## SEMINAR PAPERS: SESSION II

## SMALL-SCALE AGAROPHYTE PROCESSING

## ASPECTS ON GRACILARIA

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## ABSTRACT

Various aspects of Gracilaria are presented and discussed in this paper. While Gracilaria culture is often viewed in terms of industrial use and economic benefit, one must not forget to look at their health value for human consumption. This applies also to other species of seaweed. As regards the agar content and gel strength in Gracilaria observations show that these characteristics are related to differences in habitat and environmental factors.

\* \* \*

In southern Chile I once met a producer who proudly-claimed that his agar was the best in the world. Why was his product different? He gave two possible reasons. His Gracilaria raw material was taken for processing on the very day of the harvest without any drying and it was collected on rocky tidal flats on a fairly open coast with rough sea, while most other Gracilaria beds were found in estuaries, lagoons or sheltered bays. In the literature we will find a number of other explanations for differences in quality.

With regard to the economy of Gracilaria production I made a surprising observation: On Isla Santa Maria far out in the Pacific, fishermen and hoys in wet suits collected considerable quantities of Gracilaria in the surf of an exposed sandy beach. In the sheltered Rio Maullin estuary, on the other hand, another natural growth was collected from small boats with eight-footed "spider" drags. Here the average harvest for a day's man-effort was ten times as high as in the Santa Maria Island — and the price was a tenth! Could it really be that the agar content and quality was so inferior as the price indicated? Or is the cold truth that the price of the raw material is set by the buyers at the lowest possible level where they can find people willing to work? That the price is determined not by value with regard to quality and quantity, but by harvest per man-effort and minimum living cost for a family?

Talking of economy, we get valuable principal information in an estimate of world seaweed production (Anon, 1985). For anybody teaching phycology it can be used to test the students in the art of reading a table. Here it follows with my own breakdown and additional calculations:

## Estimate of world seaweed oroduction

Location	10 <sup>3</sup> tonnes	Value	Value per Tonne
	net weight	U S \$ Million	US\$
Japan	654 }	563.0 }	861 }
China	700 } = 69%	130.0 } = 96.5%	186 } 467
Korea (Republic)	224 }	45.0 }	201 }
USA USSR UK Others	126 } 100 } 24 } = 31% 572 }	1.9 } 5.8 } 0.4 } = 3.5% 18.9 }	15 } 58 } 17 } 33 33 }

TOTAL WORLD PRODUCTION 2400 x 10<sup>3</sup> tonnes, 765 x 10<sup>6</sup> US \$

Far eastern seaweed landings are worth 14 times as much per tonne as those of the rest of the world. An analysis will touch upon all thinkable aspects of the seaweed economy; here I will stop—seaweed production for human consumption is worth more than production for industry. Any advice for developing countries should include this basic fact.

World statistics refer to fresh weight at harvest; the value for the fishermen. They should not be confused with export values of dried seaweed which may at the time have been about US \$800/tonne. Of course industrial end products will be sold at hundreds or even thousands of times more than the raw material, but processing proceeds will not reach the fishermen.

Import and export figures are not all. We are happy to live in a time when life quality is also included in our demands, in this case health aspects. Around the Bay of Bengal there are still areas with protein deficiencies, keratomalacia (Vit.A), beriberi (thiamine), pellagra (niacin). ariboflavinoses (B<sub>2</sub>) and scurvy (C).

Gracilaria is famous for its high protein content. Some of the species are known for their quantities of, among others, provitamin A, thiamine and niacin. However, if seaweeds should go where they are needed, they should all be sent to areas afflicted with severe endemic goitre (the Himalayas and other highlands). (Michanek, 1979, 1981). Coastal populations never suffer from goitre. and vitamin deficiencies are few.

The health impact of seaweed as a food additive does not rest with its content of proteins, vitamins, minerals and trace elements. There is increasing literature on the capacity of phycocolloids to bind heavy metals and thus to decrease our body burdens of lead, cadmium, mercury. arsenic and even radioactive strontium. If ongoing research shows that we can rinse lead out of our bodies by regular intake of seaweeds, there will be an immense increase in the demand, in particular for the populations living and dying in the traffic exhausts of our cities. For this purpose agar and carrageenan are not so good as alginic acid. This may open a new use for resources of Sagassum, Turbinaria, Cystoseira and Cystophyllum.

In the extensive literature on Gracilaria, many papers deal with the question I started with: quality of agar. Let me cite a sampling of recent papers with aspects worth discussing by cultivators

From molecule chemistry we first learn that much sulphate gives low gel strength., Second. we learn that a high curling intensity of the molecule chain gives a high gel strength and that this curling of rhe chains increases with increasing amount of 3,6- anhydrogalactose (Yaphe and Ducksworth, 1972).

For the cultivator this means that in tidal areas a Gracilaria growth which is exposed during long periods will produce galactans heavily charged with sulphates, as this radical plays a role in resistance against exposure. In subtidal specimens of the same transect, sulphates show partial absence and the molecules have a high portion of 3,6-anhydrogalactose (Bodard, et al., 1984).

Our third observation could be that 3,6-anhydrogalactose and consequently gel strength has a negative correlation to chlorophyll content - or in plain words. quality is not so good during sunshine periods (Liu, et al., 1981).

The genera Gelidium and Gracilaria are low in sulphate, while Chondrus, Gigartina and Porphyra are high (Bodard, Christiaen and Verdus, 1983). Within Gracilaria the species G. sjoestedtii has lower sulphate content and higher gel strength than G. tikvahiae, G. rextorii and G. verrucosa (Craigie et al., '1984). In a certain species different clones may have very great differences in agar composition and qualities. Strains with thin thalli have a more efficient uptake of nutrients than strains with thicker fronds (Lignell and Pedersen, 1989). Even different parts of the thallus show considerable variation (Craigie and Wen, 1984).

A fourth aspect is that, in general, gel strength is higher in agar from plants grown in nitrogen-rich water and under good light conditions, while the yield of agar is higher from plants grown under poor conditions (Craigie et al., 1984, Lignell, 1988). This may be explained by the fact that plants in nitrogen-rich water grow faster than those in nutrient-poor water and therefore have a larger portion of young tissue. In young tissue the proportion of small cortical cells to large medullary cells is high (Craigie and Wen, 1984).

