.4	4.2 ±0.2	90.2 ±4.2	12.2 ±1.3	52.4		180.2	0.9	26.9	44.7 ±3.7	12.4		5.8	90.7
Pickhandle barracuda	45.9 ±2.3	5.2 ±0.5	265.7 ±7.2	14.6 ±1.3	81.5 +5.6	1.2	412.9		65.0 ±5.9	146.8 ±4.8	6.4		13.4	231.6
Pugnose ponyfish	249.6 ±16.5	28.4 ±1.2	1101.3 ±54.3	78.3 ±5.9	319.7 ±24.1		1777.3		331.9 ±14.2	346.3	15.5		54.6 ±2.8	748.3
Rainbow sardine	178.3 ±14.2	28.2 ±5.3	826.5 ±35.2	79.2 ±5.7	269.0 ±17.6		1381.2	3.3 ±0.2	188.7 ±7.5	236.7 ±7.2			45.0 ±5.0	473.9
Shortnose ponyfish	150.4 ±11.5	36.9 ±2.0	657.4 ±54.9	48.8	204.5 ±5.7	4.0	1097.9		223.3 ±6.8	187.2 ±7.1	11.3		34.1 ±1.4	455.9
Silver sillago	71.8 ±4.6	20.9 ±1.2	584.0 ±36.2	40.9 ±2.4	193.7 ±5.9		911.3		219.3 ±17.2	301.3 ±14.2	55.4 +1.4	13.0	8.6	597.7
Small scaled terapon	144.3 ±5.9		875.8 +57.8	52.7 ±3.5	252.6		1325.5	7.4 ±0.4	303.4	529.2 ±18.6	48.7 ±3.5		21.1 ±1.1	909.8
Smithurst's ponyfish	33.2 ±2.8	9.1 ±0.4	217.5 ±12.4	17.0 ±2.4	88.5 ±5.6		365.2		50.1 ±3.8	105.1 ±5.4	1.9		13.8	170.8
Spotted sardinella	179.8 ±9.2	27.3 ±2.4	728.5 ±24.9	46.8	199.1 ±8.0		1181.6		176.7 ±8.4	258.9 ±9.0			67.1 +2.9	502.7
Streaked spinefoot	296.4	34.2	2136.5	38.0	327.2		2832.3		543.8 ±25.0	703.5 ±14.2	8.3		44.1 ±2.9	1299.6
Toothpony	62.8 ±5.8	10.2 ±0.7	411.8 ±28.0	34.8 ±11.5	164.2 ±10.0		683.7	11.7 +0.4	82.9 ±5.3	180.1 ±8.1			20.3 ±1.2	295.1
Whipfin silver-biddy	56.9 ±3.3	24.4 ±2.0	356.9 ±11.8	22.7 ±2.0	138.1 ±5.6		599.0	3.6	86.8 ±5.9	199.1 ±12.8	42.9 ±3.1		20.5 +1.8	352.9
White sardine	32.1 +4.2	3.7 +0.2	137.4 ±17.6	9.7	47.3 ±2.5		230.3		31.3 ±3.4	31.6 ±2.1	4.2		8.5	75.6
White sardinella	420.9 ±21.3	40.2 ±1.5	1008.4 ±23.8	60.1 ±4 8	265.3 ±8.0		1794.9		386.3 ±14.5	271.6 ±5.9			60.9 ±2.4	718.8
White spotted sprinfoot	32.1 ±2.4	12.7 ±0.6	135.7 ±4.6	17.6	75.4 ±5.0		273.4	2.8	28.0 ±0.9	57.7 ±2.1	9.0		44.7 +2.5	142.2
Yellowstripe scad	66.2 ±5.1	10.3 ±0.8	437.1 +17.2	25.9 ±1.6	147.0 ±4.9		686.5		77.0 ±5.1	191.1 ±12.2	5.7		16.4	290.2

Table 3. Amount of fatty acids present in small pelagics (continued).

(mean ± S.D, n – 4)

Name of fish	Amount of fatty acid (mg / 100g fish)										
	16:2 n-4	18:2 n-6	18:4 n-3	20:2 n-6	20: 4 n-3	20:5 n-3	22:4	22:5 n-3	22:6 n-3	PUFA	n-3 PUFA
Baelama anchovy	10.4	21.1 ±2.0	17.8 ±1.2			136.4 ±8.0		23.5 ±1.1	223.9 ±11.8	433.0	401.5
Bhcstripe herring	38.3 ±1.9	37.6 ±2.3			16.6 ±1.1	340.6 ±21.1		80.5 ±6.9	536.6 ±18.0	1050.1	974.2
Big eye barracuda		6.7	5.8			46.3	3.1	13.8 ±1.0	138.4 ±4.8	214.1	204.3
Blacktip sardinella	17.3 ±2.1	13.4 ±0.8	16.1 ±0.8		5.3	147.0 ±6.1	8.4	27.5 ±2.1	275.1 ±10.0	510.1	470.9
Brushtooth lizardfish	12.9 ±0.8		3.5			76.8	14.8 ±0.8	24.1 ±0.9	257.0 ±13.2	389.1	361.4
Buccaneer anchovy		3.8	8.9			66.0 ±3.8		13.3 ±0.6	130.6 ±6.8	222.5	218.7
Commerson's anchovy	11.0	16.9 ±0.7	27.9 ±0.8		2.6	97.2 ±5.7		14.9 ±0.8	214.1 ±17.2	384.5	356.6
Common ponyfish	17.8 ±0.2	6.0	14.4		3.1	101.1 ±3.8	16.5 ±1.1	25.2 ±1.4	153.1 ±5.7	337.2	296.8
Dorab-wolf herring	0.0	17.7 ±1.2	16.3 ±1.3			117.4 ±6.8	8.9 ±0.1	23.0 ±1.0	361.2 ±13.1	544.4	517.9
Double-lined fusilier	22.6 ±2.7	15.0 ±1.0	26.0 ±1.9		10.7 ±0.8	161.1 ±8.1	13.9 ±1.0	23.5 ±1.3	359.8 ±14.1	632.6	581.1
Fin-stripe goatfish	19.4 ±1.8	18.7 ±1.0	14.7 ±0.8			113.3 ±2.1		24.2 ±1.2	188.8 ±7.1	379.1	341.0
Flying fish	17.3 ±1.1	30.8 ±2.1			11.4 ±0.3	120.9 ±3.5	36.1 ±2.1	60.3 ±4.3	342.3 ±19.8	619.0	534.8
Gold band fusilier	16.4 ±1.5	26.0 ±1.2	33.0 ±2.0		16.4 ±1.0	209.2 ±7.8	14.1 ±1.2	74.0 ±4.8	457.9 ±10.2	846.9	790.5
Hilsa shad	124.7 ±5.9	60.7 ±6.4	184.9 ±13.2	31.9 ±2.5	37.1 ±2.5	1753.5 ±32.1	88.7 ±7.5	178.3 ±8.7	850.9 ±21.3	3310.7	3004.7
Indian anchovy	1.9	8.0	10.1		3.5	89.5	15.4 ±0.8	12.8	160.4 ±6.4	301.6	276.4
Indian mackerel	20.3 ±2.1	6.1				136.2 ±6.0		20.9 ±1.0	161.9 ±6.8	345.4	318.9
Indian pillona	13.9 ±0.9	10.3	11.8		4.3	109.5 ±8.0		24.3 ±1.1	187.8 ±11.8	361.8	337.6
Indian scad	2.8	15.1 ±1.2	15.8 ±0.7		3.1	114.9 ±7.8	5.1	25.9 ±1.3	216.4 ±11.2	399.0	376.0
Largehead hairtail ribbon fish					14.5 ±1.4	182.4 ±6.5	13.5 ±0.6	79.0 ±9.6	499.3 ±18.2	788.7	775.2
Largescaled terapon	23.5 ±1.7	35.4 ±2.1			6.8	109.8 ±2.7	24.3 ±1.3	58.2 ±5.8	280.3 ±17.2	538.2	455.0
Long-finned herring	55.3 ±3.2	99.8 ±6.7			32.5 ±1.5	448.6 ±26.2	58.6 ±5.1	223.5 ±15.6	768.6 ±14.2	1686.9	1473.1
Malabar thryssa	18.0 ±1.5	18.1 ±1.4	1.2	5.4	2.7	75.2 ±4.5	5.3	13.7	128.6 ±5.1	268.3	221.4
Moonfish		6.4	1.5		4.9	69.8 ±4.5	8.8 ±0.2	21.9 ±1.2	145.7 ±8.7	259.0	243.9
Otomebora mullet	72.2 ±5.1	28.6 ±1.7	49.3 ±3.1	5.4	13.8 ±0.8	240.4 ±8.0	22.1 ±1.1	42.7 ±2.6	143.6 ±9.1	618.0	489.7
Pathamadiya	8.4				2.7	34.6 ±3.4	10.3	18.2 ±0.9	42.2 ±2.8	116.5	97.8
Pickhandle barracuda	9.4	11.3	6.6		1.1	59.6	4.2	15.4 ±0.7	179.7 ±9.8	287.2	262.4
Pugnose ponyfish	42.5 ±0.8	43.4 ±2.5	26.9 ±1.5		14.6 ±0.6	368.4 ±13.5	25.1	66.6 ±3.4	589.8 ±32.1	1177.3	1066.3
Rainbow sardine	25.2 ±0.8	30.9 ±3.1	50.2 ±3.1		6.6	266.8 ±14.2	8.2	35.2 ±3.1	550.2 ±15.6	973.3	909.0
Shortnose ponyfish	35.4 ±2.8	18.1 ±1.0	29.2 ±1.8		9.3	229.9 ±4.8	20.0 ±1.3	54.3 ±3.1	338.6 ±8.1	734.8	661.3
Silver sillago	18.2 ±1.4	33.2 ±2.1				53.2	94.1 ±5.8	42.5 ±4.1	48.7 ±3.9	289.8	144.3
Small scaled terapon	35.3 ±2.5	36.8 ±1.8			5.7	208.4 ±13.2	27.6 ±0	55.4 ±3.8	147.1 ±12.2	516.3	416.7
Smithurst's ponyfish	0.0	4.7	5.0		1.5	52.7 ±3.5	2.4	20.7 ±1.0	161.3 ±14.2	248.3	241.2
Spotted sardinella	35.8 ±2.1	23.8 ±1.4	41.2 ±3.4		16.9 ±1.0	255.2 ±10.2	22.0 ±1.3	37.2 ±3.1	570.0 ±8.2	1002.2	920.6
Streaked spinefoot	35.8 ±2.5	130.0 ±7.5			18.2 ±1.3	209.9 ±4.8	35.3 ±1.0	246.8 ±11.5	211.0 ±6.8	887.0	685.9
Toothpony	33.9 ±1.2	17.1 ±1.4				120.8 ±7.5	3.9	37.6 ±5.0	357.5 ±16.5	570.8	515.9
Whipfin silver-biddy	6.0	18.9 ±2.1		3.9	2.3	51.4 ±5.8	21.5 ±0.8	38.6 ±2.4	95.5 ±78	238.2	187.9
White sardine		1.7	6.5		2.2	47.0 ±4.8	4.1	11.9 ±0.7	91.8 ±6.5	165.2	159.4
White sardinella	52.2 ±3.2	41.2 ±4.5	101.1 ±6.1		18.2 ±1.8	453.5 ±15.4	33.9 ±3.5	70.3 ±3.4	648.8 ±14.1	1419.2	1291.8
White spotted sprinfoot	8.9	6.0		2.0	3.5	12.4	10.2 ±0.3	25.3 ±1.2	79.5 ±5.8	147.8	120.6
Yellowstripe scad	0.	10.2 ±0.5	14.4 ±0.5		4.2	190.8 ±11.0	11.3 ±1.1	48.1 ±3.1	321.5 ±9.8	600.6	579.0

INFLUENCE OF EXTRACTION METHODS ON QUALITY OF SHARK LIVER OILS

by

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ABSTRACT

Shark livers are considered as waste material at most landing sites in Sri Lanka, but they can be utilized as raw material for extraction of fish oil which in turn is useful for medicinal and food purposes. This study evaluated oil extraction methods namely, steam rendering, acid silage, wet rendering, incubation at 30°C and alkali digestion with 2% NaOH at 80°C of silky shark (*Charcarinus falciformis*) livers. Observations were made on the yield, free fatty acid (FFA) content, peroxide value (PV) and thio-barbaturic acid value (TBAV) of the extracts. Results were compared with the oil extracted using the Bligh and Dyer method.

The highest oil yield (44%) was reported by the silage method and this value was not significantly different ($p<0.05$) when compared with that from incubation (43.7%) and steam rendering (41%). The lowest oil yield (25.5%) was obtained from alkali digestion and the value obtained (33.1%) from wet rendering was intermediate. The lowest free fatty acid value (0.04%) was recorded by the alkali digestion and this value was not significantly different ($p<0.05$) when compared with that from wet rendering (0.12%), steam rendering (0.2%) and incubation (0.6%). However, silage gave the highest free fatty acid value (4.2%). Based on the oil yield and the quality, it could be suggested that extraction of oil using steam rendering and silage are suitable for introduction as a small scale industry for coastal communities in Sri Lanka.

INTRODUCTION

In 1997 total marine fish production in Sri Lanka was 248 000mt (Anon., 1998). Sharks contributed 13% to the total. They are a valuable fish as almost every part of the body can be utilized. However, shark livers are presently wasted. Livers contain 30 -75% of oil which is rich in omega -3 poly-unsaturated fatty acids (Jayasinghe *et al.*, 1998). Fish liver oil with its high vitamin A and D levels has been used to prevent night blindness and rickets (Hall, 1992). Liver oil is used in medicinal, food and industrial purposes. Oil composition varies considerably with a number of factors such as species, age, sex and season (Jayasinghe *et al.*, 1998). Different methods are used to extract oils from shark livers (Tanikawa, 1971; Govindan, 1985). The suitability of the extraction methods for a cottage industry has not been assessed. The aim of this study was to compare the available liver oil extraction methods to find out a suitable method for the rural community.

EXPERIMENTAL

Assessment of liver oil extraction techniques

Different oil extraction methods such as acid silage, wet rendering, alkali digestion, steam rendering, incubation and Bligh and Dyer (1959) methods were carried out. Silky shark (*C. falciformis)* liver was minced using a blender (Sumeet, India). Minced liver was divided into 100g portions and the following techniques were applied to extract oil. Yield of oil was measured and quality of oil was assessed by determination of free fatty acid value (AOCS 1992), peroxide value (AOCS, 1992) and thio barbaturic acid value (AOCS, 1990) a few hours after extraction.

Extraction techniques

All methods were tried out in triplicate.

(a) Bligh and Dyer method (control)

The quantity and quality of oil extracted using the Bligh and Dyer method (1959) was used as a control to compare the efficiency of other extraction methods.

(b) Acid silage method (Jayawardena *et al*, 1980).

Added 3.5% (w/w) formic acid to 100 g minced liver and mixed thoroughly for 10 min. Mixtures were kept for 24 hours at room temperature (28±1°C) and the oil was separated by centrifugation (MISTRAL 2000) at 2000 rpm for 10 minutes.

(c) Wet rendering method

Minced liver was mixed with water (20%) and boiled for 30 minutes. The oil was separated by centrifugation.

(d) Alkali digestion method

Added 10% water to liver mince and digested at 40-50°C until liver in liquefied. Then added 2% sodium hydroxide and continued heating at 80°C with pH adjusted to 9. Washed excess alkali with warm water and the oil was separated by centrifugation.

(e) Steam rendering at 95°C for 30 minutes.

Minced liver was placed in a round bottom flask (1000ml) and steam passed through the sample for 2 hrs. The resulting mixture was centrifuged at 2000 rpm for 10 minutes to obtain oil.

(f) Incubation at 30°C for 48 hours

Liver mince was kept in an incubator (Blue, USA.) at 30°C for 48 hours and the oil was separated by centrifugation.

Statistical Analysis (Zar, 1984)

A one-way analysis of variance (ANOVA) with replicates was carried out independently to study the quality of shark liver oil. Significance was accepted at a probability of 5% or less. Bonferroni's Multiple Comparison was used to identify the means, which were significantly different. All means were presented with their standard errors (SEM).

Results:

Table 1. Analysis of crude silky shark liver oil extracted with different techniques.

Parameter	Control (Bligh & Dyer)	Silage	Wet rendering	Alkali digestion	Steam rendering	Incubation
Yield %	63.3 ± 1.3^{1}	$44.3^{a}\pm1.0$	$33.1^{b}\pm0.8$	$25.5^{e}\pm0.7$	$41.7^{a}\pm0.8$	$43.7^{a}\pm0.9$
FFA %	0.6 ± 0.1	$4.2^{a}\pm0.1$	$0.1^{b}\pm0.0$	$0.04^{b}\pm0.0$	$0.2^{b}\pm0.0$	$0.6^{b}\pm0.0$
Peroxide (meq peroxide/g)	1.7 ± 0.0	$6.1^{b}\pm0.1$	$9.1^{a}\pm0.2$	$9.7^{a}\pm0.2$	$1.7^{c}\pm0.0$	$0.02^{d}\pm0.0$
Thio barbaturic acid value	-	$304.6^{bc}\pm5.7$	$245.3^{d}\pm5.8$	$620^{a}\pm10.0$	$274.3^{cd}\pm6.6$	$335.1^{b}\pm8.6$
Refractive index	-	1.48	1.48	1.48	1.48	1.48

[1] *Values in the same row not sharing the same superscript letters differed significantly ($p<0.05$), when analyzed by the Bonferroni's test.*

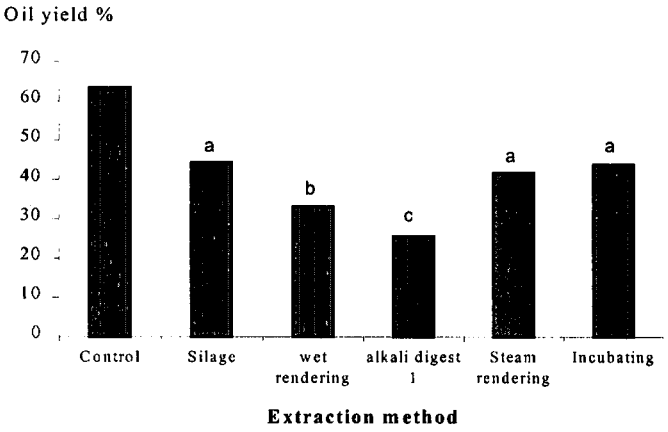

Fig. 1. Oil yields of different extraction methods.

Results of the analysis of oil, using different extraction techniques, are presented in table 1. Control (chloroform -methanol extraction method) recorded the highest oil yield 63.3±1.3%. The value given by acid silage method (44.3±1.0) was not significantly different ($p<0.05$) when compared with incubation (43.7±0.9) and steam rendering methods (41.7±0.8). Lowest oil yield was reported from alkali digestion method (25.5±0.7). Wet rendering method showed an intermediate value (33.1±0.8) (Table 1 and Figure1).

The lowest free fatty acid value was recorded from oil recovered by alkali digestion (0.04%) and this value was not significantly different ($P<0.05$) when compared to wet rendering (0.12%), steam rendering (0.18%) and incubation (0.58%). However, silage recorded the significantly highest (4.2%) free fatty acid value compared to other methods used in the experiment. Extraction methods where heat was applied such as wet rendering, alkali digestion, steam rendering and incubation showed low FFA values compared to non heating methods (Fig.2).

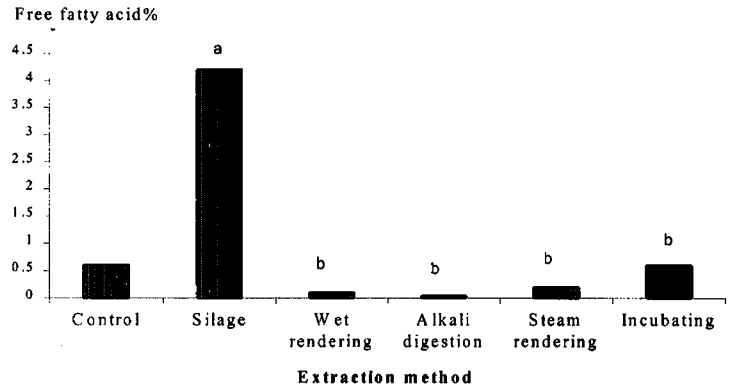

Fig. 2. Free fatty acid values of oils from different extraction methods.

Significantly ($p<0.05$) lower peroxide values were recorded by incubation 0.02 (meq peroxides/g) compared to other methods described in this experiment (Fig. 3). The highest peroxide value 9.7 (meq peroxides/g) was shown by alkali digestion and this value was not significantly ($P<0.05$) different with compared to the value (9.1 meq peroxides/g) given by wet rendering. Steam rendering and silage showed intermediate values 1.7 and 6.1 (meq peroxides/g) respectively.

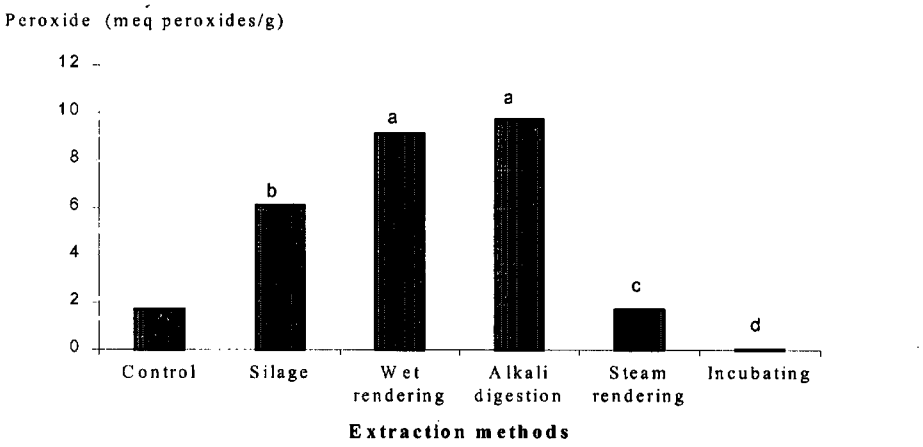

Fig. 3. Peroxide values of oils from different extraction methods.

Denaturing of peroxides was measured by thio barbaturic acid value. The lowest TBA value was shown by oil recovered from wet rendering (245) but this value was not significantly different ($p<0.05$) from oil recovered by steam rendering (274) (Fig.4). Significantly highest ($P<0.05$) TBA values were reported by alkali digestion (620) compared to other methods used in this experiment. However, silage and incubation showed intermediate values (304 and 335).

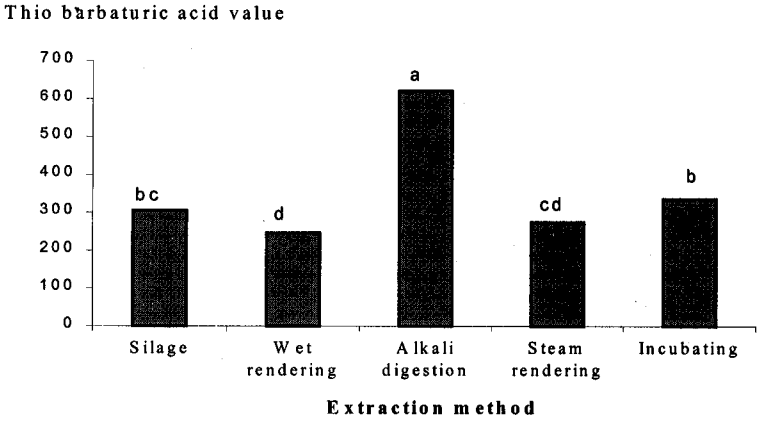

Fig. 4. Thiobarbaturic acid values of oil from different extraction methods.

Refractive index values of recovered oil were identical in all treatments. It showed that the density of the oil does not depend on the procedures, which were used to extract oil.

DISCUSSION

In this study chloroform - methanol extraction recorded the highest oil yield. These results are accordance with the Sunarya *et al.*, (1991), who reported that the oil yields from Bligh and Dyer (chloroform-nethanol extraction) and Soxhlet extraction were similar and higher than from steaming. As the organic chemicals have been used in the extraction process, this method is not recommended to extract oil for food and feed purposes. Acid silage, incubating and steam rendering method recorded the second highest oil yield. It has been explained that steaming results in the thermal rupture of the liver cells and so releases the oil. But, the oil, which is more closely held by the proteinaceous liver tissues, is not released under these conditions (Sunarya *et al.*, 1991; Hall, 1992). If fish oils are obtained from livers by means of wet rendering, a large quantity of vitamin A would remain in the residues left after pressing (Tanikawa, 1971; Govindan 1985). They also described that the amount of oil left in the screening or pressing is 10-30% of the total amount of oil in the raw livers. Therefore, the processing of vitamin rich oil by wet rendering is not recommended. Long processing procedures may also result in low oil yield (Tanikawa, 1971). Oil yield from alkali digestion in this study confirmed the above statement. However, it has been reported that the yield of vitamin A secured by alkali digestion is 70 - 95% of that in raw livers (Tanikawa, 1971).

The highest free fatty acid percentage in oil was recorded from acid ensilage. This may be due to an addition of acid in the processing technique. However, results on the free fatty acid content of the recovered oil suggested that heat treatments might produce low FFA values. A similar observation has been made by Presten (1986) who reported that the oil recovered by heat rendering gives a low free fatty acid and the resulting crude oil is non perishable. Results of the present study confirmed the findings of Summers *et al* (1991). The author demonstrated that the oil produced by heat treatment has a very low percentage of free fatty acids, and no peroxides. The absence of oxidizing peroxides may be advantageous to the quality of the crude oil during long-term storage. Furthermore, Presten (1986) explained that oil obtained by heat rendering could be stored for a long period under tropical conditions without subsequent loss of quality.

Autoxidation and oxidation of oil result in the formation of malonaldehyde, the development of which can be monitored analytically by the determination of thio barbaturic acid value (Summers *et al*, 1992). The highest thio- barbaturic acid value was recorded by alkali digestion and it may be due to the involvement of

alkali in the processing. Steam rendering showed low oil oxidation. However Gopakumar and Thankappan (1986) suggested that the oil should be recovered by steam rendering and protected from atmospheric oxygen by continuous flushing with nitrogen.

According to the cost estimates of the present study it is suggested that steaming and silage methods could be used to extract shark liver oil for food and feed purposes. The present experiment supports the suggestions of Sunarya *et al.*, (1991) that oil extraction by steaming is easier, cheaper, quicker and can probably be introduced to rural communities. It has been reported that solvent extraction methods are not employed for the preparation of vitamin A oils from fish livers, because the equipment is expensive and the recovery of the solvent is not satisfactory (Tanikawa, 1971). Hall (1992) observed that direct steaming at 80-85°C is a simple and economical technique that involves direct steaming. Some procedures require temperature of 70 -75°C. Summers *et al* (1991) reported that the cheapest way of liver oil refining was to ensile the livers with a mixture of formic and phosphoric acids. The silage product is a stable liquid with a malty odour, which has very good storage characteristics. It is a simple process and requires a little capital investment, particularly if non-oily fish are used. The advantage of ensilage for recovering oil is that the neutralized liver slurry can be used as a stock feed or fertilizer (Summers *et al*, 1991).

The centrifuged oil probably contains suspended soap or excess alkali. Therefore, further centrifugation is necessary after washing with warm water (Tanikawa, 1971). The defect of the method is the loss of vitamin A and the decomposing and dissolving of water soluble vitamins which are contained in fish livers besides vitamin A (Tanikawa, 1971). Therefore, it can be assumed that the method of alkali digestion (10% water & 2% NaOH) is not suitable for high quality oil extraction. Kreuzer and Ahmed (1978) have reported contrary results. They suggested that oil extraction by alkali digestion, the addition of 2% (w/v) sodium hydroxide and heating at 80°C for 30 to 45 minutes was very effective.

Silage also can be used in more technologically advanced fisheries. For example, the livers can be ensiled at sea or at the shore at the time of separating livers from the sharks and then subjected to oil extraction (Windsor and Barlow, 1981). Clucas and Sutcliffe (1981) reported that livers with high oil content are usually steamed (85°C) or indirectly heated at 71°C.

Livers with low oil content are treated with alkali, alkali/enzyme digestion and solvent extraction. Excessive heating must be avoided. Since vitamin A is inactivated by light, the oil must be stored in the dark. Hall (1992) recorded that the current level of alkali is important to prevent saponification of fat, and vitamin A absorption in the soap fraction. However, the result of this study suggests that steam rendering and ensilage methods can be used to recover liver oil for food and feed purposes.

Based on the quality and yield of liver oil, it is suggested that steam rendering and silage methods are suitable for extraction of liver oil. This study opens an avenue for a small- scale liver oil production industry among coastal communities in Sri Lanka.

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ASSESSMENT OF NUTRITIONAL VALUE OF PROCESSED SHARK FINS IN DIFFERENT SPECIES

by

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ABSTRACT

The aim of this study was to introduce a method for processing of shark fins and to assess the nutritional value of processed fins belonging to different shark species. Complete sets of fins of silky shark (*Carcharinus falciformis*), hammerhead shark (*Sphyrina lewini*), blue shark (*Prionace glauca*), whitetip shark (*C. longimanus*) and thresher shark (*Alopas pelagicus*) were processed using 3% acetic acid (food grade) and the total yield by weight was determined. Dry matter, ash, protein, fat, glycogen, non-protein nitrogen, amino acid profile and calorific value of processed fins were studied.

Significantly higher ($p<0.05$) processed fin yield (17.6%) was recorded by hammerhead shark compared to the other four species used in this study. Thresher shark gave significantly lower ($p<0.05$) fin yield (2%). Silky, white tip and blue sharks showed intermediate values (14, 13.5 and 12.7%) respectively. Moisture content of fins varied between 10.3-13%. Blue shark fins recorded the lowest ash content (0.16%). However, ash content varied between 1- 0.16%. Fat content in the fins of all shark species recorded negligible values. White tip shark recorded the highest nitrogen content (14.8%) and it was not significantly different ($p<0.05$) with that of hammerhead shark (14.4%), thresher shark (14.4%) and silky shark (14.2%). Significantly lowest nitrogen content was recorded in blue shark (13.4%) compared to other species. Non-protein nitrogen content was varying between 0.13 – 0.52%. The lowest glycogen content was recorded by thresher shark (0.33%) while the highest value was recorded by hammerhead shark (0.87%).

The highest total amino acid content was recorded by fins of blue shark (8795 μmoles/g) followed by that in hammerhead shark (7525 μmoles/g) and white tip shark (7415 μmoles/g). Significantly ($P<0.05$) a higher percentage of essential amino acid (of total amino acid) was obtained from white tip shark (40.2%) compared to other species. Silky shark showed the lowest value (28.1%) and this value was not significantly different from that of thresher (28.3%) and blue sharks (28.5%). Hammerhead shark recorded an intermediate value (33.3%). Thresher shark recorded the highest energy content 5525 cal/g while that of hammerhead was the lowest (4863 cal/g). White tip, blue and silky sharks showed intermediate values (5264, 5142 & 4884 cal/g).

This study demonstrated that hammerhead shark recorded the highest yield of processed fins when the acetic acid processing technique was used. However, the nutritional value of fins of white tip shark was higher compared to other species.

INTRODUCTION

Shark have soft collagen and elastin fibres in their fins which are in great demand as a raw material for food specialities. Dried shark fins are a valuable commercial product, exported from Sri Lanka. Export earnings from dried shark fins showed a rapid growth during the last decade. In 1994, Sri Lanka exported 70 mt of dried shark fins valued at Rs 20 million, and export earnings had increased to Rs 206 million in 1997.

Major markets were Singapore and Hong-Kong where dried fins would be further processed to value-added fin rays.

Amarasooriya (1993) has identified forty-six shark species belonging to five orders and fifteen families in Sri Lanka. According to the species composition there are 6-7 species which commonly occur in the catch. The highest catch was reported by silky shark (61%) followed by blue shark (12.3%), white tip shark (6.3%), hammerhead (5.3%), thresher shark (4.6%) and other requiem shark species (4%) (Joseph, 1997). In Sri Lanka shark fins from all species are accepted for export but fins fetch highly varying prices. The selling price (FOB) of large size (<30cm fin base length) dried silky, white tip and hammerhead shark fins varies between 50-70 US$ per kg. The average selling price of large fins of species such as blue and thresher sharks is 50 US$ per Kg. Medium size wet fins (25-30cm) are sold at 40 US$ per kg while smaller fins are (<25cm) sold at 25 US$ per kg in dry form by the shark fin traders.

Many researchers (Ramachandran and Madhavan, 1974; Jayawardena, 1980; Clucas, 1992; Ka-Keong, 1983; Govindan, 1985) have reported shark fin processing technologies for extraction of fin rays. However, literature on the nutritional value of fin rays related to different shark species is not available. Therefore, this study was conducted to improve and introduce a method for the preparation of processed fins and assess the yield and nutritional value of the fins from different shark species in Sri Lanka.

EXPERIMENTAL

A complete set of fins from a shark which consists of lower lobe of tail fin, two pectoral fins, 1st dorsal fin, 2nd dorsal fin, anal fin and pelvic fins (Fig.1) were used in this study.

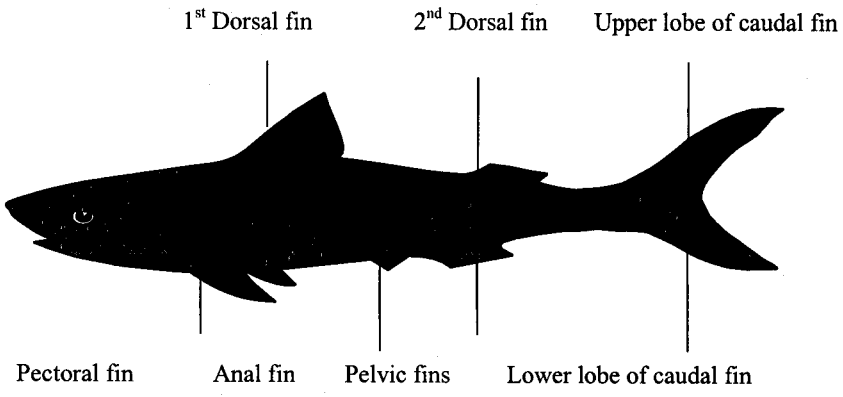
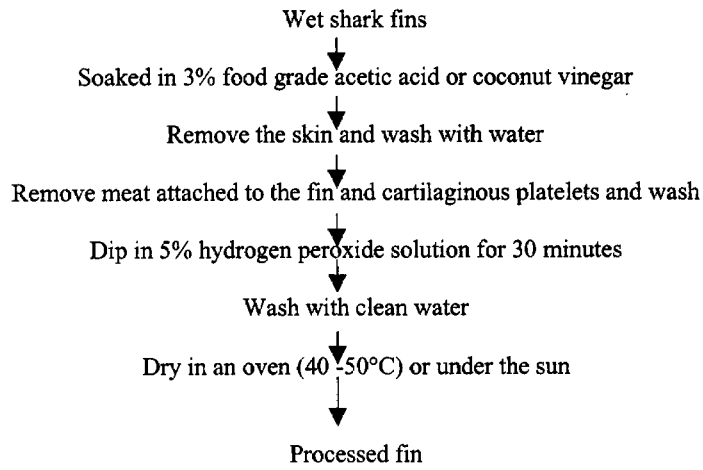


Fig. 1. Lateral view of a shark.

Complete sets of fins of silky shark (*Carcharinus falciformis*), hammerhead shark (*Sphyrina lewini*), blue shark *(Prionace glauca)*, whitetip shark (*C. longimanus*) and thresher shark (*Alopias pelagicus*) were processed using 3% acetic acid.

Flow chart for processing of shark fins



Total yield of processed fins by weight, moisture, ash (AOAC, 1980), total nitrogen, non-protein nitrogen (Kjeldhal method), fat (Soxhlet method) and acid insoluble ash were determined. Glycogen content was measured according to Giese (1967). Calorie values of processed fins of different shark species were obtained by using a bomb calorie meter (Shimadzu CA-4P, Japan).

Amino acid composition was determined by the Pico-tag method (Cohen *et al.*, 1989).

Data on yield and chemical composition were analysed by a one way ANOVA. Significance was accepted at a probability of 5% or less. Bonferroni's multiple comparison was used to identify the means which were significantly different from each other (Zar, 1984).

RESULTS

Colour of the processed fins of all shark species varied from colourless to light yellow. Yield of the processed dry fins is shown in Fig.2. Significantly higher ($p<0.05$) processed fin yield (17.6%) was recorded by hammerhead shark compared to other four species used in this study. Thresher shark gave significantly lower ($p<0.05$) yield (2%) while silky, white tip and blue sharks reported intermediate yields 14, 13.5 and 12.7% respectively.

Yields of processed fins (dried) of different fin types on a dry basis are shown in Fig.3. Highest fin yield among pectoral fins was recorded by silky sharks (13.7%) followed by white tip (12.5%) and hammerhead (10.6%) sharks. Among the first dorsal fins hammerhead sharks gave the highest fin yield (28.5%) while thresher sharks gave the lowest yield. Second dorsal fins of white tip sharks produced the highest fin yield (25.2%) among the five species. Lower lobe fins of silky sharks showed highest yield (28.0%) followed by blue (27.8%) and hammerhead (25.5%) sharks. Anal fins of whitetip sharks produced the highest fin yield (26.9%) out of five species. All fins of thresher sharks recorded the lowest fin yield compared with other shark species.

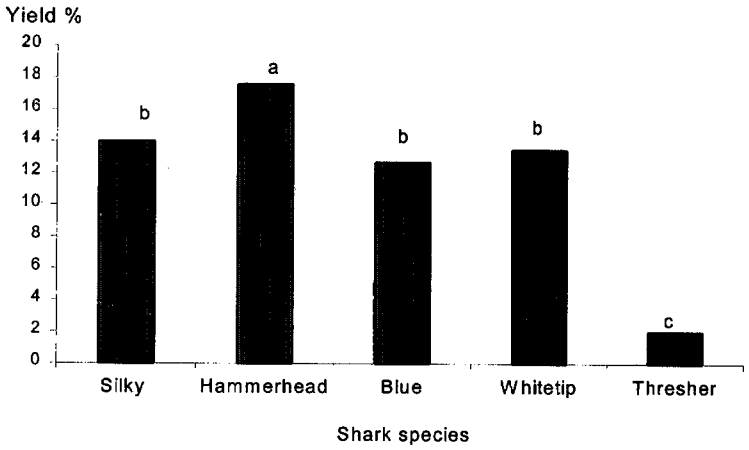


Fig 2. Fin yield % of processed fins of different shark species.

Proximate composition of processed fins are shown in Table 1. Significantly ($p<0.05$) lower moisture content in white tip shark (10.3%) compared to the other species used in the study. The highest moisture content was in hammerhead shark (13%) and this was significantly different from blue, silky and thresher sharks.

Table 1. Proximate composition of fins of different shark species.

Parameter	Silky Shark	Hammerhead shark	Blue shark	Oceanic White tip shark	Thresher shark
Moisture %	11.3^{b} *	13^{a}	11.1^{b}	10.3^{b}	11.7^{b}
Ash %	$0.5^{b}\pm.00$	$1.0^{a}\pm.22$	$0.16^{bc}\pm.01$	$0.2^{bc}\pm.02$	$0.4^{b}\pm.03$
Total nitrogen %	14.2^{a}	14.4^{a}	13.4^{b}	14.8^{a}	14.4^{a}
Non protein nitrogen %	$0.46^{ab}\pm.01$	$0.34^{b}\pm.0$	$0.33^{b}\pm.0$	$0.52^{a}\pm.05$	$0.13^{c}\pm.02$
Fat %	Nil	Nil	Nil	Nil	Nil
Acid insoluble ash %	Nil	Nil	Nil	Nil	Nil
Glycogen %	$0.73^{a}\pm.02$	$0.87^{a}\pm.07$	$0.78^{a}\pm.1$	$0.65^{a}\pm.13$	$0.33^{b}\pm.10$

* *Values in the same row not sharing the same superscript letters difference significantly ($p<0.05$), when analysed by the Bonferroni test. All means were presented with their standard deviations (SD).*

Ash content of all processed shark fins showed very low values, i.e. blue shark fins recorded the lowest ash content (0.16%). The highest value was recorded by hammerhead shark (1%). Acid insoluble ash content and fat content of all shark fins were negligible.

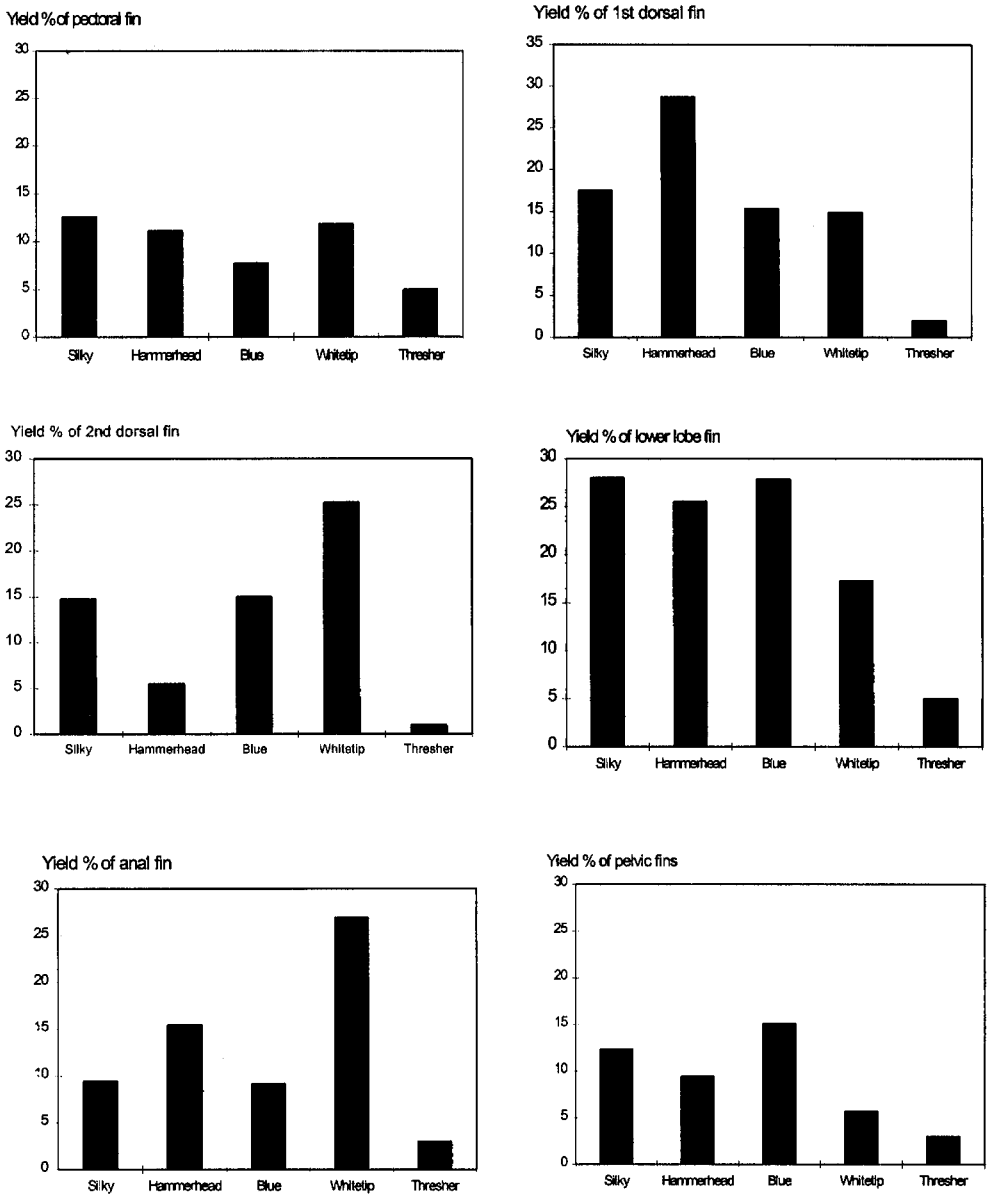


Fig. 3. Fin (wet raw fin → processed dry fin) yield % of different shark species.

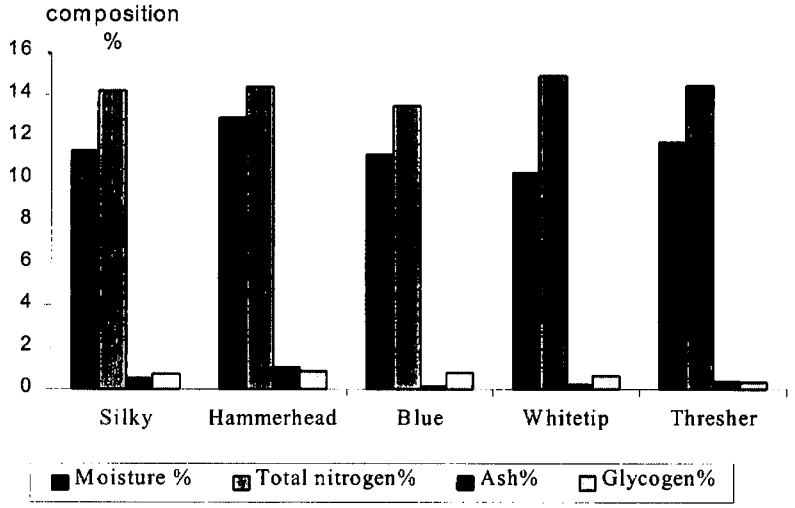


Fig. 4. Proximate composition of processed fins of different shark species.

Similar values for total nitrogen content were recorded by white tip shark (14.8%), hammerhead shark (14.4%), thresher shark (14.4%) and silky shark (14.2%) (Fig.4). The lowest nitrogen content was reported by blue shark (13.4%) ($p<0.05$) compared to other species used in the study. Non-protein nitrogen contents of all shark species varied between 0.13 and 0.52%. The lowest non protein nitrogen content was reported by thresher shark (0.13%) ($p<0.05$) compared to the other species.

A significantly higher glycogen percentages were recorded by hammerhead shark (0.87%) and blue shark (0.78%), silky shark (0.73%) and white tip shark (0.65%). The lowest value was recorded from thresher shark (0.33%) compared to the other species.

Thresher shark recorded the highest energy content of 5525 cal/g while that of hammerhead was the lowest (4863cal/g). Whitetip, blue and silky sharks showed intermediate values (5264, 5142 & 4884 cal/g) (Fig.5).

The highest total amino acid content was observed in blue shark (8795 µmol/g) followed by hammerhead shark (7525 µmol/g) and white tip shark (7415 µmol/g) (Table 2). The highest total essential amino acid content was recorded in white tip shark (2125 µmol/g) followed by that in blue (1950µmol/g) and hammerhead (1880µmol/g) sharks. A higher content of essential amino acids as a percentage of total amino acids was found in oceanic white tip shark (40.2%) ($P<0.05$). Silky shark showed the lowest value (28.1%) and this value was not significantly different from that of thresher (28.3%) and blue sharks (28.5%). Hammerhead shark recorded an intermediate value (33.3%).

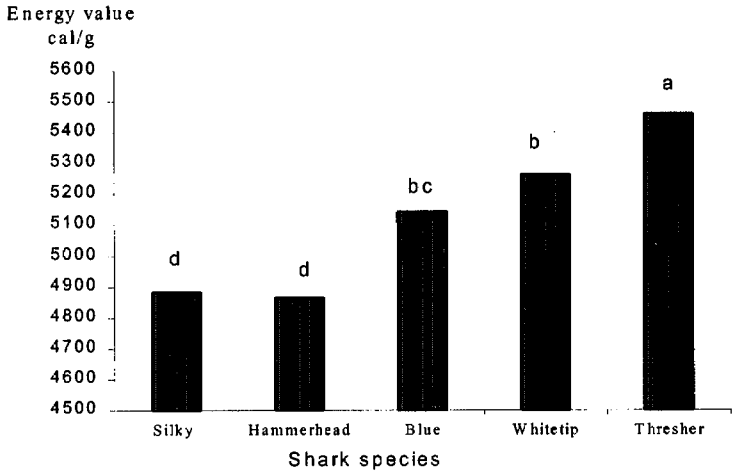


Fig. 5. Calorific value of processed fins of different shark species.

Table 2. Amino acid content of processed fins of different shark species.

Amino acid	Concentration μ mols/g				
	Silky shark	Hammerhead shark	Blue shark	Whitetip shark	Thresher shark
Aspartic acid	145	340	320	585	125
Glutamic acid	195	550	430	705	185
Serine	190	195	330	35	230
Glycine	2115	1845	2885	2095	2150
Histidine	240	325	295	260	255
Arginine + Threonine	435	535	595	630	455
Alanine	640	800	900	815	610
Proline	1220	1570	1560	550	1320
Tyrosine	260	320	385	390	245
Valine	160	250	240	265	165
Methionine	80	85	105	160	75
Cysteine	50	25	35	115	50
Isoleucine	110	160	150	190	115
Leucine	100	170	140	185	95
Phenylalanine	110	170	145	165	115
Lysine	120	185	280	270	115

*Values in the same raw not sharing the same superscript letters difference significantly ($p<0.05$), when analysed by the Bonferroni test.

DISCUSSION

The extraction technique (3% acetic acid followed by 5% hydrogen peroxide) described in this study was easy to practice and it was very useful to generate self-employment at the small-scale industry level in Sri Lanka. Tsuchiya and Nomura (1953) reported four extraction procedures i.e., water-soaking method, water boiling method, lime water/acetic acid/sodium carbonate treated method and NaOH treated method for the extraction of fin rays. Jayawardana (1980) indicated that dilute HCl solution (1%) could be safely used for quicker and easier extraction of shark fin rays from the fin. However, many authors (Clucas, 1982; Ka-Keong, 1983) recommended a water-boiling method for extraction of shark fin rays. Ka-Keong (1983) described the processing method with five steps, which are preliminary soaking, de-scaling and skinning, removing the meat, removing blood and bleaching and finally drying. Govindan (1985) also described a technique similar to the present study and reported that the proteinaceous matter left behind after separation of the rays can be concentrated to yield a glue with excellent sticking properties.

Acetic acid is useful in softening the outer skin of fin during the soaking period. It also helps to soften the flesh and the rays are separated by hand (Nair and Madhavan, 1974). This softening could be due to the greater hydrolysing effect of the collagenous threads into gelatine (Jayawardena, 1980).

Result showed that the lower lobe of silky, hammerhead and blue sharks contain higher amounts of fin rays than other fin types. Unlike other fin types, the lower lobe consists of many fin rays. The first dorsal, pectoral and upper lobe fins are heavy, but contain fewer fin rays. As these fins are supported by many bone like cartilages, the fins are heavy and produce a low fin yield. Ramachandran and Madavan (1974) showed that only the lowest portion of caudal fin contains ray though it appears bulky.

Concerning the nutritional value of the fin rays and fin yield of the sharks used in the study, the fins of oceanic white tip and hammerhead sharks could be categorized as top grade. However, Ka-Keong (1983) reported that the grading of shark fins depends on their size, thickness and their fin needle content. He also reported that hammerhead, mako and blue shark fins are top grade. White and thresher shark fins are 1st grade while oceanic whitetip and tiger shark fins are 2nd grade. Fins of smaller shark's are in grade three. Pectoral, dorsal and anal fins of sharks are exceptionally tasty and nutritious, and are therefore in great demand in the international markets (Anon, 1967)

However, according to the traditional grading system, fins are sold in a complete set of first dorsal fin, two pectoral fins and a caudal fin (whole tail). The second dorsal, ventral and anal fins and the fins from small sharks are not sold as a set but as mixed fins after processing (Ka-Keong, 1983).

The chemical composition of fin rays showed higher total nitrogen and very low ash content. These results are in accordance with Jayawardena (1980) who reported that the approximate chemical composition of fin rays of *Carcharhinus sp. and Sphyrna sp.* showed a very high crude protein content (82%), very low ash content (0.23%) (the balance may be moisture and carbohydrates). In whale shark (*Rhiniodon typus smith*), the total nitrogen content is about 15-16% (dry basis), ash content is in the range of 0.5 to 0.9% and no acid insoluble ash (Ramachandran and Sankar, 1990). This shows that nitrogen compounds are major constituents of fin rays. Tsuchiya and Nomura (1953) observed the materials used in different methods and showed that there were no remarkable differences of moisture, ash and total nitrogen content in different fin types.

Amino acid composition of the shark fin rays showed that one-third of the amino acids are essential amino acids. These results are contradictory with Jayawardena (1980) who reported that due to lack of essential amino acids shark fin rays have no food value. Shark fins are commercially valuable because of the gelatine they contain (Anon, 1975). They are used in Asia for shark fin soup, a delicacy that fetches up to US$ 150 per bowl (Lemonick, 1997).

The market for shark fins is incredibly profitable. Sharks have a primitive but highly immune system, which may play an important role. For some reason, sharks rarely get tumours, a surprising fact that could lead to new cancer treatments (Lemonick, 1997).

CONCLUSIONS

A low-cost and convenient acetic acid/Vinegar processing technique can be introduced as a cottage industry. The highest yield of processed fins is from hammerhead shark and the lowest, thresher shark. All the species as well as the types of fins are nutritious; highest nutritive value is found in white tip shark and thresher shark.

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STUDIES ON THE EXTRACTION OF PHOSPHOLIPIDS FROM MUSSEL

by

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ABSTRACT

Mussels contained 18% lipid of which about 25% was phospholipid. The extraction method involved precipitation from a dissolved protein solution by chitosan.

INTRODUCTION

Mussel is an economic resource in China with annual catches exceeding 500,000 tons, but only a small part is sold for human consumption. Most is utilized as prawn and other animal feed. If the phospholipids in mussel are extracted and the proteins are completely utilized, mussel will have a new potential use. Phospholipids are the focus of attention, not only as an emulsifier in food but also as bioactive compounds in pharmacological products [Szuhaj, 1983]. Marine resources are rich in phospholipids and will be as good a source as soybean and eggyolk.

Squid contains 2.5% lipid, of which approximately 75% is phospholipid (Koning, 1993). The total lipid content in lancelet is 1.7% of wet weight, and the phospholipids content up to 79.29% of total lipids (Svetashev, 1994). Mussel is also rich in phospholipids which oxidizes more easily than the non-polar lipids during storage (Xue, 1995), as the phospholipids contain more polyunsaturated fatty acids. This is the biggest difference between marine and terrestrial life.

There are many methods of extracting phospholipids including cloroform-methanol (Folch) and dichloromethane-ethanol (Kwan, 1991) which extracts all lipids. The phospholipids are then precipitated with acetone. The composition of phospholipids is best determined by HPLC (Zeng, 1994).

MATERIALS AND METHODS

Materials

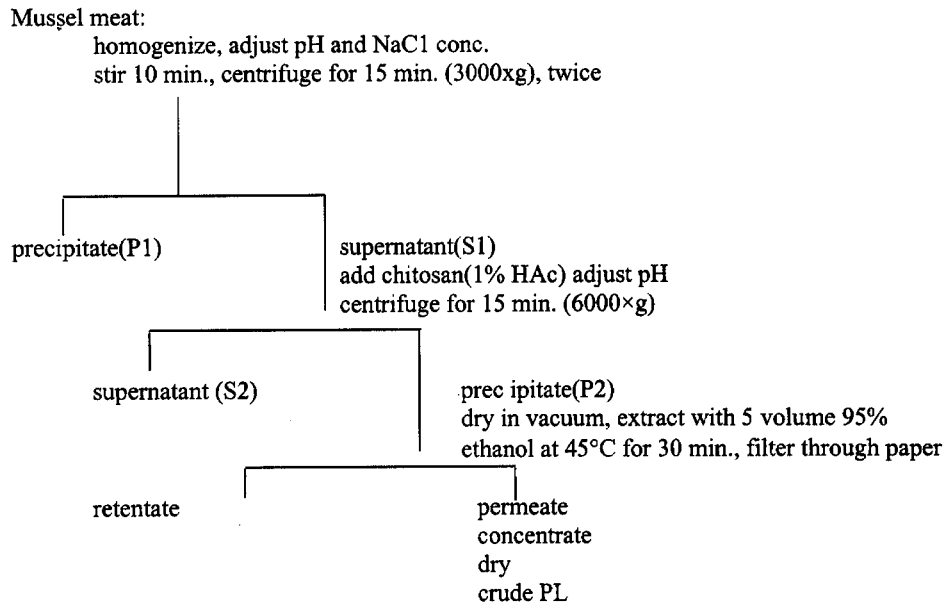
Fresh mussel samples were gathered from Qingdao in September. (Yellow Sa, China).

Determination of phospholipids

Total phospoholipids were estimated by the method of the AOAC, using a factor of 25.8 times inorganic phosphorus content.

Separation of the components was carried out in a column (5 μm, 25 cm×4.6 mm) with silica gel. Acetonitrile: phosphoric acid:methanol (CH_3CN:H_3PO_4:CH_3OH=176:1.5:2) was used as the solvent and the separated components were detected at 205 nm wave length.

Method of extracting



RESULTS AND DISCUSSION

Phospholipids dissolve in polar and non-polar solvents as they have hydrophilic and hydrophobic groups. The solubility can be adjusted according to the system characteristics.

100 g mussels contain 1862.4 mg total lipids and 489.9mg phospholipids. The extraction ratio of phospholipids was 59.2%. After centrifuging, it is difficult to increase the ratio unless the pH and NaCl conc. were adjusted (Table 1).

Table 1. The components of phospholipids in mussel.

	S1	P1	S1+P1
PL(mg/100g)	303.3	186.6	489.9
total lipids(mg/100g)	982.4	880.0	1862.4
Phospholipids/total lipids (%)	30.8	21.2	26.3
PL /total PL(%)	59.2	40.8	100

At pH 3.5 and 8.5, the amounts extracted were respectively 74.4 and 214.6 mg/100g. They were lower than at pH 5.5. If the pH is higher or lower, the phospholipids will decompose, and not dissolve in the supernatant. At pH 5.5 the protein solubility is lowest and proteins were separated from the system.

Table 2. Effect of pH on extracting phospholipids.

pH value	S1(mg/100g)	P1(mg/100g)	S1+P1
3.5	74.4	265.7	340.1
5.5	326.9	152.4	479.3
6.5	307.3	143.9	451.2
8.5	214.6	196.1	410.7

NaCl concentration affects the extraction ratio (Hatta, 1988). Table 3 shows that extraction increased with NaCl concentration, but there was no significant difference. If the NaCl concentration continued to increase, the salt-soluble protein would go into solution.

Table 3. Effect of NaCl concentration on extracting phospholipids.

NaCl (%)	S1(mg/100g)	P1(mg/100g)	Total phosphatidyl cholines	S1/Total phosphatidyl cholines
0.5	264.7	159.9	424.6	62.3
1	290.3	156.5	446.8	64.9
2	297.1	148.8	445.9	66.6
3	314.2	147.1	461.3	68.1
5	319.3	139.1	458.4	69.7

There were no phosphatidyl inositols or lyso-phosphatidyl cholines in the precipitate (Table 4), all remained in the supernatant. However, most of phosphatidyl ethanolamines and half of phosphatidyl cholines were precipitated.

Table 4. The ratio of phospholipids between supernatant and precipitate (mg/100g).

Composition	S1	P1
phosphatidyl inositols	12.2	-
phosphatidyl serines	3.2	7.6
phosphatidyl ethanolamines	85.8	178.2
phosphatidyl cholines	148.2	134.8
lyso-phosphatidyl	101.2	-
Total	350.6	320.6

Phospholipid was precipitated by chitosan addition to the supernatant and the precipitate was dried under vacuum and extracted.

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REGULAR CHANGES OF FREE AMINO ACID AND TAURINE DURING OYSTER FRESHNESS PRESERVATION

by

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ABSTRACT

Changes of various free amino acids in *Ostrea rivularis*, *Ostrea plicatula* and *Ostrea gigas* were followed at different storage temperatures. The experimental results show some common features. The content of various free amino acids decreased slightly during early storage and then increased irregularly. During storage, changes in the content of various free amino acids are closely associated with the R-group polar of the amino acids. Increase of the non-polar R-group amino acids is more than polar R-group amino acids with or without electric charge. The higher the storage temperature the faster the increase. Taurine content decreased a little during 15 days storage in *Ostrea rivularis*, and *Ostrea plicatula*, but increased a little in *Ostrea gigas*.

INTRODUCTION

Oyster is an aquatic animal of high (Tan Guili *et al.*, 1993) nutritive value. The nutritive components in the cells not only regulate osmotic pressure, but also give the oyster its special taste. According to the reports (Shen Yuyun, 1986) changes in contents of free amino acids and taurine are closely associated with the original contents, autolysis and microbial level (Michiyo Murata, 1986). In the early eighties Japanese researchers did a lot in this field (M, Sakaguchi, 1984) with cold stored fish, such as *Pneumatophorus japonicus, Katsuwonus pelamis, Thunnus* tonggol. They studied the relationship between changes in contents of FAA and indices of freshness. With further study and elucidation of the mechanisms, processing and preservation techniques were improved. Studies in this field started later in China and the information was limited.

Oysters are abundant in China. *Ostrea plicatula, Ostrea gigas* and *Ostrea rivulans* are important species. At present oysters are eaten directly without processing. The study was conducted to learn the biochemistry of free amino acids and taurine in oyster during cold storage, in order to search for new processing techniques.

MATERIALS AND METHODS

Materials: *Ostrea gigas* was obtained from Luoyuan farm; *Ostrea plicatula* and *Ostrea rivulans* were obtained from Longhai farm.

Sample preparation: shelled oyster meat was put in bags and sealed, then stored in three groups at 0~2°C, 3~5°C and 6~8°C.

Determination method

A 30g sample was homogenized with 1.5g sulfosalicylic acid and stored in a refrigerator for one hour. After centrifuging the supernatant was frozen for determination. Before analysis, the liquid was thawed, then determined with Waters' PICO-TAG method. Separation column: PICO TAO c18 3.9x150mm; mobile phase as liquid A:19.0g NaAc +0.5ml triethylamine, dissolved in 940ml distilled water, adjust pH to 6.4, with 60ml CH_3 CN; liquid B: 60% CH_3CN, flow speed: 1.5ml/min, UV detector, wave length: 254nm.

RESULTS

Changes of total amount of free amino acids

Changes of total free amino acids in *Ostrea gigas*, *Ostrea rivulans* and *Ostrea plicatula* stored at different temperature are shown in Fig.1. On the first day, the total of free amount of free amino acids in these oysters were significantly different: 225~252mg/100g in *Ostrea plicatula*, 254~285mg/100g in *Ostrea rivulans* and 472~508mg/100g in *Ostrea gigas*. The contents of amino acids in all oysters increased to different levels during storage. At 0~2°C, the total amount of free amino acids increased slowly. After 15 days the total amount of free amino acids in *Ostrea plicatula*, *Ostrea rivulans* and reached 296mg/100g, 289mg/100g and 498mg/100g respectively. At 3~5°C, total free amino acids increased more quickly, after 15 days, in *Ostrea plicatula*, *Ostrea rivulans* and *Ostrea gigas*, they reached 390mg/100g, 426mg/100g and 662mg/100g respectively. At 6~8°C, total amount of free amino acids increased most quickly. After 15 days, they respectively reached to 605mg/100g, 687mg/100g and 718mg/100g. Increase and decrease of total free amino acids in these oysters occurred during storage, but in general the main trend was an increase.

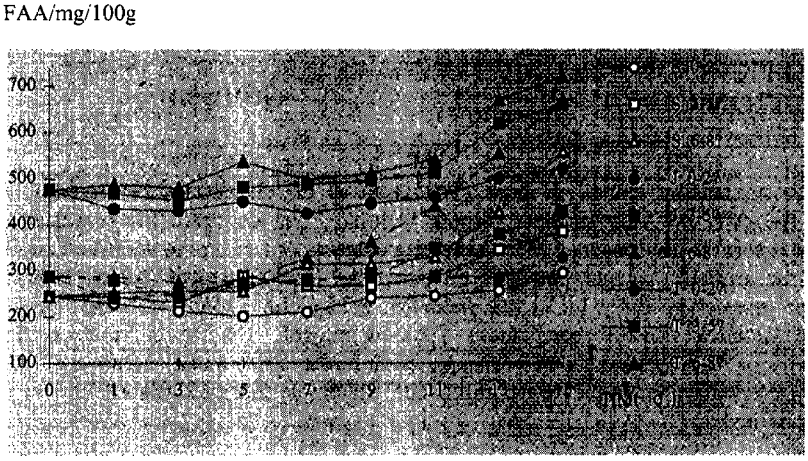

Fig. 1. Changes of total amount of free amino acids in oyster stored in several temperature ranges.

S- *Ostrea plicatula* J- *Ostrea rivulans* T- *Ostrea gigas*

Changes of various free amino acids

Free amino acids in oyster stored at different temperatures changed at different rates, more quickly at higher storage temperature. There were some differences in changes of various free amino acids. According to the presence of side chains in polar amino acids, free amino acids are classified as non-polar amino acids, or polar amino acids with electric charge (Shen Tong *et al.*, 1980). During storage, non-polar amino acids increased more quickly than polar amino acids. Changes in the non-polar amino acids of Ostrea rivulans stored at 6~8°C for 15 days were: Met increased from 1.4mg/100g to 20.2mg/100g, Phe increased from 1.1mg/100 to 29.1mg/100g. Pro increased from 9.7mg/100g to 14.7mg/100g. Iso increased from 1.8mg/100g to 31.2mg/100g. Leu increased from 2.4mg/100g to 51.3mg/100g, Val increased from 5.8mg/100g to 37.5mg/100g. Ala increased from 49.2mg/100g to 89.6mg/100g. Polar amino acids without electric charge increased more slowly. Ser increased from 36.0mg/100g to 53.1mg/100g, Thr increased from 13.2mg/100g to 52.1mg/100g, Tyr increased from 3.9mg/100g to 25.8mg/100g, Cys decreased a little; Polar amino acids carrying electric charge changed slowest: Glu increased from 61mg/100g to 98mg/100g, His increased from 2.2mg/100g to 10.3mg/100g, Arg increased from 26.2mg/100g to 41.3mg/100g, Asp increased a little, and Lys was almost unchanged after 15 days storage. Changes of *Ostrea plicatula* and *Ostrea gigas* were the same. Essential amino acids such as Met, Phe, Leu, Ile and Val are non-polar amino acids, their contents increase rapidly during cold storage.

Changes of taurine

Content of taurine is high in oyster. In *Ostrea gigas*, *Ostrea rivulans* and *Ostrea plicatula* content was 765mg/100g, 250mg/100g and 356mg/100g respectively. During storage, taurine in *Ostrea gigas* increased slightly. After 15 days storage at temperature ranges of 0~2°C, 3~5°C and 6~8°C, they increased to 760mg/100g, 850mg/100g and 854mg/100g respectively, while the content of taurine in *Ostrea rivulans* and *Ostrea plicatula* both decreased slightly, to 270mg/100g, 225mg/100g, 240mg/100g and 376mg/100g, 362mg/100g, 367mg/100g respectively at the three temperature ranges.

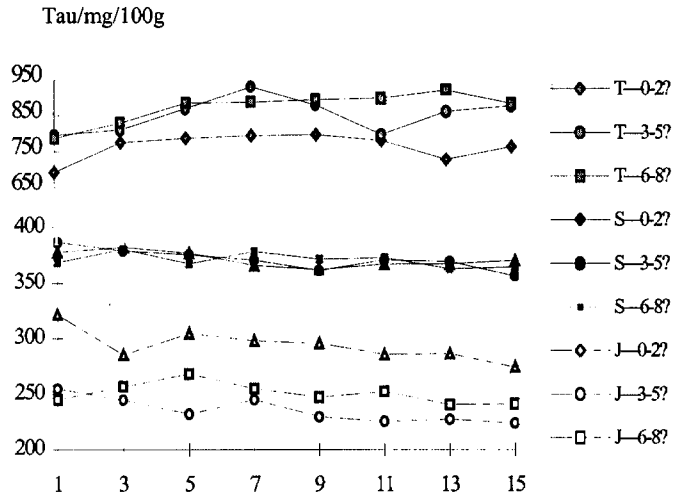

Fig. 2. Changes of taurine in oyster stored in several temperature ranges.

S- *Ostrea plicatula*□ J- *Ostrea rivulans*□ T- *Ostrea gigas*

DISCUSSION

Total amount of FAA in oyster decreased during early storage and then increased in later storage. Shown in Fig.1, total amount of FAA in oyster stored at different temperature changed at different rates. For example, at 0~2°C, total amount of FAA in *Ostrea rivulans* decreased, after 5 days of storage it began to increase, but at 3~5°C and 6~8°C, it began to increase irregularly after 3 days. Changes in *Ostrea plicatula* and *Ostrea gigas* were the same as *Ostrea rivulans*. We consider that these changes are associated with post-mortem physiological changes in the oyster. Fresh oyster meat is neutral or weakly alkaline. A series of biochemical reactions inside the oyster did not stop during storage. Firstly, glycogen was decomposed to lactate, then phytic acid was decomposed to phosphoric acids. The accumulation of lactate and phosphoric acid made the oyster acidity. When the pH of oyster meat reached 5.6~6.0, fibrillin lost solubility due to its lower moisture retention.. At the same time, some free amino acids were used iup as a nitrogen source for bacterial propagation and some free amino acids were decomposed by autolysis (Liu Yongchen, 1996; Wu Guanghong *et al.*, 1990). So FAA in oyster decreased during primary storage but the decrease varied with different storage conditions and different species of oyster. With time of storage on one hand, some proteins were decomposed to peptides, peptones and amino acids due to autolysis in the oyster meat (Wangzhang, 1990), while on the other some were decomposed due to bacterial activity. This led to a combination of amino acid decomposition and synthesis. Total amount of FAA increased when the speed of amino acid synthesis was faster than the speed of amino acids decomposition. We postulate that this is the primary factor in determining changes in total amount of FAA in oyster during storage.

There were some differences in changes of the various free amino acids during storage. These differences are associated with composition and transformation of amino acids in the oyster protein (Zhou

Runqi *et al.*, 1990), and are also associated with the polarity of amino acids. With ongoing time of storage increase of non-polar R-group amino acids is more marked than polar R-group amino acids with or without electric charge. The reason is that polar R-group amino acids with electric charge such as Lys, Arg and Asp are hydrophilic and easily dissolved in water due to the electric charge. Polar R-group amino acids without electric charge such as Cys and Gly can form hydrogen bonds with water and easily dissolve due to having dissociated polar groups in their side-chains. Among them, Ser, Thr and Tyr are polar due to their hydroxy groups (Shen Tong *et al.*, 1980). FAA are dissolved and thus spread throughout the oyster. FAA come into intimate contact with autolytic enzymes and microorganisms. They are easily autolysed and are used up as a nitrogen source for propagation by microorganisms. Non-polar R-group amino acids such as Leu, Val and Ile dissolve with difficulty due to their hydrophobic groups. Amino acids hydrolyzed from proteins attach themselves to proteins so they move more slowly and have less contact with autolytic enzymes and microorganisms. Non-polar R-group amino acids increase rapidly when the speed of hydrolysis to amino acids from proteins is faster than the speed of amino acid decomposition. This phenomenon is distinct during higher temperature storage.

The glutamate in three species of oyster is high. During storage, content of glutamate is 100mg/100g in *Ostrea gigas*, 60-70mg/100g in *Ostrea rivulans* and 50-60mg/100g in *Ostrea plicatula*. This probably relates to the biochemical process of free amino acids, which is synthesized around 2-ketoglutarate and glutamate (Zhou Runqi *et al.*, 1990). The majority of amino acids, such as Gly, SER, Thr, Cys, Val, Pro, Arg, lys, Leu and Ile are probably combined with 2-ketoglutarate to change to Glu and relevant ketonic acids. Arg is synthesized from Glu's N-2acyl intermediate product by microorganisms. This intermediate product is also a precursor of Pro for bacteria. On the contrary, various amino acids can be synthesized from Glu and the relevant ketonic acids by transamination. Some transaminations will only occur in the presence of proteolytic enzymes from bacteria (Cheng Guocheng, 1982).

Content of Asp is relatively stable in oyster, and is about 40mg/100g in *Ostrea gigas*, about 10mg/100g in *Ostrea plicatula* and *Ostrea rivulans*. Synthesized from Asp, Thr can also be changed to Ile. Except for Val, Leu, aryl amino acids and His, the majority of amino acids can be synthesized from Glu, Asp and Gly (Zhang Qianheng *et al.*, 1995).

Like amino acids, taurine will be used up as a nitrogen source for bacterial growth after the oyster's death. Taurine is a non-protein amino acid. It is considered that taurine is changed from a sulphur-bearing amino acid (Zhang Qianheng *et al.*, 1995). During storage, content of taurine in *Ostrea rivulans* and *Ostrea plicatula* decreased slightly, while in *Ostrea gigas* it increased slightly. According to Li Deming (1995), contents of sulphur-bearing amino acids such as Cys and Met are high in oyster protein. They are 510.4mg/100g and 641.6mg/100g respectively. Shown in Fig.2, Met increased slightly, and Cys changed little during storage. For example, at 0-2°C, 3-5°C and 6-8°C, Met in Ostrea plicatula increased to 6mg/100g, 6.2mg/100g and 22mg/100g from 3mg/100g respectively, while Cys in *Ostrea rivulans* and *Ostrea plicatula* was only 6mg/100g, Cys in *Ostrea gigas* was only 9mg/100g. This illustrates that taurine is probably derived from cysteine and leucine which are in turn derived from protein by hydrolysis. Cys in *Ostrea plicatula*, *Ostrea gigas* and *Ostrea rivulans* can be changed from Met and Ser. The sulphur in Cys is from Met by transsulfuration, and the carbon frame in Cys is from Ser. It was reported by Zhang Qianheng that taurine can be formed from Cys through two ways. One way is that Cys can change to sulfonic acid alanine by oxidation, then change to taurine by de-carboxylation. Another pathway is for Cys to change a sulphuric acid group by oxidation and decomposition. Cys loses mercapto- and amino- directly to change to pyruvic acid, ammonia and H2S, then H2S is oxidised to sulphuric acid. The mercapto- in Cys can be oxidised to sulfonic-, then change to sulphuric acid.

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FISH INSPECTION AND CONTROL SYSTEMS IN THE ASIA PACIFIC REGION

by

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ABSTRACT

The Asia Pacific countries are both major suppliers and consumers of fish and fishery products. In the past decade, there has been a worldwide trend towards demanding safer food and thus a more stringent control of food safety and quality. Governments as key players in food safety control systems have tightened up regulations and introduced new control systems that are applied to imported and exported products. Other players in the system such as industry, buyers and consumers have an increasing role in the control system. Those roles can be identified as monitoring, inspection, verification, audit and education.

The purpose of the fish inspection and control system can be identified as providing safe food, increasing market access and protecting consumers. The system is usually based on end product analysis, certification of products and establishment registration. The approaches used are control of GMPs, sanitation, hygiene and HACCP. There are both voluntary and mandatory systems applied. Official bodies responsible for fish inspection and control in most cases involve more than one agency. In the Asia Pacific region, the official control body is normally the Ministry of Health or Fisheries rather than veterinary control systems as in case of European or South American countries. Responsibilities are, in some cases, divided to imports plus domestic control and exports control. The demand of major countries for a single competent authority has forced governments towards a one-agency control over the commodity. However, there are movements within countries for agreement among control agencies and modernization of inspection and control system toward a single agency.

Since 1990, the region has adapted to the worldwide trend of HACCP application with demands for control from raw material production to processing establishment and products. Many countries are able to provide the overall control, to meet the demands of importing countries. Training on HACCP in the region since 1991 has accelerated and concentrated in 1995 – 1996, on preparation for the EU own check system and USFDA Seafood HACCP regulation. The applications of HACCP in Asia Pacific countries are very progressive because of the export nature of the industry, though different levels of understanding and application exist. It is believed that the same HACCP principles as identified by the Codex Committee on Food Hygiene are applied but there are vast difference in the approach and procedures, causing confusion in practices and assessment of the efficiency of the system. In addition there are differences in standards for products and manufacturing practices, that make fish inspection and control systems very complex. It is recognized that harmonization and recognition of equivalency of fish inspection system are necessary for the development of trade. Countries need to be transparent in their inspection and control systems as well as standards applied to species, process and products. They should also be prepared to accept the equivalency of another system that may be different but produces acceptable levels of protection. The harmonization and recognition of equivalency requires trust and co-operation between the involved parties. This approach should be initiated among countries in the region.

INTRODUCTION

The Asia Pacific countries are both major suppliers and consumers of fish and fishery products. In the past decade, there has been a worldwide demand for safer food and thus a more stringent control of food safety and quality. Governments as key players in food safety have tightened up regulations and introduced new controls that apply to imported and exported products. Other players in the system such as industry, buyers and consumers have an increasing role in the control system. Those roles can be identified as monitoring, inspection, verification, audit and education.

The purpose of the fish inspection and control system is to provide safe food, increase market access and protect consumers. The system is usually based on end product analysis and certification of products

and establishment registration. The approaches used are control of GMP's, sanitation, hygiene and HACCP. There are both voluntary and mandatory systems. A number of ministries may share the responsibility of fish inspection. In the Asia Pacific region, official control bodies are normally Ministries of Health or Fisheries not veterinary control as in Europe or South America. Responsibilities are, in some cases, divided to imports plus domestic control and export control. The demand of the major importing countries for a single competent authority has encouraged the trend towards a one-agency control over the commodity. However, there are also movements within countries for agreements among control agencies and modernization of inspection and control system as well as towards a single agency.

Since 1990, the region has been adapting to the worldwide trend of HACCP application, covering control of raw material, processing establishment and products. Many countries are able to apply overall control, to meet the demands of importing countries after training on HACCP that has been available in the region since 1991. In 1995 – 1996,it has concentrated on preparation for the EU's own check system and USFDA Seafood HACCP régulations. The application of HACCP in Asia Pacific countries is very progressive because of the export nature of the industry, though different levels of understanding and application are apparent. Although the HACCP principles as identified by the Codex Committee on Food Hygiene are generally applied there are differences in the approach and procedures, which cause confusion in practice and in the assessment of the efficiency of the system. In addition there are differences in standards for products and manufacturing practices that make fish inspection and control systems very complex. It is recognized that harmonization and recognition of equivalency of fish inspection system is necessary for the development of trade. Countries need to be transparent in their inspection and control systems. They should also be prepared to accept the equivalency of another system, which may be different but produces an acceptable level of protection. The harmonization and recognition of equivalency requires trust and co-operation between the parties involved. This approach should be stimulated among countries in the region.

The need for fish inspection and control system in the Asia Pacific countries

World fish production increased to reach 112.4 million tones in 1994, this was entirely due to higher aquaculture production. Asian countries are major fish producing countries. China, Japan, India, Indonesia, Thailand the Republic of Korea and the Philippines are among the world top ten fish producing countries. (Table1)

The roles of Asian countries as exporters of fishery products continues to grow, with Chinese exports growing particularly strongly. Thailand, China, Korea, Indonesia and Japan are among the world's big fish exporting nations, earning more than US $ 700 million annually. (Table 2) While Asian nations are also importers of fishery products, among those are Japan, China, Hong Kong, Singapore, Thailand, Republic of Korea and Malaysia. (Table 3)

Consumption of fish in the Asian region is already high and growing. Intra regional trade is increasingly significantly. Food safety for intra regional trade is also very important and cannot be ignored neither can food safety for domestic consumption.

Products traded internationally are moving toward convenience food (ready to eat or ready to cook), that require more stringent control to ensure food safety. Moreover, preserved traditional products are now traded more widely, and need to be examined for manufacturing practices and product safety standards.

Table 1. World Fisheries Production.

World Production(in million tonnes)			
	1993	**1994**	**1995**
World total	102.2	109.6	112.4
China	17.6	20.7	24.4
Peru	8.5	11.6	8.9
Chile	6.0	7.8	7.6
Japan	8.1	7.4	6.8
United States	5.9	5.9	5.6
Area of Former USSR	5.5	4.6	5.3
India	4.3	4.5	4.6
Indonesia	3.7	4.0	4.1
Thailand	3.3	3.4	3.6
Norway	2.6	2.6	2.8
Korea, Rep.	2.6	2.7	2.7
Philippines	2.3	2.3	2.3
Denmark	1.7	1.9	2.0
Others	30.1	30.2	31.6

Many Asian countries, such as Bangladesh, Indonesia, India, and Thailand, derive considerable benefit from the fish/shrimp producing sector as an export earner and as a generator of income, especially for the small-scale rural industries. Major markets remain the USA, the European Union, Australia and Canada. The future for fish exports is being further seriously threatened by new regulations progressively imposed by these countries.

As a result of consumer pressure for a safer food supply, the EU, introduced regulations that fish imports will only be permitted from countries or companies which have convinced the EU Commission that the fishery products have been produced in establishments which comply with EU Directive 91/493. The EU has issued a Commission Decision drawing up a list of third countries from which import of fishery products is authorized (97/564/EC). Only 8 countries in Asia appear on the list for permanent authorization status. Those are Indonesia, Japan, Malaysia, Philippines, Singapore, South Korea, Taiwan Province of China and Thailand. While another 4 Asian countries are given an interim status, they are Bangladesh, China, India, Maldives and Vietnam. However, this list is updated frequently. Countries that have not been approved are not allowed to export fishery products to the EU after 1 July 1998. The USA introduced Seafood HACCP regulations, effective December 18, 1997, requiring that fishery products, processed domestically or imported, be processed by processors that operate quality assurance systems, based on the principles of Hazard Analysis Critical Control Point (HACCP). Canada recently amended the Fish Inspection Regulation to incorporate HACCP Principles to the existing Quality Management Program. The new arrangements for quality assurance place the responsibility for consumer safety on the producer, that is they transfer the inspection function from the point of export/import to the producer.

Table 2. World Fishery Products Exports *(in million $)*.

	1993	1994	1995
World total	41 494	46 798	49 249
Thailand	3 404	4 190	4 449
Norway	2 302	2 178	3 123
United States	3 179	3 230	3 582
China	1 542	2 320	2 854
Denmark	2 151	2 359	2 460
Canada	2 055	2 182	2 314
Korea, Rep.	1 335	1 411	1 565
Chile	1 125	1 304	1 704
Indonesia	1 149	1 583	1 667
Netherlands	1 129	1 436	1 447
Iceland	1 138	1 265	1 343
Russian Federation	1 471	1 191	1 628
Spain	814	1 021	1 191
India	836	1 125	1 125
United Kingdom	1 037	1 180	1 019
France	858	910	993
Germany	653	790	899
New Zealand	648	692	814
Morocco	539	620	786
Ecuador	574	724	724
Japan	767	743	713
Mexico	431	481	691
Others	11 919	13 322	12 157

Table 3. World Fishery Products Imports *(in million $)*.