Chemical observation number five is that gel strength of agar deteriorates markedly with increasing content of 4-0-methyl-L- galactose, which simply means that aged tissue is inferior to young tissue (Craigie and Wen, 1984, Cote and Hanisak. 1986, Lahaye and Yaphe, 1988).

From laboratory culture experiences we could note that Gracilaria is extremely sensitive to low concentrations of nitrogen (Edelstein et al. 1976).

Wang et al. (1984) observed that local plants doubled the fresh weight of their thalli in considerably less time than transplants.

What are the problems of seaweed culture? In one of the discussions at the Symposium of Useful Algae, which we know from the volume "Pacific Seaweed Aquaculture" (Abbot, Foster, and Eklund Eds. 1980), a participant stated that

- \* Problem no. 1 is grazing and overculture.
- \* Problem no. 2 is weeds and epiphytes.

The problem of epiphytes, when there is no grazing, was studied by Brawley and Fei (1978).

At the 13th International Seaweed Symposium in Vancouver last August there was a workshop on Engineering Aspects of Algal Cultivation, where various participants noted that:

- \* each time a culture system is scaled up, new problems arise
- \* production limits are probably set by the ability to agitate (circulate), rather than by self-shading.
- \* a given system can be made more economic by reducing the cost of production rather than by increasing productivity. (Applied Phycology, September 1989).

  For the farmer there are many problems which we see nothing of in the literature, e.g.:
- \* Is an upgrading of living material through nitrogen starving feasible?
- \* For a sea farmer, is the way to gain more only to produce more or would it pay better to sit down and do a lot of hand-rinsing of epiphytes, other species and plastic fragments or to rinse a clay-rich harvest from a muddy bottom in clean water of the open sea?
- \* How much is lost in quality if there is a transport delay between harvest and processing?
- \* Does the buyer ask for quantities only, while the factory primarily asks for quality?

#### References

ANON. (1985) Industrial Biotechnology Oct/Nov: 73

BODARD. M.. CHRISTIAEN. D. and STAEDLER. T. (1984). L'agar, phycolloide des algues rouges. Plantes et Alques en Cosmetologie et en Phytotherapie. Edited by Y. de Roeck-Holtzhauer, Nantes. 57-69

BODARD. M., CHRISTIAEN, D. and VERDUS M.V., (1983). Mise au point sur les phycocolloides, Bull. Soc. Bot. N. France, 36 (1-2): 1-14.

COTE. G.L. and HANISAK. M.D.. (1986). Production and properties of native **agars** from Gracilaria tikvahiae and other red algae. Bot. Mar. 29: 359-366.

CRAIGIE. J.S. and WEN. Z.C. (1984). Effects of temperature and tissue age on gel strength and composition of agar from Gracilaria tikvahiae (Rhodophyceae). Can J. Bot. 62: 1665-1670.

CRAIGIE. J.S.. WEN. Z.C. and VAN DER MEER J.P.. (1984). Interspecific intraspecific and nutritionally-determined variations in the composition of agars from Gracilaria spp. Bot. Mar. 27: 55-61

LAHAYE, M. and YAPHE. W. (1983). Effects of seasons on the chemical structure and gel strength of Gracilaria **pseudo-** verrucosa agar (Gracilariaceae, Rhodophyta). Carbohydr. Polym. 8: 285-301.

LIGNELL. A. (1988). Physiology and cultivation of marine seaweeds with emphasis on Gracilaria secundata (Rhodophyta. Gigartinales). Acta Umv. Ups., Comprehensive summaries of Uppsala dissertations from the Faculty of Science 120. Uppsala: 48 pp.

LIGNELL. A. and PEDERSEN. M. (1989)). Agar composition as a function of morphology and growth rate. Studies on some morphological strains of Gracilaria secundata and Gracilaria verrucosa (Rhodophyta) Bot. Mar. 32: 219-227.

LIU. C.Y.. WANG. C.Y. and YANG. S.S. (1981). Seasonal variation of the chlorophyll contents of Gracilaria cultivated in Taiwan. Proc. Int. Seaweed Symp. 10, Walter de Gruyter & Co. Berlin: 455-460.

MICHANEK. G. (1979). Seaweed resources for pharmaceutical use. In: Marine Algae in Pharmaceutical Science, de Gruyter. Berlin: 203-235.

MICHANEK. G. (19X1). Getting seaweed to where it is needed. Ceres: FAO. Rome: 41-44.

YAPHE. W. and DUCKWORTH. M. (1972). The relationship between structures and biological properties of agars. Proc. Int. Seaweed Symp. 7, Univ. of Tokyo Press: 15-22.

# SEMINAR PAPERS : SESSION II SMALL-SCALE AGAROPHYTE PROCESSING

## AGAROPHYTE HANDLING AND PROCESSING WITH SPECIAL EMPHASIS ON *GRACILARIA*

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## **ABSTRACT**

This paper reviews the technologies of agar extraction from agarophytes, with special emphasis on *Gracilaria*. The normal sequence of steps in handling and processing agarophytes are described as dehydration, pressing, evaluation, removal of undesired products, pre-treatment prior to extraction and the control of molecular weight during extraction. While small-scale cottage industry can feed the local market, it does not generally produce agar that meets international quality standards. Home industries may be developed for local demand. There is a lack of marketing information in the Bay of Bengal region.

#### 1. Introduction

**Gracilaria**spp. are agarophytes or agar-yielding red seaweeds belonging to the class Rhodophyceae. They give rise to a hydrophilic colloid. agar, which on processing is insoluble in cold water but soluble in hot water. Other agar-producing seaweeds are **Gelidiella, Gelidium**, and **Pterocladia.** 

"Agar-agar" is the original Malay word for a food gel obtained from seaweed. Chemically, agar consists of neutral agarose and charged agaropectin. Agarose is a long-chain polymer of neutral galactose and its derivatives, while the charged agaropectin chain has some sulphated substitutes.