	1993	1994	1995
World total	44 549	51 009	54 088
Japan	14 187	16 140	17 853
USA	6 290	7 043	7 145
France	2 556	2 797	3 221
Spain	2 630	2 639	3 106
Germany	1 884	2 136	2 479
Italy	2 131	2 257	2 281
United Kingdom	1 629	1 880	1 910
Hong Kong	1 377	1 642	1 828
Denmark	1 094	1 415	1 574
Netherlands	792	1 192	1 139
Canada	821	913	1 034
Belgium	730	921	1 035
China	576	856	941
Thailand	830	816	826
Korea, Rep.	537	718	825
Portugal	628	670	763
Singapore	567	620	660
Australia	360	428	494
Switzerland	354	390	418
Norway	310	322	490
Malaysia	265	304	325
Other	3 999	4 728	3 740

The need for market access pressured Asian countries to step up and strengthen their fish inspection systems to guarantee their market position. In these countries strengthening of systems was undertaken through bilateral and multilateral assistance. The quality assurance systems must be at least equivalent to those used in the importing countries in order to facilitate import/export controls.

However, as a result of perceived poor quality of processed fishery products, and also lack of recent information on production hygiene, inspection and quality control efforts of exporting countries, rejection, automatic detention, bans on products or processors and bans on countries still occur.

Therefore, it is necessary for countries in the Asia Pacific region to build up the fish inspection and control system to guarantee control of food safety, quality and fair trade for both local and export markets. In addition, good control will give confidence to consumers, buyers and control authorities of trading partners, thus market access and confidence will be gained.

Present Structure of Fish Inspection and Control System

Existence of a National Fish and Fishery Products Inspection and Control System

The author conducted survey on Who is Who in Fish Inspection in selected ASIA – Pacific Countries in 1995. (APEC, 1995) The study revealed that Asian countries are active in fish inspection and quality control in all 17 countries surveyed. Though some indicated that there are no fish inspection system such as Brunei Darussalam and Hong Kong but fish inspection is part of food inspection in those countries. Profiles of the fish inspection systems of selected countries are attached. Countries in South Asia and the Arab countries, were not included in the survey, however, fish inspection in those countries is active. In most Asian countries, quality assurance systems have developed as a result of market requirements.

Legislative Framework

Laws and regulations that empower fish inspection agencies to conduct fish inspection and control system are crucial for the control of food safety, quality and fair trade.

The inspection agencies in Asian countries are supported by Food Acts, Public Health Services Acts, Food Standard Acts, Commodities Act, Fisheries Acts, The Sale of Food Act, Import and Export Control Act. While no specific fish inspection act is applied in the region the authority for conducting inspection and quality control is in various sections of those Acts.

Ideally, the fish inspection agency should have the authority, based on adequate legislation, to establish and enforce regulatory requirements.

Legislation should provide the necessary authority to carry out controls at all stages of raw material holding, handling, transporting, processing, packaging, and trade (domestic, import and export) in fish and fishery products. It is also crucial that the law authorizes the fish inspection agency to control food safety, and prevent unsafe food from entering markets or export. It appears that there are very few countries in Asia that have clear legislation to support activities at all stages of fish production, resulting in a rather complex government structure for fish inspection.

Governmental Structures

The inspection authorities in Asia define the purposes of fish inspection and control as to provide safe food, increase market access and protect consumers as well as to enhance development of the fishery industry.

Official control bodies include Ministries of Health, Fisheries, Industry or Commerce. Controls are of public health and standards rather than a veterinary control system as in European or South American countries.

Responsibilities are, in some cases, divided to imports plus domestic control and exports control. In many countries, such as India, Indonesia, China, Malaysia, the Philippines, Thailand, Vietnam and Taiwan Province of China different authorities in the same country have jurisdiction over different parts of the fish inspection and control system. The official body responsible for fish inspection in most cases involves more than one agency. This leads to problems of duplication of roles and results in improper control on production, manufacturing process and product safety.

The demand of major importing countries, such as the EU, for a single competent authority has encouraged the movement towards one-agency control over the commodity. There are also trends within countries for agreement among control agencies such as in the Philippines and Indonesia and modernization of inspection and control systems toward a single agency.

Resources/Tools

The fish inspection authority should have in place: the necessary controls, procedures, standard setting mechanisms, enforcement options, facilities, equipment, laboratories, transportation, communications, personnel and training to support the objectives of the fish and fishery products inspection and control.

In Asia Pacific countries, the above vary from country to country depending on financial support, priorities, competency and government policies. However, again, because of the export oriented natures of many countries, the resources and tools are in place. Moreover, in the past decade countries in the region have received assistance from World Bank, Asian Development Bank, Canadian International Development Agency, USAID, JICA, FAO and UNDP to improve their programmes.

Controls:

Inspection in the region involves the control of production (sanitation, environment and produce), handling, processing, and products. These controls include registration, approval and certification.

However, only in a few countries does the fish inspection authority have overall control. Examples are India, Indonesia, Philippines, and Thailand. Most inspection agencies in the region concentrate on inspection of facilities and end product certification such as Sri Lanka, Oman, Vietnam, Singapore, Korea, Hong Kong, China and Taiwan Province of China.

It is important that the control system covers pre harvest control in terms of food safety, in order to ensure safety and quality of raw material, sources and traceability.

Procedures

Asian countries appear to lack specific procedures for fish inspection. The inspection procedures need to be formulated and documented both in general for sanitation and hygiene and also specifically for products. Working methods and techniques should be formally documented. In addition procedures should be in place to ensure that inspections are carried out using priorities based on risk, in order to address known or suspected non-compliance situations; and in a coordinated manner between different regulatory authorities, if several exist.

Standard setting mechanism

In Asian countries, standards are usually drafted (under food act, product commodities act, industrial standard acts etc.), by a group of experts from government and industry using international standards as a model. Standard setting still concentrates on product standards rather than manufacturing practices.

Personnel

The strength of countries in this region is the qualification and training of personnel. Most countries identify the minimum qualification for an inspector as Bachelor in Science. Training on various aspects of inspection has been designed for the inspectors. Inspectors have opportunities for training on inspection, quality control and HACCP. Many international organizations and importing countries have worked closely with countries in the region on training on inspection and quality control. However, practical experience and specific training on process and product or even HACCP are still needed.

Enforcement options

Enforcement options depend very much on the legislative power of the authority, the enforcement options of countries in this region include:

- for processing facilities, formal warnings, implementation of voluntary closures, and suspension of registration, withdrawal from approved list and legal action as appropriate.
- for product, detention of product, culling, suspension of inspection, re-inspection, seizure of product, disposal of violative product; legal action

In Asian countries, where, different authorities in the same country have jurisdiction over different parts of the fish inspection system, or more than one body has jurisdiction, conflicting requirements and enforcement options could occur. This could create legal and commercial obstacles to trade.

Laboratory capacity

The laboratory is an important support to fish inspection. Exporting countries in the region have fairly well equipped laboratories. However, it is difficult for the laboratories to cope with all the demands of importing countries, as the list of analyses keeps expanding. For example, the Ministry of Health, New Zealand requires that frozen crustaceans, both cooked or uncooked, be examined for the presence of pathogens (*Salmonella* and *Listeria*) and heavy metal contamination (cadmium, copper and selenium). For shipment of molluscan bivalves, it requires that PSP, DSP, ASP and NSP be tested. This means that the laboratory must have the capacity for mouse bioassay and other complicated equipment such as HPLC.

It is also necessary that inspection laboratories operate under good laboratory practices and participate in inter-laboratory collaboration programmes. (Report of CODEX Committee in Method of Analysis and Sampling. 1997) Often the laboratory is heavily loaded with product inspection for certification, rather than verification testing to support implementation of HACCP. Good Laboratory Practice is therefore neglected. Personnel at different levels still lack training and experience, especially on analysis procedures, operation and maintenance of complicated equipment and laboratory quality management.

Inter-laboratory testing is also neglected. Check programmes are not available for all tests and a limited testing scheme is available to date. The region should co operate on this issue.

Certification system

Fish inspection services in the region concentrate on certification of export products as this is still required by most importing countries.

The certificate requirements vary greatly. Certificates commonly used for export shipment include sanitary certificate, plant hygiene certificate, heath certificate or certificate of analysis, stating the test results of the product inspected. Most countries have their own format. Countries trading with the EU have to use certificates in the format and language of EU member states.

When required, certification of the conformity of a product or batch of products may be based on:

- regular checks by the inspection service;
- analytical results;
- evaluation of quality assurance procedures;
- any inspections specifically required for the issuance of a certificate.

Responsible authorities should take all necessary steps to ensure the integrity, impartiality and independence of certification systems. Personnel empowered to validate certificates must be appropriately trained and fully aware of the significance of the contents of each certificate which they complete.

Private sector involvement

Other players in the system such as industry, buyers and consumers have an increasing role in the control system. Those roles can be identified as monitoring, inspection, verification, audit and education.

Processors are involved directly in fish inspection and control, they have responsibility to produce safe products. The minimum requirement is a quality control program based on the CODEX General Principle of Food Hygiene. Once HACCP is required, their responsibility for control is more obvious In the US requirements, processors may use a written guarantee together with HACCP plan as objective evidence of their application of HACCP. In addition to the use of a HACCP certificate and inspection report from a third party has paved the way for private third party audit.

Since the announcement of the US Seafood HACCP regulations, private consulting firms offering auditing and training from the USA and the EU have been working rather actively in the region.

HACCP Based Fish Inspection System

Countries in the region such as Thailand, Indonesia and India have been among the early starters in implementing HACCP. Too date HACCP systems are being implemented at both government and industry level, mainly for export. Countries reporting active HACCP implementation by government and private sector include Bangladesh, Indonesia, India, Philippines, Malaysia, Singapore, Thailand, Korea. In some countries, the private sector is leading the way in HACCP implementation *e.g.*, Japan, China, Hong Kong and Taiwan Province of China. The application of HACCP in Asia Pacific countries is very progressive because of the export nature of the industry, though there are different levels of understanding and application.

Although training is ongoing there are still training needs to increase understanding on hazard analysis, formulating control measures and corrective actions, critical limit verification, internal audit, documentation of plans, and a prerequisite program for industry.

In order to promote the application of HACCP, the strategic activities of the government may include:

- facilitating training programs for industry and government personnel
- implementing the necessary infrastructure in terms of guidance, expertise and where appropriate, legislation
- developing the necessary support and training materials for industry, government and interest parties
- formulating an overall program to assess HACCP system in place.

These activities may be carried out by different bodies of government or by the private sector. Conflict of interest should be avoided such as when activities are conducted by the inspection authority's assessors who provide advice on how to implement HACCP and are then responsible for assessment. This is true for most Asian countries where in one agency carries out all activities.

The inspection authorities in the region need to look into the inspection and verification of HACCP system of the industry to assess the effectiveness of the program. The role of government will have to shift from traditional inspection methods towards the assessment of HACCP systems. The assessment of HACCP systems by government may include:

- assessment of: HACCP management,
- the basis for HACCP plan development,
- Hazard Analysis,
- effectiveness of control measures,
- verification procedures,
- documentation and
- Implementation.

Harmonization

There are differences in standards for products and manufacturing practices, that make fish inspection very complex. These differences can become major barriers to trade.

Harmonization is necessary for the development of trade. To this end countries need to be transparent in their inspection and control systems as well as in the standards applied to species, process and products.

Differences in standards and acceptable practices could be dealt with through regional or international harmonization. The best forum is the CODEX Alimentarius. Yet involvement of Asian countries in CODEX is minimal, when seen in comparison to the importance of fish trade to the region. As a result codes of practice and standards do not appropriately cover Asian tropical conditions such as temperature and species of fish e.g. tuna and sardine standards do not cover species found in the region. There are few participants from Asian countries in the CODEX Committee on Fish and Fishery Products. At the 23rd CCFFP meeting where Codes of Practice for Fresh, Frozen, Minced and Canned Fish, Frozen Shrimp and Prawns, Molluscan Shellfish, Salted Fish, Smoked Fish and the Products of Aquaculture; as well as a Standard for Salted Anchovies and a Standard for Fish Crackers were under review, there were very few countries from Asia present at the Meeting.

As CODEX standards will be used as reference standards in international trade, countries should participate and give more input to international standard development.

Equivalency

The WTO – SPS Agreement encourages Governments to accepts the SPS measures of other countries, where they meet the importing countries level of protection. Negotiation of equivalence agreements is encouraged.

They should also be prepared to accept the equivalency of another system that may be different but produces an acceptable level of protection. The harmonization and recognition of equivalency requires trust and co-operation between the involved parties. This approach should be initiated among countries in the region.

According to the WTO SPS Agreement, a country is obliged to consider a request for an equivalency agreement once asked.

Guidelines for equivalency

There is a need for common language to measure equivalency. As a result, the Codex Committee on Food Import and Export Inspection and Certification Systems has established draft guidelines for establishing equivalency. The guidelines outline steps for equivalency determination that include a side by

side comparison of regulations, inspection procedures, resources in inspection etc; following by on-site visits to verify equivalency of the systems.

The USFDA contemplates a process that will involve a paper review, an on-site verification review, and public notice and comment in making a determination that a foreign country's system is equivalent.

The EU Commission specified steps for determining equivalency of trading partners to include document review and on-site visits and approval from Commission members to be published in Council Directives.

APEC has laid down guidelines for determining equivalency on food control systems as well.

The above guidelines are identical in that there shall be at least document review of inspection and control systems and on-site visits to audit the agency having jurisdiction of control and audit. There should be focus on government control not the individual industry's performance.

Entering into equivalency agreements is possible but rather time consuming.

Assessment of Equivalency

The determination of equivalency between fish and fishery products inspection and control systems is usually based on an assessment of the following criteria:

1. Existence of a National Fish and Fishery Products Inspection and Control System:

a) Legislative Framework
b) Governmental Structures
c) Adequate Resources/Tools
d) Appropriate Implementation of Mandate
e) Training for Inspectors and Laboratory Personnel
f) Inspection procedures and Sampling Plans
g) Certification Systems
h) Enforcement History.

2. Ability to identify Fish Processing Establishments

Enforcement should that seafood processors who are included under the Agreement have adopted a system of controls that prevent the occurrence of food safety hazards or other regulatory infractions in fish and fishery products exported to the other Party. This system of preventive controls should be based on internationally recognized principles of HACCP.

3. Ability to Perform Audit Procedures on the Inspection Control System

4. Verification of Equivalence.

System Audit

Audit of a fish inspection system will be based on procedures of the CCFICS. Assessment and verification should concentrate primarily on effectiveness of the inspection and certification system in operation in the exporting country rather than on specific commodities or establishments.

The equivalency approach is not appropriate for countries that lack legislative support, clearly defined inspection and control and qualified personnel in both government and the private sector

Possibility of Equivalency agreement among Asia Pacific Countries

Countries in the Asia Pacific region may consider establishing equivalency agreements with trading partners in the region such as Japan and their major trading partners such as EU, USA, Australia, New Zealand and Canada. So far, there is no initiative to establish equivalency agreements among Asia Pacific exporting nations. This is mainly because the similarities of the products traded makes countries compete for the market and the agreement is needed to gain market access.

Since CODEX and many importing countries such as EU, USA and Canada have developed procedures for determining equivalency, the process is becoming clearer but it is also complex and time consuming. In any case, the exporting countries have to prove that their systems are equivalent and have to document this. From the experience of Thailand, this is the most time consuming part: preparation of all necessary documents and making the system clear to the partner. The scope of document review is very extensive while the review is very intensive.

If countries in the region would like to establish agreements with one another, there is no choice but to follow the CODEX guidelines and make the agreement international.

It may not be necessary to establish equivalency agreements if the value of trade does not justify it. It is of utmost important for countries in the region to have fish inspection systems in place. These should be effective and sufficient to provide the necessary assurance that products have been produced under good hygienic practices and HACCP control and in addition that the products are safe, wholesome and meet the requirements of importing countries.

RECOMMENDATIONS

Asian countries should keep themselves up to date with the new requirements of the market and make an active contribution to CODEX

Priority should be given to improving or establishing an effective fish inspection and regulatory system, development of personnel, GMP's and HACCP for the industry.

Cooperate to strengthen laboratory services and laboratory quality management.

Countries or agencies who wish to establish equivalency agreements should recognize the steps and procedures for equivalency determination. The country or agency should have a well established and effective fish inspection and control system. Capabilities and competency is vital to the development of trust and gaining of recognition. The support of industry is very important for the development of equivalency, as industry will have to take responsibility for their quality program.

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CHINA

According to Standardisation Law, State Bureau of Technical Super-vision (SBTS) unitedly manages quality inspection standardisation work of whole country. The Ministry of Agriculture (MA) is in charge of quality inspection and standardisation work of fishery industry. Under the leading of MA and SBTS, the work is carried out concertedly by National Fishery Standards Technical Committee, Sub-committee on Fish and Fishery Pro-ducts (NFSTC SFFP) and National Center for Quality Supervision and Test of Aquatic Products (NCQSTAP).

Name of Organisation :

National Center for Quality Supervision and Test of Aquatic Products, P.R. China

Full Address :

106 Nanjing Road
Qingdao 266071
Shan Dong Province,
P.R.China
Tel. 86 532 582-6579
Fax. 86 532 581-1514

Products of Discipline :

- ✓ Fishery products for export/ import/domestic use
- ✓ Fresh and frozen fishery products only
- ✓ Chilled fishery products
- ✓ Dried fishery products only
- ✓ Others

Mandatory or Voluntary:

The supervision, selective examination for the quality of products which are pointed by the government are mandatory. Intrusted by Department concerned, examination of products quality for implant production licence and quality certification is mandatory. Examination of arbitration about quality dispute is mandatory. Examination and appreciation of high quality products are voluntary.

Description of Organisation:

NCQSTAP is one of the national agencies for quality supervision and test of products in fishery aspect. It is set up in Yellow Sea Fishery Research Institute (YSFRI). It processes perfect physical, chemical and microbial laboratory. It is equipped with many advanced analysis instruments such as ICP-AES (made in USA), GC and HPLC (made in Japan), etc. Twenty specialists are in the centre. Certified by the State Bureau of Technical Supervision 1988, the NCQSTAP set on working in April, 1989.

Policies and Regulations that Support the Inspection System:

The competent policies and regulations are two basic government laws "The Standardisation Law of the People's Republic of China" in article 19. It is ordered that the competent standards of the country level and above may in the light of needs. According to article 11, "Products Quality Law of People's Republic of China", the centre is supported by the following description. The quality test agencies must have test conditions and ability responding to its work area. They cannot take quality test only until they are past the examination made by Quality Supervision Department of SBTS.

Description of Inspection and Certification Systems:

Practised and Accreditation System Quality Certification System of Fishery Products are being established.

HONG KONG, CHINA

Name of Organisation:

Agriculture & Fisheries Department
Hong Kong Special Administrative Region
of China

Full Address:
Canton Road Government Offices
12/F, 393 Canton Road
Kowloon, Hong Kong SAR
Tel. (852) 2733-2208, 2873-8326
Fax. (852) 2311-3731, 2814-0018

Products of Discipline:

All fish and food products

Mandatory or Voluntary:

Hong Kong does not have a specific fish inspection and quality control programme. Currently, there are no standards for the quality of any of the export/import/domestic use fish products. There is however a code of practice for export frozen prawns. The processing plant can apply for an export health certificate of their frozen prawns products from the Urban Services Department or the Regional Services Department on a voluntary basis.

Description of Organisation:

Inspection of fish processing plants and of fish products comes under the general food laws. This is part of Hong Kong's comprehensive food inspection service jointly run by the Urban Services Department, the Regional Services Department and the Department of Health. The food laws are oriented towards the control of food for domestic consumption and they do not have a section specialising in quality control of fish products. In addition to the food laws, the Urban Services Department issued the Code of Practice for processing of frozen prawns. This code is very specific and applies to food factories which process frozen shrimps for which an export health certificate is required.

Policies and Regulations that Support the Inspection System:

The existing legislation to control the wholesomeness and quality of food derives from two sets of basic regulations: the Public Health and Municipal Services Ordinance and the Food Business By-laws. These laws control the handling, processing, storage, distribution and sale of all food commodities, including fish but would not include live fish and along with the Preservatives in Food Regulations, regulate their safety and composition. The food laws require that all food processing factories which include fish processing plants must operate with a licence renewable on an annual basis. There are some 740 qualified public health inspectors working in the above three departments and they are responsible for the day-to-day enforcement work. Food processing establishments such as fish processing plants are inspected, fish products are sampled and tested if required.

Description of Inspection and Certification Systems:

Export health certificates will only be granted for frozen prawns which are processed in the licensed premises and the quality of the products must comply with the Code of Practice for processing of frozen prawns. The products must also be sampled, inspected and where applicable subject to chemical and/or bacteriological analysis. Visual and sensory testing are carried out in the processing plant, chemical analysis in the Government Laboratory and bacteriological analysis in the Institute of Pathology. The health certificate is signed by the Chief Health Inspector of the Health Certificate Section of the Department of Health. Fees are charged for the testing.

The Department of Agriculture and Fisheries has a Veterinary Health Certification Section which can provide veterinary public health certification for live fish or for manufactured foods containing fishery products when this is required on animal and public health grounds by importing countries. The health certificate is signed by an Official Veterinary Surgeon.

INDONESIA

Name of Organisation:

Sub Directorate of Fish
Inspection and Quality Control,
Directorate General of Fisheries,
Ministry of Agriculture

Full Address:

Jl. Harsono R.M. no. 3,
Pasar Minggu,
Jakarta 12550, Indonesia
Tel. (6221) 789-1479
Fax. (6221) 789-1479
Telex: 47318 djikan ia

Products of Discipline:

- ✓ Fishery products for export/ import/domestic use
- ✓ Fresh and frozen fishery products only
- ____ Chilled fishery products
- ✓ Dried fishery products only
- ✓ Others :Curing, Fish/shrimp crackers, Traditional processed products

Mandatory or Voluntary:

Mandatory inspection for export and import used products and voluntary inspection for domestic used products.

Description of Organisation:

The Directorate General of Fisheries is designated as a *Competent Authority* in which the Directorate of Fisheries Enterprise and Processing Development, viz. Sub Directorate of Fish Inspection and Quality Control is responsible for conducting periodically mandatory sanitary and hygienic assessment as well as the operational aspects of handling and processing techniques. *Certificate of GMP* and *Certificate of Competence* (as Fish Technology Supervisor or as Quality Controller) are issued by the Directorate General of Fisheries.

Day-to-day plant inspection and supervision is carried out by local inspectors employed by the Provincial Fisheries Services and the Laboratories of Fish Inspection and Quality Control at the provincial and district level.

The Provincial Laboratories of Fish Inspection and Quality Control conduct inspection and issue *Certificate of Quality/Health* for final fish and fishery products prior to export. The Laboratories are also designated, under the European Commission Decision no. 94/324/EC, to issue the EC-version *Health Certificates.*

The National Centre for Fish Quality Control and Processing Development designated as *reference laboratory* to provide supervision to the provincial laboratories, in terms of analytical methods, processing technology development and training packages.

The Directorate General of Fisheries has also developed a HACCP-based Integrated Quality Management Programme (IQMP), for the past three years, to the industry.

The Directorate General of Fisheries also set up standards of fish and fishery products which shall then be promulgated by the National Council of Standardisation through the Centre of Standardisation and Accreditation at the Ministry of Agriculture.

Policies and Regulations that Support the Inspection System:

A number of legislative decrees concerned with re-regulation of Fish Inspection and Quality Control have been issued, namely:

1) European Commission Decision no. 94/324/EC of 19 May 1994, laying down specific conditions for importing fishery and aquaculture products from Indonesia

2) Government Regulation no.5/1991 of 1 March 1991, laying down the Indonesian National Standardisation

3) Presidential Decree no. 12/1991 of 1 March 1991, setting forth the implementation, application and control of the Indonesia National Standard

4) Presidential Decree no. 2/1990 of 28 May 1990, setting forth the Inspection and Quality Control of Fresh and Frozen Fish and Fishery Products

5) Government Regulation no. 15/1990 of 28 May 1990, laying down Fisheries Enterprise

6) An implementation decree issued by the Minister of Agriculture no. 815-Kpts/IK. 120/11/90 of 1 November 1990, setting forth regulation on Fisheries Enterprise

7) A decree issued by the Minister of Agriculture no. 303 Kpts/OT. 210/ 94 of 27 April 1994, setting forth Standardisation, Certification and Accreditation of Agriculture Products

8) A Memorandum of Understanding between Minister of Agriculture, of Health and of Trade on May 1990, setting forth the implementation of inspection and quality control of fresh and frozen fish and fishery products according to respective Ministry's jurisdiction

9) An implementation decree issued by the Minister of Health no. 397/Menkes/ SK/VIII/1990 of 24 August 1990, setting forth regulations on additives permitted in handling and processing of fish and fishery products

10) Presidential Decree no. 47/1986 of 17 September 1986, setting forth the improvement of post-harvest technology of agriculture products

Description of Inspection and Certification Systems:

1) Certificate of Quality for final fish and fishery products intended for export can only be issued by the Laboratories of Fish Inspection and Quality Control that have been accredited according to criteria drawn-up by the National Council of Standardisation.

2) EC-version Health Certificate for fish and fishery products intended for export to EC is under designation of Laboratories of Fish Inspection and Quality Control and is issued if the result of inspection meets the requirement as stipulated in the Commission Decision no. 94/324/EC of 19 May 1994 which refers to the Council Directive no. 91/493/EEC of 22 July 1991.

3) Certification of GMP is conducted by the Directorate General of Fisheries based on the result of inspection carried out by fish inspectors engaged at the Sub Directorate of Fish Inspection and Quality Control and monitoring report submitted by the local inspectors employed by the Provincial Fisheries Services and the Laboratories of Fish Inspection and Quality Control.

4) Certificate of Competence (for fish processing supervisors and/or quality control personnel at processing plants) is issued by the Directorate General of Fisheries.

5) Verification for the implementation of Integrated Quality Management Programme which applied by fish industries, is provided by the Directorate General of Fisheries, Provincial Fisheries Services and the Laboratory of Fish Inspection and Quality Control.

JAPAN

Name of Organisation:

Veterinary Sanitation Division
Environmental Health Bureau
Ministry of Health and Welfare

Full Address:

2-2, Kasumigaseki 1-Chome
Chiyoda-ku, Tokyo 100-45
Japan
Tel. (813) 3503-1711 (ext.2436)
Fax. (813) 3503-7964

Products of Discipline:

- ✓ Fishery products for export/ import/domestic use
- ____ Fresh and frozen fishery products only
- ✓ Chilled fishery products
- ____ Dried fishery products only
- ____ Others

Mandatory or Voluntary:

In principle, fish inspection is applied mandatorily for export/import/ domestic use products.

Description of Organisation :

(1) Inspection system for export

For example, the fish products for EU are inspected under the guidance of each prefecture. Sanitary Research

Institute in each prefecture performs its role as the reference laboratory.

(2) Inspection system for import

Inspection is conducted by the quarantine office at each port or the Centre of Quarantine and Inspection (only at Kobe City and Yokohama City).

(3) Inspection system for domestic use products

Inspection is conducted by the Sanitary Research Centre or the public health centre in each prefecture.

Policies and Regulations that Support the Inspection System:

"Food Sanitation Law"

The purpose of this law is to protect the people from health hazards which may be caused by eating or drinking, and to help improve and promote public health.

Description of Inspection and Certification Systems:

The inspection and certification system varies with items. However, in case of import, the results of inspection by authorities of exporting countries are accepted in principle.

In some case, inspection and certification system is based on bilateral arrangements, for example ;

(1) The certification system for the processing facilities of the scallops for EU.

(2) Arrangement on sanitary controls of fresh or frozen oysters, clams and mussels intended for shipment between US and Japan.

KOREA

Name of Organisation:

National Fishery Products Inspection Service (NFPIS)

Full Address:

National Fishery Products
Inspection Service
Wonnam Dong 103, Chongrogu,
Seoul, 110-450 Korea
Tel. (02) 762-9214
Fax. (02) 765-1755

Products of Discipline:

- ✓ Fishery products for export/ import/domestic use
- ✓ Fresh and frozen fishery products only
- ✓ Chilled fishery products
- ____ Dried fishery products only
- ____ Others

Mandatory or Voluntary:

The NFPIS has received a mandate from the Ministry of Health and Welfare to inspect the raw fisheries materials and simply processed products. Among the products for export and domestic consumption (including government saving for emergency), 13 items are subject to compulsory inspection.

Description of Organisation:

The NFPIS consists of the main office in Seoul and 11 branch offices throughout the country.

Inspection I Division in the main office controls the overall inspection system and Inspection II Division runs a laboratory for scientific analysis of the fishery products.

Policies and Regulations that Support the Inspection System:

Inspection for fishery products is implemented under the Fishery Products Inspection Law and Food Sanitation Law.

13 fisheries product items for export are subject to inspection in accordance with Fishery Products Inspection Law and some simply processed fishery products such as frozen, dried and salted fishery products are subject to inspection under Food Sanitation Law.

Description of Inspection and Certification Systems:

The NFPIS issues Inspection Certificates for exported products and Import Notification Certificates for imported products for the customs clearance.

Establishment of fishery processing plants for frozen and chilled products, agar-agar, fish liver oil and fish oil products and fishery processing industry on factory vessel are subject to licensing from the mayors or governors of the province, and the establishment of processing plants for dried, salted products and other fishery products is subject to report to the competitive authority.

MALAYSIA - 1

Name of Organisation:

Malaysian Fisheries Development Authority (MFDA)

Full Address:

8th Floor Wisma PKNS,
Jalan Raja Laut,
P.O. Box 12630
50784 Kuala Lumpur, Malaysia
Tel. (03) 292-4044
Fax. (03) 291-1931

Products of Discipline:

- ✓ Fishery products for export/ import/domestic use
- ____ Fresh and frozen fishery products only
- ____ Chilled fishery products
- ____ Dried fishery products only
- ____ Others

Mandatory or Voluntary:

Mandatory for export/import of fish and fishery products whereas voluntary for domestic use.

Description of Organisation:

Mainly physical inspection and observation of fish and fish products brought into the country. The inspection is conducted at the entry points and is done manually. The use of equipment for inspecting the quality of fish is presently being tested by MFDA and will be incorporated into the inspection system.

Policies and Regulations that Support the Inspection System:

The Fish Marketing Regulation 1988 empowered MFDA to enforce programmes that support inspection for fish and fish products.

Description of Inspection and Certification Systems:

Presently, MFDA only enforces on the importers/exporters to make declaration on certain items/information relating to importation of fish and fish products required by MFDA by using pre-printed forms.

MALAYSIA - 2

Name of Organisation:

Fisheries Research Institute
Department of Fisheries Malaysia

Full Address:

Fisheries Research Institute
Department of Fisheries Malaysia
11960 Batu Maung,
Penang, Malaysia
Tel. (04) 626-3925/6
Fax. (04) 626-2210

Products of Discipline:

- ✓ Fishery products for export/ import/domestic use
- ✓ Fresh and frozen fishery products only
- ✓ Chilled fishery products
- ✓ Dried fishery products only
- ✓ Others

Mandatory or Voluntary:

Voluntary

Description of Organisation:

Based on importing country requirements. Analyses are conducted by accredited private laboratories upon request. The Veterinary Services and Health Ministry Food Quality Control unit conduct inspection of premises. The *Competent Authority* for Malaysia is the Ministry of Health. Whilst the reference laboratory is based at the Fisheries Research Institute, Penang.

Policies and Regulations that Support the Inspection System:

- Food Regulations 1985
- Fisheries Act 1985

Description of Inspection and Certification Systems:

Inspection is normally carried out on seafood processors by inspectors of local authorities, Ministry of Health and the Department of Veterinary Services. Samples are sent to accredited laboratories for analyses. Accredited private laboratories are also providing analytical services with health certificates being issued by the Ministry of Health.

REPUBLIC OF THE PHILIPPINES

Name of Organisation:

Bureau of Fisheries and Aquatic Resources

Full Address:

860 Quezon Avenue, Arcadia Bldg.
Quezon City, Metro Manila,
3008 Philippines
Tel. (632) 973-617
Fax. (632) 987-871, 973-617

Products of Discipline:

- ✓ Fishery products for export/ import/domestic use
- ✓ Frèsh and frozen fishery products only
- ✓ Chilled fishery products
- ✓ Dried fishery products only
- ____ Others: Wildlife species (Sea snakes, frogs and by- products)

Mandatory or Voluntary:

Fish inspection for export has become voluntary with the implementation of Executive Order No. 1016 "Withdrawing the Inspection, Commodity, and Export Clearance Requirements for Philippine Exports". However, inspection, commodity clearances/health certificates are mandatory for those covered by international trade agreement; commodities which are restricted, regulates and when required by the importing country.

Description of Organisation:

The Fish Inspection Section of the Fisheries Post Harvest Technology Division, Bureau of Fisheries and Aquatic Resources is responsible for the inspection, issuance of commodity clearance and/or health certificates and the quality control programme for fish and fishery/aquatic products.

Fish processing establishments are inspected every quarter to verify compliance with the provisions of existing rules and regulations on plant structures and equipment, production requirements, transport facilities and hygiene and sanitation of plants and workers. For plants with serious and critical deficiencies regular follow-up is undertaken on a monthly basis.

For plants located in the regions, there are at least two (2) deputised inspectors responsible for the follow-up inspection of plants located in each respective region. This is an ongoing activity to upgrade the condition of fishery establishments.

Fish inspectors draw and collect samples based on AQL 6.5 sampling plan for sensory, chemical and microbiological analysis. Health certificates are issued based on the result of the product analysis conducted either by BFAR and/or government and private laboratories recognized by BFAR.

Imported fresh, chilled and frozen fishery products are inspected upon arrival at the port for quality control measures. Samples are randomly taken/drawn for sensory evaluation, chemical and microbiological tests. Results are used as a basis for the sale and distribution of the products.

For products locally produced and intended for domestic consumption, regulations on the sale and distribution are under the jurisdiction of the local government units. The Bureau of Food and Drug is the agency mandated to implement the rules and regulations on the production, distribution and sale of locally manufactured fishery products.

The One Stop Export Documentation Centre, Fisheries Unit is responsible for the inspection, processing and issuance of documentation requirements for fishery products for export in coordination with Fish Inspection Section. CITES Permit for Wildlife Species and those included in the Convention and International Trade in Endangered Species of Wildlife Flora and Fauna (CITES) regulations are issued in this Unit. Commodity clearance requirements for other fishery products are issued after inspection is conducted.

Policies and Regulations that Support the Inspection System:

Policies, rules and regulations that support the inspection system for export/import/domestic trade of fishery products include:

a. Presidential Decree (PD) 930 and its implementing Guidelines. "Simplifying Export Procedures and Documentation by Realigning Functions of certain Government Offices/Agencies involved in processing export documents, by authorizing the issuance of periodic clearances, by adoption of standardized export documents and for other purposes".

   The decree prescribes inspection and issuance of commodity clearances by government agencies to safeguard the quality of Philippine exportable products but stipulate simplified export procedures by reassigning and/or realigning overlapping functions of certain specialized agencies of the government.

b. Presidential Decree 704 known as the "Fisheries Decree of 1975". Revising and Consolidating all Laws and Decrees Affecting Fishing and Fisheries.

   The decree declares the policy of the state for optimum utilization of the country's fishery resources, conservation and protection, acceleration and promotion of the integrated development of the fishery industry and the promotion of exportation of fish and fishery/aquatic products.

c. Fisheries Administrative Order No. 117 series of 1975 (FAO 117). Rules and Regulations Governing the Operation of Processing Plants for Fish and Fishery/ Aquatic Products and Prescribing/Requiring Standards, Quality Control and Inspection of Processed Fish and Fishery Aquatic Products.

d. FAO No. 117-1 series of 1994. Amendment of Sections 3, 4, and 5 of FAO 117s. 1975. Certain provisions on the minimum requirements for processing plants and the standards for shrimps and tuna were amended to conform with the present international standard of specifications.

e. Republic Act (R. A.) 7394 known as the "Consumers Act of the Philippines" declares the policy of the state to protect the interest of consumers, promote the general welfare and establish standards for business and industry.

   Department of Agriculture Administrative Order No. 9 series of 1993 Rules and Regulation to achieve the objectives of protecting the consumers against hazards to health and safety and deceptive, unfair and unconscionable sales acts and practices and providing information and education to facilitate sound choice and proper exercise of rights by the consumer.

f. Executive Order No. 1016. "With-drawing the Inspection, Commodity & Export Clearance Requirements on Philippine Exports" - The order simplifies the export documentation requirements of commodity agencies.

Description of Inspection and Certification Systems:

The inspection and certification system practised is based on the approved rules and regulation of FAO 117 and 117-1; patterned after the Quality Management Programme (QMP) of Canada and the principles of Hazard Analysis and Critical Control Point (HACCP). The accreditation system implemented especially for the European Union will be in coordination with the Department of Health approved legislation in the operation of food plants.

SINGAPORE

Name of Organisation:

Veterinary Public Health &
Food Supply Division Primary Production Department
Ministry of National Development

Full Address:

5 Maxwell Road, #02-00/#03-00
Tower Block, MND Complex
Singapore 069110
Republic of Singapore
Tel. (65) 265-0622
Fax. (65) 265-0784
Telex RS 28851 PPD

Products of Discipline:

- ✓ Fishery products for export/import/ domestic use
- ✓ Fresh and frozen fishery products only
- ✓ Chilled fishery products
- ✓ Dried fishery products only
- ____ Others

Mandatory or Voluntary:

All fish, including finfish, crustaceans, molluscs, and fishery products which are wholesome and fit for human consumption may be imported or exported with a licence. Additional requirements on product quality and live fish quarantine may be imposed on selected fish/fish products as and when necessary.

Fish inspection is mandatory for import consignments of high-risk seafood such as frozen oyster, frozen raw blood cockle meat, frozen cooked prawns / shrimps and frozen crabmeat. End product testings are also carried out to ensure fitness for human consumption. These high-risk seafood also require health certificates from competent authority in the country of origin. Prior approval from the Primary Production

Department (PPD) is required before importation of these products.

Import of chilled forms of the above products are prohibited.

Description of Organisation:

The Veterinary Public Health & Food Supply Division (VPHFS) of the Primary Production Department (PPD) is charged with the responsibility of providing an efficient and effective inspection and testing services to ensure primary produce are safe and whole-some in Singapore and to assure the foreign buyers that Singapore's export of primary produce conforms to their health requirements. VPHFS is also responsible for ensuring a stable and adequate supply of fresh produce in Singapore.

These responsibilities are discharged through VPHFS's four branches, viz.; Food Inspection Services Branch (FISB), Veterinary Public Health Laboratory Branch (VPHL), Food Sup-ply Branch (FSB) and Development & Compliance Branch (D&C).

The VPHL is an internationally recognized laboratory able to detect food-borne diseases as well as major chemical contaminants and drug residues in food. The VPHL is also the National Reference Laboratory for pesticide residues.

Policies and Regulations that Support the Inspection System:

- The Slaughterhouses and Meat Processing Factories Act (Chap 307)
- The Animals and Birds Act (Chap 7)
- Fisheries Act (Chap 294)
- Control of Plants Act 1993
- The Sale of Food Act (Cap 283)
- The Environment Public Health Act (Cap)

* Relevant Rules/Orders under above Acts.

Description of Inspection and Certification Systems:

Traders have to submit the declarations for all imports and exports of fish and fishery products (including finfish, crustaceans and molluscs) through TradeNet for processing by the PPD.

They also need to submit (fax acceptable) the invoices, bill of loading/ airway bill and other relevant supporting documents.

Health certificate for the high-risk seafoods issued by the authority of the country of origin has to be submitted to the D&C, Level 2, MND Building, Maxwell Road, Singapore 069110 for approval of an import permit. Upon importation, these products will be subject to inspection, sampling and testing by the FISB and VPHL.

All local fish processing plants are licensed by the PPD. PPD's enforcement strategy is that of a system audit, inspecting and testing at critical control points of the production processes. The whole system is backed up by a comprehensive laboratory monitoring programme.

Each export consignment, which needs certification, would be inspected by the FISB. Samples are collected for laboratory examination in accordance with the sampling and testing protocols of the export certification programme depending on destination. The VPHL would issue the health certificate when the testing criteria are met.

TAIWAN PROVINCE OF CHINA 1

Name of Organisation:

Department of Health,
The Executive Yuan

Full Address:

No. 100 Ai Kuo E. Road
Taipei, Taiwan Province of China
Tel. (8862) 393-8209
Fax. (8862) 392-9723

Products of Discipline:

- ✓ Fishery products for export/import/ domestic use
- _____ Fresh and frozen fishery products only
- _____ Chilled fishery products
- _____ Dried fishery products only
- _____ Others

Mandatory or Voluntary:

Mandatory

Description of Organisations:

Import

(1) National Quarantine Service, Department of Health

(2) Bureau of Commodity Inspection and Quarantine, Ministry of Economic Affair (Commissioned by the Department of Health)

Domestic

(1) Department of Health

(2) Health Bureaus of Local Governments

Policies and Regulations that Support the Inspection System:

(1) Quarantine Regulations for Cholera on Imported Fishery Products

(2) Law Governing Food Sanitation

Description of Inspection and Certification Systems:

Import

(1) Sampling test of toxigenic *Vibrio cholerae* may be conducted at the entry ports by the quarantine authorities. For fishery products imported from districts infected with cholera, a certificate of *Vibrio cholerae* negative issued by the exporting country must be submitted.

(2) Inspection is conducted to check if fishery products meet the requirements of the Law Governing Food Sanitation.

Domestic

Fishery products is free for sale on market unless they do not conform to sanitary standards, which are prescribed by the Department of Health, the Executive Yuan. The products on market are under regular monitoring by health bureaus of local governments.

TAIWAN PROVINCE OF CHINA 2

Name of Organisation:

Bureau of Commodity Inspection
and Quarantine (BCIQ)
Ministry of Economic Affairs
(MOEA)

Full Address:

No. 4, Chinan Road,
Sec. 1, Taipei,
Taiwan Province of China
Tel. (8862) 343-1700
Fax. (8862) 393-2324

Products of Discipline:

- ✓ Fishery products for export/ import/domestic use
- ✓ Fresh and frozen fishery products only
- ✓ Chilled fishery products
- ✓ Dried fishery products only
- ____ Others

Mandatory or Voluntary:

Inspection for fishery products subject to inspection is applied mandatory, inspection for those not subject to inspection is applied voluntarily.

Description of Organisation:

BCIQ has been established as the national agency of commodity inspection. One of BCIQ's responsibilities is to conduct export/import inspection for agricultural products (including fishery products), machinery/electronic apparatus, chemical products, and mineral/ metal products. BCIQ has headquarters in Taipei and six branch offices throughout the island. Each office has its own laboratories, including testing room for fishery products, to conduct inspection work.

Policies and Regulations that Support the Inspection System:

According to Commodity Inspection Act, fishery products which have been promulgated by the Ministry of Economic Affairs (MOEA) to be subject to inspection must pass the inspection of BCIQ before they can be exported or imported.

Description of Inspection and Certification Systems:

Commodities subject to inspection shall not be exported or imported unless they have passed inspection and been certified by BCIQ. For these commodities, the application for inspection shall be filed with BCIQ by the owner of the commodities or the agent thereof by filling out an application form and submitting it together with inspection fees.

THAILAND - 1

Member Economy:

Thailand

Name of Organisation:

Fish Inspection and Quality
Control Division
Department of Fisheries

Full Address:

Kasetsart University Campus
Phaholyothin Road, Chatuchak,
Bangkok 10900, Thailand.
Tel : (662) 579-6729, 579-6915
Fax : (662) 579-6687

Products of Discipline:

- ✓ Fishery products for export/ import/domestic use
- _____ Fresh and frozen fishery products only
- _____ Chilled fishery products
- _____ Dried fishery products only
- _____ Others

Mandatory or Voluntary:

Mandatory (export only)

Description of Organisation:

The Department of Fisheries is the competent authority in conducting inspection and quality assurance for fish and fishery products. Inspection and certification of fish and fishery products is the responsibility of the Department of Fisheries. The Department of Fisheries conducts mandatory inspection of processors and packers of fish and fishery products and final products prior to exporting. Certificates will be issued, if the processing plants and products are in compliance with standard requirements set by the Department and standard requirements of importing countries. Such services are backed up by research and development work including international cooperations.

The current seafood safety and quality control programme is based on Good Manufacturing Practices and the Hazard Analysis and Critical Control Point (HACCP) principles.

The Department has established 6 regional services as follows:

- Fish Inspection Centre (Bangkok)
- Fish Inspection Centre (Songkhla)
- Fish Inspection Centre (Surattha-ni)
- Fish Inspection Centre (Samutsa-korn)

Policies and Regulations that Support the Inspection System:

- Fisheries Act
- Import/Export Control Act

Description of Inspection and Certification Systems:

1. Fish processing establishments intend to export fish and fishery products are subject to plant inspection. Plant inspection is carried out at the rate of 2 - 4 times/plant/year. The inspection is made up of three inspectors. The inspection involves raw material, conditions and maintenance of construction, equipment, processing operations, plant hygiene and personnel. The plant must be in compliance with the standards established by the Department of Fisheries. The Department will maintain the list of approved establishments, during this period of approval the processors/exporters can apply for preshipment inspection of products. If the plant is found not in compliance to the requirements, corrections of defects are allowed time frame, usually not more than 3 months, for re-inspection by inspectors.

2. A company requesting certificates must provide in advance the following information:

   - certificate type
   - lot location
   - lot size
   - product description
   - consignee
   - consignor
   - manufacturer

   The inspector of the Certification Sub-division will inform the inspector supervising the sampling of the sampling schedule (date/time) and details from the requesting company.

3. The inspector of sampling section will determine plan, calculate lot size, sample size and withdraw sample from the lot. Samples will be withdrawn by authorized fishery officers only.

5. Within 24 hours, the sample will be prepared and laboratories will perform analysis according to AOAC methods unless specified by import authorities.

6. Once the analysis is done, the supervisor of the laboratory will review and verify results and identify whether the sample is conformed to the requirements or not.

7. The lot will pass or fail based on results of laboratory inspection. If the lot passes, a certificate will be issued by Certification Sub-division to be reviewed by the Director of FIQD and then be submitted for the approval of the Director of the Department of Fisheries (DOF). The Director of DOF or the designated staff may sign the certificate. If the lot fails to comply with the standards, a certificate will not be issued for the lot, resampling may be conducted based on reason of failure.

THAILAND - 2

Name of Organisations:

Department of Medical Sciences
Division of Food-for-Export Analysis

Full Address:

Division of Food-for-Export Analysis
693 Bamrung Muang Road,
Bangkok 10100 Thailand
Tel. (662) 223-3772
Fax. (662) 222-

Products of Discipline:

- ✓ Fishery products for export/ import/domestic use
- ✓ Fresh and frozen fishery products only
- ✓ Chilled fishery products
- ✓ Dried fishery products only
- ✓ Others

Mandatory or Voluntary:

In case of canned products are mandatory base and the rest are voluntary base.

Description of Organisations:

The Department of Medical Sciences (DMSc.), one of the agencies of 14 Divisions located in Bangkok and Nonthaburi Province and 9 Regional Medical Sciences Centres. Its major responsibilities are research, services, training and others in the field of medical and health sciences. One important responsibility of the Department is to provide services concerning food safety and quality standards assessment and certification of food for export according to the requirements of importing countries and or importers. These activities are performed to facilitate international trade of food at the Division of Food-for-Export Analysis in Bangkok and the Regional Medical Sciences Centres in Songkhla, Chonburi, Trang and Chiengmai Provinces. The Department plans to expand food export certification services to other Regional Medical Sciences Centres as required by the industry.

The Division of Food-for-Export Analysis (DFEA) has the responsibilities in providing laboratory services for exporters and food producers for export; issuing certificates for food export consignments in the fields of food safety and health standards according to the requirements of importing countries and/or importers and promotion of food export industry with reference to health requirements.

The Division of Food Analysis is responsible for laboratory checking in domestic and imported foods, to ensure the consumer safety according to the Food Act B.E. 2522 (1979).

Policies and Regulations that Support the Inspection System:

Food Act B.E. 2522 (1979).

Legal Food Control in Thailand: Current Laws and Regulations

Food Control System in Thailand is exercised in accordance with the Food Act B.E. 2522 under the administration of the Ministry of Public Health. The Act is composed of 8 chapters describing administrative procedures for legal food control operations. They are the establishing of Food Commission, applications of licences and issuance of licences, duties of licensees with regard to food, control of food, licensing and advertising of foods, competent officers, suspension or revocation of licences and penalties. The Ministry of Public Health Notifications have been issued in pursuance of the Food Act B.E. 2522 describing quality standards and relevant information on Foods, labelling including labelling for exported foods, processing procedures and food packaging materials.

Prepackaged processed foods including canned foods, milk, dairy products, bottled drinking water and some traditional foods have been declared to be specific-controlled foods by the Minister of Public Health. Their quality standards and labellings have been described in the Ministry of Public Health Notifications.

The scope of food control system covers all types of foods both for domestic consumption and import.

The Food and Drug Administration (FDA) is responsible for the legal control of food products in Thailand, and imported into the country.

Who is Who in Fish Inspection of APEC Economies

Economies	No. of Agency	Agency Name	Address	Main Contact Person(s)
Australia	1	Australian Quarantine and Inspection Service AQIS)	Edmund Barton Building, Blackall St., Barton ACT 2600, GPO Box 858 Canberra ACT 2601 Tel. (616) 272-4725; Fax. (616) 272-3682	Mr. Steve Bailey
Brunei Darussalam	1	Department of Fisheries Ministry of Industry and Primary Resource	5th Floor Athirah Plaza, Jalan Kubah Makam Diraja, Negara Brunei Darussalam 2069 Tel. (6732) 242-067; Fax. (6732) 242-069	Director General
Canada	1	Canadian Food Inspection Agency (Fish, Seafood and Production Div.)	59 Camerot Drive, Napean, Ontario K1A 0Y9 Canada Tel. (613) 990-0144; Fax. (613) 993-4220	Mr. Cameron Prince Director
Chile	1	Servicio Nacional de Pesca	Yangay N° 1731, 4° piso, Valparaiso, Chile Tel. (5632) 217-390, 212-090; Fax. (5632) 259-564	Dr. Juan Rusque Director
China	1	National Center for Quality Supervision and Test of Aquatic Products	106 Nanjing Road, Qingdao 266071, Shan Dong Province, P.R. China Tel. (86 532) 582-6579; Fax. (86 532) 581-1514	Li Xiao Chuan Director of Center
Hong Kong, China	1	Agriculture and Fisheries Department	Canton Road, Government Offices, 12/F, 393 Canton Road, Kowloon Hong Kong SAR Tel. (852) 2733-2208, 2873-8326 Fax. (852) 2311-3731, 2814-0018	Veterinary Officer Health Certification
Taiwan Province of China	2	1. Department of Health, The Executive Yuan	No. 100 Ai Kuo E. Rd., Taipei, Taiwan Province of China Tel. (8862) 393-8209; Fax. (8862) 392-9723	Dr. Shu-Kong Chen Director, Bureau of Food Sanitation
		2. Bureau of Commodity Inspection and Quarantine (BCIQ), Ministry of Economic Affairs (MOEA)	No. 4, Chinan Rd., Sec. 1, Taipei, Taiwan Province of China Tel. (8862) 343-1700; Fax.(8862) 393-2324	Mr. Chin-Ping Houng Director of 1st Department
Indonesia	1	Sub Directorate of Fish Inspection and Quality Control	Jl. Harsono R.M. No. 3, Pasar Minggu, Jakarta 12550 Indonesia Tel. (6221) 789-1479; Fax. (6221) 789-1479 Telex. 47318 djikan ia	Dr. Josephine Wiryanti

Economies	No. of Agency	Agency Name	Address	Main Contact Person(s)
Japan	1	Veterinary Sanitation Division, Environmental Health Burcau, Ministry of Health and Welfare	2-2, Kasumigaseki 1-Chome, Chiyoda-ku, Tokyo 100-45 Japan Tel. (813) 3503-1711 (ext. 2436) Fax. (813) 3503-7964	Mr. Toshiaki Kuwasaki D.V.M. Technical Official
Korea	1	National Fishery Products Inspection Service	Wonnam Dong 103, Chongrogu, Seoul, 110-450 Korea Tel. (02) 762-9214 Fax. (02) 765-1755	Mr. Roe Seong-Man Director Inspection 1Division
Malaysia	2	1. Malaysian Fisheries Development Authority (MFDA)	8th Floor Wisma PKNS, Jalan Raja Laut, P.O. Box 12630, 50784 Kuala Lumpur, Malaysia Tel. (03) 292-4044 Fax. (03) 291-1931	Mr. Mustafa Hj. Ahmad Marketing Director
		2. Fisheries Research Institute, Department of Fisheries Malaysia	11960 Batu Muang, Penang, Malaysia Tel. (04) 626-3925/6 Fax. (04) 626-2210	Mr. Hamdan Bin Jaafar, Fish Inspection & Quality Control Center
Mexico	2	1. Ministry of Fisheries	Lateral Anillo Periferico Sur 4209, 5º piso, col. Jardines en la Montana 14050 Mexico, D.F.	Mara Angelica Murillo Correa, General irector of International Fishery Affairs
		2. Ministry of Health	Donceles 39, col. Centro, C.P. 06010 Mexico, D.F. Tel. (52-5) 521-9134/3050 Fax. (52-5) 512-9628	Mr. Jose Meljem Moctezuma, General Director of Sanitary Control of Services and Goods
New Zealand	2	1. Ministry of Agriculture	**Postal :** P.O. Box 2526, Wellington, New Zealand **Physical :** ASB Bank House, 101-103 The Terrace Wellington, New Zealand Tel. (644) 474-4100 Fax. (644) 474-4239	John Lee, National Manager Fish or Phil Busby, National Manager Shellfish
		2. Ministry of Health	**Postal :** P.O. Box 5013, Wellington, New Zealand **Physical :** 133 Molesworth Street, Wellington, New Zealand Tel. (644) 496-2000 Fax. (644) 496-2340	Manager Food Administration

Economies	No. of Agency	Agency Name	Address	Main Contact Person(s)
Philippines	1	Bureau of Fisheries and Aquatic Resource (BFAR)	Arcadia Bldg., 860 Quezon Avenue, Quezon City, Metro Manila, 3008 Philippines Tel. (632) 973-617 Fax. (632) 987-871, 973-617	Dennis B. Araullo Director
Singapore	1	Veterinary Public Health & Food Supply Division Primary Production Department, Ministry of National Development	5 Maxwell Rd., #02-00/#03-00 Tower Block, MND Complex, Singapore 069110 Republic of Singapore Tel. (65) 265-0622 Fax. (65) 265-0784 Telex : RS 28851 PPD	Dr. Paul Chiew King Tiong, Head/Food Inspection Services Branch or Dr. Chew Siang Thai, Head/Veterinary Public Health Laboratory Branch
Thailand	2	1. Fish Inspection and Quality Control Division, Department of Fisheries	Kasetsart University Campus, Paholyothin Rd., Chatuchak, Bangkok 10900 Thailand Tel. (662) 579-7738 Fax. (662) 579-6687	Mr. Montri Klitsaneephai-boon Director
		2. Departement of Medical Sciences	693 Bamrung Muang Rd., Bangkok 10100 Thailand Tel./Fax. (662) 223-3730, 589-9862	Director General
USA	2	1. Food and Drug Administration (FDA), Office of Seafood, Department of Health and Human Services	HFS-400, 200 C St., S.W. Washington D.C. 20204 Tel. (202) 418-3150 Fax. (202) 418-3196	Director, Division of Programs and Enforcement Policy
		2. National Marine Fisheries Service (NMFS), Inspection Services Division, Department of Commerce	1315 East-West Highway, Silver Spring, MD 20910 Tel. (301) 713-2355, (301) 713-1081	Mr. Richard Cano Chief Inspection Services Division

HISTAMINE IN TUNA IN THE PACIFIC ISLAND REGION

by

TONY CHAMBERLAIN
Marine Studies Program, University of the South Pacific
Suva, FIJI ISLANDS

ABSTRACT

Fisheries are of crucial importance to many Pacific Island countries and for several, one of the largest earners of foreign exchange. The most significant fish and fishery products exported from the region are tuna and tuna products, i.e. sashimi, canned tuna and dried tuna. With the introduction of US Seafood HACCP Rule 1995 there is a strong incentive for countries that export seafood products, particularly to North America and Europe, to maintain trade access to these large and extremely valuable markets. Even considering the larger businesses, the degree of HACCP preparation is very limited. SPC and FAO have made some efforts to evaluate and commence the development of HACCP in the Pacific Island region. More training, assistance to industry and governments, and research is still needed. Good regional data are not always readily available to justify currently used endpoints or to permit appropriate detection of the hazard. Research is needed to determine the appropriateness and effectiveness of current quality control criteria in reducing the incidence of scrombrotoxin in the Pacific Island region. To this end the University of the South Pacific has refined a highly quantitative chemical test for histamine analysis. A critical review of the USFDA stand on histamine control in tuna reveals that contentious issues exist concerning temperature and time limits, sampling regimes, critical limits and whether to control histamine by a HACCP program or by SSOPs. Recommendations for future research include validation of histamine test kits, regional studies into temperature and time affects on histamine production in fresh tuna and studies on histamine production in tuna jerky products.

INTRODUCTION

Tuna fisheries are of crucial importance to many Pacific Island countries and for several, one of the largest earners of foreign exchange. The annual commercial catch for tuna in the region in 1992 was in excess of one million tonnes, of which 67% was skipjack and 24% was yellowfin.

The main tuna exports are fresh/frozen tuna (especially sashimi grade tuna), dried tuna and caned tuna. The high prices offered in the highly competitive and demanding sashimi markets justifies the special treatment of the product and strict adherence to stringent trading requirements. Successful tuna jerky operations are Teikabuti Fishing Company, Tarawa, Kiribati and Ocean Traders at Pacific Harbour Fiji. Both have found successful export markets in Australia, New Zealand, Hawaii and Japan. Canned tuna has had a very important role in Fiji and throughout the Pacific in the past half century. There are large canneries established at a number of Pacific Island countries including: Fiji, American Samoa and the Solomon Islands. Canned tuna is extremely popular, with various species used such as albacore, skipjack, bluefin and yellowfin.

The importance of tuna exports is apparent if we look at Pacific islands by country. In Tonga most major exporters export fresh and frozen tuna or bottom fish (Gillet, 1997). In Samoa most major exporters export fresh, frozen and smoked tuna. According to the Secretariat for the Pacific Community (SPC) Tuna Fishery Yearbook (1995), Fiji Islands domestic long line fleet (less the US/Vietnamese vessels) landed 2,465 mt of tunas and other species in 1995. Most major exporters export fresh, frozen and smoked tuna. In the Solomon Islands fresh, frozen, smoked and canned tuna make up the major share of fish exports by weight and by value; although the reef fish exports have proven to be lucrative exports also. Sixty percent of the canned tuna production is exported to the United Kingdom, virtually all of the fresh tuna and katsuobushi is exported to

Japan and the frozen tuna are exported to a variety of locations including Japan, Thailand, Fiji, American Samoa, Philippines, and Latin America (Gillet, 1997).

HAZARD ANALYSIS AND CRITICAL CONTROL POINT

Introduction

On December 18, 1995 the USFDA passed a regulation Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products. This is now known as the "Seafood HACCP Regulation". The US Food and Drug Administration (FDA), for instance, hopes to prevent 30,000 to 60,000 cases of illnesses attributed to the consumption of seafood by implementing the US Seafood HACCP Rule on the safe and sanitary handling and processing of all fish and fish products.

Current status of HACCP in Pacific Island Countries

Introduction

Food processors all over the world, particularly in the least developed countries are having to come to terms with a different more rigorous and effective quality assurance systems. The promising evolution of the export fish trade from the Pacific Island region is considered to be under threat as a result of regulatory changes that were introduced by the major fish importing countries. The loss of these important markets, in particular the US, could have potentially lead to closures of businesses and loss of employment opportunities (McDorman, 1998). The incentive for countries that export seafood products, particularly to North America and Europe, is not only to maintain trade access to these large and extremely valuable markets, but also to become even more competitive and successful in the international market place (Roberts, 1998).

Tonga

There is currently no testing of fish exports. There are no laboratory facilities at the Ministry of Fisheries, although some laboratory facilities exist in the Ministry of Health where officials state that coliform bacteria and salmonella tests can be performed (Gillet, 1997).

Samoa

There is some testing of food and water carried out by the National Health Laboratory, but it is apparently not in response to a legal requirement. The Director of the Laboratory and a technologist stated they occasionally carry out tests on food and water.

Fiji

According to Ministry of Health officials, the testing requirements and procedures are those requested by the overseas client. The Ministry of Health uses the laboratory facilities of the Ministry of Agriculture, Fisheries, Forests, and ALTA at Koronivia, but those facilities do not have international accreditation. PAFCO maintains laboratory facilities at their cannery that, according to the General Manager, are able to do histamine analysis. Those facilities are not accredited but if testing in an accredited laboratory is required, samples can be forwarded to the B C Packers lab in Canada.

The Institute of Applied Science (IAS) at the University of the South Pacific (USP) has testing facilities. Although they have no accreditation at present, they expect to obtain such certification in about a year when they transfer to new facilities. The Director of IAS expects they could take on a

verification/auditing role and because USP is a regional body, this function could be extended to other countries.

Federated States of Micronesia

The only analytical test the National Food Inspectors are able to carry out is that for E.coli and this test is only carried out when contamination is suspected. The Food Safety Act has provision for the appointment of Food Analysts to "perform laboratory and field tests upon food and other articles". According to the National Food Inspectors, there are plans to build a national laboratory in Chuuk for the testing of food and water. Officials of the Pohnpei Fisheries Corporation (PFC) stated that the PFC laboratory is able to do most standard food and water quality tests (but not histamine tests) and that PFC has had some role in training government health workers.

Solomon Islands

There do not appear to be any specific testing requirements under the Pure Food Act (to date, no regulations under the Act have been made). Under Section 23 of the Act, a food exporter can apply to have products inspected and analysed by a laboratory certified by an approved laboratory certification scheme". The specific tests are not stipulated.

The Environmental Health Division is responsible for the Public Health Laboratory (distinct from the hospital laboratory). According to the Director of the Environmental Health Division, the laboratory is able to carry out the HACCP-related tests, water analysis (BOD, COD), and histamine tests.

General conclusions

There are varying degrees of dependence on marketing of fishery products from Pacific Island Countries to countries where HACCP has been put in place.

Table 1. Dependence on EU, Canada, and US Markets.

Country	Market Dependence (%of total value of exports)
Tonga	72%
Western Samoa	95%
Fiji	73%
FSM	10%
Solomon Islands	30%

Although there were exceptions, in 1997 it appeared that in most countries the government fishery departments had passed little or no information about HACCP to the private sector fish exporters. Only a few government fisheries officials charged with HACCP responsibilities had a good understanding of HACCP principles or specific details of US FDA HACCP regulations.

Table 2 gives a subjective evaluation of the degree of preparation by the major fish exporters in five countries. The Table considers only the major exporters, few, if any, of the smaller exporters (especially those operated by Pacific Islanders) had made any HACCP preparations.

Table 2. HACCP preparations in a number of Pacific Island countries.				
	Number of exporters			
Country	contacted	with well-advanced HACCP preparations	with some substantial HACCP preparations	with little or no substantial HACCP preparations
Tonga	5	1	1	3
Western Samoa	5	1	1	3
Fiji	7	2	0	5
FSM	4	1	0	3
Solomon Islands	4	1	0	3
Total	25	6	2	17

Even considering the larger businesses, the degree of HACCP preparation is very limited. It appears as though only four exporting firms have any sort of HACCP plans in place (two of these are industrial-scale canneries), with two additional firms making some progress on plan formulation and implementation (Gillet, 1997).

Advances of Pacific Island countries towards HACCP

A Regional Workshop hosted by SPC and FAO on the Implementation of HACCP-based Quality Assurance for Seafood was held in Fiji in 1997. The workshop provided valuable grounding in the legal and technical aspects of HACCP implementation by the USA (and to a lesser extent, the EU) and at identifying strategic options which governments could pursue in meeting the US requirements.

In response to recommendations made at the Regional workshop in Fiji, FAO Sub-Regional Office in Apia (FAO SAPA) and SPC Coastal Fisheries Program, continued to collaborate by organising certified HACCP workshops that met the US HACCP training curriculum as drawn up by National Seafood HACCP Alliance for Training and Education.

The workshops ensured that recognised HACCP trained individuals are available to exporters to undertake key activities described in the HACCP Rule. Such activities include developing, reviewing and modifying as necessary written HACCP plans and procedures; monitoring, reviewing and maintaining written records; undertaking basic verification procedures; etc. In all 60 participants were certified (18 in Samoa, 24 in Fiji, 18 in Tonga and 18 in the Federated States of Micronesia). Most of those certified were staff belonging to export operations. A number of different Government departments were also represented.

Assistance to seafood exporters to meet HACCP requirements

In 1997 five Fijian, five Samoan and three Tongan seafood exporters were assisted by an Australian HACCP consultant who evaluated the processing establishments and helped develop Good Manufacturing Practice (GMP), Sanitation Standard Operating Procedures (SSOP), HACCP plans and training advise.

All companies that received assistance in 1997 had implemented HACCP at least to a basic but satisfactory level of compliance and that there were genuine commitments to improved processing. One or two companies had made genuine attempts to meet all requirements and almost every company had significantly improved their premises during 1998 (Roberts, 1998).

Future Efforts

- further visits to seafood exporters
- more national HACCP workshop are needed

- assistance to establish effective national Controlling Authorities
- development of a protocol on how best to establish an effective Controlling Authority, training of inspectors and auditors
- more in-depth training to exporters
- top-up training to the senior HACCP staff
- training for production line staff
- more applied and pure research on seafood safety and quality in the region

Handling, processing and distribution methods can be monitored by a Hazard Analysis Critical Control point (HACCP) system. However good data are not always readily available to justify currently used endpoints or to permit appropriate detection of the hazard. Research is needed to determine the appropriateness and effectiveness of current quality control criteria in reducing the incidence of human disease (Ahmed, 1992).

HISTAMINE

There has been considerable international attention paid to histamine in canned, chilled, frozen and dried fish. High histamine levels in seafood products exported from the Indo-Pacific region have, in the past, resulted in economic losses and now a number of importing countries have introduced regulations on maximum allowable histamine content.

Temperature/Time

The mechanism of histamine formation is similar between species. Histamine will develop if there is a fish with copious amounts of free histidine, and there are bacteria (from whatever source) that can convert histidine to histamine, and there is time where the fish are stored at elevated temperatures, its about that simple.

High levels of histamine (approaching 50 mg% and beyond) are normally associated with high temperature/shorter time abuse of the fish. This is even mentioned on p 69, Chapter 7 in the Fish and Fishery Products Hazards and Controls Guide (USFDA, 1998). The higher temperature abuse results in the bacteria that decarboxylate histadine to histamine being able to proliferate and produce substantial amounts of the active agent - the enzyme histidine decarboxylase.

High levels of histamine indicate that the fish has been stored under conditions that could give rise to toxicity, and effective temperature control during handling and processing is the main and practical way of minimising the hazard. The main problem associated with distribution of seafood products is time-temperature abuse. Most bacteria are not capable of growth at temperatures <4°C, thus proper cooling of seafoods during transportation becomes an important consideration. Chilled product should be loaded at internal temperatures below 4°C and frozen product at or below –18°C (Ahmed, 1992).

Any time above 4.4°C significantly reduces the expected safe shelf-life. For this reason, fish should not be exposed to temperaturs above 4.4°C for more than four hours, cumulatively, after chilling on board the harvest vessel. The safety of this limit is dependent upon proper handling at sea. Fish that have been handled particularly well on-board the harvest vessel may be able to safely withstand somewhat more exposure to elevated temperatures during post-harvest handling (USFDA, 1998). Fish should not be exposed to temperatures above 4.4°C for more than twelve hours, cumulatively, after chilling on board the harvest vessel. An uninterrupted period of exposure should not exceed six hours. Intermittent refrigeration breaks the cycle of rapid bacterial growth and slows the formation of histamine. The safety of these limits is again dependent upon proper handling at sea (USFDA, 1998).

The only realistic place or point where we can see high levels of histamine forming would be between the time the fish are caught and die on board the fishing vessel, until the temperature is reduced to

approximately 4.4 - 5.0°C. Fresh fish should be kept at the 4.4-5°C temperature or below until ready for processing.

Relative to the use of 4.4°C as a critical limit for backbone temperature in incoming fish, Section K, p 4175 of the preamble to the Proposal to Establish Procedures for the Safe Processing and Importing of Fish and Fishery Products (USFDA, 1994) states the harvesters goal should be to bring the fish to an internal temperature of 4.4°C or below as soon as possible after the fish dies to minimise the risk of histamine production. Cooling fish below 10°C, greatly reduces the growth of populations of the bacteria which are the most likely to cause histamine formation. Once bacterial growth has begun, temperature at or below 5°C halts bacterial growth, although enzymatic histamine formation may slowly continue (p 95)". The agency goes on to say that they were dropping the temperature recommendations in the proposal to 4°C, to make it consistent with other temperature recommendations in the proposal, and invited comments on the appropriateness of this temperature. In reviewing the preamble to the Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products (USFDA, 1995) there are no comments discussed by FDA where there was an objection to the use of this temperature. The agency does acknowledge, however, that a large number of comments were received relative to the appropriateness of the 4.4°C temperature proposed. The agency was not going to discuss the comments received on the appropriateness of 4.4°C as a refrigeration temperature.

The Refrigerated Foods and Microbiological Criteria Committee (RFMCC) (1988) supports the use of 4.4°C as an appropriate refrigeration temperature. The RFMCC is aimed primarily at establishing good manufacturing practices for finished products which are to be stored and distributed under refrigeration, it basically supports the use of 4.4°C (and below, as appropriate) as being recommended for products requiring refrigeration.

Burns (1985) states that the rate of histamine formation decreases markedly at temperatures below 10°C. The International Committee on Microbiological Specifications for Foods (1996) appears to offer some degree of corroboration with this by indicating the control of the histamine hazard in scombrotoxic species is enhanced by bringing the temperature of the fish to 10°C within at least 2 hours after death and maintaining it at <10°C. These authors also stated that from the standpoint of spoilage (i.e. quality factors), the fish temperature should be maintained at 0°C. Ahmed (1992) recommends bringing the temperature below 15°C and, preferably below 10°C in four hours, a time factor that was not listed by the USFDA in the proposed seafood HACCP rule reference quoted above. They also state that from the standpoint of spoilage (i.e. quality factors) the fish temperature should be maintained at 0°C.

Perhaps the only practical way to determine the time/temperature and histamine/decomposition relationship is to conduct some actual real-time studies. It is noteworthy, moreover, that the ICMSF states that what they term a "dangerously toxic condition" occurs in scombrotoxic species only when they are held for several hours in the temperature range >15-20°C. Another possibility is to conduct studies where conditions on board the fishing vessels and/or during transportation to the factory could be realistically simulated. Once the time/temperature relationship to a level of histamine of 50 mg% or greater was determined one might then have established a scientific basis for critical limits. The same would apply if a particular backbone temperature could be related to a particular level of time/temperature abuse, leading to high histamine levels. Exceeding such critical limits, established in this manner, would present, therefore, a real possibility that scombrotoxic levels of histamine could be present, and would necessitate true HACCP-type corrective action.

It should be noted that the time required to lower the internal temperature of fish after capture will be dependent upon a number of factors, including (USFDA, 1998):

- The harvest method;- delays in removing fish from a longline may significantly limit the amount of time left for chilling and allow the fish to heat up as it struggles; the size of the sets;
- The size of the fish;
- The chilling method; how fast is they chilled after catch?

CRITICAL LIMIT

The USFDA has established decomposition limits for histamine in raw, frozen tuna and mahi-mahi, canned tuna and related species of 50 ppm (5 mg/100g or 5 mg%) or less. They have also specified a health hazard (scombrotoxic) limit of 500 ppm (50 mg/100g or 50 mg%) and above (USFDA, 1996).

In 3 August 1995 Federal Register notice, the decomposition limit was lowered by the USFDA from 100 ppm **and** evidence of organoleptic decomposition; to the current 50 ppm **or** evidence of organoleptic decomposition. The notice also eliminates the provision in the original CPG that states findings of less than 200 ppm histamine need to be confirmed by organoleptic evaluation. Chemical analysis now is deemed to be sufficient for confirmation.

Histamine is considered a poisonous or deleterious substance because, when ingested at sufficiently high levels, it is known to cause scrombroid poisoning, FDA noted. The agency is not changing the 500 ppm action level at this time because the threshold toxic dose of histamine is not known.

Furthermore, the presence of other amine decomposition products in fish may have a synergistic effect on histamine toxicity. The synergism may dramatically lower the threshold toxic dose."

> "Although the agency intends to use this defect action level in deciding whether to recommend regulatory action, it does not consider that the fact that a fish or fishery product has a histamine level below 50 ppm establishes that the fish or fishery product is acceptable. Other spoilage mechanisms are possible that do not result in the formation of histamine. Thus a finding of histamine levels between 20 and 50 ppm should be viewed as indicating that the fish or fishery product has deteriorated and should cause a producer to further evaluate or test the product."

The critical concentration for poisoning through histamine in spoiled fish is then about $100mg100g^{-1}$ of muscle. Amounts found as high as 450 to $500mg100g^{-1}$ do not, however, always produce poisoning. It is believed that related substances (e.g. TMAO, TMA) could intensify histamine activity or even be more toxic than histamine.

In most cases histamine levels in illness-causing fish have been above 200 ppm, often above 500 ppm. However, there is some evidence that other chemicals (*e.g.* biogenic amines, such as putrescine and cadaverine) may also play a role in the illness (USFDA, 1998).

Finally, it should be emphasized that although the USFDA seafood HACCP regulation does not require documentation supporting the establishment of critical limits (or hazard analysis for that matter), the Codex "recommend it for science as well as for due diligence." While it is important to have documentary support for any limits - critical or otherwise - is essential that it be there for true critical limits because, by definition, compliance with critical limits is necessary for direct public health reasons. Qualified persons with expert knowledge in the establishment of the specific critical limit(s) in question, and having any necessary facilities to establish such limits.

DECOMPOSITION LIMIT OR SAFETY LIMIT

Historically, the major problems involving histamine in tuna have been the result of reaching or exceeding the limits for decomposition, rather than the critical limit for safety (Cole, 1998). Why then use HACCP to control factors other than those affecting the safety of the product. This happened in the food industry in the US in the 1970s, with unfortunate results. We are seeing evidence of the same inclination again today, among certain organisations. What happens eventually is that since HACCP is such a science-based, intensely focused system, it quickly loses that necessary focus when it is used to control quality and economic factors. In the 1970s several firms in the US tried turning HACCP into a Total Quality Control System and it

became so burdensome and so unwieldy, that a large segment of the industry gave up on it. While HACCP is a science-based system, it does not need to be made complicated beyond the realm of human comprehension.

In cases of high temperature abuse fish may still appear organoleptically sound, which means it could be used in processing. Staruszkiewicz (undated), it was stated that for fresh/frozen fillets of mahi-mahi, allowed to decompose at temperatures above 21°C, sixty-five (65%) of those which contained scombrotoxic levels of histamine were found acceptable by organoleptic expert analyst, based solely on odours in the cleaned fillets.

It is important to keep in mind that the hazard of scombrotoxin must be in product which can continue to be processed and is deemed fit for consumption by the consumer, that is, scombrotoxic levels of histamine **combined with** acceptable organoleptic quality. Since decomposition is one of the most often reported problems with seafood, and since scombrotoxin-formation in certain species of fish is considered by the USFDA to be a hazard which is "reasonably likely to occur"; the question arises as to whether both quality and safety related decompostion might be considered under a HACCP plan.

Lower levels of histamine are usually associated with the abuse of fish at lower temperatures and for longer periods of time. This also often results in organoleptic decomposition and is, of course, why fresh fish, kept under refrigeration (not frozen) spoils after a few days. Even if the histamine levels increase over time at these lower temperatures, the fish normally become noticeably spoiled organoleptically, before scombrotoxic levels of histamine (>=50 mg% or 500 ppm) are reached. If the lot cannot be used in processing because of quality deterioration, then, rationally, it becomes a quality control point, not a critical control point. You can only rationally have a critical control point **if** the fish can be further processed; **and** the hazard (500 ppm) is **not** prevented, eliminated or reduced to acceptable levels; **and** the product can reach the consumer with the hazard potentially, or, in fact, present.

Most tuna processors attempt to control histamines from a decomposition standpoint (a quality issue), rather than the critical limit of 500 ppm. With the advent of the Procedures for the Safe and Sanitary Processing and Importing of Fish and Fishery Products (USFDA, 1995), tuna processors have not been sure whether to control histamine in a sanitation standard operating procedure (SSOP) or in a HACCP plan. Most have tended to locate their controls for histamine in a HACCP plan, while listing decomposition limits as their critical limits. This is contrary to the intent of HACCP, which is to control the hazards which most critically and directly affect the safety of the product. This confusion was compounded, no doubt, by information contained in USFDA (1998), for example, in Chapter 7 "Scombrotoxin (Histamine) Formation (A Chemical Hazard)" Table 7-3 lists Receiving as critical control points; the critical limits however, appear to be pertinent to decomposition not safety, at least for Receiving.

With respect to establishing a critical limit for incoming raw tuna, where the preventative measure is other than time/temperature control, the only other reasonable possibility that presents itself would seem to be to establish 500 ppm as the critical limit. If 500 ppm were the limit then a sufficient number of fish in each incoming lot for histamine would have to be analysed, to assure that at the 95% confidence level that there were no fish with histamine levels above the critical limit. This, however, might require a large number of samples to achieve that confidence level.

DETECTION

Organoleptic evaluation and chemical testing are the accepted methods for detecting histamine. There is a lot of mileage in a chemist looking for rapid methods of measurement (D. James, pers. comm., 1998). Neogen's Veratox test Kit is a recently developed quick, portable and reasonably quantitative method for determining histamine. Another good area to be developed would be a meat probe specific for *Histadine decarboxylase* concentration. Computer modelling in predictive microbiology may also have the potential to be developed as an accurate detector of histamine.

Chemical testing is an effective means of detecting the presence of histamine in fish flesh. However, the validity of such testing is dependent upon the design of the sampling plan. For this reason, chemical testing alone will not normally provide adequate assurance that the hazard has been controlled (USFDA, 1998).

Association of Official Analytical Chemists (AOAC) methods are available for histamine analysis. The University of the South Pacific's (USP) Institute of Applied Science (IAS) has been developing a method of histamine analysis that combines and modifies two of the known methods. This quantitative method is to be developed for use with miniaturised chromatographic columns capable of very precise applications (P. Ravi, pers. comm., 1998).

Principle

The sample is extracted with methanol as histamine is soluble in alcohol and water. The extract is passed through a purified ion-exchange column. O-Phthaldialdehyde solution is reacted with eluate to form fluorescent histamine derivatives. Flourescence intensity is measured on HPLC using a fluorescence detector. Quantification is done using external standards.

Standards

- Stock solution (1000 ppm) - 0.1656 g of histimine dihydrochloride dissolved and made to 100 ml in water.
- Dilution 1 – 10 ml to 100 ml gives 100 ppm.
- Dilution 2 – 5 ml (100 ppm) to 100 ml gives 5 ug/ml.
- Working solutions – 0.0, 0.1, 0.2, and 0.5 ml of 5 ppm solution is diluted to 25 ml giving 0.0, 0.02, 0.06 and 0.1 ug/ml histamine. Reagents must be added to produce fluorescent derivatives before diluting to mark.

Sample treatment

- Fish sample is homogenised in food processor/blender.
- 5 g of duplicate sample is weighed accurately but separately into 50 ml centrifuge tubes.
- Blanks and a few recoveries are also included. Recoveries are a third fish sample with 1.0 ml of 1,000 ppm stock added and followed through the procedure.
- 20 ml methanol is added to all solutions, shake vigorously 5 min, centrifuge for 5 min at 2,500 rpm. Transfer extract to 50 ml volumetric flask via funnel. Repeat extract twice more similarly each time using 15 and 10 ml of methanol. Finally make volume of extract to 50 ml using methanol.
- Convert the resin from Cl^- form to OH^- form by treatment with 2M NaOH. Swirl resin (in ratio 15 ml NaOH for each gram of resin) with NaOH for at least 15 min then decant NaOH. Repeat process one more time then wash resin with excess distilled water each time until pH of water is < 9.0.
- Fill resin into chromatographic columns (20 cm X 1.0 cm diameter). Resin height should be about 10 cm. Plug the end of the column using cotton wool. Store filled resin under distilled water and never let it go dry.
- Load 1.0 ml of extract onto the column and immediately direct column flow into a 50 ml volumetric flask containing 5 ml O.1 N HCl. Collect eluate till about 35 ml using water to elute.
- Once about 35 ml remove purified solution and dilute to 50 ml using 0.1 N HCl.

Derivatisation

- Should be done with care.
- Pipette 2.0 ml of purified and diluted solution into a 25 ml volumetric flask.
- Add 4.0 ml 0.1 N NaOH solution and mix.
- Add 0.2 ml of O-Phthaldialdehyde solution (OPA) (1% solution in methanol m/v).

- Allow 5 min for OPA to react.
- Add 2.0 ml of citric acid solution (0.2 M in water), mix and make volume to 25 ml using 0.1 N HCl.
- [Note: standards are derivatised exactly the same way by taking appropriate volumes of stock 1000 ppm histamine solution into 25 ml volumetric flasks].
- Inject appropriate volumes (usually 30 to 40 ul) of derivatised standards and sample solutions.
- [HPLC specifications: mobile phase is water filtered and degassed; flow rate is 1.0 ml/min; detector is a scanning fluorecence 470, excitation wavelength 340 nm, emmission wavelength is 425 nm, sensitivity 64 Gain 100; integrator – water 746, chart speed 0.25 cm/min, attenuation 32 or 16.
- Calculate peak height as follows:

$$\frac{\text{Peak Height Sample}}{\text{Peak Height Standard}} \times \text{Standard conc. ug/ml} \times \frac{\text{Vol Std Inj}}{\text{Vol Samp. Inj}} \times \text{DF} \times \frac{\text{Vol of Extract}}{\text{Wt. Fish Samp.}}$$

RESULTS

Fresh tuna analysed from the Pacific Fishing Company (PAFCO) tuna cannery, Levuka, Ovalau, Fiji had histamine levels of 4.0 to 25.0 mg/kg tuna. Only two batches of old canned tuna (i.e.; material that had passed its "use by" date) gave unacceptable results of levels of 300 mg/kg.