A higher charged agaropectin chain leads to increased viscosity but reduced gel strength. Reducing sulphate content helps increase gel strength. According to Chandrkrachang and Chinadit (1988). the most important properties in determining the quality of agar are gel strength, gelling and melting temperature, sulphate and metoxyl content. clarity of the solution, and ash content.

Apart from seaweed powder and dried seaweed from which agar is extracted, other product forms are strip-agar, agar flakes, and agar powder. High-grade agar is white, while light yellow is acceptable for lower grades.

## 2. Handling and Processing

Armisen and Galatas (1987) provided an excellent review of the handling and processing methodology for agarophytes. This paper while drawing on their work, attempts to summarise the technologies used.

Preservation of seaweeds between the time of harvest and processing is very important to minimise spoilage, facilitate long distance transportation and extended periods of storage before processing. Before preservation, the seaweed is washed in sea or fresh water to remove adhering sand, mud, snails, barnacles or other foreign material.

Factors to be considered in agar processing:

The extract from the agarophyte has to contain the maximum possible quantity of agar from within the seaweed. In addition, the agar obtained should also have the best physico-chemical properties to satisfy the standards expected of the end-product. The normal sequence of steps in handling and processing agarophytes are dehydration, pressing, evaluation, removal of undesired products, pre-treatment prior to extraction and the control of molecular weight during extraction. There is a need to work with large volumes of dilute extracts. and to consider the economics of dehydrating.

## 2.1 Dehydration

Immediately after harvest, the seaweed is dried to a moisture content of less than 20%. Seaweed may be air-dried in the open, preferably off the ground, to keep it clean or dried artificially. Bamboo slats, plastic sheets on the ground, or concrete surfaces are used. Sun-drying also bleaches the seaweed. The seaweed must be sufficiently dry to prevent anaerobic fermentation resulting in spoilage or even carbonisation of the bales during storage.

The yield achieved for Gracilaria under experimental conditions in New Zealand is 40 tonnes of dried seaweed from 240-280 tonnes wet raw material. One tonne of the same dried Gracilaria yields 250-300 kg of agar (Hollings, 1985). In Taiwan, the drying ratio obtained is a similar 1:7 (Chen. 1978).

Preservation of Gracilaria by dehydration is difficult as enzymatic hydrolysis of the agar occurs even at relatively low moisture content. This rate is, however, variable depending on the species and its origin. Gracilaria harvested in Sri Lanka, India, Venezuela, Brazil and other warm waters has an agarose less resistant to enzymatic hydrolysis than the more stable Chilean Gracilaria. Agar in Gracilaria, which can undergo hydrolysis because of endogenous enzymes or the growth of Bacillus cereus, is less stable than that of Gelidium (Armisen & Galatas, 1987).

## 2.2 Pressing

The **second** step is pressing the weed in bales of about 60-100 kg with a hydraulic press, in order to reduce the volume and consequently transportation and/or storage costs.

The seaweed may then be packed into sacks, either for export or sale locally. Alternatively, it may be ground into powder or subjected to agar extraction at the small-scale processor level.

## 2.3 Evaluation

Because of variations in harvesting and processing methods, chemical properties and product utilisation, it is not possible to consider seaweed and, therefore, the seaweed industry, as a homogeneous entity. Agarophytes from different growing areas should be evaluated for their agaryielding properties before processing, so that their potential can be assessed.

**In** principle, evaluation of agar properties involves combining some preliminary treatments with different extraction methods. An important consideration is sampling from a large growing area and the proper treatment of dirt-free samples until they are ready to be processed. A sample size of about 400-500 g of dried seaweed should be used. Provided good results have been obtained, a pilot laboratory which follows closely the actual operations that would take place on an industrial scale should be set up.

Next, aliquots of seaweed representing a homogeneous composition should be taken, and tested for moisture determination, pure seaweed determination, and extraction of agar. Moisture determination is caried out in a drying oven at 65°C.

With Gruciluriu, a sulphate alkaline hydrolysis pre-treatment is usually carried out to change the L-galactose 6-sulphate into 3, 6-anhydro-l-galactose. This is usually done by diffusion with sodium hydroxide solution (0.1M) for one hour at a temperature of 80-97°C taking care not to extract the agar. The agar extraction which follows is carried out while stirring **the** slurry at neutral pH without pressure (95–100°C), for a period that varies depending on the type of Gracilaria. This can take several hours.

A manufacturer of good quality agar should be able to spot variations in yields in a laboratory trial before embarking on an industrial-scale production with a new batch of seaweed. The properties of seaweed from different areas vary greatly. This has resulted in the failure of many processing factories attempting to process batches of seaweed from different sources.

## 2.4 Removalof undesired products

In order to obtain the purest possible extract, seaweeds are generally hand selected and washed prior to alkaline treatment to eliminate a large quantity of foreign substances.

#### 2.5 Pre-treatment

A variety of pre-treatments are available. Treatment differs depending on source, growth stage, environmental conditions and species.

Pre-treatment should achieve maximum desulphation, while avoiding yield loss through agar dissolving in the solution. The disadvantage is that long-chain polymers may break (as discussed in the following section on "molecular weight control") leading to a reduction of gel strength.

An alternative technique to pre-treatment is *strong alkali post-treatment* at relatively low temperatures soon *after* agar extraction. Ry this means, relatively low-grade crude agar is refined to produce high-grade agar. Alkali post-treatment of traditionally extracted low-grade agar from *Gracifaria* has yielded a two-to-three-fold increase in gel strength, from 300-400 g/cm<sup>2</sup> to 700--1000 g/cm<sup>2</sup> (Chandrkrachang & Chinadit, 1988).

Shengyao et *al.*, 1988, studied the effects of alkali treatment on agars from Chinese *Gracifaria* species using a *cold concentrated alkali pre-treatment* in which *Gracilaria* is treated with 32% NaOH at room temperature for five days. Agars extracted with sodium hexametaphosphate *after* cold concentrated alkali treatment were much better than those extracted with water or any other alkali treatment. The gelling and melting temperatures of agar treated by alkali increased while the viscosity decreased.

The 3, 6-anhydro-1-galactose content of agar isolated from alkali-treated *Gracilaria* was higher than that without alkali treatment, and the reverse relation was observed about the sulphate and galactose content. The increase in gel strength lay in the degree of the sulphate reduction and the 3, 6-anhydro-1-galactose increased.

In another study on pre-treatment by Minghe, 1986, *Gracilaria* was *pre-treated with dilute alkali* prior to extraction at a temperature of 78-80°C for three hours. The yield and gel strength of the agar produced this way were higher than that of agar produced with the usual concentration of sodium hydroxide solution.

## 2.6 Molecular weight control

Within the seaweed, agar is insoluble in both warm and cold water. Extraction has to take place within certain pH, temperature, and redox conditions. This results in some hydrolysis taking place, thereby increasing its solubility.

During this fractionation, it is necessary to minimise molecular weight reduction. Since all agar extraction works on the principle that agar dissolves in hot but not cold water, excessive molecular weight reduction would cause loss in yields for low-temperature water soluble agar. On the other hand, all molecules that have weights at the higher extreme will not be extracted and will remain in the seaweed.

Thus, a manufacturer has to develop a means of obtaining maximum yield of particle size in the mid-range. This translates mto a high gel strength with more uniformity in particle size and reduced losses at the extremes. It is difficult to modify the percentage of molecular weights dissolving below 20°C but the extraction of molecular weights dissolving above 125°C can be increased by raising the water temperature under pressure whenever the seaweed permits.

To further strengthen the agar product the acid concentration should be adjusted at pH 2-2.5, and for increasing productivity pH 4.0 – 5.0 was best. A pH of 3.5 is ideal, resulting in an agar yield of 21.6% and 650 g/cm2 gel strength (Longchang, *et al.*, 1986).

## 2.7 Dehydration of dilute extracts

Agar extracts of 0.8-I 5% are considered to be the optimal concentration for subsequent dehydration. The more the agar extracted, the larger the quantity of water required. Generally factories working with *Gracifaria* have a higher water consumption than those using other raw materials. Water consumption also increases when better quality agar is required.

Working with a 1% solution, 99 litres of water have to be eliminated to produce | kg of agar. *Evaporation* or *precipitation* is recommended where freezing/thawing is not practicable.

**Freezing** and thawing the extract is employed both at industrial and cold country household level. Industrial freezing should be slow, to maximise both the growth of ice crystals and the separation of agar. This is usually followed by draining with a water-extracting centrifuge.

This freezing-thawing method. known as syneresis, followed by washing the gel matrix, also helps to purify the gel. Sulphated galactan portions of agar, salts, pigments and other organic compounds are soluble in the thaw water, and are partially removed from the insoluble agarose by filtration.

Factories which use syneresis usually have high water consumption. Some **Gracilaria** agars form soft gels which on washing disintegrate into fine particles. Similarly, low-temperature soluble agarose is lost when washing some agars. Thus, washing the gel matrix after the freeze-thaw cycle can only be performed with high gel strength agars or when alkaline pre-treatment is employed (Yaphe, 1984).

## 3. Small-Scale Agar Manufacture

Small-scale agar manufacturers with basic technology do not generally produce agar that meets international quality standards. There is little or no scientific control over processing, bacteriological contamination is usually too high, and prices offered too low. Consumption is usually local.

Seaweed is washed and then boiled for agar extraction in water and, possibly, sulphuric acid for about four hours. Washing facilities differ from factory to factory.

To obtain a clear agar solution after extraction, some processors adopt pressure filters, or use high speed centrifugal machines; others depend on sedimentation. After filtration, the hot extract is poured into wooden boxes to gel. The gel is removed from the boxes, sliced into strips, spread on mats and exposed to freezing cold weather to dry.

In order to produce agar strips, some use air-freezing rooms; others make use of brine tanks in small ice-making plants to freeze the agar gel strips inside ice moulds.

The processing of agar sheets is simpler than that of other products. it may be made in large or small plants. To produce agar powder from dried Gracilaria, the processing technology is similar until the freezing stage. The frozen blocks of agar are then crushed, and the pieces washed to remove impurities. When the crushed pieces are melted, the agar is bleached and washed again before dehydration by low speed centrifuge. It is then dried to a moisture content of 20%. The pieces of dried agar are again crushed mechanically into powder and packed in polythene in quantities ranging from 100 g to 10 kg (Chen, 1978).

A simple cottage industry agar manufacturing process is common in the Philippines. Seaweeds (Gracilaria verrucosa, G. eucheumoides, Gelidium sp. and Eucheuma sp.) are washed with fresh water, sun-dried, and then re-soaked for 5-10 minutes. They are dried again until yellow, and then bleached in dilute vinegar until the colour turns olive green. They are then further dried until they become light brown.

Extraction is done by boiling in vinegar or even sulphuric acid with constant stirring. It is strained in cheese-cloth while still hot and cooled to gel at room temperature. Once set, the agar is cut into bars or strips, sprinkled with salt and ice, and frozen. It is then thawed and dried at room temperature or under the sun, after which it is ready for the local market (Guzman & Guiang, 1987).

## 4. Comment

It appears that the seaweed industry in the Bay of Bengal region suffers from a lack of marketing information and a need to improve technical know-how on processing. The establishment of processing facilities within the region would help stabilise the industry. The large exports of dried agar-yielding seaweeds, and the exports of high-valued agar products from importing countries such as Japan, should further encourage agar processing. Home industries should be developed with an eye towards product development, and also to suit the local demand.

## References

ARMISEN. R. & GALATAS. F. (1987). Production. properties and uses of agar in production and utilisation of products from commercial seaweeds. edited by D J McHugh. FAO Fisheries Technical Paper (28X): 189 pages.