More work is still being done on this method to verify it. Quality assurance and quality control procedures are still being incorporated into testing schemes. The four recoveries tested in a batch of fifteen produced a test accuracy in the range 89 to 102 %.

Blanks gave peaks due to fluorescing OPA. Therefore this needs to be taken into consideration in the calculation.

Replicates were within < 10 % variation.

Both the AGX and Dowex resin were tested. There was no difference between resins as long as the resin treatment is correct and the resin is not overloaded with sample.

It was found that there was no difference to the peak height when standards were either passing through, or not passing through, the columns. Therefore it was concluded that standards need not be passed through columns in the future (P. Ravi, pers. comm., 1998).

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HISTAMINE PRODUCING *MICROCOCCUS* AND *FLAVOBACTERIUM* SPP. FROM FISH

by

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ABSTRACT

Histamine produced by bacteria on fish cause allergic reactions in some consumers. Histamine producting bacteria were isolated and identified in this study.

Of 103 bacterial cultures isolated from fish, 30 produced histamine. They consisted of *Micrococcus* sp. (3) of the family Micrococcaceae and *Flavobacterium* sp. (4), *Hafnia* sp. (5), *Enterobacter* sp. (1), *Klebsiella pneumoniae* (3), *Klebsiella* sp. (8), *Hafnia alvei* (1), *Morganella* sp. (4) and *Proteus* sp. (1) of family Enterobacteriaceae. They produced mean histamine concentrations of 234±88, 110±6, 950±50, 209±8, 354±50, 480±127, 382±10 and 700±100 mg/kg, respectively, in Niven's medium. *Micrococcus* and *Flavobacterium* spp have not previously been reported to produce histamine. *Micrococcus* and *Flavobacterium* spp carry intermediate and low potential to produce histamine, respectively. Low and intermediate potential histamine producers represent only 7%, while high potential histamine producers represent 22% of the total spoilage bacterial flora of the 103 isolates.

INTRODUCTION

Fish carry a variety of microorganisms responsible for spoilage of fish. They include *Morganella morganii*, *Hafnia alvei*, *Clostridium perfringens*, *Aeromonas aerogenes*, *Klebsiella pneumoniae* and *Vibrio alginolyticus*. A major food safety problem associated with spoilage of fish is the production of histamine. Histamine is produced more commonly by *Morganella morganii*, *Hafnia alvei* and *Klebsiella pneumoniae* (Taylor, *et al.*, 1984).

Histamine producing bacteria usually grow rapidly at temperatures of 20-45 °C with optimum production at 38 °C (Yoshinaga and Frank, 1982). Several growth media have been investigated to isolate and identify histamine producing bacteria. The formulation of Niven's medium is considered most suitable to identify these bacteria currently (Niven *et al.*, 1981). In the studies where Niven's medium was developed *Proteus morganii*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Proteus* sp, *Edwardsiella* sp and *Vibrio* sp have been identified (Niven *et al.*, 1981).

Modifications of Niven's medium have been tested by others for the ability to isolate histamine producing bacteria. They found that Niven's medium was superior to three other modifications in achieving the isolation of bacteria (Chen *et al.*, 1989). Estimation of histamine concentrations in fish and isolation and identification of histamine producing bacteria continue to be an important approach in assessing safety of fish. Further studies reported several new histamine producing bacteria (Ababouch *et al.*, 1991; Lopez-Sabater *et al.*, 1994; Lopez-Sabater *et al.*, 1996). This study examines the histamine producing bacteria in fish.

MATERIALS AND METHODS

Isolation of bacteria

From fish available at the market, approximately 2 g of inside with the skin was aseptically transferred into 10 ml of sterile 1% peptone water and shaken for two min. to mix it well. A loopful of this solution was inoculated into violet-red-bile-glucose-agar (VRBGA) in petri dishes and incubated at 37°C for 5 days. Colonies showing a pink reaction were transferred into Niven's agar medium and incubated at and 37°C for 2 days (Niven *et al.*, 1981). Colonies showing a purple halo, indicating probable histamine production, were isolated and maintained on nutrient agar slants at 5°C for further studies.

Histamine production by bacteria

The bacteria isolated from Niven's agar were activated in sterile trypticase soy broth supplemented with histidine (TSBH) at ambient temperature of (25±2) °C for 24 h. A loopful of activated bacteria were inoculated into 25 ml of sterile Niven's broth in triplicate and incubated at 37 °C for 10 days (Chen *et al.*, 1989; Ababouch *et al.*, 1991). Histamine concentration in 5 g of the incubated broth was estimated by TLC (Lieber and Taylor, 1978) and fluorometry (AOAC, 1990).

Identification of bacteria

The cultures showing histamine production were maintained on nutrient agar at 37°C and identified using the following tests; Gram's stain, motility, oxidase, catalase, MR-VP, Koty, Hugh and Leifson test for cocci and bacillii, presumptive urease, citrate, indol ring, indol nitrate, nitrate reduction, utilization of sugars (for bacillii), gelatin liquefaction, growth at 10 °C and 44 °C (Cowan, 1974; Baird Parker, 1974; Baird Parker, 1979; Chinivasagam, 1988). The identified bacteria were subcultured and stored at -10 °C.

RESULTS AND DISCUSSION

Identification of histamine producing bacteria

One hundred and three (103) cultures showing positive reaction on the VRBGA medium were transferred to Niven's agar and 63 out of them showed a purple halo indicating probable production of histamine. These bacteria were tested for histamine production in Niven's broth. Thirty (30) histamine producing cultures were identified based on biochemical tests. Of the 30 cultures identified, three (3) were *Micrococcus* sp from the family Micrococcaceae while 27 belonged to the family Enterobacteriaceae. In the family Enterobacteriaceae, the bacteria identified were *Flavobacterium* sp (4), *Hafnia* sp (5), *Enterobacter* sp (1), *Klebsiella pneumoniae* (3), *Klebsiella* sp (8), *Hafnia alvei* (1), *Morganella* sp (4) and *Proteus* sp (1).

Histamine producing ability of identified bacteria

Micrococcus sp, *Flavobacterium* sp, *Hafnia* sp, *Enterobacter* sp, *Klebsiella pneumoniae*, *Klebsiella* sp, *Hafnia alvei*, *Morganella* sp and *Proteus* sp produced mean histamine concentrations of 234±88, 110±6, 950±50, 209±8, 354±50, 480±127, 382±10 and 700±100 mg/kg, respectively when incubated in Niven's broth at 37 °C for 10 days (Table 1). Histamine production has not been reported, to our knowledge by the species *Micrococcus* and *Flavobacterium* isolated from fish.

Of the species examined *Flavobacterium* sp produced low concentrations of 110±6 mg/kg of histamine. In contrast *Micrococcus* sp which produced 234±88 mg/kg of histamine which is comparable with the histamine concentration produced by species commonly accepted as histamine producers in fish. *Micrococcus* and *Flavobacterium* represent 7% while other bacteria represent 22% of the total spoilage

bacterial flora of 103 isolated from fish in this study. The histamine contribution by commonly known bacteria in fish is higher than the two new histamine producers identified in this study. In this study, histamine concentrations were estimated quantitatively by fluorometry in most instances while at a later stage of the research estimations were done by TLC.

Table 1. Histamine producing bacteria isolated and identified from fish, their sources and concentrations of histamine produced in Niven's broth at 37° C.

Type of bacteria	No. of cultures	Code No.	Sources of cultures	Histamine (µg/ ml) Mean ± SD
Klebsiella pneumoniae	3	A1	dried anchovies	325±05
		A2	fresh tuna	318±18
		A3	fresh tuna	420±07
Klebsiella sp	8	A4	fresh tuna	409±08
		A5	maldive fish	200±12
		A6	fresh skipjack	500-600
		A7	fresh tuna	500-600
		A8	fresh tuna	500-600
		A9	fresh skipjack	500-600
		A10	dried skipjack	500-600
		A11	fresh skipjack	500-600
Hafnia alvei	1	B1	fresh tuna	382±10
Hafnia sp	5	B2	fresh crabs	900-1000
		B3	fresh prawns	900-1000
		B4	fresh tuna	900-1000
		B5	fresh tuna	900-1000
		B6	fresh tuna	900-1000
Enterobacter sp	1	C1	fresh tuna	209±08
Flavobacterium sp	4	D1	fresh carp	110±02
		D2	fresh flying fish	112±02
		D3	fresh skipjack	102±05
		D4	fresh mahseer	115±04
Morganella sp	4	E1	fresh skipjack	600-800
		E2	fresh tuna	600-800
		E3	fresh tuna	600-800
		E4	fresh herring	600-800
Proteus sp	1	F1	fresh tuna	500-600
Micrococcus sp	3	G1	fresh trevally	269±08
		G2	dried anchovies	313±13
		G3	fresh skipjack	121±09

Sources of histamine producing bacteria

The sources of the identified bacteria were mainly fresh fish, while several dried-fish samples and a Maldive fish sample also contained histamine producing bacteria. Three cultures of *Micrococcus* sp were isolated from fresh trevally (*Caranx stellatus*) and skipjack (*Katsuwonus pelamis*) and dried anchovies (*Thrissocles* sp). Four cultures of *Flavobacterium* sp were isolated from fresh carp (*Cyprinus carpio*), flying fish (*Exocoetus volitans*), skipjack and masheer (*Tor khudree longispinis*). The available evidence does not show any preference by the two new histamine producing species for any type of fish or processing method for fish such as drying or smoking.

Effectiveness of Niven's medium

The capability of Niven's agar medium to isolate histamine producing bacteria needs further investigation. Out of 65 cultures of bacteria which produced purple coloured colonies with a purple halo on the yellow background of Niven's agar medium, only 30 were able to produce histamine in Niven's broth at detectable concentrations of above 5 mg/kg. It has been reported that Niven's agar medium has a tendency to show positive results even though the bacterial cultures do not produce histamine concentrations in Niven's broth detectable either by TLC or fluorometry. This disparity between the reactions in Niven's agar medium and Niven's broth has led to the assumption that Niven's agar medium tends to give false positive results (Chen *et al.*, 1989; Ababouch *et al.*, 1991).

The reason for the difference between the histamine observed in Niven's broth and agar medium may be attributed to the fact that not only histamine but other alkaline products such as tyramine, putrescine, cadaverine, spermine and spermidine can be produced in Niven's medium and cause the colour change from yellow to purple. In such situations, when the Niven's broth is analyzed for histamine it may not show histamine (Lopez-Sabater *et al.*, 1996). On the other hand there could be low potential histamine producing bacteria which may show positive results in Niven's agar medium due to formation of several alkaline amines along with negligible quantities of histamine. If inoculated into Niven's broth these bacteria may not show positive results for histamine due to formation of low quantities which cannot be detected by TLC or fluorometry. Thus it is important to combine both the microbiological method (Niven's medium) and the chemical assessment in studies on histamine production by bacteria.

It appears that a wider variety of bacterial species, from among the spoilage micro-organisms, commonly found in fish, are capable of histamine production. Therefore more efficient mechanisms are needed in minimizing bacterial activity to reduce formation of histamine in fish during handling and storage.

CONCLUSIONS

Flavobacterium sp and *Micrococcus* sp which have not previously been reported as histamine producing bacteria before, were isolated from fish. *Micrococcus* sp appears to produce comparable histamine concentrations as other histamine producing bacteria in fish. *Flavobacterium* sp appears to be a low histamine producer.

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INCIDENCE OF SALMONELLA IN FISHERY PRODUCTS

by

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ABSTRACT

Contamination of Salmonella in various processed and non-processed fishery products was surveyed during May 1996-1997. Samples of 5 219 fishery products were collected from 72 processors in the central and eastern region of Thailand. Contamination by *Salmonella* spp. was found in 91 samples (1.74 percent). Twenty two serovars were detected. The top ten serovars found were *Salmonella weltevreden*, representing 45 percent, *S. enteritidis* 5 percent, *S. senftenberg* 5 percent, *S. brunei* 4 percent, *S. anatum* 3 percent, *S. virchow* 3 percent, *S. stanley* 3 percent, *S. hadar* 3 percent, *S. paratyphi* 3 percent and *S. caen* 3 percent. Products contaminated were frozen raw shrimp 1.6 percent, frozen processed shrimp 1.9 percent, fillets of marine fish 3.3 percent, frozen freshwater fish 5.8 percent, frozen cephalopods 1.4 and other products 1.2 percent. The sources of the serovars isolated from this survey were not only from fish handlers and process environment, but also from raw material and culture sites, particularly of aquacultured fish and shrimp.

INTRODUCTION

Frozen fishery products are important exports from Thailand worth at least two billion US dollars each year. Shrimp, cephalopods and fish fillets, in various types of ready to eat and processed products, are the main items. Quality problems, particularly pathogen contamination is still a factor resulting in product detention by some importing regulatory authorities, for instance, the United State Food and Drug Administration (USFDA). Frozen fish and shrimp from some Thai exporters have been placed on import alert for suspected adulteration with Salmonella (USFDA 1996). As a result, products are automatically detained at the point of entry and released thereafter if the importers are able to prove that the products are free from Salmonella. This automatic detention procedure has resulted in extra-expenses for sample analysis, storage while awaiting for the results, and above of all loosing competitive opportunities in markets.

During 1990-1993, frozen cephalopods from five processors were rejected by the Italian health authority due to Salmonella contamination and subsequently the said processor was banned from exporting to Italy. This incident also impacted the Thai fish industry to a certain extent.

Salmonella are found naturally in the intestinal tracts of mammals, birds, amphibians and reptiles but not in fish, crustaceans or molluscs. Salmonella is able to transfer to seafood through several means, for instance by sewage pollution of the coastal environment, contamination after harvest, or contamination during processing.

Bangtrakulnon et al. (1997) reported that Salmonella serovars commonly found in humans were *S. enteritidis*, S. 1, 4, 5, 12; I:-, *S. weltevreden*, *S. derby*, *S. typhimurium*, *S. anatum*, *S. agona*, *S. stanley*, *S. virchow*, *S. paratyphi A*, and *S. typhi*. The study also found that *S. enteritidis* has been increasing in humans and is the most common cause of Salmonella infection. This finding is more or less similar to the report of Gomez et al. (1997) who stated that serovars which commonly cause outbreak of Salmonellosis were *S. enteritidis* and *S. typhimurium*.

Several investigations on isolation of Salmonella in animals have been conducted. Bangtrakulnon *et al.* (1989) reported that serovars naturally found in lizards were *S. weltevreden, S. brunei, S. lexington, S. Newport, S. havana, S. derby, S. typhimurium, S. I8,20:y:-, S. IV43,Z_4:Z_{23}, S. stanley, S. anatum, S. saintpaul, S. agona, S. montevideo*, and *S. virchow*.

Sonsanit *et al.* (1989) also reported serovars commonly found in cockroaches were *S. Lexington, S. weltevreden, S. brunei, S. agona*, and *S. IV43,Z_4:Z_{23}*.

Moreover, a survey on Salmonella naturally found in reptiles and other animals in Thailand conducted by Bangtrakulnon (1997) indicated that 50.67 percent of lizards, 4.6 percent of cockroaches and 46.2 percent of rats had Salmonella. The study also stated that serovars isolated from lizards and cockroaches were similar to those found in humans and chicken. In addition, the epidemic study of Salmonella infection from chicken and its products revealed that contamination from this source was as high as 22 percent. The important serovars concerned were *S. enteritidis, S. anatum, S. muenchen, S. blockley, S. agona*, and *S. typhimurium*.

Bangtrakulnon *et al.* (1991) isolated Salmonella from different internal organs of poultry such as spleen, intestine, liver, heart, and excrement. A total of 1 410 different strains were found. The majority were *S. Java, S hadar, S virchow, S typhimurium* and *S blockley*.

A review of the occurrence of Salmonella in cultured tropical shrimp by FAO (Reilly *et al.*,1992), demonstrated that Salmonella in final products from shrimp aquaculture originate from the environment rather than as a result of poor standards of process hygiene and sanitation. This conclusion co-related with the study of Iyer and Varma (1990) on occurrence of Salmonella in shrimp processing operations. Results were shown as follows:

Source	**Serotype isolated**
Water from culture pond	*S. farmsen, S. weltevreden*
Mud from culture pond	*S. newport*
Coastal sea water	*S. havana*
Sea beach sand	*S. weltevreden, S. bareilly*
Process water	*S. typhimurium*
Ice	*S. bareilly*
Floor, pre-processing unit	*S. bareilly, S. weltevreden*
Utensils	*S. weltevreden*
Lizard droppings	*S. weltevreden*
Rodent droppings	*S. weltevreden, S. typhimurium*

An incidence of Salmonella in frozen fishery products for export from Thailand was studied by Sajjapala *et al.* (1987). Contamination in shrimp was found to be 1.2 percent, marine fish 2.5 percent, cephalopods 0.8 percent, shellfish 2.7 percent, and freshwater fish 8.5 percent. However, there was no serovar isolation in this study.

According to a report of USDA (1988), 40 percent of frozen poultry and its products were found to contain Salmonella. It is accepted that, despite research over the past twenty years, Salmonella incidence in chicken cannot be overcome. It is, therefore, the consumer's responsibility to destroy Salmonella by proper cooking before consumption.

The objective of this study is to identify the contamination rate of Salmonella in exported fishery products of Thailand and to locate the sources of contamination which would be of use in establishing appropriate preventive measures for Salmonella.

MATERIAL AND METHODS

A total of 5 219 fishery product samples was collected from 72 processors in the central and eastern region from May 1996 to May 1997. Samples were transported to the laboratory in insulated containers to keep them frozen.

Salmonella analysis was carried out by the procedure of the Compendium of Methods for the Microbiological Examination of Foods (Vanderzant and Splittstoesser,1992). Twenty-five gram of each sample was weighed, pre-enriched with lactose broth and incubated at 35°C for 24 hours. 1 ml of mixture was transferred to 10 ml tetrathionate broth (with brilliant green added) and selenite cystine broth, and incubated at 43°C and 35°C for 18 and 24 hours, respectively. The incubated samples were then streaked onto 3 selective media as follows:

1. Brilliant green agar (BG), incubated 22-24 hours at 35°C.
2. Bismuth sulfite agar (BS), incubated 24-48 hours at 35°C.
3. Xylose lysine desoxycholate agar (XLD), incubated 22-24 hours at 35°C.

After incubation, typical characteristics of Salmonella colonies were selected to inoculate into the slants of triple sugar iron agar (TSI) and lysine iron agar (LIA) together with McConkey agar plates. The inoculated media were incubated at 35°C for 22-24 hours. The positive results of Salmonella were retained for biochemical tests and confirmed by using antiserum. The confirmed culture were further sent to the WHO Salmonella and Shigella Center, Department of Medical Science, Ministry of Public Health for specific serological identification.

RESULTS AND DISCUSSION

The results of this survey revealed that 91 of 5 219 samples were found to be positive for Salmonella (1.74 percent) (Table 1). Products positive for Salmonella varied from month to month but included both raw and processed products such as black tiger shrimp, marine fish, cuttlefish, squid, octopus, freshwater fish, cooked black tiger shrimp, dim sum, fish ball, imitation crab meat, and mixed seafood. August was the month with the highest rate of salmonella incidence (3.3 percent). However, export products were different in each month.

Twenty-two serovars were identified from 91 positive samples. The top ten serovars are ranked in Table 2. *S. weltevreden* was found to be the principal serovar (45 percent) contaminated in fishery products. The rest, with a lesser extent, were *S. enteritidis* (5 percent), *S. senftenberg* (5 percent), *S. brunei* (4 percent), *S. anatum* (3 percent), *S. virchow* (3 percent), *S. stanley* (3 percent), *S. hadar* (3 percent), *S. paratyphi* (3 percent), and *S. caen* (3 percent).

Table 1. Salmonella contamination in fishery products.

Month /Yr	No. of samples	No. detected	%	Product types (& Salmonella)
May 96	631	4	0.63	B/T(*,*,*), M/R fish (W)
Jun	508	8	1.57	M/R fish (*,W,K,W), B/T (W,W), cooked B/T (W), Dimsum (W)
Jul	491	11	2.24	fish ball (W), M/R fish (S,W,W,W), B/T(W,W,Z), squid (W,W), imitation crab meat (W)
Aug	590	20	3.38	cuttlefish(A,B), mixed seafood (W,W,*), M/R fish(*,H), cooked B/T(W,Br), crocodile meat(V,H), squid(*), F/W fish (E), B/T(W,T,W,W,W), scallops (St), tuna loins(K)
Sep	561	10	1.78	mixed seafood (*,W), B/T(W,G,W), processed B/T(W), M/R fish (S), octopus (W), F/W fish (P,W)
Oct	690	13	1.88	processed B/T©, crocodile meat (M,Hv,M,A), F/W fish (Ty,V), M/R fish (W), B/T(St,*), F/W shrimp(W,*), imitation crab meat(W)
Nov	460	11	2.39	squid©, B/T (W,W), cuttlefish (B,B,S), M/R fish (L) F/W fish (Pa,*), dimsum (W,W)
Dec	311	3	0.96	processed shrimp (Mb), cuttlefish (*), B/T (*)
Jan 97	224	3	1.33	S/W shrimp (P), F/W fish (E,E)
Feb	125	1	0.80	fish ball (Mt)
Mar	235	3	1.28	S/W shrimp (W), B/T(S), F/W fish (E)
Apr	162	1	0.62	F/W fish (Ty)
May	231	3	1.30	B/T (W,Ha), crocodile meat (H)
Total	**5,219**	**91**	**1.74**	

*= not identified
W = Weltevreden
K = Kentucky
S = Senftenberg
A = Anatum
B = Braenderup
V = Virchow
P = Paratyphy B Bio Java

E = Enteritidis
St = Stanley
T = Thomson
H = Hadar
Br = Brunei
G = Give
Z= Subspecies IV 43:Z4 Z23
Ha= Haardt

M = Muenchen
Ty= Typhimurium
Hv= Hvittingfoss
L = London
Pa= Panama
Mb= Mbandaka
Mt= Montevideo
C = Caen

B/T = black tiger shrimp
F/W fish = freshwater fish

M/R fish = marine fish
S/W shrimp = sea water shrimp

Table 2. Top ten serovars isolated from fishery products (expressed as percentage of total Salmonella).

Serovars	%
S. weltevreden	45
S. enteritidis	5
S. senftenberg	5
S. brunei	4
S. anatum	3
S. virchow	3
S. stanley	3
S. hadar	3
S. paratyphi	3
S. caen	3

From Table 3, it appears that frozen freshwater fish has the highest contamination (5.8 percent) whereas marine fish, raw black tiger shrimp, and cooked shrimp were found to be at the rate of 2.9, 1.7 and 1.6 percent, respectively.

Table 3. Percentage of Salmonella contamination based on product types.

Product types	%
Frozen raw black tiger shrimp	1.7
Frozen cooked shrimp	1.6
Frozen marine fish	2.9
Frozen freshwater fish	5.8
Frozen cuttlefish	1.4
others	1.02

S. weltevreden was also the main serovar found in contaminated shrimp and marine fish products, while *S. enteritidis* was dominant in freshwater fish and *S. Braenderup* in cephalopods (Table 4). This finding is similar to a review of Salmonella incidence in aquaculture shrimp by Reilly *et al.* (1992). The review concluded that *S. weltevreden* was identified as the principal species found in aquacultured shrimp due to its culture site environment such as mud, pond, water being inherently contaminated with Salmonella.

Table 4. Serovars isolated from fishery products.

Products	No. of samples	No. positive samples	Serovars
Frozen shrimp	1,435	28	
black tiger shrimp		24	Weltevreden(13), #(5), give, Stanley, Senftenberg, Haardt, Subspecies IV 43:24:Z23, Thomson
freshwater shrimp		2	Weltevreden, #
pink shrimp		2	Weltevreden, Paratyphi
Processed and cooed shrimp	568	9	Weltevreden(6), Caen, Mbandaka, Brunei
Frozen marine fish fillets	479	14	Weltevreden(7), Senftenberg(2), #(2), Hadar, London, Kentucky
Frozen freshwater fish	190	11	Enteritidis(4), typhimurium(2), Weltevreden, Paratyphi B Bio Java, Virchow, Panama, #
Frozen cephalopods	795	11	
squid		3	Weltevreden(2), #
cuttlefish		6	Braenderup(3), Anatum, Senftenberg, #
octopus		1	Weltevreden
crumbed squid ring		1	Caen
Others	1,752	18	
crocodile meat		7	Muenchen(2), Hadar(2), Virchow, Hvittingfoss, Anatum
mixed seafood		5	Weltevreden(3), #(2)
imitation crab stick		2	Weltevreden(2)
fish ball		2	Weltevreden, Montevideo
scallop roe off		1	Stanley
tuna loins (skipjack)		1	Kentucky
Total	**5,219**	**91**	

 # : not confirmed
serovars stated without brackets equal to one sample

The incidence of Salmonella in raw material potentially exists in Thailand, since the climate and culture environment are suitable to promote the growth of this organism. Types of raw material used and products including processing environment and transportation conditions also influence the contamination rate of final products. Freshwater fish tends to be more contaminated than others due to its culture condition and the feed used.

Contamination of freshwater fish was found to be at the rate of 5.8 percent. *S. enteritidis* was the majority (4 in 11 positive samples, or 36 percent). *S. paratyphi* and *S. typhimurium* were also detected. USFDA reported the incidence of Salmonella in freshwater fish from Thailand in 1988 to be 40 percent of samples taken for analysis. As a result, USFDA have placed this product on an automatic detention.

As the matter of fact, freshwater fish are mostly from farms where raw feed such as chicken intestines, other internal organs, and chicken excreta may be used for feeding. There is no doubt about the existence of Salmonella in the culture site, since *S. enteritidis* is transmitted to fish through chicken manure and intestinal organs as fish feed (USDA, 1988). Additionally, the limitation of water circulation and accumulation of feed in ponds also increases the potential for Salmonella contamination. The only means to eliminate the incidence

of Salmonella in the final product is to avoid buying raw material from farms that use the practices mentioned above.

For frozen marine fish, 1.7 percent were positive for Salmonella; *S. weltevreden* was the most frequent, 7 in 14 positive samples (50 percent), then *S. senftenberg*, 4 in 14 (29 percent). These serovars could be transmitted to fish through the contaminated coastal sea water, sea birds at the fishing port, or unhygienic practices in fish handling at processing plants. The hygienic practices can be improved by application of HACCP, but contamination from the culture environment is more difficult to control.

Salmonella contamination was found in 1.6 percent of raw black tiger shrimp. Again *S. weltevreden* was the main serovar detected in 13 out of 24 positive samples (54 percent). As noted, sources of *S. weltevreden* are commonly reptiles and birds, and transfer to shrimp could be during the farming period. Other serovars found were also correlated to those found in various fishery products in the study of Reilly *et al.* (1992). In comparison with the study carried out by Sajjapala *et al.* (1987), it was found that the contamination rate of Salmonella in shrimp had increased to 1.2 percent. This is probably because the export of shrimp from aquaculture has been increasing compared to wild caught shrimp. The chance for farmed shrimp to become contaminated is higher than for the wild caught product, especially by *S. weltevreden,* the dominant serovar inherently found in shrimp ponds (Reilly *et al.*, 1992). The shrimp culture environment is one of the main factors affecting the control of Salmonella, as animals such as lizards and birds are commonly seen. A group of experts from FAO/NACA/WHO (1997) concluded that Salmonella contamination in cultured shrimp is mainly from birds and reptiles and is unavoidable. It is not because of unhygienic practices in processing plants.

In processed shrimp and cephalopods, 1.6 and 1.4 percent, respectively, were positive with *S. weltevreden* as the main serovar for processed shrimp (6 in 9 samples, or 67 percent). For cephalopods, the serovars varied, possibly indicating contaminated raw material for shrimp and contamination during processing, due to poor sanitation and personal hygiene, for squid. These consequences are clearly reflected particularly for cooked products.

To minimize the incidence of Salmonella in products, many practical preventive measures have been broadly recommended. Examples are listed below.

1. Pest control to eliminate *S. weltevreden, S. brunei, S. lexington, S. newport, S. havana, S. derby*, etc.;
2. Sanitation control to avoid *S. weltevreden*, *S. brunei*, *S. panama, S. anatum, S. montevideo*, *S. virchow* (Iyer and Verma, 1990);
3. Personal hygiene control to eliminate *S. enteritidis*, S. *paratyphi*, *S hadar* (Bangtrakulnon, 1997);
4. Appropriate temperature control during transportation and on line processing. USFDA (1996) has suggested product temperature should be maintained below 5°C throughout processing, since exposure of raw material above 21°C for 2 hours could allow Salmonella to grow.

CONCLUSION

FAO/NACA/WHO (1997) and Gomez *et al.* (1997) quote reports of studies on the epidemiology of Salmonella conducted by the health authorities in Europe and America. These reveal that the epidemiological spread of Salmonella from fishery products is low compared to chicken and dairy products. Also that the rate of Salmonella contamination in fishery products was low and hence the risk to public health less.

Results of the present study indicate that the contamination rate in finished products is still low at 1.74 percent. *S. weltevreden* was the dominant serovar found in fishery products, specifically black tiger shrimp and marine fish, while *S. enteritidis* was the main serovar in freshwater fish. Other serovars isolated were shown to vary depending on the type of products. However, they were similar to those found in humans, lizards, cockroaches and other animals. These indicate that Salmonella in final products could come both from the

processing plants, as a result of unhygienic practices, and from beyond the processors' responsibility such the cultures environment.

It must be accepted that no preventative measures can be taken by the processor to achieve absolute control of Salmonella if the organism is present in the raw material as a result of environmental contamination. This is true even if a well managed HACCP system, and QMP, is in place. However, for raw products that will be cooked before consumption, Salmonella is not a significant hazard and should be accepted as a part of the natural flora as it is in chicken.

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DISTRIBUTION OF CLOSTRIDIUM BOTULINUM IN CURED FISHERY PRODUCTS

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ABSTRACT

A total of forty cured fish samples procured from local retail markets in and around Cochin were analysed for the moisture content, salt content, water activity (aw) and for the presence of Clostridium botulinum. Wide variations in the salt concentration and moisture level were observed in the cured fish samples examined. Fifty five percent (22/40) of the samples had water content in the range of 45-51%. The sodium chloride content of the cured fish samples varied between 16-25% in only 60 percent of the samples. The sodium chloride content of less than 7% was noticed in 8 prawn samples. The aw of the samples varied greatly. Clostridium botulinum was detected in 13% of the cured fish samples and type D was predominant (4/5) followed by type C (1/5). Clostridium botulinum spores remain viable at aw level 0.75. The incidence of C. botulinum in cured fish should emphasize the need for adequate brining and drying to protect these products until their final use.

INTRODUCTION

Cured fish is the only traditional fish product of overall commercial importance in India accounting for utilization of about 30 percent of marine fish landings (Sripathy 1983). The process of curing fish imparts a degree of microbiological stability to the product but their stability is a function of several factors including the salt level reached after brining and the amount of heat applied. Since these factors vary from product to product, the microbiological quality and safety of cured fishery products vary.

Clostridium botulinum is one of the food safety hazards associated with fish. There are several botulism hazards associated with seafoods or fish (Sakaguchi 1979, Huss 1981, Dodds 1993). Even though the salt added during the production of cured fish is inhibitory to microbial growth, there have been outbreaks of botulism associated with salt dried fish (Sakaguchi 1979, Centre for Disease Control 1987, Hauchild 1989, Weber *et al.* 1993, McClure *et al.* 1994).

Incidence of *Clostridium botulinum* was reported in fish from India (Lalitha and Iyer 1990, Lalitha and Surendran 1992). The mere presence of *C. botulinum* on a food product will not cause illness, conditions much be such that viable *C. botulinum* spores are present and are given the opportunity to germinate and produce toxin. At present there is paucity of data on the occurrence of *C. botulinum* in cured fish. This paper describes a survey on the distribution of *C. botulinum* in cured fish on sale in markets in Cochin, India.

MATERIALS AND METHODS

Cured fish/shellfish samples from the retail outlets, in and around Cochin, were aseptically collected in sterile polythene bags and transported to the laboratory for the estimation of chemical parameters such as salt content, moisture content and water activity and for the detection of *C. botulinum.*

Cured fish/shellfish samples procured from local retail outlets were analysed for sodium chloride and moisture contents. Moisture and sodium chloride contents were determined according to the methods

followed by AOAC (1990). The water activity of cured fish samples were estimated using a water activity meter (Lufft a_w - Wert-Messer, Germany).

Cured fishery products obtained from retail outlets in and around Cochin were examined for the presence of *C. botulinum*. A 3-5g sample of cured fish was inoculated into 25 ml Cooked Meat Medium (CMM) after removing dissolved oxygen by steaming 10-15 min and cooling quickly without agitation. Sterile paraffin oil was used to cover the surface. Inoculated tubes were incubated at 30°C for 6 days.

After incubation for 6 days at 30°C, cultures were centrifuged at 10000 x g and 4°C for 20 min (Remi, India) and each supernatant was adjusted to pH 6.2 with 1N HCl and frozen at -15°C overnight to eliminate non-specific mouse deaths (Baker *et al.* 1990). Supernatants were stored at -15°C until tested. Toxicity of the supernatant was tested in mice and the toxin type was identified using a procedure followed by the US Food and Drug Administration (Solomon *et al.* 1995).

RESULTS AND DISCUSSION

A total of 40 cured fish/shellfish samples procured from local retail markets were examined for the presence of *C. botulinum* and the data are summarised in table 1 and 2. *Clostridium botulinum* was detected in 13% of the samples with type D predominating (4/5) followed by type C (1/5).

Table 1. Chemical characteristics of cured fish.

Common English name of sample	Scientific name	No. of samples examined	Moisture %		Salt %		a_w	
			Average	Range	Average	Range	Average	Range
White sardine	*Escualosa thoracata*	10	45.07	39-49	16.75	13-19	0.76	0.75-0.80
Malabar Tongue sole	*Cynoglosus macrostomus*	5	45.84	40-49	17.32	10-25	0.79	0.75-0.83
Ribbon fish	*Lepturacanthus savala*	7	46.13	37-51	17.10	15-21	0.76	0.75-0.80
Malabar anchovy	*Thryssa malabarica*	8	45.35	44-48	16.69	14-20	0.75	0.75-0.76
White bait	*Stolephorus bataviensis*	2	45.76	45-46	21.51	20-22	0.75	0.75
Prawn	*Metapenaeus dobsoni*	8	18.39	12-22	4.61	3-6	0.75	0.65-0.88

The water activity (a_w) of samples varied greatly (Fig. 1). Eighty eight percent of the samples had a_w values in the range of 0.71-0.80 while 10% had a_w levels of 0.81 to 0.88. The analysis of the water content of the cured fishery products revealed that 55% (22/40) samples had water content in the range of 45-51% and in 25% of the samples, water content varied between 36-45%. In cured prawn samples, water content was still lower (<22%). Sodium chloride content of the cured fish samples varied between 16-25% in only 60 percent of the samples. 20% had sodium chloride content in the range of 11-15%. Sodium chloride content of less than 7% was noticed in 8 prawn samples in the present study. Wide variations in the salt concentration and moisture level were observed in the cured fish samples examined (Fig. 2 and 3).

Table 2. Occurrence of *Clostridium botulinum* in cured fish.

Sample	No. of samples examined	No. of samples positive for C. botulinum	C. botulinum Type identified
Escualosa thoracata	10	1	C
Cynoglossus macrostomus	5	1	D
Lepturacanthus savala	7	1	D
Thryssa malabarica	8	1	D
Stolephorus bataviensis	2	-	-
Metapenaeus dobsoni	8	1	D

Hauschild (1989) reviewed the prevalence of *C. botulinum* in fresh processed fish. Salted fish from the Caspian Sea were 29 percent positive while smoked fish carried spores in 1.1-20 percent of samples. Dodds (1993) reported incidence of *C. botulinum* in 63% of salted carp from the Caspian sea and type E spores were found at the level of <60/10g in salted fish from the Caspian sea and 490/10g in salted carp. A low incidence of *C. botulinum* (13%) was found in cured fish in the present study.

The viability of *C. botulinum* spores at water activity level 0.75 was shown in the present study by the toxic enrichment cultures of cured fish with water activity 0.75. The viability of *C. botulinum* spores for long periods in many foods of low water activity have been reported earlier (Troller and Christian 1978; Genigeorgis and Riemann 1979; ICMSF 1980). These spores are of great concern after rehydration of such foods (ICMSF 1980). There have been outbreaks of botulism associated with salt dried fish (Troller and Christian 1978; Sakaguchi 1979; Huss 1981; McClure *et al.,* 1994).

It is noted that 56% of the cured fish samples (18/32) had moisture content in the range of 46-51% and 44% (14/32) had moisture contents in the range of 37-45%, values higher than the Bureau of Indian Standards (BIS) (IS: 2882, 1964; IS: 5198, 1969 and IS: 2883, 1976) which prescribe a range of 10-35% moisture normally and 40-45% moisture in respect of certain big fish (Gopakumar and Devadasan 1983). Most of the samples do not conform to the BIS levels of salt (IS: 594, 1962). It is evident from the results of the present study that the majority of the cured fish at the retail level contained inadequate salt levels and high moisture levels as reported earlier (George Joseph *et al.,* 1986, 1988; Kalaimani *et al.,* 1988; Prasad *et al.,* 1994). Kalaimani *et al.,* (1988) reported a_w levels of less than 0.80 in 30 percent of the cured fish examined from Quilon, Vizhinjam and Tuticorin. In the present study nearly 90% of the samples had a_w values in the range of 0.71 to 0.80. Wide variations were also observed in the a_w levels of cured fish examined in the present study.

A survey of the cured fish sold at the retail markets in Cochin revealed wide variation in the salt concentration indicating inadequate control of the brining process in a number of products. Recently outbreaks of botulism from *Kapchunka* (salt-cured, air dried uneviscerated white fish) in USA and Israel (CDC 1987; Slater *et al.,* 1989; Telzak *et al.,* 1990) and from aseikh (uneviscerated fish) in Egypt (Weber *et al.,* 1993) have been reported and attributed to a poorly controlled salting process. *C. botulinum* types B and E were involved in the above outbreaks.

The predominance of *C. botulinum* types C and D in fresh fish have been reported from tropical areas (Tanasugarn 1979; Haq and Suhadi 1981; Lalitha and Surendran 1992). *C. botulinum* types C and D are the predominant types in sediment samples from Bangladesh, Indonesia, Thailand and India (Huss 1980; Haq and Suhadi 1981; Tanasugarn 1979; Lalitha and Surendran 1993) and soil samples from Indonesia and Thailand (Tanasugarn 1979; Hayashi *et al.*. 1981). The finding that types C and D are the predominant types in cured fish as shown by the present study is as expected.

Clostridium botulinum types C and D have been reported to cause intoxications only in animals (Smith 1990; Hauschild 1993). However, cases of human botulism due to types C and D have occurred earlier (Roberts and Gibson 1979; Hauschild 1993). Sonnabend and Sonnabend (1981) detected *C. botulinum* type D and toxin in patients who died unexpectedly with severe respiratory difficulty and dysphagia. Fastidiousness of type C in experimental and captive monkeys (Dolman *et al.*, 1961; Barns and Mead 1986) and recent infant botulism by type C in Japan (Oguma *et al.*, 1990) indicate a potential risk. The possible hazard due to salted fish is highlighted by the incidents of botulism associated with it.

The present study indicates a low incidence of *C. botulinum* (13%) and predominance of type C and D in cured fishery products. Clostridium botulinum spores remain viable at water activity (a_w) level 0.75 and are able to grow and produce toxin after rehydration of such foods. The detection of *C. botulinum* in cured fish indicate either post processing contamination or the ability of *C. botulinum* strains to survive the salting process during the manufacture of cured fish. Wide variations in the salt concentration of cured fishery products indicate inadequate control of the brining process. Therefore, the incidence of *C. botulinum* in 13 percent of the cured fishery products should emphasize the need for adequate brining and drying to protect these products until their final use. Proper hygienic conditions in the curing yards are to be encouraged for the production of good quality products.

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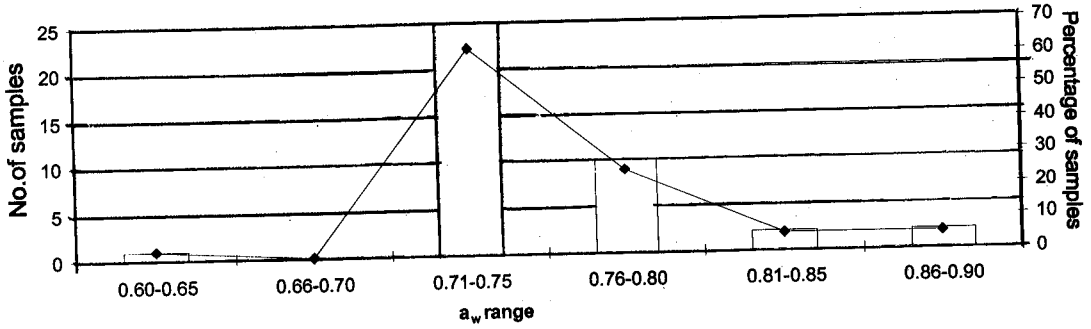

Fig.1. a_w in cured fish.

No. of Samples %of Samples

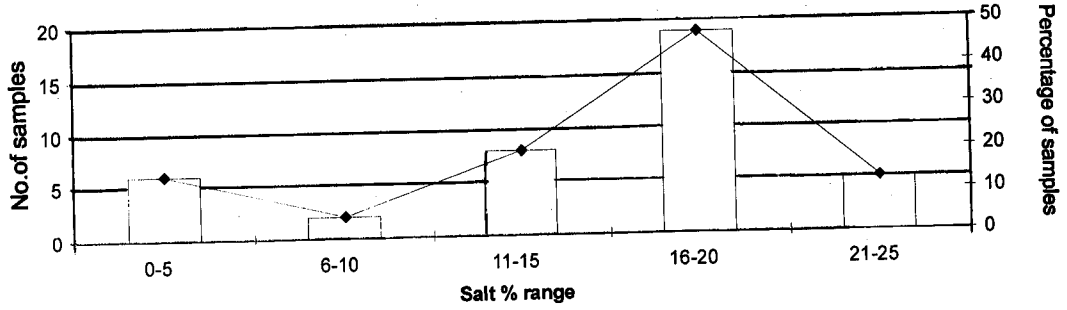

Fig.2. Percentage of salt in cured fish.

No. of Samples %of Samples in each salt range

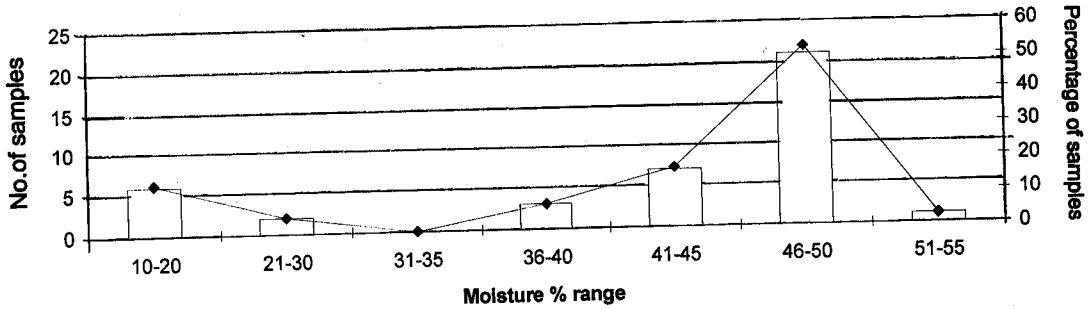

Fig.3. Percentage of moisture in cured fish.

No. of Samples %of Samples

INCIDENCE OF *LISTERIA* IN FISH AND SEAFOOD IN INDONESIA

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ABSTRACT

Fish and seafood samples (124) were collected from fish landings, local markets and processors and tested for the presence of *Listeria*. Of these, 14 were positive with *L. innocua* dominating the positive samples followed by *L. welshimeri*. No *L. monocytogenes* isolated. It was found that competing micro organisms such as *Corynebacterium* and *Enterococcus* did not necessarily compete with *Listeria*. There is no clear reason for the absence of *L. monocytogenes* as the minimal growth requirements were suitable in some samples tested.

INTRODUCTION

Fish industries in Indonesia supply both local consumption and export markets. Some countries, particularly the United States, have a "zero-tolerance" policy for *Listeria monocytogenes* in ready to eat seafood from national and foreign producers. This is due to the pathogenic nature of *L. monocytogenes* which has been associated with several food outbreaks and sporadic cases in many parts of the world (Schuchat *et al.*, 1991; De-Bess, 1993; Goulet, 1995; Jacquet *et al.*, 1995). Seafood was responsible for two outbreaks (Lennon *et al.*, 1984; Riedo *et al.*, 1990), and three sporadic cases of listeriosis (Facinelli *et al.*, 1989; Frederiksen, 1991; Baker *et al.*, 1993).

Infection caused by *L. monocytogenes* can produce several distinct symptoms including meningitis, central nervous system infection, stillbirths, abortions, premature labour, and septicaemia (Doyle, 1985; Gellin and Brome, 1989; Lund, 1990; Ryser and Marth, 1991).

Within the genus *Listeria*, *L. monocytogenes* is the major pathogen in humans and animals. *Listeria* spp. other than *L. monocytogenes*, e.g. *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. murrayi*, *L. grayi* and *L. ivanovii* are not pathogenic to humans. *L. ivanovii* and *L. seeligeri* are pathogenic to animals, and have been reported to occasionally cause disease in humans (Lovett and Twedt, 1988).

Listeria is widely distributed, being commonly found in the environment and isolated from a wide variety of foods. In fish and seafood, the presence of *Listeria* is common. However, there is a very little information on the incidence of *L. monocytogenes* in tropical fish and seafood. Where data is available, the prevalence of *L. monocytogenes* is very low (Wong *et al.*, 1990; Ng and Seah, 1995).

Since there is not much information regarding *Listeria* in fish and seafood in Indonesia, this study was carried out to determine the prevalence of Listeria particularly *Listeria monocytogenes* in a wide range of fish and seafood.

MATERIALS AND METHODS

Samples

124 fish and seafood samples (Table 1) were collected from local market, fish landing and processors around Cirebon, Semarang, Surabaya, DKI Jakarta, Lampung and Palembang. Fresh samples were kept on ice, while frozen samples were kept frozen. All samples were stored at 4°C before analysis.

Methodology

The USDA method described by Mclain and Lee (1988) was used to detect the presence of *Listeria*. A 25-gr. portion of each sample was homogenized with 225 ml UVM1 (Oxoid) as primary enrichment. After 24 h incubation at 30°C, 0.1ml of culture was transferred into 10 ml of Fraser broth (Difco) for secondary enrichment and incubated at 35°C for 24 h. The culture was then streaked onto PALCAM (Difco) and Oxford (Difco) agar and incubated at 35°C for 48 h. Suspect colonies on plates were identified by Gram staining, catalase test, MR-VP test, nitrate reduction, indol, urease, growth on TSI agar, motility on SIM medium, hemolyisis test by stabbing sheep blood agar, as well as carbohydrate fermentation tests using rhamnose, xylose and mannitol.

Samples were also detected for the presence of competing micro organisms such as *Staphylococcus aureus*, *Corynebacterium* and *Enterococcus*. Detection of *S. aureus* was only applied to the processed samples, while *Corynebacterium* and *Enterococcus* were applied to all samples.

Analysis of samples for the presence of *S. aureus* used the spread plate method on Baird Parker agar (BPA) as described by Bennet (1992). A 25-gr unit was homogenized with 225-ml Butterfield's phosphate-buffered dilution water and appropriate dilution was made. 0.1 ml of homogenate was inoculated in duplicate onto BPA plates. The plates were then incubated at 35°C for 48 h. Suspected colonies were identified on the basis of coagulase reaction and thermonuclease production.

Detection of *Corynebacterium* used Tinsdale agar as described in Oxoid Manual 1982. Before inoculating, a 25-gr sample was enriched in 225 ml brain heart infusion broth and incubated at 35°C for 24 h. Inoculation of homogenates was made by stabbing Tinsdale agar plates at interval. Plates were then incubated at 35°C for 48 h. The presence of *Corynebacterium* was only detected presumptively for browning colonies.

The presence of *Enterococcus* used procedures described in the Difco Manual 1984. A 25-gr unit sample was enriched in 225 ml brain heart infusion broth and an appropriate dilution was made. Pour plate method was applied by transferring 1 ml of homogenate into plates in duplicate and then KF Streptococcus agar was added. The plates were then incubated at 35°C for 48 h. and the pink colonies counted.

RESULTS

Out of 124 samples tested, 14 were positive for *Listeria*. The positives were fresh fish (4 samples), squid (3 samples), oyster (1 sample), shrimp (4 samples) and smoked fish (2 samples). It was found that *L. innocua* dominated the positive samples followed by *L. welshimeri*. However, *L. monocytogenes* was not detected. Details can be seen in Table 1.

Table 1. Type of samples and prevalence of *Listeria* in the samples.

Type of samples	No. of samples	Positive samples		% positive
		L. innocua	*L. welshimeri*	
1. Fresh fish	31	–	–	13.0
- silver pomfret	1	–	–	
- mackerel	6	3	–	
- red snapper	6	1	–	
- little tuna	4	–	–	
- black pomfret	3	–	–	
- white snapper	3	–	–	
- catfish	2	–	–	
- trevally	1	–	–	
- milkfish	3	–	–	
- yellowstripe trevally	1	–	–	
- yellowtail fusilier	1	–	–	
2. Squid	8	2	1	37.5
3. Oyster	10			10.0
- cockle shell	5	1	–	
- ark shell	2	–	–	
- green shell	5	–	–	
4. Shrimp	31			13.0
- tiger prawn	10	1	–	
- yellow white shrimp	8	–	–	
- brown shrimp	1	1	–	
- giant shrimp	8	1	1	
- cooked shrimp	4	–	–	
5. Salted boiled fish	9			0.0
- milk fish	4	–	–	
- mackerel	2	–	–	
- sardine	2	–	–	
- little tuna	1	–	–	
6. Smoked fish	9			22.2
- cawtail ray	4	1	–	
- catfish	1	1	–	
- canine catfish eet	1	–	–	
- milk fish	3	–	–	
7. Fermented fish	4			0.0
- *bekasam*	1	–	–	
- fish paste	1	–	–	
- mackerel	2	–	–	
8. Salted fish	3			0.0
- anchovy	1	–	–	
- marine catfish	1	–	–	
- red snapper	1	–	–	
9. Surimi-based products	10	–	–	0.0
10. Frog legs	8	–	–	0.0
11. Frozen snail	1	–	–	0.0

The main competing micro organism detected in the samples was *Enterococcus* spp., while *Corynebacterium* was isolated in some samples. However, no *S. aureus* was detected in the samples tested. Even in *listeria-po*sitive samples, *Enterococcus* spp. and *Corynebacterium* spp. were also found. Competing micro organisms isolated from positive samples can be seen in Table 2.

Table 2. Type of competing micro organisms isolated from positive samples.

Class of sample	Type of sample	*Listeria* species	Competing micro organisms /25 g unit analysis		
			S. aureus	*Coryne-bacterium* spp.	*Entero-coccus* spp.
Fresh fish	mackerel	*L. innocua*	*	–	–
	mackerel	*L. innocua*	*	–	–
	mackerel	*L. innocua*	*	+	–
	red snapper	*L. innocua*	*	+	+
Squid	squid	*L. welshimeri*	*	–	–
	squid	*L. innocua*	*	+	+
	squid	*L. innocua*	*	–	+
Oyster	cockle shell	*L. innocua*	*	–	–
Shrimp	fresh giant shrimp	*L. welshimeri*	*	–	–
	fresh giant shrimp	*L. innocua*	*	–	+
	frozen tiger prawn	*L. innocua*	*	–	+
	frozen brown shrimp	*L. innocua*	*	+	+
Smoked fish	cawtail ray	*L. innocua*	–	–	–
	catfish	*L. innocua*	–	–	–

* not detected

DISCUSSION

That positive samples were dominated by *L. innocua* was not surprising. According to several reports, *L. innocua* is the *Listeria* spp. most often isolated from seafood (Weagent *et al.*, 1988; Masuda *et al.*,1992; Ryu *et al.*, 1992). This species was also most often detected in fish from tropical countries (Fuchs and Surendran, 1989: Fuchs and Sirvas, 1991; Karunasagar *et al.*, 1992, Adesiyun, 1993). Studies by Curiale and Lewus (1992) and Petran and Swanson (1993) may explain why *L. innocua* is isolated more frequently than *L. monocytogenes*. These authors found that *L. innocua* grew faster than *L. monocytogenes* in enrichment broth.

The presence of competing micro organisms may inhibit the growth of *Listeria*. Natural microflora commonly found in fish such as *S. aureus*, *Corynebacterium* and *Enterococcus* (Jay, 1992) were found to be competitors to *Listeria* (Tran *et al*, 1991; Dallas *et al*, 1992). In this study, however, it seems that there is no correlation between *Listeria* and competitors. Table 2 shows that the presence of *Corynebacterium* spp. and *Enterococcus* spp. did not affect the growth of *Listeria innocua* isolated in some fish, squid and shrimp. It might be that the competitors were present in those samples in low number, resulting in the detection of *Listeria innocua*. The presence of *Listeria* in smoked fish which contains no competitors is usual. Contamination of *Listeria* in this sample may be caused by post-process contamination since a hot-smoking process usually smokes the fish.

Although the possibility of *Listeria* contamination in fresh water is higher than in marine fish (Fuchs and Reilly, 1992), in this study it seems that there is no correlation between shrimp from fresh water and marine water. Both of them were positive to contain *Listeria*, even in frozen samples. In contrast, *Listeria* was absent in cooked shrimp, in which they should be present since contamination may occur after cooking via the hands in the peeling process.

The present study differs from Rorvik and Yndestad (1991) who found *L. monocytogenes* in brined shrimp, as no *Listeria* spp. was found from salted fish either dried or boiled. However, this study agrees

coincidence with Fuchs and Surendran (1989) who did not isolate any *L. monocytogenes* from 11 samples of dried salted fish in India. High concentrations of salt may be the cause of the absence of *L. monocytogenes* in salted fish since a salt content of 15-30 % is usual in salted fish in Indonesia (Pratiwi *et al.*, 1997).

The conditions mentioned above reflect that *Listeria monocytogenes* could be present in the samples tested since the growth requirements are suitable. However, *Listeria monocytogenes* was absent, which is in agreement with previous studies (Fuchs and Surendran, 1989, Manoj *et al.*, 1991, Karunasagar *et al.*, 1992). Even if *L. monocytogenes* was detected, the number of positive samples was very low. Wong *et al* (1990), detected 57 fresh and frozen fish and seafood in which only 10.5 % were positive for *L. monocytogenes*, while Ng and Seah (1995) isolated 3 and 1 *L. monocytogenes* from 16 fish fingers and 2 smoked salmon respectively. This study, along with others, confirms that the prevalence of *L. monocytogenes* in tropical fresh fish and seafood is very low.

A clear reason of the absence of *L. monocytogenes* in this study is not known although according to Ben Embarek (1994), it may due to high ambient temperature and microbial competition which inhibits the growth of the psychrotrophic *L. monocytogenes* in tropical fresh fish and seafood.

CONCLUSION

The pathogen *L. monocytogenes* was not found in 124 samples tested. This finding adds to the argument for review of the microbiological standards for *L. monocytogenes* in fish and seafood products for export purposes from tropical countries. In addition, a collaborative study among tropical countries might be useful to give more data on the incidence of *L. monocytogenes* in tropical seafood products.

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ANALYSIS OF PARALYTIC SHELLFISH POISON (PSP) USING MOUSE BIO-ASSAY TO SUPPORT THE INDONESIAN SHELLFISH SANITATION PROGRAM

by

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ABSTRACT

Analysis of paralytic shellfish poison (PSP) by mouse bio-assay was undertaken to support the Indonesian Shellfish Sanitation Program. Samples of shellfish were taken once a year during 1996 to 1998 from fourteen provinces, considered to be either suspected as potentially contaminated by PSP producing algae or commercial production areas of shellfish.

The results showed that most samples did not contain PSP, however, samples taken from two areas (i.e. Lampung Bay in Lampung and Ambon Bay in Maluku) were positive for PSP up to 250 *u*g/100 gram. This is higher than the recommended maximum level for human consumption (80 *u*g/100 gram).

INTRODUCTION

Shellfish is one of Indonesia's fishery products that has, recently, increased quite markedly either for local consumption or export (Murdjijo, 1997). In general, shellfish is consumed raw or half-cooked, therefore the risk to human health is high when shellfish from uncontrolled growing or fishing areas is not handled properly after harvesting.

Currently, one of quality requirements of shellfish being exported, particularly to European Union Countries, is that the product must not contain more than 80 *u*g/100 gram PSP. This is a basic part of the requirements, besides microbial content and other toxic chemical residues, that must be implemented through a comprehensive shellfish sanitation programme (Anon, 1997).

Paralytic shellfish poisoning is a biological intoxication caused by consumption of shellfish containing saxitoxin (STX) and its derivatives which are naturally produced by certain microalgae of dinoflagellates (Halstead, 1984). Motohiro (1995) reported that the main genus of dinoflagellates producing PSP are *Gonyaulax, Pyrodinium* and *Prorocentrum.* Shellfish that have been found to contain PSP are taxonomically grouped in eleven genus including Mytilus and Modiulus, as well as Crassostrea, Ostrea, in relatively low levels.

The map of harmful algal blooms published by the Asean Canada Cooperative Program on Marine Science (Anon,1995) indicates that certain Indonesian waters have potential for PSP due to the content of harmful algae producing PSP. This reflects that a quality control program on PSP in Indonesia is urgently needed.

This paper describes the result of PSP analysis of shellfish in Indonesia in the last three years. In order to support our Indonesian Shellfish Sanitation Program.

MATERIALS AND METHODS

Samples (mainly bivalves) were taken from catching or harvesting areas of fourteen provinces in Indonesia once a year during 1996 to 1998. For the first two years (i.e. 1996 and 1997), sampling sites were determined by considering the potency of harmful algal blooms in the area. In 1998, sampling was done in areas where the potential for shellfish production was relatively high. The species taken in each area were those caught or harvested by fishermen at the time of sampling.

Sampling points varied between coastline and up to $\pm$ 2 km seaward depending upon the condition of each area. The samples were all frozen and packed in styrofoam box and sent to the laboratory of the National Centre for Fishery Quality Control and Processing Technology Development (NCQC), Jakarta.

All the chemical reagents were analytical grade (PA) of E. Merck, whereas the standard for PSP was saxitoxin dihydrochloride (STX) obtained from te Washington Seafood Laboratory of the FDA - USA. Mice of ddY strain were provided by National Quality Control Laboratory of Food and Drug, Jakarta.

Analysis of PSP was carried out by mouse bio-assay using the AOAC method (AOAC, 1984) where standardization of the mice is done by injecting ten mice intra-peritonially (ip) with STX standard at a certain concentration in triplicate. Samples were extracted using 0.1 N hydrochloride acid solution at pH at 3.5 $\pm$ 0.3. One ml extract was then injected into at least 3 mice. PSP content was calculated using the following formula :

ug PSP/100 gram = MU x CF x DF x 200

where MU = mouse unit (obtained from Sommer Table)
CF = correction factor
DF = dilution factor

RESULTS AND DISCUSSION :

The results of PSP analysis including location and time of sampling as well as species of shellfish are tabulated in Table 1. Nine out of fourteen provinces were chosen because those areas are main sites of commercial production of shellfish in Indonesia, whereas the rest were those with potential for the occurrence of algal bloom.

From Table 1, it can be seen that most of samples did not contain PSP. However, samples taken from Lampung and Maluku were positive up to 250 ug/100 gram. It is important to note that limit detection of the analytical method was approximately 40 ug/100 gram (Sullivan and Wekell, 1986). Therefore, not detected (ND) result did not always mean that samples were negative to contain PSP, but they might contain PSP at the level of less than 40 ug/100 gram.

Praseno (1996) reported that certain areas in Indonesia were suspected of being sources of toxic algal blooms, including Malaka Bay (North Sumatera), Jakarta Bay (Jakarta) and Ambon Bay and Kao Bay (Maluku) and that 20 species of microalgae-producing toxin had been identified of which one species (*Pyrodinium bahamense* var *compressum*) was found to be a PSP-producing dinoflagellate. Monitoring of red tides in Ambon Bay have also been reported by Sidabutar (unpublished) and *Pyrodinium bahamense* var *compressum* was found to be one of microalgae that causes red tide in the area. PSP was found in the samples taken from Lampung Bay (Lampung) although this area has not been confirmed to contain toxic algae. This may be because the life cycle of the microalgae was influenced by season as reported by Halstead (1984). In other words, only during certain seasons and conditions will toxic microalgae grow and subsequently be consumed by shellfish in the area.

Table 1. Sampling location, species, sampling time and results of PSP analysis.

No.	Province	No. of Sampling Location	Species	Sampling time	Result (ug/100gr)
1.	Aceh [A]	1	*Anadara inequivalvis, Anadara granosa*	September 1997	ND
2.	North Sumatera [A,B,C]	2	*A. inequivalvis, Meritrix-meritrix*	September 1997 August 1998	ND
3.	Riau [A, C]	1	*A. granosa*	August 1998	ND
4.	South Sumatera [C]	1	*A. granosa*	August 1998	ND
5.	Lampung [A, C]	2	*A. inequivalvis, Vena piridis, Crassostrea sp.*	October 1996, September 1997, August 1998	56 - 137
6.	Jakarta [A,B,C]	2	*V. piridis, A. inequivalvis, A. granosa*	October 1996, September 1997, August 1998	ND
7.	West Java [C]	3	*V. piridis, A. inequivalvis, A. granosa*	August 1998	ND
8.	Central Java [C]	2	*A. granosa*	August 1998	ND
9.	East Java [C]	2	*A. granosa,* Common windowpen	August 1998	ND
10.	Bali [A]	1	*A. granosa* , Kerang batik	September 1997	ND
11.	East kalimantan [A,C]	1	*A. granosa*	August 1998	ND
12.	South Sulawesi [A]	1	*A. inequivalvis, A. granosa*	October 1996	ND
13.	Maluku [A,B]	2	*V. piridis, Crassostrea, sp Pinctada, sp*	October 1996, September 1997, August 1998	43-250
14.	Irian Jaya [A]	1	*A. granosa*	October 1996	ND

Note : A : Site of algal bloom
B : Site of toxic algal bloom
C : Site of commercial production of shellfish

Table 2 gives results of samples taken from Lampung and Maluku and shows that the positive samples containing PSP were those taken in the last two years (i.e. 1997 and 1998). In addition, certain samples contained more PSP than the maximum level for human consumption recommended by WHO which was 80 ug/100 gram (Halstead, 1984). In those areas, the risks of paralytic shellfish poisoning were very obvious. In relation to the shellfish sanitation programme which has started in Indonesia, a recommendation to close the area, particularly Lampung Bay and Ambon Bay, for catching or harvesting shellfish has been made until the area proven to be safe from PSP.

Table 2. Result of PSP analysis of samples from Lampung and Maluku.

No.	Province	Sampling Location	Sampling time	Species	Result (ug/ 100gr)
1.	Lampung	Lampung Bay	October 1996	*Anadara inequivalvis*,	ND
				Crassostrea, sp	ND
			September	*Vena piridis*	137
			1997	*Crassostrea*, sp	82
				A. inequivalvis	56
			August 1998		
2.	Maluku	Kao Bay	October 1996	*Anadara granosa*	ND
				Pina bicolor	ND
		Ambon Bay	October 1996	*V. piridis*	ND
				Crassostrea, sp	ND
			September	*V. piridis*	250
			1997	*Crassostrea, sp*	51
				Pincdata, sp	125
			August 1998	*Crassostrea*	43

Note : ND = Not Detected

The results of this work indicate that certain areas must be closely controlled in terms of PSP because they contained quite high levels of PSP. It is not only PSP that must be controlled by a shellfish sanitation programme, but special attention must be paid to microbiological quality of the shellfish. For this reason, catching and harvesting areas are classified into 3 classes; A, B, and C, depending upon the level of contamination of coliforms, *E. coli* and *Salmonella*.

CONCLUSION

From this work, it can be concluded that shellfish from two areas (i.e. Lampung Bay and Ambon Bay) contained more than maximum level of PSP for human consumption (80 ug/100 gram). However, not all areas with toxic algal blooms were positive for PSP. Every PSP analysis higher than 80 ug/100 gram must lead to a recommendation to close the catching or harvesting area, particularly commercial production areas.

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STUDY OF A HAZARD ANALYSIS CRITICAL CONTROL POINT SYSTEM RELATED TO THE PROCESSING OF FISH AND MEAT PRODUCTS

by

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ABSTRACT

A Hazard Analysis Critical Control Point (HACCP) system was developed for Chinese fish rolls and skinless chicken sausages. The production process of the above products were studied at the processing factory. Microbiological tests were carried out to assess the status of the products during the production process. The tests were also repeated after taking some corrective measures.

The processing stages, namely blending, chilling, battering, crumbing and packing of the final products showed some level of microbial contamination with *E. coli*, in Chinese fish rolls. In chicken sausages the production line receiving area and equipment workers hands, mincer plates, meat chopper, filled sausages and many other areas showed contamination with *E. coli*. *Staphylococcus aureus* contamination was also noted in certain stages but no *Salmonella* contamination was detected at any stage of processing.

Based on the results of monitoring the chilling, battering and crumbing equipment in the production line and during packing was considered important in Chinese fish roll production while in skinless chicken sausage production, cooking, casing and packing were considered for further improvement of quality.

The suggested modifications: chilling temperature, thorough cleaning and disinfecting of the production line and hygienic practices of the food handlers in the processing line for Chinese fish rolls and washing the peeling machine with boiling water, providing effective disinfectants for hand washing and cleaning the equipment of the chicken sausage processing line led to drastic reduction of *E. coli.* and *Staphylococcus* contamination resulting in very satisfactory improvement of the quality of products. Use of disinfectants illuminated *E. coli.* and *Stapylococcus* from work surfaces.

INTRODUCTION

It is accepted that the traditional quality control methods cannot eliminate hazards at the correct time. The development of the Hazard Analysis Critical Control Point (HACCP) system, which is based on a preventive strategy based on a thorough study of existing conditions, is considered a better method to ensure quality and to reduce the cost of production. The HACCP system has seven main elements identifying potential hazards including determining the critical control points, establishing controls, establishing monitoring systems, establishing corrective actions when the CCP is not under control (Huss 1994).

During this study a food processing factory producing Chinese fish rolls and chicken sausages was selected to continuously monitor production process using a HACCP system with a view to improving the quality. Special emphasis was given to cleaning and disinfection procedures.

Procedure

Flow charts for the production of:

(a) Skinless chicken sausage
(b) Chinese fish rolls

were prepared. Each step was carefully analyzed with available data and more analytical results obtained by further examination of samples taken at different control points.

MATERIALS AND METHODS

In a month, ten samplings were carried out at following points in the production lines of Chinese rolls and skinless chicken sausages.

Sampling method

Details of the product/swab samples taken for microbial analysis of each processing step in skinless chicken sausages processing line are given in Table I. Sampling details on Chinese fish roll processing line are given in Table II.

Based on the results of analysis from samples taken in the chicken sausage processing line corrective actions were taken at following points:

a) Cooking
b) Showering
c) Peeling
d) Packing

The sample taken from the Chinese fish rolls did not imply any major corrective action except for the disinfection procedure.

Both production lines were subjected to an alteration in cleaning procedures using two disinfectants:

(a) Sumabac 3% (Quaternary Ammonium Compounds);
(b) Halamid 0.2% (Chloramine T).

Cleaning procedure

The detergent and disinfectant were used together. The contaminated surfaces were rinsed with water and then the detergent and the disinfectant mixture was applied. For Halamid disinfectant mixture, 0.9 g of Halamid was mixed with 100 ml of water and 50 ml of M-100 soap was added and made up to 4.5 L.

For Sumabac disinfectant mixture 135 ml of Sumabac was mixed with water and filled up to 4.5 L(Sumabac has detergent in it).

This mixture was applied on the contaminated surfaces and left for 20 min. Then the surfaces were rinsed off with water.

Method of sampling

Swab samples were taken from packing tables, processing machines, baskets etc. in the sausage processing plant as well as in the Chinese fish roll processing plant.

Microbiological analysis

(a) Product Sample Analysis

For sampling of the product for total bacterial counts (TBC), *Escherichia coli* and *Staphylococus aureus*, 10 g samples were blended with 90 ml pre sterilized peptone water in sterile stomacher bags for 30 seconds. The serial dilution were made by aseptic transfers.

(a) Total bacterial counts - determined by plating appropriate dilution in nutrient agar plates;
(b) Total coliform counts - were determined by plating appropriate dilution in duplicate plates of MacConkey agar.
(c) *Escherichia coli* - determined using MPN - (3-tube) technique, using MacConkey broth. Gas and acid positives at 35°C for 24 hr incubation were transferred to brilliant green bile broth and incubated at 44°C. Gas positives were further confined with IMVIC tests.
(d) *Staphylococcs aureus* - was detected by plating appropriate dilutions on Baird Parker plates incubated at 37°C for 48 hr. Black colonies with a clearing zone were considered positive for *S. aureus*.
(e) *Salmonella* - Samples were pooled to get a composite sample and 25 g were added to lactose broth 225 ml.

Pre enrichment	-	lactose broth 30oC - 24 hrs
Enrichment	-	tetrathionate broth
Selective Agars	-	brilliant green agar and SS agar

Presumptive colonies were confirmed with following bio-chemical tests
TSI slants, urease test, ONPG test.

(b) Cotton Swab Sample Analysis

Cotton swabs were prepared and sterilized at 121oC for 20 min.

Sterilized swabs were taken aseptically and rolled over the surface once and inserted into peptone tube (sterilized).

Sterilized 1 ml of inoculated peptone was used for swabbing was introduced to 9 ml sterilized peptone. This constituted the 10^{-1} dilution. The serial dilution prepared were used for testing for *E. coli* , *S. aureus* and *Salmonella*.

RESULTS

Fig. 1 represents the Flow chart for processing of skinless chicken sausages while Fig. II represents the Flow chart for processing of Chinese fish rolls.

Table I represents the results of the samples analyzed in the chicken sausage processing line.

Table II represents the results of microbial analysis of samples taken from the Chinese fish roll processing line.

Table III shows comparison of the results of microbial analysis of chicken processing plant before and after the same corrective measures.

Fig. III shows the results of cleaning with and without disinfectant on the processing equipment of chicken sausages processing plant when assessed for the presence of *E coli*.

Fig. IV gives the details of the results of cleaning with and without disinfectant on the processing equipment of the chicken sausage processing plant when assessed for the presence of *Staphylococcus aureus.*

Fig. V represents the details of the results of cleaning on tables with and without disinfectant in the Chinese fish rolls processing plant in relation *E. coli*, while Fig. VI represents the same details in relation to *S. aureus*.

DISCUSSION

In the processing of skinless chicken sausage, cooking, peeling, vacuum packing, blast freezing and frozen storage were assessed as critical control points. Cooking completely eliminated *E. coli.* and *S. aureus*

present in the sausage. Extension of cooking time by 3 min. also reduced the total bacterial count by about 1 log cycle. All steps prior to cooking are control points, while all the steps from then should follow good manufacturing practices. As cross-contamination cannot be eliminated, in later steps, extra precautions for consumer safety and product acceptability are a must.

In the processing of Chinese fish rolls steps prior to flash frying was considered control points. The process of flash frying tended to eliminate most of the microorganisms. All steps following the frying should be done with extra care to prevent any contamination and cross contamination.

The results analysis of swab samples after cleaning without using disinfectants do not seem to have much effect on removal of *E. coli* and *S. aureus* on work surfaces in the chicken processing areas as well as in fish processing areas. However the use of Halamid and Sumabac both had a remarkable effect in the cleaning and disinfectant process. None of the swab samples tested was positive for *E. coli* and *S. aureus* after use of disinfectant.

The active agent in Sumabac is a Quaternary Ammonium Compound while that of Halamid is chloramine-T. Most quaternary ammonium compounds are classed as germicidal cationic-detergents. The bacteriocidal power of the quaternaries is exceptionally high against Gram-negative organism. Bacteriocidal concentrations range from dilutions of one part in a few thousand to one part in several hundred thousand. They are also known to manifest bactoriostatic action far beyond their bacteriocidal concentration (Pelezar *et al.*, 1986). QUATS are reported to be less effective than those of halogens while they are less affected by pH and organic matter (Shapton and Shapton, 1991).

The chloramines represent another category of halogen compounds used as disinfectants, sanitizing agents or antiseptics. They are stable (more stable than hypochlorite) in terms of prolonged release of chlorine (Pelezar *et al.*, 1986). However, QUATS are more expensive than chloramine compounds but both cleaning and disinfection were found to be equally effective for cleaning of both processing plants.

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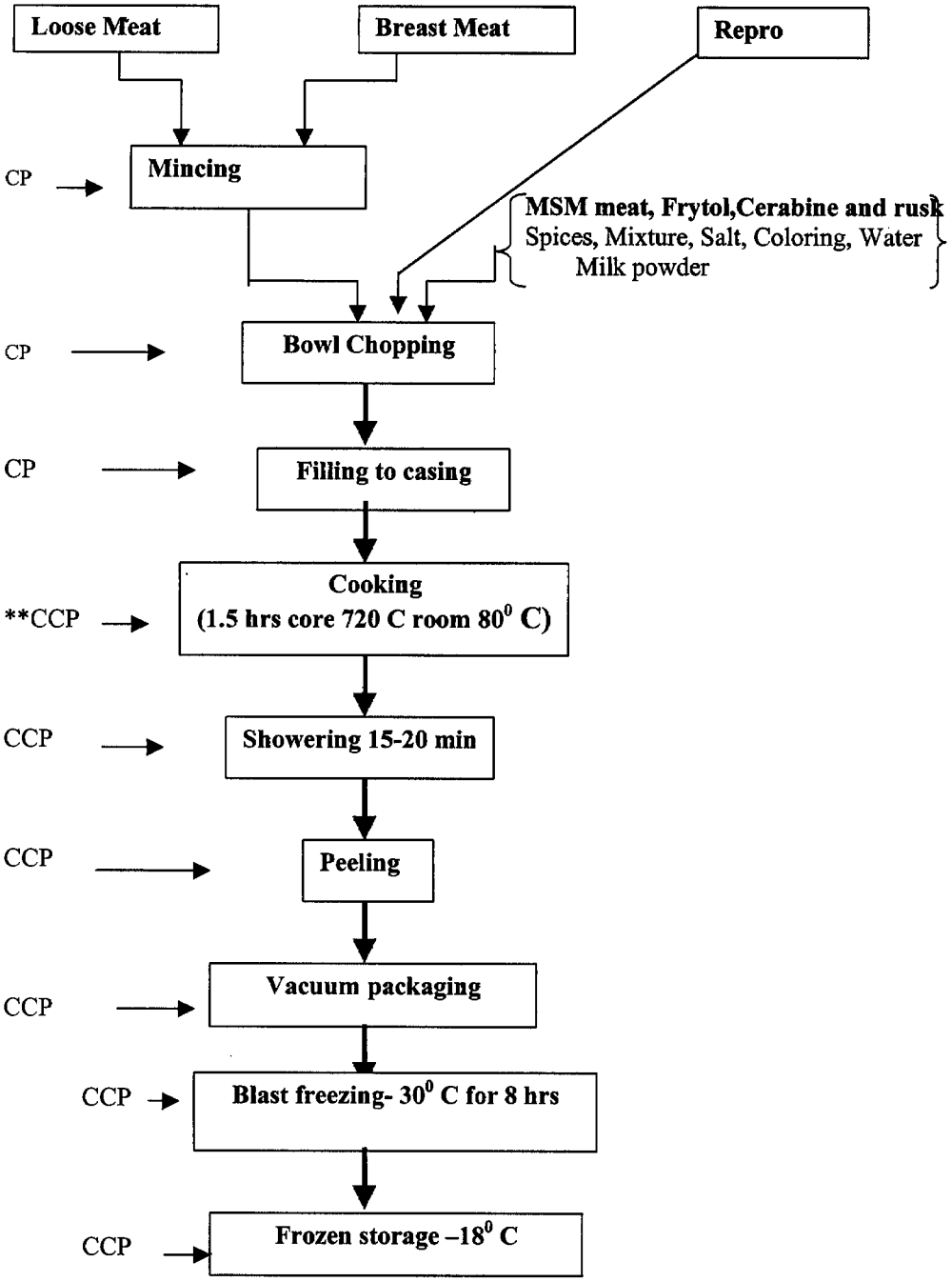

Fig. 1. Flow diagram for skinless chicken sausage.

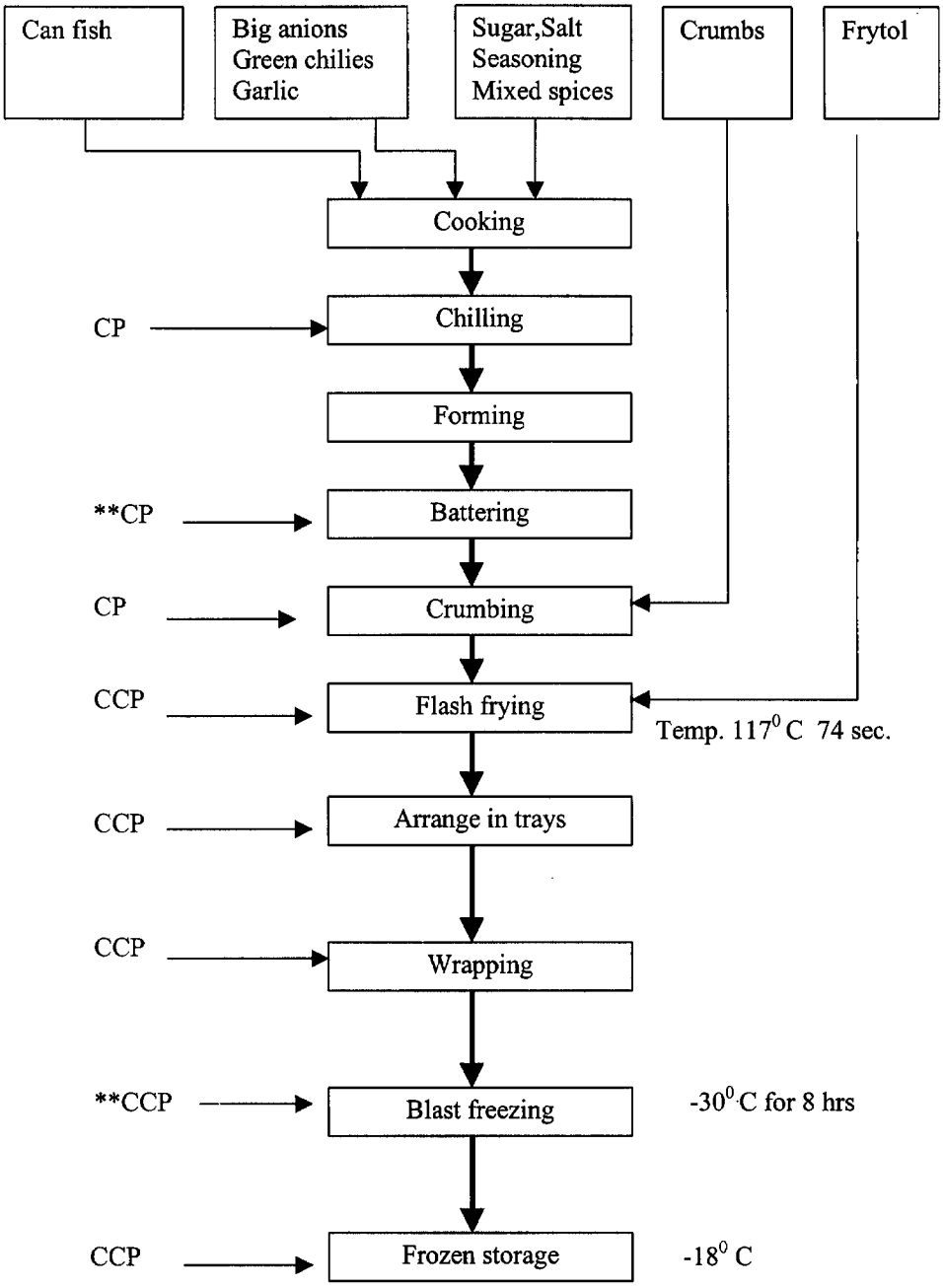

Fig. II. Flow diagram for fish rolls.

Table 1. Results of the samples analysed from the chicken processing line.

Process step	# of samples	Microbial analysis		
		E. coli	Staphs.	*Salmonella*
1). Receiving and storage				
1.1. MSM/MDM, breast meat loose meat	10	<10 *E.coli* for all samples	3 present	Absent
2). Receiving area and equipment				
2.1. Work surfaces	20	15 samples for >10 E. coli, other <10 E. coli	5 present	-
2.2. Workers hands	20	18 samples >10 and others<10	18 present	-
2.3. Knives and baskets	20	Baskets – all >10	absent	-
3). Mincer plate	10			
3.1. Before work		5 samples <10 others absent	absent	-
While working		all present	absent	-
After cleaning		all absent	absent	-
3.2. Minced meat	10	all >10 *E.coli*	absent	-
4). Bowl chopper – working	10	all >10 *E.coli*	absent	-
4.1. After cleaning	10	all absent	absent	-
4.2. Chopped meat	10	all >10 *E.coli*		-
5). Filler nozzle				
While working	10	all <10 *E.coli*	absent	-
After cleaning	10	all absent		
6). Filled raw sausage	15	all >10 *E.coli*	2 present	-
7). After cooking	30	all absent	All absent	-
8). After shower	30	all absent	All absent	-
9.1) Before peel	30	All absent	All absent	-
9.2 After peel	30	20-<10 E. coli other absent	5 present	-
9.3 Environmental	10	3 present	8 present	-

9.4. Peeling table before work	20	10 - <E.coli Others absent	3 present	-
While working	20	All present	10 present	-
After cleaning	20	All absent	all absent	-
9.5. Workers hands	40	All present	20 present	-
10).1.Transfer tube	10	All present	2 present	-
10.2. Transfer worker hands before work	10	5 present	absent	-
11).1. Packing :-				
Before pack	20	all >10 E.coli	3 present	-
After pack	20	all >10 E.coli	5 present	-
11.2. Hands :- Before work	30	all absent	absent	-
While working	30	all >10 E.coli	absent	-
11.3. Baskets, Table				
before use	30	20 present	10 present	-
after clean	30	all absent	all absent	-
11.4. Packing materials	10	all absent	all absent	-

Table II. Results- Microbial Analysis of Processing Steps - Fish Chinese Rolls.