- CHANDRKRACHANG. S & CHINADIT. CJ. (1988). Seaweed production and processing a new approach. INFOFISH International No.4/88: 22-25.
- CHEN, G.C. (1978). Agar-agar manufacturing. Specialist. Fisheries Division. JCRR. Taipei. Taiwan (Publisher unknown): 56-67.
- GUZMAN de. D.L & GUIANG. G.S. (1987). Making gelatin from seaweeds. Aqua Farm News. SEAFDEC Aquaculture Department. Vol. V. No: 10. November-December: 13.
- HOLLINGS. T. (1985). Gracilaria economics. New Zealand Fishing Industry Board Newsletter. November/December: I 1.
- LONGCHANG. W.. PING. F. & CHANGSHUN. Z. (1986). The technology of acidifying and bleaching in processing of Gracilaria agar. Journal of Fisheries of China. 1985-1986: 23.
- MINGHE. W. (1986). Studies on a new method in production of Gracilaria agar with dilute alkali treatment. Journal of Fisheries of China: 52.
- SHENGYAO. S. YANXIA. Z., XIAO. F.. ZHI'EN. L. & WANGQUNG. L (1988). The effectsof alkali-treatment on agars from Chinese species of Gracilaria. Journal of Fisheries of China, 12 (2): 156-155.
- YAPHE. W. (1984). Chemistry of agars and carrageenans. Hydra Biologica 116/1 17. 186: 171-174

## SEMINAR PAPERS: SESSION II

## SMALL-SCALE AGAROPHYTE PROCESSING

## PRODUCTION OF AGAR FROM SEAWEED WITH SPECIAL REFERENCE TO INDIA by J J W Coppen

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## **ABSTRACT**

Agar production in India is about 75 tonnes annually, and takes place mostly in Tamil Nadu. Species from the genera *Gelidium, Gelidiella* and *Graciluria* are utilized for agar production. Two grades of agar are manufactured in India: food grade and IP grade (Indian Pharmacopoeia standards). The common processing is as follows: acid treatment, hot water extraction, freeze-thaw cycle, bleaching and sun-drying. Plant capacity ranges from 2 to 60 kg agar per day.

## World production

Marine algae provide a rich and diverse source of raw material for the production of seaweed gums, polysaccharides that find wide application in the food, pharmaceutical and industrial sectors. The three most important polysaccharides, in terms of volume and value, are sodium alginate (and its derivatives), carrageenan and agar. A recent estimate (Anon. 1988) has put annual world production of agar at between 7,000 and 10,000 tonnes. The production of carrageenan and alginates, by comparison, is approximately two and three times as great, respectively.

Seaweed gums: Annual world production (tonnes)

Agar	7,000-10,000
Carrageenan	12,000-15,000
Alginates	22,000-25,000

According to Armisen and Galatas (1987), of around 7,000 tonnes of agar produced in 1984, approximately half came from *Gracilaria*, the remainder coming mainly from *Gelidium*. The breakdown by country was as follows:

Agar: World production, 1984 (tonnes)

115	world produc	tion, 1501 (tonne	25)
Japan	2,440	Taiwan	275
Spain	890	Argentina	200
Chile	820	Indonesia	150
S. Korea	600	China	140
Morocco	550	Others	300
Portugal	320	TOTAL	6,685

## Indian production

Observations in India and discussions with all but a few producers, indicate that current agar production is about 75 tonnes annually. If the expansion of output planned by several companies is realised, and the commissioning of other factories now under construction also comes about, total production could almost double within the next few years to around 140 tonnes per annum.

Collection of seaweed destined for agar production, as also that of Sargassum and Turbinaria for production of alginates, is confined at present to the southern part of the Tamil Nadu coastline, between Cape Comorin in the south and the peninsula that stretches out towards Sri Lanka and forms the Gulf of Mannar.

Species of the genera Gracilaria. Gelidium and Gelidiella are utilised for agar production\*.

<sup>\*</sup> The terms Gelidium ανδ Gelidiella are often used interchangeably within the Indian agar industry. In practice, of the two general it is probable that Gelidiella is the main one utilized.

Indian agar: species of seaweed used

Gracilaria	Gelidium	Gelidiella	
G. edulis <sup>(1).</sup> G. verrucosa <sup>(2)</sup> G. crassa G. corticata G. multipartita <sup>(3)</sup>	Gelidium spp.	G. acerosa	

Notes: (1) Syn. G. lichenoides (2) Syn. G. confervoides

(3) Syn. G. foliifera

Of the Gracilaria species, **G.** edulis, collected from the waters off the mainland coast and those surrounding the off-shore islands, is the principal agarophyte. G. *verrucosa*, found in less salty estuarine areas, is used by a few agar producers. The other **Gracilaria** species are collected more by accident than design and do not form any significant part of the raw material utilised.

After landing the seaweed, the collector sells his haul to an agent who then dries it and sells it to the processor. A small agent may subsequently sell it to a larger one. Seaweed prices paid both by the agent to the collector and by the processor to the agent reflect the higher quality of the agar obtained from *Gelidium* and *Gelidiella* compared to that from *Gracilaria*.

Indian agar: seaweed prices

Price paid (Rs	Price paid (Rs./tonne)		
Agent to collector(')	Processor to agent(2)		
1800	5,000-8,000 2,500-3,500		
	Agent to collector(')		

Notes: (1) Wet weight (2) Dry weight

Unlike *Gracilaria* from other sources, Indian material appears not to respond to alkali treatment as a means of increasing gel strength. Agar derived from Indian *Gracilaria* typically has a gel strength in the range 100-150 g/cm2, while that from *Gelidium* or *Gelidiella* is around 300 g/cm2.

Two types or grades of agar are manufactured in India: food grade, which is usually produced in mat form, and IP grade, which conforms to Indian pharmacopoeia standards and is usually sold in powdered form. For food use, paleness of colour is invariably considered more important than gel strength, and *Gracilariu*alone (which is cheaper and easier to bleach than *Gelidium*or Gelidiella) or a mixture of *Grucilaria*with *Gelidium*is commonly employed as the raw material. In a few cases, or where high gel strength is required, *Gelidium*alone is used to produce food grade agar, often in the form of shreds or so-called 'individuals' (strands of larger dimensions than shreds). IP grade agar is produced wholly or mainly from *Gelidium*or Gelidiella.

Prices of the agar also, of course, reflect the raw material used and, for IP grade, the more stringent processing requirements. Within the Muslim community, demand for agar, and therefore also its price, is high during the Ramzan season.

Indian agar: prices for agar obtained by producer

Grade	Туре	Price (Rs./kg)
Food	Mat	140-160 180-210 <sup>(l)</sup>
IP	Shreds/'Individuals' Powder	200-300 <sup>(2)</sup> 270-280 300-400 <sup>(3)</sup>

Notes: (1) During Ramzan season

- (2) Prices quoted by a few producers
- (3) One producer quoted Rs.600/kg

The factories that produce the agar are located for the most part in Tamil Nadu, in close proximity to their raw material source. A few, however, are more distant from the seaweed belt, in Kerala and Andhra Pradesh.

The term 'factory' is used in the broadest sense to cover everything from the smallest family unit producing 2 kg of agar per day to the larger units producing 60 kg. Recently constructed factories plan to produce up to 100 kg/day.

Indian agar:production levels

Scale	Production		
	kg/day	tonnes/year	
Large	up to 60	up to 25	
Medium	10-40	2-10	
Small	2-4	0.5-1	

Almost without exception in India, the same basic method of processing is followed by all producers: acid treatment of the cleaned seaweed followed by hot water extraction; primary dewatering and purification of the agar by means of a freeze-thaw cycle; bleaching; and sun drying. Some adjustment is made according to whether Gracilaria or Gelidiella is being processed or whether it is G. edulis or G. verrucosa.

Initial handling and cleaning of the seaweed involves laying it out in the sun to dry and to bleach, removing epiphytes and other foreign matter by hand, and washing or soaking the weed several times in water. The seaweed may or may not be dried in between washes.

Acid treatment, to soften the weed in preparation for extraction, is accomplished by immersing it in cement tanks containing dilute hydrochloric acid for periods from 10 to 30 minutes, depending upon the species of seaweed.

After washing the seaweed free of acid, it is boiled in water at normal pressure without the addition of chemicals. Small units may employ direct heat by wood fire to boil the water; otherwise it is done by the use of steam. The extraction vessel may be fabricated of wood, aluminium or stainless steel. The length of time needed to extract 'the agar is dependent on the quality and nature of the raw material, but is usually somewhere between 1.5 and 3 hours. Gelidiella requires a somewhat longer time than Gracilaria. Yields of agar are around 10%.

After filtering, the extract is run into aluminium trays and allowed to gel. The trays are then transferred to a freezer, where they are kept, usually for 20 to 24 hours but sometimes longer. After removal from the freezer and thawing/draining, the crude agar gel is washed and then bleached by immersing briefly in hypochlorite solution. After washing again, the gel is laid out on mesh screens in the sun to dry. For IP grade agar, particular care is taken during handling and drying to avoid contamination by specks of dirt and other foreign matter. The sun-dried agar may be further dried in a hot-air drier.

## References

ANON. (1988). Seaweed. Globefish Highlights. 3,29-33, FAO, Rome

ARMISEN, R. and GALATAS. F. (1987). Production, properties and uses of agar. From: Production and Utilisation of Products from Commercial Seaweeds, FAO Fisheries Technical Paper 288. FAO. Rome. 1-57

## SEMINAR PAPERS: SESSION II SMALL-SCALE AGAROPHYTE PROCESSING

## PROSPECTS OF AGAR INDUSTRY IN INDIA

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#### **ABSTRACT**

This paper summarizes the status of previous and present work on utilization of agarophytes for agar extraction in India. Present national agar production does not meet demand. Standards are given for food and pharmaceutical grades. Physical properties of 1.5% agar are tabulated for *Gelidiella acerosa, Gracilaria edulis* and G. verrucosa. Yields range from 12 to 55% and gel strength varies between IS and 300 g/cm<sup>2</sup> depending on species.

\* \* \*

Utilisation of seaweeds in India for the extraction of soda ash, alginate and iodine started during the II World War. Although the importance of seaweeds was realised during this period, the production of agar did not start until the 1960's. The export of seaweeds continued until 1975 when, in order to meet the requirements of the local agar industry, the Government of India banned the export of seaweeds. However, it should be noted that the local industry does not produce sufficient agar to satisfy the ever increasing domestic demand. Consequently, India imports a large quantity of agar.

The important agarophytes of India are *Gelidiella acerosa*, *Gracilaria edulis*, *Gracilariarassa*, *Gracilariacorticata* and *Gracilariafolifera*. The seaweeds used for commercial extraction of agar are *Gelidiella acerosa*, *Gracilaria edulis* and G. crassa..

**Gracilaria**edulis is widely exploited for industrial utilisation in India. Collection is possible throughout the year around the islands in the Gulf of Mannar, Tamil Nadu. The, **Gracilaria**collected invariably contains various other plants. Fresh seaweed is sold at the rate of Rs.0.50/kg. When dried, the yield is 15% of the fresh material Commercial dried material is 60% pure, with a moisture content of 22%.

Since **1983**, **Gracilaria**crassa has been collected from Pampan, Vedalai and Kilakarai, Tamil Nadu. G. crassa grows in shallow areas attached to pebbles and stones; collection is done by hand picking. Only negligible quantities are harvested, and then only when there is no collection of G. **edulis**. It fetches a price of Rs. 1,000 per tonne (dry weight).

Agar is a complex mixture of polysaccharides obtained from certain species of red algae. It is mainly a mixture of two polysaccharides, agarose and agaropectin. Humm (1951) and Yaphe (1959) have defined agar as a gel-forming substance soluble in hot water and requiring a 1% solution to set as a gel on cooling.

The yield of agar and its gel strength vary from species to species and also on the method of extraction. Processing conditions play a significant role in the quality of the end product.

In India, Bose *et al* (1943) soaked dried seaweeds in water for 18 hours prior to treatment with acetic acid. Chakiaborty (1945) did the same, but used the process of freezing and thawing for purifying the agar gel, and used activated carbon to decolourise the gel. Karunakar *et al* (1948). and Joseph and Mahadevan (1948) purified the gel by soaking it in water of low salt content for 96 hours and finally washing under pressure. After freezing, the gel was dried with acetone. Thivy (19.51) soaked the seaweeds for 24 hours prior to extraction. Kappanna & Rao (1963) studied the method of Thivy, and found that soaking and extraction under pressure reduced the quality of agar gel. Srinivasan and Santhanaraj (1965) washed the seaweeds in sea water and then in fresh water several times, and dried them in the sun until they were completely bleached. Extraction was carried out by boiling the seaweeds at pH 6 and the extract was finally filtered and frozen.