Process step	# of samples Tested	Microbial parameters		
		E.coli	Staphs.	Salmonella
1). Bowl chopping	5	All absent	All absent	-
2). Blending	5	1 present	All absent	-
3). Chilling before	5	4 present (<10)	All absent	-
While chilling	5	4 present	All absent	-
After chilling	5	4 present	All absent	-
4). Chilling baskets Before and after	10	all present	All absent	-
5). After battering	5	all present	2 present	-
6). Crumbing	5	all present	2 present	-
7). Flash frying	10	all absent	all absent	-
8). Arranging workers				
gloves – before work	10	all absent	all absent	-
While working	10	8 present	3 present	-
9). Wrapping working gloves				
before work	5	all absent	all absent	-
while working	5	all present	4 present	-
10). Final product	10	4 samples <10 *E.coli*	absent	-

Table III. Results of Microbial Analysis - Chicken Sausage Processing Line.

	Corrective action	# of sample tested	Results.	
			Before monitoring	After monitoring
1).	Extension of cooking time	10	TPC - 10^4 *E.coli* – absent Staphs. – absent	TPC – 10^3 - 10^4 *E.coli* – absent Staphs. – absent
2).	Water treatment at showering			
	Before shower	20	TPC - 10^4 *E.coli* – absent Staphs. – absent	There was no monitoring
	After shower	20	TPC - 10^4 *E.coli* - absent Staphs. - absent	Water quality is high.
3).	Peeling Workers' hands	20	*E.coli* < 10 all samples Staphs. present	
	After dipping hands with Halamid	20		*E.coli* absent in all samples. Staphs. also absent
	Before boil, peeling machine samples	25	*E.coli* <10 all samples Staphs. absent	
	After boiling	25		All samples absent for *E.coli* and Staphs
	After ½ hr from boiling	5		All samples absent for *E.coli* and Staphs
	After 1 hr from boiling	5		All samples absent for *E.coli* and Staphs
	After 1½ from boiling	5		2 samples positive for *E.coli*
	After peeling sausages	15	TPC - >10^4 *E.coli* <10 Staphs. – absent	TPC - 10^3 - 10^4 *E.coli* – absent Staphs. - absent

Table III. continuation...

Corrective action	# of sample tested	Results.	
		Before monitoring	After monitoring
4). Packing After one hour gloves	20	>10 *E.coli* Staphs. – 10 samples present	
Due to changing of gloves : after 1 hr Baskets and tables	25	>10 E.coli Staphs. - 10 samples	*E.coli* absent Staphs. absent
After practised one way cleaning Baskets and table	25		*E.coli* absent Staphs. absent
5). Final product	20	15 samples were >10 *E.coli* and 3 were staphs Present TPC $>10^5$	Only 3 samples <10 *E.coli* present. Rest of others absent. TPC = 10^4

Table IV. Results of Microbial Analysis - Fish Chinese Rolls Processing Line.

Corrective Action	# of sample	Results	
		Before monitoring	After monitoring
1). Change of gloves after 1 hr	10	*E.coli* >10 in all samples	*E.coli* absent
utensils and table		Staphs. positive in 7 samples	Staphs. absent
2). Disinfecting utensils before battering	10	*E.coli* >10 in all samples Staphs. positive in 2 samples	*E.coli* negative in all samples Staphs. negative in all samples
3). Use of Halamid hand dips before commencement of battering	10	*E. coli* >10 in all samples Staphs. positive in 2 samples	*E.coli* negative all samples Staphs. absent in all samples

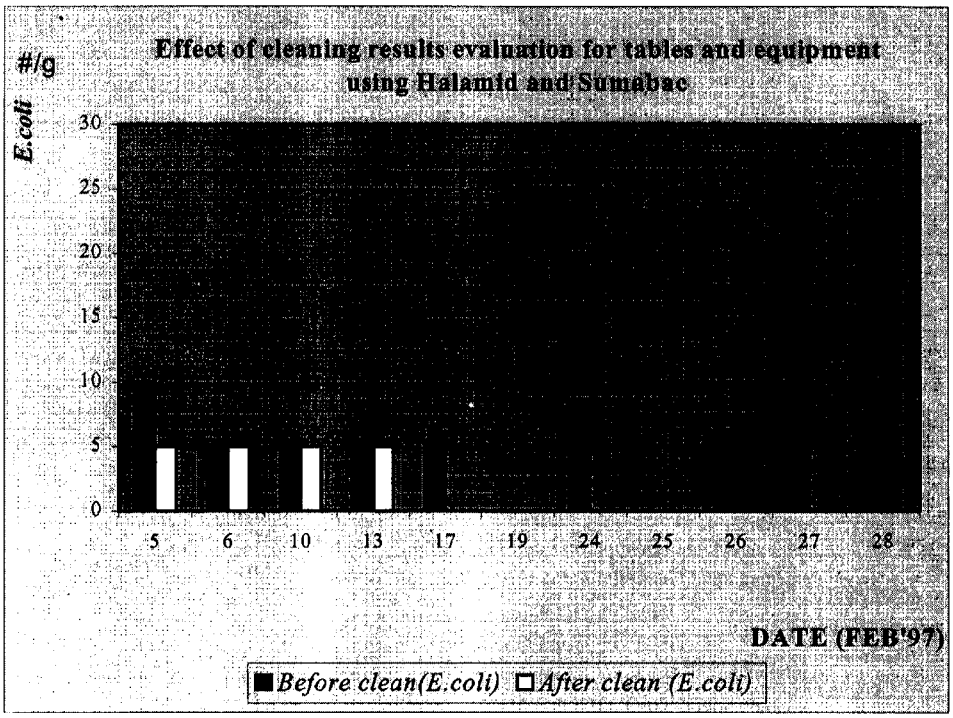

Fig. III. Effect of cleaning with and without disinfectant on the processing equipment of the chicken sausage processing plant when assessed for *E. coli.*

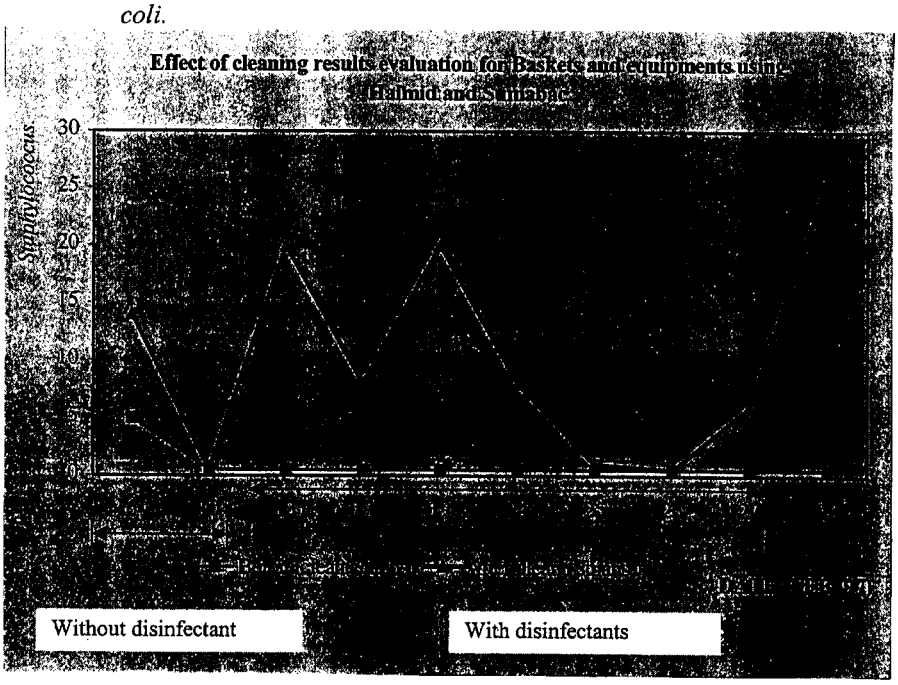

Fig. IV. Effect of cleaning with and without disinfectant on the processing equipment of the chicken sausage processing plant when assessed for *S. aureus.*

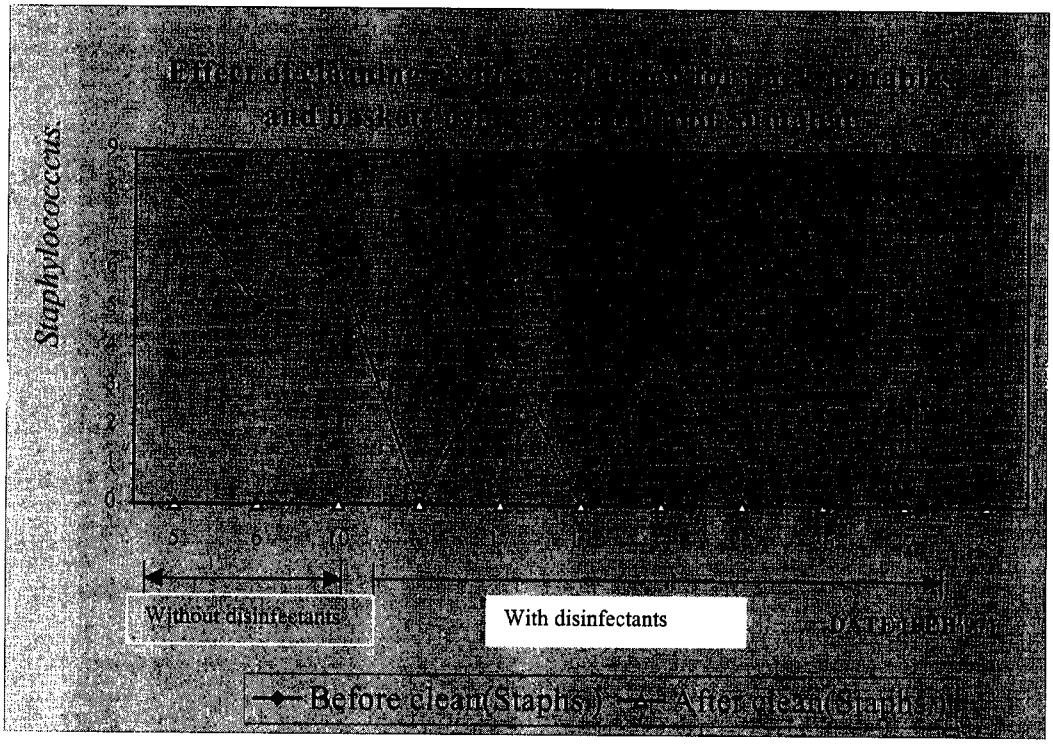

Fig. V. Chinese Rolls Processing Plant.

QUALITY DETERMINATION OF COMMERCIALLY FROZEN PRAWNS USING NUCLEOTIDE-BASED PRODUCTS, SENSORY ASSESSMENT AND TEXTURE MEASUREMENTS

by

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ABSTRACT

The nucleotide degradation profile of two species of farmed shrimps, kept in ice and frozen storage showed high AMP and IMP accumulation for a prolonged period. Quality of frozen shrimp during storage was evaluated by measuring the levels of AMP, IMP, inosine, hypoxanthine and K-value. Sensory assessment and instrumental texture measurements were also employed to assess the quality of prawns. Prawns in their prime quality had AMP and IMP levels >3μmol/g each. K-value below 25% indicated prime quality. The overall acceptability score was closely related to IMP level or the sum of (AMP+IMP),Hx and also K-value. For accepTablequality prawns, K-value should be in the range of 40-46% and the sum of (AMP+IMP) values should be >2μmol/g. Based on these findings, a number of commercial frozen prawns from the industry were evaluated for their quality. These samples were grouped into grade I, II and III based on organoleptic assessment, K-value and levels of nucleotide components. Thus, 30% of the frozen shrimp (HL, PD or PUD) from the industry was rated as grade I (K-value≅25%), 50% as grade II (K-value=36.5 to 43.6%) and 20% as grade III (K-value≅60%). Instrumental texture measurements were compared with organoleptic rating.

INTRODUCTION

Frozen shrimp is one of the most valuable fishery products in international trade. Through the export of frozen prawns, India is realizing a huge amount of foreign exchange. Frozen prawn exports from India earned over 750 million US dollars during 1996-97. However, the quality of seafood is of paramount importance in the global trade. In the fish industry quality is generally assessed by standard sensory methods. However, in international trade, objective methods of quality evaluation are considered to be more reliable. Nucleotide based methods have assumed importance in the assessment of freshness of fish in recent years (Amu and Disney, 1973; Martin *et al.,* 1978; Jacob and Rand, 1982). Individual nucleotides and nucleotide ratios (K-value) have been used to indicate quality in many species of fish (Gill *et al., 1987;* Green *et al., 1990;* Price *et al.,* 1991, Hattula and Kiesvara, 1992; Ryder *et al.,* 1993 and Lakshmanan *et al.,* 1996). This is based on the fact that in most finfish species nucleotide breakdown proceeds as follows:

ATP→ADP→AMP→IMP→Inosine→Hx→Uric acid.

Ehira(1976) remarked that K-value as proposed by Saito *et al.* (1959) was one of the most appropriate indicators of freshness.

However, very little information is available on the nature of nucleotide degradation in shrimp and how it affected the quality and undesirable flavours in shrimp. Arai (1966) indicated that marine shellfish degrade ATP in a different way compared to finfish. He proposed two pathways for the breakdown of ATP in the Japanese prawn, *Pandalus hypsinotus.* One involves the direct deamination of AMP to IMP; while the second involves the dephosphorylation of AMP to adenosine followed by deamination to inosine. Both

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changes would lead to the production of bitter, off flavour hypoxanthine. A reliable, objective test to assess the quality of shrimp when it reaches the factory or at the time of processing would be useful to the industry.

The objective of the present study was to follow the kinetics of nucleotide degradation in the two species, *Penaeus indicus* and *P. monodon* during storage in ice and the frozen state so as to assess the quality before freezing and the changes taking place under frozen storage. The objective indices would be used to validate the sensory assessment and to evaluate the quality of frozen prawns from the industry..

MATERIALS AND METHODS

The commercially important prawns, *P. indicus* and *P. monodon* were procured from the culture farm of Kerala Agricultural University at Puthuvypin. The samples were caught live, headed and iced immediately. One lot from each sample was frozen in a block of 500 g. and kept in cold storage below -18°C. *P. monodon* had weights in the range of 60-70 g and *P. indicus* weighed in the range of 10-15 g. Prawns were iced in the ratio 1:2 and re-iced every day during storage, in an insulated box. Samples were drawn at different intervals of time for the determination of nucleotide catabolites, volatile base-nitrogen (VB-N), pH, texture measurement and sensory evaluation. Frozen material was sampled - every month for a period of 1 year for the analysis. Commercial frozen prawns of different forms, namely headless shell on (HL), peeled (PUD), peeled and deveined (PandD) and grades were procured from eight fish processing factories in Cochin - over a period of one year. A total of 142 samples were collected under different forms. They were evaluated for quality following the same procedure adopted for experimentally frozen prawns.

The nucleotides and related compounds in the muscle were determined by High Performance Liquid Chromatography (Ryder, 1985). Extraction of the muscle was done using 0.6M perchloric acid at 0°C and neutralised using IM KOH. It was then filtered through a Millipore (.45μm) syringe filter. Nucleotide standards and potassium phosphates were obtained from Sigma Chemical Company. All other reagents and water used were of HPLC grade. A Hewlett Packard HPLC (model 1090) and a computing integrator (model 3392 A) were used. Other conditions were the same as reported earlier (Lakshmanan, *et al.*, 1996). The individual nucleotides were calculated and K-value was computed as defined by Saito et al. (1959). Volatile base nitrogen (VB-N) was determined by the micro-diffusion method of Conway (1962). The sensory evaluation was carried out in raw and steamed samples by a panel of 6-8 trained staff members. The samples were tasted for flavour, texture and appearance and scored on a 10 point hedonic scale. The texture (cutting and piercing strength) was measured using a Rheotex (SD-305).

RESULTS AND DISCUSSION

The results of the study are presented in Tables 1 to 7. The values are a mean of three determinations. The initial levels of nucleotide concentration in the two species of cultured prawns were respectively, 13.45 μmoles/g (*P. indicus*) and 16.03μmol/g (*P. monodon).*

a) Iced storage:

Table1 describes the sensory characteristics including taste panel results of iced prawns. The variations in AMP, IMP, inosine, hypoxanthine and K-value in *P. indicus* and *P. monodon* during iced storage are given in Table2.

The initial levels of ATP and ADP in *P. indicus* were respectively, 1.72 and 2.39 μmol/g and in *P. monodon* higher levels of 4.69 and 4.86 μmol/g were obtained. However, after 1 day storage in ice, both ATP and ADP declined rapidly, and reduced to insignificant levels in both the species. They are not further discussed, but the values were used in the calculation of K-value.

During the same period, AMP increased rapidly in both species and reached maxima, (9.93±1.59) and (10.68±0.8) μmole/g in *P. indicus* and *P. monodon*, respectively. Unlike in finfish there was high accumulation of AMP in prawns. By 2 days in ice IMP level also increased (Table2) and reached its peak.

In *P. indicus*. IMP level reached 5.75μ mol/g in 3 days and in *P. monodon* 5.12 μmol/g at 2 days. After 24 hr AMP level declined sharply to around 5.6μmol/g in both the species. After 4 days in ice, both AMP and IMP levels decreased gradually, and were running in parallel (Table2). By 10 days storage in ice AMP level fell to 2.19 and 1.8 μmol/g in *P. monodon* and *P. indicus*, respectively. The IMP concentration also decreased gradually, in both the species, however, retained >1μmol/g at 14 days in*P. Monodon* and 12 days in *P. indicus*. At the end of 18 days ice storage, IMP concentrations were reduced respectively to 0.53 (*P. monodon)* and 0.42 μmol/g (*P. indicus*).

Hypoxanthine content increased slowly from an initial level of 0.04 μmol/g, in *P. monodon* to 1.03 in 10 days, 1.71 in 14 days and reached a limiting value of 2 μmol/g in 18 days ice storage. A similar pattern of accumulation of Hx was found in *P. indicus* as well. Thus, Hx level reached 1 μmol/g in 10 days 1.60 in 14 days and a higher value of 2.59 μmol/g in 18 days storage. A rapid increase of Hx in *P. indicus* at 18 days might probably be attributed to spoilage microbial activity. The inosine content also increased in the same pattern as Hx, at least upto 14 days and then decreased to 1.14 and 0.89 μmol/g at 18 days storage, in *P. indicus* and *P. monodon*, respectively.

The K-value (KV) in the prawn muscle increased linearly with time. However, the rate of increase of KV was faster in *P. indicus* than in *P. monodon*. Thus, in *P. indicus*, KV reached around 20% in 6 days, 30% in 8 days, 40% in 10 days, 58% in 14 days and over 70% in 18 days ice storage. In *P. monodon*, the rate of increase of KV was relatively slower than in *P. indicus*. Thus, KV reached 20% in 8 days, 28% in 10 days, 46% in 14 days and over 60% in 18 days ice storage. In both species, KV increased linearly with time and hence form an index of quality. In general, KV below 25% indicated very good quality in these prawns.

According to taste panel, *P. indicus* and *P. monodon* retained their prime quality up to 6 and 8 days on ice when muscle KV reached 21.72 and 19.68% respectively. After these periods, they had reduced freshness, however, were rated good and accepTableup to 12 (*P. indicus)* and 14 days (*P. monodon)* when their K-values were respectively 44.22 and 46.52%. The prawns in prime quality had high levels of AMP and IMP, and the minimum quantity should be above 3μmol/g. The decline in the levels of AMP and IMP seemed to reduce flavour in the prawn muscle. Hashimoto(1965) and Jones *et al.* (1964) had observed that purine nucleotide, nucleosides and bases contribute to the flavour of fish. The prawns were acceptable to the panelists when both AMP and IMP levels were >1μmol/g eaçh of muscle. Hence we would suggest that knowledge of the levels of AMP, IMP and K-value of raw prawns would determine their initial quality. For acceptable quality prawns, the K-value was in the range 40-45% in combination with (AMP+IMP) values above 2 μmol/g. The VB-N values did not indicate any particular trend in these species. The value remained between 14 -21 mg/100g. of muscle for about 8 days and then increased to a value around 30mg/100g. However, the increase was not regular.

Saito(1964) and Suwetja *et al.*(1989) studied the accumulation of IMP and AMP in crustaceans. With no or less AMP deaminase in marine invertebrates, these authors concluded the major pathway of ATP degradation in marine invertebrates proceeds, via the adenosine pathway. Saito (1964) stated that crustaceans accumulate relatively high amounts of AMP at ice storage; which compliments the present findings. Fatima *et al.* (1981) observed an accumulation of IMP in the shrimp *P. merguensis* and established a correlation between IMP concentration and flavour as well as total sensory score.

The relatively low initial levels of IMP observed in the two species seemed to be due to low AMP deaminase activity. From the processing point of view, it has a beneficial effect in that AMP would form a continued reserve for IMP and hence would retain the sweet meaty flavour for a longer period. Sakaguchi *et al.* (1991) while studying the ice storage characteristics of oyster, observed the accumulation of AMP and IMP in the adductor muscle and proposed that two pathways are operating in ATP degradation. One involves the direct deamination of AMP to IMP, while the second involves the dephosphorylation of AMP to adenosine, then to inosine and Hx.

The high level of AMP accumulation in prawn muscle observed in the present study might be attributed to a low level of AMP deaminase in these species and we presume that both the above pathways operate in these species as AMP and IMP conentrations ran in parallel during ice storage.

b) Frozen storage:

Table3 shows the average variations in AMP, IMP, Hx and K-value in *P. indicus* and *P. monodon* during frozen storage. In frozen samples also there were high AMP and IMP accumulations, in both the species. The maxima were observed during the first month and thereafter both the components started declining, AMP more sharply than IMP. The pattern of nucleotide changes were similar in both species. AMP and LMP levels remained high during frozen storage and in *P. monodon* their levels were around 1.5μmol/g each and in *P. indicus*, their levels were respectively, 1.16 and 0.74 μmol/g. Hx concentration increased steadily, but slowly in *P. indicus* from an initial level of .08 to 2.2μmol/g in 12 months storage. In *P. monodon* it rose from 0.06 to 1.78 μmol/g during the same period. K-value in both the prawns increased slowly and steadily; reached around 20% in 3 months (Table4). In *P. indicus* KV reached 32.8% in 5 months 46.3% in 8 months and over 60% during 1 year of frozen storage. In *P. monodon*, the KV was 33% in 5 months, reached 40.31 in 8 months and 58.6% during 1 year frozen storage. Instrumental texture measurements showed toughening of texture in prawn muscle during storage (tables 3). The muscle pH also increased in both the prawns during frozen storage - from 6.60 to 7.60 during 1 year period (Table3). TVB-N values did not exhibit a regular increase; after an initial increase during the first month, the values fluctuated between 25 to 30mg/100g upto10 months in both the species (Table5), probably due to the inactivation of spoilage organisms. Cutting and piercing strength in cooked prawns increased considerably during storage period (Table3).

Sensory evaluation (Taste panel study) of the steamed samples judged them as good with sweet meaty flavour and soft firm texture, when the samples had high IMP and AMP levels (>2μmol/g) in them. Both the species retained their characteristic flavour and texture upto 8 months when the K-values were 40.3% in *P. monodon* and 46.3% in *P. indicus*. However, the samples were accepTableto the panelists up to 12 months.

Regression analysis of storage time and overall acceptability score with K-value showed significant relations in both the species (Table7). KV showed significant positive correlation with storage time and negative correlation with sensory scores. A good correlation exists between overall sensory score and IMP level, the sum of (AMP+IMP) and Hx level. K-value and the concentrations of IMP and AMP seemed good indices of prawn quality. The level of AMP and IMP in the muscle of frozen prawns would also indicate the initial quality of the raw material before freezing. Kiesvaara et al (1990) also remarked that K-value and IMP concentrations can determine the quality of fish.

c) Commercial frozen prawns

The quality of commercial samples of frozen prawns was evaluated by determining nucleotide degradation products, K-value and sensory assessment and classified into three grades. The criteria used in grading the prawns were sensory characteristics including score and objective parameters, namely AMP, IMP, Hx levels and K-value. Samples with K-value <25% with AMP and IMP levels ≥3μmol/g are classified as grade I. These samples are excellent in appearance and have, sweet flavour and soft firm texture. In grade II samples K-value is proposed to be in the range of <25 to ≤45% with good sweet meaty flavour soft firm texture. Samples with K-value in the range of <45 to ≅60% was classified as grade III. The sample is fair to bleached appearance, has slight rancid flavour and tough texture. In all cases, the K-value in combination with AMP and IMP levels helps validate the sensory scores.

The results of the study are presented in tables(6 aandb). In general, frozen prawns from the industry consists in 30% grade I; 50% grade II and 20% grade III, taking the three forms together. The HL prawns had retained much of the quality attributes compared to PUD and PD prawns. Around 40% of the Frozen HL prawns were rated grade I quality. They had higher levels of both AMP and IMP (>4μmol/g each) and an average sensory score of 8.4. The Hx content (≅0.74μmol/g) and TVB-N (28.4 mg/100g) were below the threshhold limit. In grade II, (47.41%) HL prawns, the average K-value was 43.6% with significant amounts of AMP and IMP; and had sweet meaty flavour. However, 12.6% of Fz HL prawns were rated grade III, with bitter flavour and tough and rubbery texture. The bland taste may be due to the low levels of AMP and IMP (<1μmol/g) in the muscle. K-value had reached around 60% with high TVB-N value (average=37.13 mg/100g). The percentage contributions of PUD and PandD prawns to the three grades indicate that more

samples were shifted to grade II and III compared to HL prawns (Table 6a and b). The levels of nucleotide products and TVB-N were relatively lower in PUD and PD prawns and consequently a reduced sensory score. In general, frozen HL prawns from the trade had higher levels of flavour attributes (AMP and IMP content) and hence a better sensory score than the other two forms.

Matsumoto and Yamanaka (1990) found that there was a linear increase of K-value in Kuruma prawns, with storage and hence could be useful as index for freshness of prawns. Yamanaka and Shimada (1996), while studying the ice storage life of Japanese spiny lobster, observed that AMP accumulated in the muscle, immediately after storage, while IMP, Inosine and Hx increased during storage. They proposed that K-value and ornithine were useful as potential indices for freshness and decomposition of Japanese spiny lobster.

The overall acceptability score in the prawns was closely related to IMP level or sum of (AMP+IMP) and K-value. A high level of IMP and AMP indicated shrimp of good quality and as Hx content or % K-value increase, the quality tend to become poorer. It would appear that measurement of IMP, AMP and K-value would prove good objective quality indices for the Seafood Industry to validate the sensory assessment.

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Table 1. Changes in the sensory characteristics of *P. indicus* and *P. monodon* during iced storage.

Days	0	2	4	6	8	10	12	14	18
P. indicus									
Raw	Bright, shining, fresh sea	Bright, shining	Appearance and odour very good	Slightly tough, good odour	No discoloration	Slightly faded, no off odour	Slightly bleached appearance	Bleached appearance, slight off odour, few black pieces	Bleached appearance, soft blackening
Cooked	Sweet, juicy, soft and firm	Sweet, meaty, soft and firm	Flavour and texture good	Appearance and odour very good	Appearance and odour good	Good appearance	Slightly faded appearance	Odour and appearance satisfactory	Bleached appearance, few black spots, slight off odour
Overall score	9.4±.5	9.10±.6	8.50±.5	8.0±.4	7.6±.5	6.8±.6	6.1±.6	5.6	4.4±.5
P. monodon									
Raw	Bright, shining, fresh sea, weedy odour	Bright, shining	Appearance and odour very good	Appearance and odour good	Appearance and odour good	Good appearance	Slightly faded appearance	Odour and appearance satisfactory	Bleached appearance, few black spots, slight off odour
Cooked	Sweet, juicy, soft, firm	Very good flavour and texture	Sweet, soft and firm	Good flavour and texture	Sweet, soft and firm	Good flavour and texture	Slightly tough, bland	Bland, no off odour, tough texture	Tough texture, slight off odour
Score	9.6±0.4	9.0±0.5	8.6±0.6	8.2±0.4	7.9±0.5	7.1±0.4	6.6±0.5	6.0±0.6	4.8±0.5

Table 2. Changes in AMP, IMP, inosine, hypoxanthine (μmol/g±S.D) and K-value (%) in prawns, *Penaeus indicus* and *P. monodon* during ice storage.

Parameter	Time (days) 0	1	2	4	6	8	10	12	14	18
P. indicus										
AMP	5.82 ±1.90	9.93 ±1.60	5.60 ±0.72	3.82 ±0.60	3.09 ±0.47	2.71 ±0.43	1.80 ±0.12	1.09 ±0.11	0.68 ±0.10	0.45 ±0.03
IMP	3.19 ±2.32	3.79 ±2.21	5.14 ±0.81	4.07 ±0.55	3.43 ±0.51	2.68 ±0.20	1.79 ±0.27	1.26 ±0.14	0.72 ±0.08	0.42 ±0.02
Inosine	0.33 ±0.29	0.42 ±0.14	0.48 ±0.18	0.55 ±0.16	0.77 ±0.10	1.14 ±0.08	1.33 ±0.10	1.17 ±0.30	1.26 ±0.40	1.14 ±0.20
Hypo-xanthine	0.12 ±0.10	0.19 ±0.12	0.36 ±0.12	0.62 ±0.	0.85 ±0.05	0.89 ±0.08	0.98 ±0.04	1.19 ±0.06	1.60 ±0.10	2.59 ±0.18
K-value	2.59 ±2.73	4.28 ±2.15	7.35 ±0.94	13.35 ±2.44	21.72 ±1.45	32.18 ±3.70	40.71 ±1.62	44.22 ±1.65	49.10 ±2.10	74.57 ±1.54
P. Monodon										
AMP	5.45 ±0.17	10.68 ±0.82	5.58 ±0.75	4.21 ±0.31	3.49 ±0.45	3.08 ±0.11	2.12 ±0.23	1.75 ±0.12	1.45	1.12
IMP	0.60 ±0.24	3.52 ±2.00	5.12 ±0.25	3.70 ±0.14	3.42 ±0.30	2.94 ±0.32	2.29 ±0.40	1.87 ±0.12	1.38	0.53
Inosine	0.04 ±0.02	0.21 ±0.11	0.57 ±0.19	0.68 ±0.20	0.80 ±0.15	0.88 ±0.07	1.03 +0.24	1.31 ±0.09	1.44	0.89
Hypo-xanthine	0.04 ±0.12	0.12 ±0.05	0.31 ±0.06	0.57 ±0.04	0.60 ±0.07	0.94 ±0.12	1.03 ±0.10	1.25 ±0.05	1.71	1.96
K-value	0.30	2.10 ±1.54	7.13 ±0.14	12.74 ±0.40	16.10 ±0.24	19.68 ±1.28	27.90 ±0.50	35.85 ±1.64	46.50 ±1.30	63.40 ±1.45

Table 3. Changes in the physical and sensory characteristics of *P. indicus* and *P. monodon* during storage at -18°C.

Months	0	1	2	3	5	6	8	10	12
P. indicus									
1. pH	6.60	6.94	7.05	7.14	7.20	6.98	7.18	7.3	7.40
2. Textro meter reading									
a. Cutting strength, g									
Raw	54±8.6	66 ±6	48.8±6.5	56.7±5	50±4	46±6	44±11	40±10	36±8
Cooked	365±18.4	460±12	416±6	487±12	501±8	494±7.5	514±16	519±11	526±14
b. Piercing strength, g									
Raw	54±6.5	46±8	56±4	44±6	52±5	48±6	47±8	41±8	42±6
Cooked	78±5.6	98±5	101.4±10.2	93±8	96±6	94±5	97±7	103±14	97±8
3. Sensory characteristics	Very good flavour, soft and firm	Very good flavour and texture	Good flavour, soft and firm	Sweet flavour, soft and firm	Good flavour and texture	Good flavour and texture	Less sweet, slightly tough	Bland, Slightly tough	Tough Slightly bitter
4. Average score	9.4±.5	8.8±.6	8.4±.5	8.0±0.7	7.6±0.8	7.1±0.6	6.8±0.5	6.0±.6	4.8±.6
P. monodon									
1. pH	6.68	7.04	7.12	7.16	7.20	7.24	7.30	7.4	7.6
2. Textro meter reading									
a. Cutting strength, g									
Raw	58±13.7	56.3±12.5	48.6±4.8	52.6±5	59.7±9.9	64±7	440.7±6.5	9.7±9.8	54.7±16.5389
Cooked	320±25	345±18	449±38.6	480±36	477±44	493±23	511±18	523±12	±17
b. Piercing strength, g									
Raw	44±3.4	35.4±9.8	40±7.9	39.6±6	40.4±4.1	25.7±5.9	30.5±6	44±7.9	40.4±7.6
Cooked	78±6.3	86.7±21	90.4+15.7	88.4±7	104.2±12	94.2±12	102.45±27	105+6.5	93.4±21
3. Sensory characteris-tics	Sweet meaty flavour, soft and firm	Very good flavour and texture	Sweet meaty flavour, soft and firm	Sweet flavour, soft and firm	Good flavour and texture	Good flavour, soft and firm	Less sweet, slightly tough	Slightly tough	Bland, tough
4. Score	9.6±0.4	9.0±.6	8.5±0.7	8.2±0.5	8.0±0.55	7.5±0.5	6.8±0.6	6.1±0.4	5.1±0.5

Table 4. Changes in AMP, IMP, inosine, hypoxanthine (μmol/g ±S.D) and K-value (%) in the prawns *Penaeus indicus* and *P. monodon* during frozen storage below (-18C).

Para-meter	Storage time, months								
	P. indicus								
	Initial (O)	1	2	3	5	6	8	10	12
AMP	8.41 ±1.60	6.69 ±2.12	5.67 ±1.30	4.48 ±1.15	2.81 ±0.75	2.24 ±0.35	1.48 ±0.21	1.20 ±0.24	0.74 ±0.13
IMP	4.60 ±1.20	5.74 ±0.80	5.11 ±0.60	4.64 ±0.78	4.03 ±0.65	3.64 ±0.44	2.75 ±0.38	1.92 ±0.50	1.16 ±0.12
Inosine	0.28 ±..08	0.81 ±0.20	1.33 ±0.28	1.34 ±0.40	1.67 ±0.50	2.09 ±0.60	1.90 ±0.48	2.02 ±0.45	1.58 ±0.34
Hypox-anthine	0.08 ±0.03	0.35 ±0.08	0.62 ± 0.12	0.85 ±0.20	1.16 ±0.24	1.35 ±0.30	1.60 ±0.31	1.94 ±0.40	2.20 ±0.42
KV	2.60	8.2	14.6 ±1.1	21.7 ±1.2	32.8 ±1.4	38.4 ±1.4	46.3 ±1.6	52.2 ±1.5	60.2 ±2.1
	P. monodon								
AMP	5.40 ±1.23	6.80 ±1.35	5.20 ±1.10	4.12 ±0.83	2.38 ±0.46	1.88 ±0.44	1.65 ±.30	1.52 ±0.24	1.48 ±.20
IMP	0.46 ±.10	4.57 ±0.80	4.01 ±0.65	3.56 ±0.74	2.74 ±0.38	2.30 ±0.24	1.96 ±0.20	1.70 ±.20	1.53 ±.28
Inosine	0.04	0.66 ±0.10	0.90 ±.22	1.60 ±0.25	1.46 ±.30	1.44 ±0.20	1.44 0.20	2.01 ±.22	3.33 0.24
Hypox-anthine	0.06	0.31 ±0.06	0.56 ±0.10	0.82 ±0.16	1.10 ±.22	1.25 ±0.21	1.40 ±0.18	1.63 ±0.20	1.78 ±.34
KV	1.50	7.57 ±0.74	12.92 ±0.80	22.60 ±1.45	33.11 ±1.8	35.90 ±1.6	40.30 ±2.4	48.74 ±2.8	58.60 ±2.60

Table 5. Changes in TVB-N (mg/100g) values in *P. indicus* and *P. monodon* duringice and frozen storage.

Time (days)	Iced storage		Time (months)	Frozen storage	
	P. indicus	***P. monodon***		***P. indicus***	***P. monodon***
Initial(0)	16.60±0.8	14.59±0.5	Initial (0)	16.60±0.8	14.59.0.5
1	17.82±0.6	16.45±0.4	1	18.40±1.2	21.67±0.7
2	16.90±0.5	17.10±0.6	2	23.64±0.7	24.75±0.6
4	18.45±0.4	17.84±0.5	3	28.30±1.1	26.67±0.5
6	20.60±0.5	21.75±0.7	5	30.43±0.5	28.50±0.5
8	22.47±0.6	20.90±0.8	6	29.60±0.8	29.70±0.7
12	25.71±0.8	22.40±0.7	8	30.50±1.1	28.60±0.6
14	27.50±0.6	25.36±0.4	10	33.70±1.3	32.40±0.7
18	30.25±0.7	28.94±0.6	12	37.85±1.2	38.75±0.6

Table 6 a. Quality levels of commercially frozen prawns collected from the Industry.

Physical and Sensory Characteristics

Product	Sensory characteristics (Cooked)	Texture Measurements		Overall score	Frequency (% sample)
		Cutting strength 'g'	Piercing strength 'g'		
HL Prawns (n=38) Grade I	Excellent appear. sweet meaty flavour. soft and firm texture	*R =58±13.7 *C=420±25	R=54±03.4 C=78±6.3	8.4 ±.3	40
Grade II	good. Sweet and meaty flavour . Sl. tough texture	R= 66±6 C=460±12	R=46±8 C=98±7	6.8 ±.4	47.4
Grade III	Fair to poor. sl. bitter flavour tough and rubbery	R=75±6.8 c=510±6.5	R=28±6 C124±8	4.8 ±.3	12.6
PUD (n-60) Grade I	Very good appear. Sweet meaty flavour firm and soft texture.	R=72±11 C=408±8	R=35±4 C=87±6	8.10 ±.34	30
Grade II	Good. Less sweet sl. Soft texture	R=89±13 C=509±10	R=47±5 C116±7	6.60 ±.35	51.7
Grade III	Fair to bleached appear. Sl rancid flavour rubbery texture Fair to poor.	R=86±14 C=511±12	R=45±4 C=128±6	4.5 ±.4	18.3
PandD (n=44) Grade I	Very good appearance. Sweet and meaty flavour good texture	R=69±7 C=416±6	R=56±8 C=1014	8.14 ±.20	18.2
Grade II	Good, less sweet. Sl. Tough texture	R=74±8 C=469±11	R=47±4 C=126±12	6.20 ±.31	54.5
Grade III	Fair to poor sl. Rancid flavour hard and rubbery texture	R=77±8 C=530±14	R=48±5 C=148±6	4.10 ±.20	27.3

**R - raw sample*
**C - cooked.*

Table 6 b. Quality levels of commercially frozen prawns collected from the Industry.

Chemical characteristics

Product	µmol/g				KV(%)	mg/100g TVB-N
	AMP	IMP	Inosine	Hx		
HL-Prawns (n=38)						
Grade I	5.97 ±1.56	4.15 ±1.60	1.24 ±0.40	0.74 ±.25	24.6 ±7.2	28.4 ±2.1
Grade II	2.00 ±.72	1.74 ±0.84	1.76 ±0.44	1.23 ±.34	43.6 ±8.5	35.2 ±2.5
Grade III	0.77 ±0.48	0.62 ±0.40	2.08 ±0.36	1.70 ±0.64	59.5 ±7.8	37.1 ±3.3
PUD(n=60)	3.76	4.06	1.50	0.42	22.9	21.5
Grade I	±1.36	±1.50	±0.30	±.22	±6.2	±2.2
Grade II	1.68 ±0.57	1.96 ±1.60	1.86 ±0.44	0.92 ±0.48	38.5 ±7.6	25.3 ±3.4
Grade III	0.64 ±.40	0.46 ±0.38	1.78 ±.54	1.72 ±.67	61.5 ±11.3	38.5 ±3.7
PandD (n=44)						
Grade I	3.54 ±1.30	3.94 ±1.40	1.58 ±0.28	0.58 ±.30	24.5 ±5.2	23.7 ±3.2
Grade II	1.60 ±0.60	1.68 ±1.30	1.74 ±.80	0.96 ±.54	36.5 ±8.6	28.4 ±3.5
Grade III	0.72 ±.38	0.52 ±0.32	1.69 ±0.76	1.78 ±0.60	57.84 ±9.2	34.2 ±5.4

Table 7. Correlation coefficient of storage time, sensory score, K-value and nucleotide degradation products in *P. indicus* and *P. monodon* during ice storage.