A cottage industry method for agar extraction from *Gracilaria edulis*was worked out by Thivy (1958). Umamaheswara Rao (1970) has given a comprehensive account of aspects of Indian

seaweeds and their utilisation. Much work has been reported on the chemistry of Indian seaweeds (Pillai, 1955a. 1955b). A comparative study was made by Chennubhotla et al (1977) on the yield and physical properties of agar from different agarophytes. The results are given in Table 1.

Table 1: Physical properties of 1.5% agar in water

Species	Yield %	Gel strength (g/cm <sup>2</sup> )	Setting temp °C	Melting temp
Gelidiclla acerosa	40	125	46	73
Gracilaria edu1is	55	<i>63</i>	48	65
G. verrucosa	23	41	40	55

Various authors have reported the yield and physical properties of agar obtained from Gelidiella and Gracilaria species (Table 2).

Table 2

Agarophyte	Yield %	Gel strength (g/cm <sup>2</sup> )	Setting Temp °C	Melting Temp.
Gelidiella acerosa	45	300	40	92
Gracilaria lichenoides	<i>33</i>	120	45	84
G crass a	23	140	48	84
G. corticata	38	20	44	68
G. folifera	12	15	40	

<sup>\*</sup> Gel strength of 1 5% concentration agar in water at  $28 \pm 2^{\circ}$ C

In 1970, the Bureau of Indian Standards laid down specifications for food grade agar, an extract of which is given in Table 3.

Table 3: Specification for food grade agar

Characteristics	Requirements
Colour	White or pale yellow
Odour	Odourless
Taste	Mucilaginous
Solubitity	Soluble in boiling water
Moisture, after drying at 105°C for 5 hours	20.0%
Total ash by weight, maximum	6.5%
Acid insoluble ash by weight, maximum	0.1%
Insoluble matter by weight, maximum	1.0%
Arsenic (as As) maximum	3.0 mg/kg
Lead (as Pb), maximum	1.0 mg/kg

The use of agar as a water-soluble thickening, emulsifying and gelling agent has become established worldwide in industries ranging from foods, pharmaceuticals, cosmetics, paper, textiles, petroleum, and to the new industry called biotechnology (Glicksman, 1986). In India, agar is used as a medium for tissue culture of ornamental and other plants. It is also used in bacteriological laboratories as culture media. It is used as a stiffening agent in a number of food products, as a sizing material, as mucilage, and in clarifying beverages. It is employed in canning meats, in laxative preparations, as a constituent in medical pills and capsules, in dental impression moulds, and as a lubricant for drawing tungsten in electric bulbs. It is also incorporated into the formulation of silk worm food.

Much remains to be done on the technological aspects of commercial production of phycocolloids from seaweeds. India has a vast seaweed resource which at present is not fully utilised. Concerted efforts, on a national level, are needed for the proper utilisation of available resources. Agar extraction can also be done on a small industrial scale, providing employment opportunities to a number of people. Fresh water availability and sufficiently low night temperature to freeze the agar blocks suggest places like the Nilgiris and other high ranges as ideal locations for such factories. This will, however, involve transportation of raw materials from the coast. Alternatively, freezing facilities will have to be provided in coastal areas. The relative economics of these two possibilities remains to be studied.

As the food and bio-technology industries gain momentum in India, the prospects. for producing various grades of agar from seaweeds are bright. A detailed study on the technology of production is essential in order to make production economical and to make the technical knowhow available to new entrepreneurs entering the field. An in-depth study to locate new raw material sources is also required. India needs a large number of viable small industries to provide subsidiary employment for the local fisherfolk.

#### References

- BOSE, J.L. KARIMULLAH and SIDDIQUE. S. (1943). Manufacture of agar in India. J. Sci. Indus. Res. (India) 1.
- CHAKRABORTY. D. (1945). Agar-Agar manufacture from Gracilaria conferroides. J. Proc. Inst. Chem (India). 17.
- CHENNUBHOTLA. V.S.K.. NAJMUDDIN. M. and BIDYADHAR. N. (1977). A comparative study of the yield and physical properties of agar-agar from different blends of seaweeds. Seaweed Res. Util. 2 (2): 87-90
- GLICKSMAN M. (1986). Utilisation of seaweed in hydrocolloids in the food industry. 12th International Seaweed Symposium edited by M.A. Ragan and C.J. Baird. Netherlands. 31-47.
- HUMM. H.J. (1951) The red algae of economic importance agar and related phycocolloids. Marine Products of Commerce. Ed. Tressler. D.K.. New York.
- JOSEPH. I and MAHADEVAN, S. (1948). Production of agaragar. Dept Res. Unit. Travancore Rep. 55-60
- KAPPANA. A.N. and RAO. A.V. (1963). Preparation and properties of agar-agar from Indian seaweeds. Ind. J. Technol. 1. 222-224.
- KARUNAKAR. P.D.. RAJU. M.S. and VARADARAJAN. S. (1948). Manufacture of agar-agar from seaweed Gracilaria lichenoides. Ind. Vet. J. 24. 274-282.
- PILLAI. V.K. (1955a). Utilisation of natural byproducts for the cultivation of hlue green algae. Curr. Sci. 24. 21.
- PILLAI. V.K. (1955b). Water soluble constituents of Gracilaria lichenoides. J. Sci. Ind. Rcs. (India). 14B. 4731177.
- SRINIVASAN. R. and SANTHANARAJ. T. (1965). Studies on the extraction and properties of apar-agar from seawed, Gracilaria species. in Madras State. Madras J. Fish. 3. 146-151.
- THIVY. F. (1951). Investigation of seaweed products in India with a note on properties of various Indian agars. Proc. Indo-Pacific. Council Sec. 2. 173-175
- THIVY. F. (1958). Economic seaweeds. In Fisheries of west coast of India, Bangalore. 74-80
- UMAMAHESWARA RAO. M. (1970). The economic seaweeds of India. Bulletin 20. CMFRI Cochin. 1-68
- YAPHE. W. (1959). The determination of carrageenan as a factor in the classification of Rhodtrphycae. Can. J. Rot. 37. 351-357.