	Overall score	Storage time
P. indicus(n=27)		
K-value	-0.9605[a]	0.9886[a]
IMP level	0.9220[a]	-
(IMP+AMP)	0.9150[a]	-
Hx	-0.8890[b]	-
P. monodon(n=27)		
K-value	-0.9624[a]	0.9896[a]
IMP level	0.8276[b]	
(IMP+AMP)	0.8535[b]	
Hx	-0.9549[a]	

Level of significance a = 0.1%, b = 1.0%.

OVERVIEW OF A SIMPLE, SYSTEMS BASED APPROACH TO THE REDUCTION OF BLOWFLY INFESTATION OF CURED FISH

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ABSTRACT

Insect infestation of traditionally cured fish continues to be an important cause of post-harvest losses in many developing countries and has resulted in the widespread abuse of unsuitable insecticides by fish processors in some areas (Walker, 1987; Esser, 1994). Although, this practice is highly undesirable for both health and environmental reasons, processors are unlikely to change their practices until a viable alternative method of insect control is provided.

This contribution describes a prototype strategy for controlling blowfly infestation in traditionally cured fish in the tropics. Unlike previous programmes of insect control, the strategy is systematic in its approach. This enables the underlying causes of the infestation to be identified and addressed, thereby reducing the long-term need for continuous application of control measures. The flexibility of the systematic approach enables the strategy to be fully transferable between locations, and allows for seasonal variations in the severity of infestation.

INTRODUCTION

Studies have shown that the cause and likely severity of infestation at any processing site, is subject to large variations (Walker, 1988; Esser, 1987; Johnson, 1997). Relying on the use of any single control measure to combat infestation at all sites is therefore impractical. By studying the timing, mode and cause of the infestation relative to each processing step, it is possible to select control measures which are appropriate to the nature of the infestation occurring at each and any site (Johnson, 1997; Johnson and Esser, 1998). As socio-economic considerations can be taken into account when control measures are selected, the limitations which hindered the uptake of previous non-insecticidal control measures may be overcome.

THE CONTROL STRATEGY

The strategy follows a logical system of control based upon the prevention of infestation "risk factors". These risk factors are associated with the processing practices being followed and the external conditions at the site, and are known to influence the level and occurrence of blowfly infestation in traditionally cured fish (Johnson and Esser, 1998).

HOW TO APPLY THE CONTROL STRATEGY

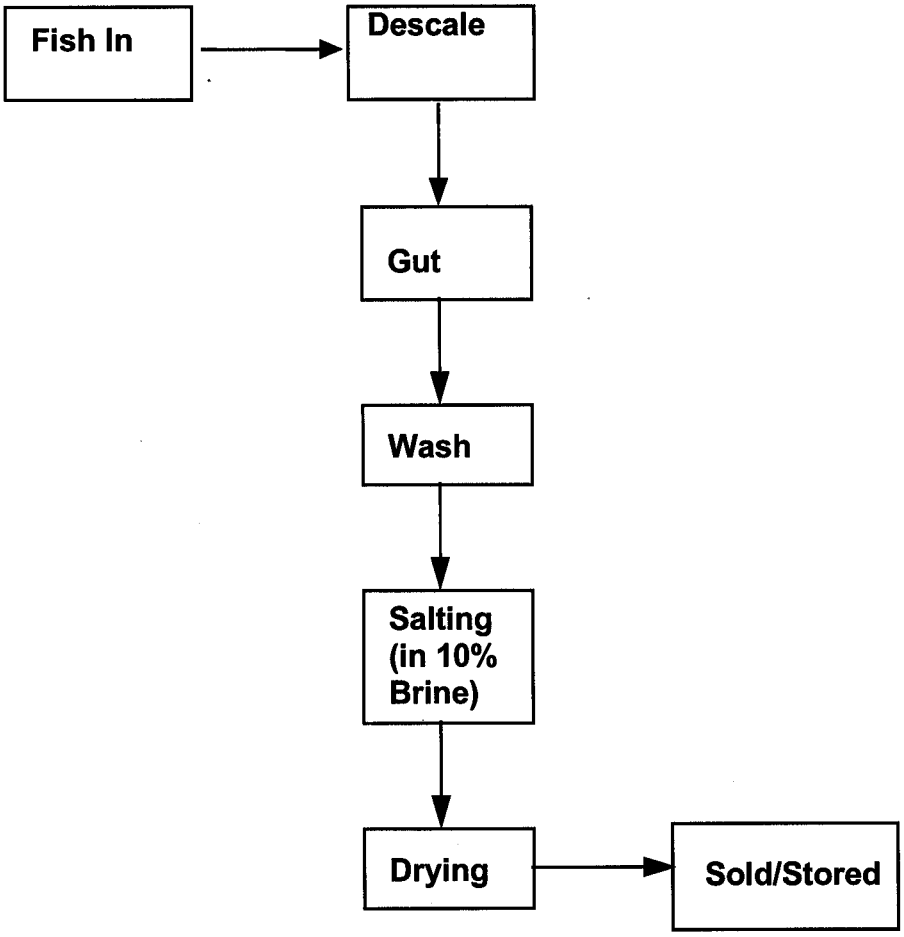
The strategy is implemented by considering the curing process systematically, from the receipt of the raw fish, through processing to the dispatch of the finished product. This takes the form of an audit conducted at the processing site, comprising 5 steps:

1. identify the processing sequence;
2. determine the timing and mode of infestation relative to the identified processing steps;
3. consider which processing risk factors may be influencing the infestation;
4. consider which external risk factors may be influencing the infestation;
5. select control measures appropriate to the circumstances of the infestation at the site.

By separating the process into discrete stages, it is possible to quantify the severity of the infestation at each infestation point. This enables the processor to prioritise where the application of control measures is most critical to the reduction of blowfly related losses at his/her site.

THEORETICAL APPLICATION OF THE STRATEGY

Example Flow Diagram for the Production of Salted, Dried Fish



APPROPRIATE CONTROL MEASURES

Fish In:

1. a) hold fish in ice and process ASAP
   b) where possible, only process fresh fish
   c) if spoiled fish must be processed, grade the fish for freshness then separate each grade during processing.
2. a) always follow good hygienic practices. Wash hands and knives regularly, and stack fish on clean surfaces, raised off the floor.

Preparation:

1. a) all fish waste should be collected and disposed of away from the site (see above)
   b) in some areas, there may be a market for the fish waste for conversion into poultry feed or fertilizer.

Salting

1. a) fly-proof lids should be fitted to all salting tanks
   b) if tanks or boxes are not available for dry salting, netting should be erected over the stacks of fish to prevent blowflies from gaining access
2. inspect all fish prior to brining. Any which are found to be infested should either:

- have the eggs and larvae manually removed
- be thoroughly washed under clean running water to wash off any eggs or larvae present
- try to minimize the level of infestation by applying large amounts of salt to the infested areas. This measure will not be effective if larvae have already burrowed into the flesh
- be processed separately from non-infested fish and losses in these fish accepted.

Drying

1. a) fly-proof netting should be erected over areas where fish are to be dried.
   b) if the level of oviposition is not high, eggs can be manually removed from the fish
   c) applying large amounts of salt to areas where eggs are likely to be deposited may deter oviposition
   d) whenever possible, fish should be dried on raised racks constructed of an open material which will allow air to circulate beneath the fish
   e) turn fish regularly to increase the rate of moisture loss from the underside of the fish
   f) where fish must be dried upon the ground, clean concrete is the most suitable surface
   g) never dry fish upon sand or loose earth as larvae can burrow into the ground in such areas to escape the intensity of the sun, and to pupate, thus sustaining the blowfly population at a site.
2. a) fish should never be overlapped during drying
   b) infested fish should be discarded or dried away from the other fish
   c) ensure the surface on which the fish are to be dried is free of larvae and debris containing eggs or larvae.

CONCLUSIONS

The strategy described offers processors of traditionally cured fish a flexible and sustainable way to control blowfly infestation in their product. By providing processors with the means to select control measures appropriate to the particular circumstances of infestation at their site, a high level of control should be achieved. Furthermore, the underlying causes of the infestation will be tackled thereby reducing the need to apply some control measures over time. Prioritizing where control is most needed, and, where available, offering a choice of effective control measures, processors will be in a position to adopt those measures applicable to their particular socio-economic circumstances. In this way, the economic limitations which hindered the application of previous control measures may be overcome.

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STUDIES ON THE ORGANIZATIONAL STRUCTURE, LEADERSHIP STYLES AND COMMUNICATION IN THE SEAFOOD INDUSTRY IN KERALA (INDIA) WITH RESPECT TO THEIR APPLICABILITY FOR INTRODUCING TOTAL QUALITY MANAGEMENT

by

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ABSTRACT

India has been exporting frozen seafood since 1953. The country now has 439 seafood factories of which 33 % are located in Kerala. Seafood constitutes 4.5% of the total export earnings of the country contributing about US$ 1125 per annum. Two hundred thousand people are directly involved in the business and the country exports to over 50 markets worldwide, the main markets being Europe, Japan and the United States. With the expansion of the markets and with the increase in the number of suppliers, product variation became a major problem. Contamination with harmful microbes and filth have been reported as the major problems associated with seafood exports from India. Even though the country tried different quality control systems from end product inspection to in-plant quality control system to self-inspection in approved units, rejection and block listing has been a regular bottleneck in the expansion of the market. The total ban on the import of Indian marine products by the European Commission (EC) has led to the introduction of HACCP concepts for quality management in India. Countries like Japan have successfully adopted Total Quality Management System in their factories with great success.

A study was conducted on the possibility of introducing Total Quality Management Systems incorporating HACCP as the core concept in Kerala for successful and sustainable development of seafood markets for Indian exporters. The paper reports the results of the study conducted on the organizational structure, leadership styles and communication systems prevailing in the various seafood factories in Kerala with respect to their applicability to Total Quality Management Systems in these factories. The study shows that 43% of the seafood factories in Kerala are partnership companies, 28% are public limited companies and the remaining 29% proprietary concerns. Typical organizational structures of the partnership companies, public limited companies and the proprietary concerns are reported. The organizational structures of the public limited companies show that 50% are based on typical line functions and the remaining 50% have line and staff functions. The management in all the factories studied adopt autocratic or feudal leadership styles. This is not suitable for introducing Total Quality Management in these factories. An egalitarian leadership model is developed to suit the seafood industry in Kerala. The paper also discusses the type of communication prevailed in the seafood industry in Kerala. The communication system in the seafood industry in Kerala is also not suitable for successful implementation of Total Quality Management.

INTRODUCTION

Organizational structure is one of the most important elements of management for study in any field of human activity. Organization makes use of specialization, by allocating specific jobs to those who are considered most suitable for the specific jobs and also establishes effective communication between workers, supervisors and managers in the factories. It helps to establish the relationship between different departments engaged in similar jobs so as to integrate the efforts of various departments towards a common objective. The term organization is defined by many authors differently, Allen (in: Lal, 1990) defined organization as "the process of identifying and grouping the work to be performed, defining and delegating

authority and establishing relationships for the purpose of enabling people to work most effectively together in accomplishing objectives".

The Total Quality Management Approach identifies basic organizational problems. Effective problem solving at the early stage of a TQM process is advisable in order to understand the organization's strength and weaknesses. Kangi and Asher (1995) stressed the necessity of practical methods for developing a TQM approach for solving the basic problems affecting the organization's activities. Effective problem solving at the early stage of a TQM process is advisable in order to understand the organization's strength and weaknesses. An analysis of the organizational structure and design of seafood plants is important to understand the level of adaptability of TQM. Even though there is not even a single study on the organizational structure and design of seafood plants in India, a number of studies have been carried out elsewhere on the three main organizational design approaches: Classical, Behavioural and Contingency approaches.

There have been a number of theoretical perspectives that have emerged over the years that can be classified as belonging to the classical approach. Two separate branches of classical school have developed. The first one gave emphasis on the management of jobs (Scientific Management). Among these, Taylor's work on Scientific Management is important (Taylor, 1911). The second gave emphasis on management of organizations (Administrative Theory). Another important organizational design approach is the Bureaucratic approach, conceptualized by Max Weber, who believed that the key to the survival of an organization was through mechanisms that increased the efficiency of the organization's activities. The Weberian model is based on a number of important characteristics as: (1) Division of labour, (2) Well-defined rules and procedures, (3) Authority, (4) Impersonality, and (5) Careers. These characteristics of an "ideal" bureaucracy established a major organizational movement (Weber, 1947). Bennis (1966) assumes that bureaucracy will wither and become less prevalent in organizations because managers will be unable to manage the tension, frustration and conflict between individual and organizational goals. In addition, bureaucracy will fade because of the scientific and technological revolution in industrialized nations.

The management processes are different in various industries and environments. Burns and Stalker (1961) studied twenty industrial organizations to assess the pattern of managerial activities in planning, organizing, controlling and their relations to the external environment. They found that each firm in their study sample could be viewed as an information-processing network. Lawrence and Lorsch (1969) conducted field studies to determine what kind of organizational design was best suitable with various economic and market environments. Davis and Lawrence (1977) gave a detailed description about Matrix Design, a product structure superimposed on a functional structure, that is fast becoming the most popular form of organizational structure.

The organizing function acts as one of the major sources of development for the needed skills and roles performed by the manager. The influence component relates strongly to developing conceptual skills, and the coordination component is a determinant of diagnostic skills. In a similar manner, the various interpersonal, informational and decisional roles are affected by how the organization is structured (Szyilagyi, 1981).

Quality improvement demands the total commitment of the whole management system. According to Deming (1986) it is not enough that top management commit themselves for life to quality and productivity. They must know what it is that they are committed to – that is what they must do.

According to Vardaman and Halterman (1968), communication means the flow of material, information, perception, and understanding between various parts and members of an organization. It also means all the methods, means and media of communication (communication technology), all the channels, networks, and systems of communication (organizational structure) and all the person to person interchange (interpersonal communication). It includes all aspects of communication: up, down, lateral; speaking, writing, listening, reading; methods, media, modes, channels, networks, flow; interpersonal, intra-organizational and inter-organizational.

It is mere common sense that the intelligence of a handful of technocrats, however brilliant and smart they may be, is no longer enough to take them up with a real chance of success. Only by drawing on

the combined brainpower of all its employees can a firm successfully survive in today's world of changing environment and markets. Team building is the only solution in this respect. Only by carrying out planning and causal analysis in work groups, like quality improvement groups or quality circles, a full utilization of the intellectual resources of the firm is possible. Quality goals and quality policies are the basis of quality plans. Most of the seafood firms in India do little in this area. Quality goals and quality policies have a definite function in TQM.

Sadgrove (1996) classified the leadership structure in an organization into four categories; autocratic, feudal, egalitarian and anarchic. According to him, an egalitarian company is a much more liberated place to work. Here people communicate both up and down their own department and across their departments. Teams can be formed to solve particular problems. Because the structure is flexible, it can grow and contract in response to the market. This is the culture expected by TQM.

METHODOLOGY

Twenty-four seafood factories out of the total of 120 factories were randomly selected for the study using a stratified random sampling technique for collecting general information of the seafood factories in Kerala. Out of this, ten factories were further selected at random for detailed study on the organizational structure and design, leadership style, communication and motivational aspects. To collect information on these aspects from three levels in the hierarchy (top management, middle level, floor level) pre-tested questionnaires were used. Rapid rural appraisal technique was used to get first hand information from the employees and management as described by Ward (1996). The classification of the organizational structure of the factories has been done based on the procedure described by Sadgrove (1996) to find out the leadership style and communication prevalent in the factories.

RESULTS AND DISCUSSION

The study shows that 43% of the seafood factories are partnership companies, 28% are public limited companies and remaining 29% proprietary concerns. The public limited companies are all of recent origin and came into existence in the late 1980s and early 1990s. Entrepreneurs who were already in the seafood business for a long time established 90% of the public limited companies. On critical examination of the public limited companies it could be found that most of the shareholders are belonging to a single family or their relatives. This status of the company reduces the cosmopolitan climate in the decision making process of the public limited companies. Family members or their relatives run most of the partnership companies.

Coordinating the units and departments of an organization is an extremely important but massive and costly managerial activity. The organizing function acts as one of the major sources of development for the needed skills and role performed by the manager. The influence components relate strongly to developing conceptual skills, and the coordination component is a determinant of diagnostic skills. In a similar manner, the various interpersonal, informational and decisional roles are affected by how the organization is structured (Szilagyi, 1981) Kossen (1991) defined an organization as a group of individuals structured by specialized activities and levels of authority The organizational structures of the public limited companies show that 50% are based on typical line function and the 50% have line and staff function. According to Szilagyi (1981), as organizations increase in size and complexity, it is necessary to introduce personnel with specialized knowledge and skills. This creates the arrangement of line – staff functions.

The organizational structure of public limited companies consists of Board of Directors at the helm of affairs, and a Managing Director appointed by the Board of Directors to look after the day-to-day affairs of the company and report to the Board of Director from time to time. A General Manager or senior manager assists the Managing Director. The line functions are broadly classified into operations, administration and finance. Operation is the major function in the seafood factories. Senior manager (operation) who is further assisted by a purchase manager in the production section, and production control manager in the production control and quality assurance section completes the operation division. The general administration is a small department in most of the factories. A finance manager assists the general

manager in the financial aspects. Recently some companies have also introduced a personnel section to look after the welfare of the employees with personnel manager in charge of the section and directly reporting to the general manager. Technologist and supervisors who directly report to the respective managers further support the functional lines. The supervisors directly control the floor level workers in different sections. Line groupings are those units that are directly involved in producing the products or services but staff groupings are those units that perform in support of the line function Szilagyi (1981). According to Kossen (1991), staff members don't ordinarily have direct authority over line members, although there are numerous exceptions. Because of their technical or professional knowledge, staff members commonly provide assistance or advice to line members, which helps to free line managers from details that are neither directly related to daily operations nor require specialized skills and knowledge.

In case of partnership companies, the major partner acts as the managing partner and the remaining partner look after the various functions of the organizations. In most of the partnership companies a clear-cut delegation of powers has been noticed with respect to the authority of partners. The majority of the partnership companies have three functional departments namely – production, administration and marketing. A production manager heads the production department. The production manager looks after both production and quality assurance of the company. Technologists and supervisors assist the production manager and quality assurance manager. The organizational structure of partnership companies shows typical line authority structure Kossen (1991).

The pattern of organizational structure in proprietary companies is more or less similar to the partnership companies except the fact that the proprietor heads the company and functions as an autocratic leader.

Sadgrove (1996) discussed leadership structure in an organization into four categories – autocratic, feudal, egalitarian and anarchic. The study shows that 71% of the seafood factories in Kerala are following an autocratic style of leadership. Here the proprietor functions as a dictator with little authority delegated to lower levels. According to Sadgrove (1996) in an autocratic set-up one person is at the centre of the business, nothing happens without his approval. The present study in the seafood factories in Kerala was in perfect agreement with Sadgrove's findings. The remaining 29 % follow a feudal leadership style (Figure 7). These factories are all public limited companies. In the feudal company, the people at the top were far away from the bottom and the two never communicated each other. Sadgrove (1996) also reported similar findings. So the structure is rigid. Public limited companies in Kerala show typical feudal leadership style. An egalitarian style of leadership model is recommended for the seafood factories in Kerala to transform them into TQM factories. This is a basic necessity to apply any quality management measures in the seafood factories. With out this transformation all other efforts to improve quality through the modern concepts like HACCP or ISO 9000 would not be successful. According to Sadgrove (1996) an "Egalitarian" company is a much more liberated place to work. Here people communicate both up and down their own departments. Teams can be formed to solve particular problems. Because the structure is flexible, it can grow and contract in response to the markets. This is the culture expected by Total Quality Management.

In the seafood processing industry in Kerala, communication from top management to floor level employees is not satisfactory. Top managers are unable to communicate with floor level employees. According to Dahlgaard et al. (1995), a central element of leadership is the ability to communicate the goal to employees so perfectly that they unequivocally accept it as common goal of the group. Leadership's most important role is to help people do a better job.

The overall communication in the factories is not satisfactory as 40% of the information is passed through oral communication. This has every chance of distorting the information in the communication channels. Only 10% of the communications are found to be participatory in nature. This shows a very high communication gap in the factories with autocratic style of leadership. The main media of communication are oral or through notice boards. Informal channels dominate the communication network, but meetings are also occasionally called to settle disputes. The horizontal communication is all informal. The employees have upward communication only to report their grievances. No official upward channel is provided for the lower level employees to express their ideas about products or quality assurance.

A communication system that only gives specific directives about job instructions and procedures and fails to provide information about job performance or rational-ideological information about the job has a negative organizational impact. According to Luthans (1977), this type of downward orientation promotes an authoritative atmosphere, which tends to inhibit the effectiveness of the upward and horizontal system of communication.

The following communication channels are used by the management to communicate with the floor level employees in the seafood processing industry in Kerala. In the case of permanent employees they use formal channels like issuing notice and calling meeting for direct communication. The written communication is usually routed through the hierarchical levels. In the case of contract employees, the communication is passed through the respective managers and the contractors. Usually informal oral communication is passed to the contract employees through the contractors. Kossen (1991) point out that a principal advantage of oral communication over written communication is that spoken message allow you to receive instant feedback. With written communication, feedback may be delayed or even non-existent. Telephone communication, although not face to face, have some of the same advantages as face to face communication.

Traditionally, one of the dominant themes of communication has been the so-called downward system. The downward system is more accurately portrayed as superior-subordinate communication. There are personal linkages, not just information flow, in the downward systems (Luthans, 1977). Katz and Khan (1966) identified five general purposes of superior-subordinate communication in an organization. They are, to give specific task directives about job instructions, to give information about organizational procedures and practices, to provide information about the rationale of jobs, to tell subordinates about their performance, to provide ideological–type information to facilitate the indoctrination of goals.

In the seafood processing industry in Kerala, if the top management notices any quality defects or problems this is usually communicated to the lower level employees only through oral communication or through a meeting. The employees feel that the exact facts are not properly communicated. Inadequacy has been noticed in all the factories regarding proper communication. Bedeian (1986) discuss the major precautions that can be taken and approaches used to minimize communication breakdown. They are: obtain feedback, encourage upward communication, use face to face communication where possible, engage in deep sensing, avoid credibility gaps, anticipate rumours, write for understanding, watch your timing, be sensitive to needs and feeling of others. Simpson (1959) points out, in today's increasingly large and complex organization, communication across the chain of command has become quite important to organizational performance. This type of communication is referred to as lateral communication. The need for lateral communication is created primarily because of problems with the organization's structure. First, there is the issue of time. Frequently, information must be transmitted across organizational functions for decision making purposes, such as a customer complaint on product quality that is received by a sales representative, but must work its way to production unit manager. Under normal circumstances, this information would be transmitted along the chain of command of the marketing function, and then down the manufacturing chain of command to the responsible manager. When time is critical, as in solving a serious customer problem, this form of communication can be less than satisfactory.

In the seafood processing industry in Kerala, all the employees interviewed at different levels feel that the communication gap at different levels is the main cause for difference of opinion and conflict in the factories. Szilagyi (1981) points out that mutual personnel exchange (conflict resolving strategy) involves increasing the communication and understanding between groups by exchanging personnel for a time. The assumption underlying this strategy is that the exchanged personnel can learn about the other group and communicate their impression to their original group.

Employees in the seafood processing industry in Kerala also feel that improving the open and efficient communication in the factories will lead to improved quality and productivity of the industry.

The study shows that the main type of communication in the factories is only informal, that is oral communication. Szilagyi (1981) reported that the open plan design of the work place improves communication and performance between organizational members when there is a need for frequent verbal (face-to-face communication).

The top management of the seafood processing industries in Kerala considers the production turn over as the important contribution of the employees and does not provide any opportunity for the employees to participate actively for the improvement of the quality of the products or quality of the factories. Ramachandran (1990) reported that the fish processing industry in Kerala and elsewhere in India has totally neglected the contribution of workers in the productivity, quality and overall growth of the industry. This total neglect of workers is one of the main reasons of low productivity, low quality of the output in the industry.

Ninety percent of the seafood processing factories do not have any quality team comprising employees from different levels. Only 10% of the factories have an HACCP team, which includes floor level employees. These 10% of the factories have instituted HACCP teams as a result of pressure from the EC Directive (91/493/EEC) and have obtained EC approval. Many researchers and authors have pressed the need for workers involvement in productivity and establishment of quality circle (Udapa, 1983; Smith, 1983; Rai, 1984; Chandreshekharan 1986; Ramachandran, 1990). This will improve the productivity and quality of the product.

The major source of communication between the middle managers and the top-level managers is through informal channels, 50% of them oral. Other important means of communication are written memos, letters, meetings, display of information in the notice boards, or direct discussion. The horizontal communication mainly takes place through direct communication or through informal networks. Similarly, the downward communication originated from the middle level also takes place through direct communication or through informal channels. In fact the main communication link between the lower employees and the middle level are found to be the supervisors. They are found to be the linking pin between the lower level employees and the middle level managers.

Open communication with participatory type of management can definitely improve the commitment of the employees and improve the quality and productivity of the seafood factories. For this there is a need for change in the existing pattern of leadership styles, organizational structures and communication systems in all the factories.

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INLAND FISHERIES AND AQUACULTURE FOR FOOD SECURITY IN SRI LANKA

by

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ABSTRACT

Total food demand in Sri Lanka has been projected to rise by 1.2% annually between 1993 and 2010, largely as a result of population growth and changes in population structure. Significant levels of under–nutrition exist in Sri Lanka. According to recent estimates, 36% of the population show symptoms of stunting and 18% of wasting. Another 5% show concurrent symptoms. According to World Bank estimates, about 22.4 % of the population, that is nearly 3.8 million, were poor. Poverty is greatest in the rural sector, followed by the urban sector and the estate sector.

In Sri Lanka, the number of people directly engaged in fisheries is estimated at 120,000; most of them are relatively poor. Sri Lanka is producing 206,000 mt of fish from the marine sector and 22, 250 mt of fish from inland water bodies.

Fish and fisheries products are important to ensure food security of resource poor communities, Fish and fisheries products also provide much–valued foreign exchange. Earnings from total fisheries exports from Sri Lanka during the year 1996 were worth Rs. 4297 million.

More than 95% of the fish production in Sri Lanka is from capture fisheries, the rest is from aquaculture. The Sri Lankan Government has realized the importance of carefully managed exploitation of natural fish resources and has made rules and regulations to switch over from open access fisheries to community managed fisheries. While coastal fish resources are approaching their maximum sustainable exploitation limits, there is a considerable potential in Sri Lanka for expanding inland aquaculture in order to attain food security for the nation. Sri Lanka has 3 hectares of inland water for every square kilometer of land, perhaps the highest density of water for any island in the world. The total area of reservoirs in Sri Lanka will rise to over 250,000 hectares when construction of all the reservoirs has been completed as planned.

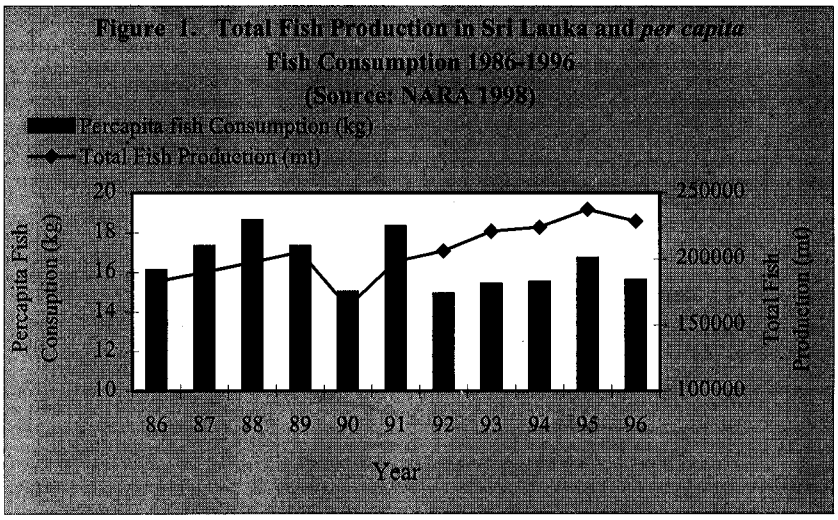
The introductions of exotic fish *Tilapia spp.* has increased the fish yields from 1 kg/ha to 227 kg/ha and has contributed towards establishment of capture based culture fisheries. Considering these trends, the Government policy is to concentrate on the culture-based capture fisheries in inland reservoir to ensure the food security of the poor communities.

INTRODUCTION

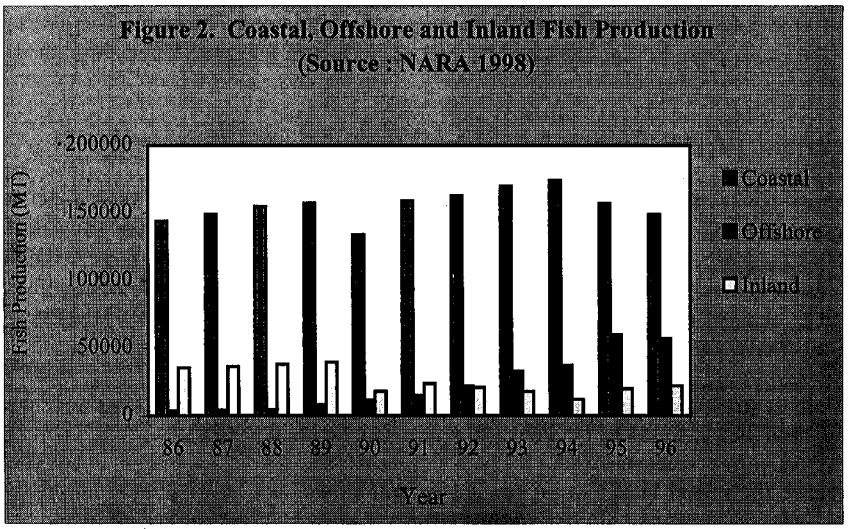
The food demand in Sri Lanka rose by 1.5% per year and an annual rise of 1.2% between 1993 and 2010 is projected. The population growth and changes in population structure is said to be responsible for this change (FAO, 1996). The rate of rise is greater in urban areas and is expected to be 3.3% annually till the year 2010. Analysis of households achieving different levels of calorie requirement show that about 30% of the households consume less than 90% of calorie requirements, while about 40% of households consume over 115% of calorie requirements.

Fish production in Sri Lanka

Coastal fish production has contributed between 65.3% and 78.8% during the period 1986 to 1996 to the total fish production while inland fish production has contributed only between 19.3% and 5.4% during the same period. Figure 1 gives the coastal, offshore and inland fish production during the period 1986 to 1996.

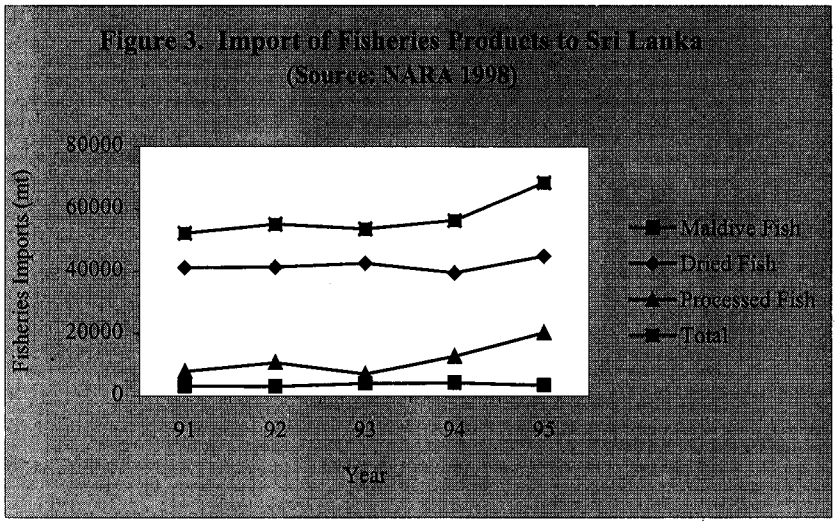


During this period coastal fish production has varied between 174 500 and 134 132 mt while inland fish production has fluctuated between 12 000 and 39 721 mt The drastic reduction in inland fisheries production from the year 1990 is a result of the withdrawal of state patronage for inland fisheries. The country's total fish production has not fluctuated very widely but has stabilized around 230 000 mt. during last few years (Figure 2).



Fish imports

To supplement the fish and fish based protein requirement, considerable quantities of Maldive fish, dried fish and preserved or processed fish are imported to Sri Lanka (Figure 3). During the year 1995, Sri Lanka imported 20 196 mt of preserved or processed fish, 44 798 mt of dried fish and 3371 mt of Maldive fish. The imported fisheries products also contribute significantly to the *per capita* fish consumption in Sri Lanka.



Fish consumption.

Around 56.5% of the total animal protein consumption comes from fish. During 1986 to 1996 period national *per capita* fish consumption has fluctuated between 18.6 and 14.9 kg, while it reached a value of 15.6 kg during 1996. (Fig 2). Although our *per capita* fish consumption is higher than that of India, Bangladesh and Indonesia it is lower than in the Maldives, Malaysia and Thailand. The average intake of fish protein in the rural areas can be increased by promoting inland fish production through culture based capture fisheries.

Food insecurity and rural poverty in Sri Lanka

Significant levels of under–nutrition exist in urban and rural areas of Sri Lanka; 36% of the population show symptoms of stunting and 18% of wasting (FAO 1996). Another 5% show concurrent symptoms. Nutritional status data by age group and sex show that the stunting affects males and females equally (36%), but more males show wasting (20%) than females (17%). Incidence of stunting in different age classes shows similarity among males and females, but females show higher rates of wasting than males in the early stages (FAO 1996).

According to World Bank estimates, about 22.4% of the population (3.8 million) were poor. Poverty is greatest in the rural sector, followed by the urban sector and the estate sector.

To enhance the inland food fish production several exotic species have been introduced to inland waters Three African cichlids, *Oreochromis mossambicus*, *O. niloticus* and *Tilapia rendalli* have successfully colonized inland waters and contribute significantly to the inland fish production. The common carp (*Cyprinnus carpio*) Chinese carps, *(Hupothalimichthys molitrix* and *Aristichthys nobilis*) and Indian carps, (*Catla catla, Labeo rohita* and *Cirrhinus mirigala*) grow well in our inland waters. There is evidence

that the trout, common carp and Indian carp (Rohu) are spawning in wild. There are records on the introduction of brackishwater indigenous species *Etroplus suratensis, Chanos chanos* and *Mugil* spp. to inland fresh water bodies.

Capture based culture fishery in inland waters

Sri Lanka's freshwater fisheries have historically not been considered to be of any economic importance. Only subsistence level fishing activities have been evident in both inland freshwater and brackishwater bodies. It was only during 1949/1950 that a concerted effort was made to utilize inland waters to increase fish production. The origin and the gradual development of the inland fishery which to date is essentially the reservoir capture fishery attributed to the introduction of *O. mossambicus,* an African cichlid fish. Within a few years of introduction, *O. mossambicus* had a tremendous impact on fish production, particularly from the larger irrigation reservoirs (Fig 4). By 1956, the introduction of *O. mossambicus* proved to be a tremendous success and small scale commercial fisheries had begun to operate.

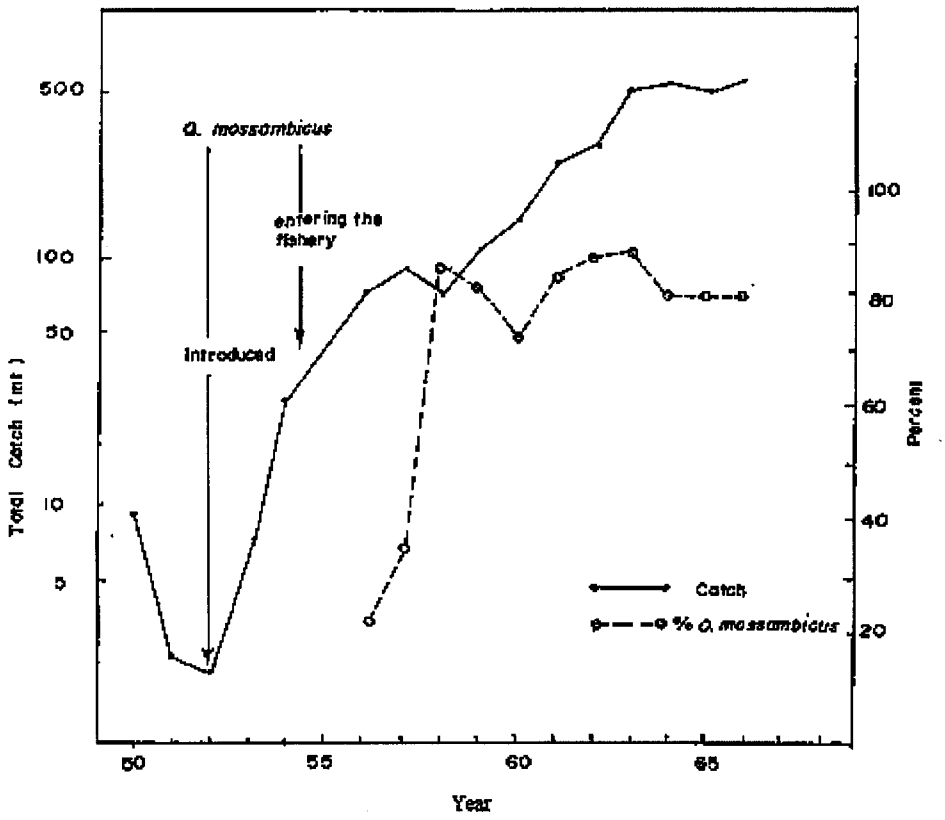


Fig. 4 . Impact of introduction of Tilapia on reservoir fishery

The annual fish yields before the introduction of *O. mossambicus* was around 1 kg/ ha in Parakrama Samudra. The average annual yield increased upto 227 kg/ ha since *O. mossambicus* dominated the fishery. The indigenous fish comprise a sizeable proportion of the catch consisting of herbivorous carps, carnivorous catfishes, snake heads and an indigenous cichlid, *Etroplus suratensis*. Most large shallow reservoirs have the same trends in production as Parakrama Samudra. Fish yields are low in deeper reservoirs like Senanayaka Samudra and in deep upcountry reservoirs with low temperatures, Tilapias have established themselves and are self-propagating. In highland reservoirs relative abundance of *O. Mossambicus* is the highest (30-58%), followed by *O. niloticus* (7-21%) and *T. rendalli* (0.65-3.0). Important factors that affect their colonization success include the food and nesting site availability.

Exotic carps have been introduced to these waters and there is evidence that common carp and rohu are self-propagating in upcountry reservoirs. Seasonal tanks are relatively smaller in size and they are dry for periods of 2-10 months of the year. These reservoirs can be used for culture based capture fisheries. Exotic fish in the reservoirs have potential to maintain natural self-sustaining populations if stocked in adequate numbers (Nathaniel and Silva, 1992).

The present estimated annual inland fisheries production is approximately 18 000 mt. This production is mainly based on capture fisheries of major and medium scale irrigation reservoirs. The highest production (39 700 mt) was recorded during the year 1989 before the withdrawal of state patronage for inland fisheries. According to the estimates of De Silva (1988) the annual fish production is around 307 kg/ha in reservoirs. Sri Lanka's reservoir fishery is mainly carried out by gill nets. The permissible mesh size is over 75 mm. In addition to gill netting, sporadic cast netting and angling takes place in most reservoirs. With the reestablishment of state patronage in 1994, a trend has been observed to increase inland fish production.

Utilization of seasonal reservoirs for culture based capture fishery was initiated in the latter part of the 1970's. High production levels (890-kg. ha^{-1}) can be achieved by polyculture of exotic carps. The combinations used included different percentages of catla/big head carp, rohu, grass carp, mrigal and common carp. Stocking densities recommended are 2 000 to 3 500 fingerlings ha^{-1} (Chandrasoma, 1996).

Government objectives, policies and programmes

The five-year development plan of the Ministry of Fisheries and Aquatic Resources has set the objectives and target for the development of the fisheries sector and aquaculture. There are clear set policies and strategies, and an investment plan to achieve these objectives and targets.

Recognizing the importance of aquaculture and inland fisheries to improve the nutritional status, and to reduce poverty by increasing, gainful employment and income opportunities, the Government of Sri Lanka has included the following development policies in its five year plan:

- Development of aquaculture and inland fisheries as a means of increasing domestic production, employment and nutrition.

- Development of a national programme for the exploitation of the fisheries and aquatic resources on sustainable bases.

- Provision of fiscal and other incentives for aquaculture.

- Implementation of appropriate scheme to provide welfare facilities to fishing communities.

The Ministry of Fisheries and Aquatic Resources will shortly establish an independent authority to be in charge of development, management, production and extension in inland fisheries and aquaculture. It will also be actively involved in transfer of aquaculture technology to the private sector, fish disease control, fish feed preparation, brood stock development and implementation of inland fisheries regulations (MOFAR, 1995).

The common property resources such as reservoirs will be managed in a more effective way by providing fishery rights to fishing communities to ensure greater participation by communities in managing their resources. Seasonal tanks which form an important resource for aquaculture development will be managed through established rural level organizations allotting rights for aquaculture in their tanks to members of such organization.

The new Fisheries Act through rules and regulations controls mesh size, environmental destruction, and fishing pressures on inland fish resources. The same act bans the use of destructive fishing methods.

Considering the resource availability and the present policies, programmes and strategies aquaculture and culture based capture fisheries will play a positive role in future, ensuring food security of the resource poor rural communities of Sri Lanka.

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