## SEMINAR PAPERS: SESSION II

SMALL-SCALE AGAROPHYTE PROCESSING

## AGAR PRODUCTION ADAPTED TO RURAL AREAS

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## **ABSTRACT**

A programme has been set up to stimulate seaweed production and processing in Thailand. In this connection, the Biopolymer Research Institute has developed a simplified method of agar extraction from many types of *Gracilaria* and *Polycavernosa* species available in the coastline of Thailand. By utilizing ordinary kitchen utensils in agar processing, it has been possible for the Institute to transfer this simple technology to rural villages in coastal areas. Two processes are proposed: the first is to produce crude agar strips and local agar desserts'; the second is a two-tier system under which crude agar strips are either used for orchid tissue culture or further refined in a processing plant. Increased interest in seaweed collection, culture and processing by rural folk may lead to expanded production and the setting up of modern processing plants to produce high quality agar.

## Introduction

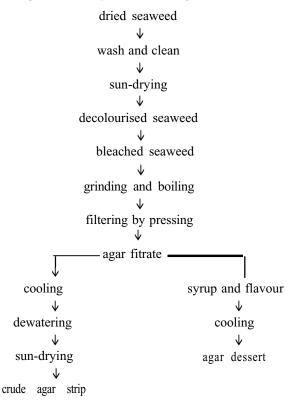
Agar-bearing seaweeds, agarophytes, are naturally abundant along more than 2,600 kilometres of Thailand's coastline. Production of natural stock is rather seasonal along the Gulf of Thailand and less so along the Andaman Sea coast. The cage culture of marine fish has become a wellestablished industry, and agarophytes are appearing as fouling organisms attached to the net cages. These potentially valuable resources remain unexploited in most areas. However, Thailand exports raw seaweeds to developed countries and imports processed agar in increasing quantities every year. As a result, Thailand faces a trade deficit in agar. To reduce the country's dependence on agar imports, the Royal Thai Government recently started a vigorous drive to produce and process seaweed. It focussed on stimulating private investment in seaweed processing for the local manufacture and marketing of agar, while at the same time creating employment for the impoverished coastal population. Within the framework of the programme, the Biopolymer Research Unit (BRU) was established and equipped to work on the identification of seaweeds and the analysis and process development of phycocolloids in Thailand. As a result of long-term fundamental research, the BRU has developed a simplified method of agar extraction from many types of Gracilaria and Polycavernosa species in Thailand. This simple technique is suitable for introducing small-scale crude agar production to rural areas. These can be used locally in food and tissue culture. The crude agar produced in rural areas can be collected and further refined with more sophisticated techniques in a central process&g plant to produce high-grade agar for different applications. This appropriate technology can be transferred to the rural people to make them aware of the wealthy seaweed resource on their doorstep.

## Materials and methods

Agar-bearing seaweeds were collected from different locations in Thailand and identified by Dr I Abbot of the Department of Botany, University of Hawaii. Some former members of the genus *Gracilaria*have been moved to the genus *Polycavernosa*. The natural stock of seaweeds collected from Songkhla Bay were identified as *Polycavernosa fishery*. The agarophytes growing attached to the net of marine fish cages along the canal of Trung were *Polycavernosa* changii while the samples collected from pond culture in Pattani were identified *as Gracilaria tenuistipitata*.

The collected seaweeds were placed on a clean surface and sun-dried for 2-3 days. Dry agar-bearing seaweeds are rather dark in colour and can be kept for a long time before extraction. The procedure starts with cleaning the seaweed by washing with fresh water 2-3 times and then soaking for 2-3 hours. The cleaner the seaweeds, the cleaner will be the agar produced. After cleaning it is dried in the sun; the colour becomes paler, usually yellowish or light-brown. Before extraction, the dry and clean seaweeds are soaked in water in a ratio of 1:20 by weight for 2-3 hours until full hydration is reached. When the seaweed is completely swollen, the mixture is gently boiled in a water bath for 45 minutes. The seaweed is then ground using a traditional stone mortar or an electrical blender. The slurry is boiled gently for another half an hour. It is then poured into a simple hand-operated press which is lined with a two layered cloth bag, and which has been preheated by rinsing with hot water. This simple press is a traditional local design used for extracting oil from coconut. After pouring the seaweed slurry into the press, the cloth bag is firmly tied and pressure is quickly applied. The filtrate flows into the tray, and the residue in the bag can be used as fertiliser. At this stage, desserts can be made by adding syrup and flavours. The filtrate is left to cool to allow the agar gel to set. The agar gel is put into a freezer for two days. The tray is then thawed at room temperature and the liquid is removed. The agar residue is sun-dried and stored for further use. If no freezer is available, the agar gel is put into a thick cloth bag and gently squeezed, either in the press or by putting a heavy stone on top of the bag overnight. The damp agar residue is then sun-dried and stored. A summary flow chart of this simple agar extraction is shown in Figure 1.

Figure 1: A simple method of agar extraction



A mobile team of BRU staff arranged the transfer of this simple technology to the rural villages of Songkhla, Satul and Trung provinces during April 1989. Ordinary kitchen tools--cooking pot, blender, gas stove and coconut press there-were put into a compact package which could be adapted for mobile training facilities in three different rural areas during a 5-day operation. More than 100 local villagers participated in the seaweed production and processing training programme. Extension aids comprising video tapes, slides, leaflets and booklets were distributed.

## Results and recommendations.

The crude agar extracted by this simple method is rather soft, but it can be used for local food. If a stronger gel is needed, more agar can be added. The mobile training programme appeared to be a

great success and beneficial to the local population who responded well. Requests for more training were received from leaders of local communities. The crude agar produced in the rural areas can be used locally, or else collected for further refining to produce high-grade agar. The scheme of such a two-tiered production system is shown in Figure 2.

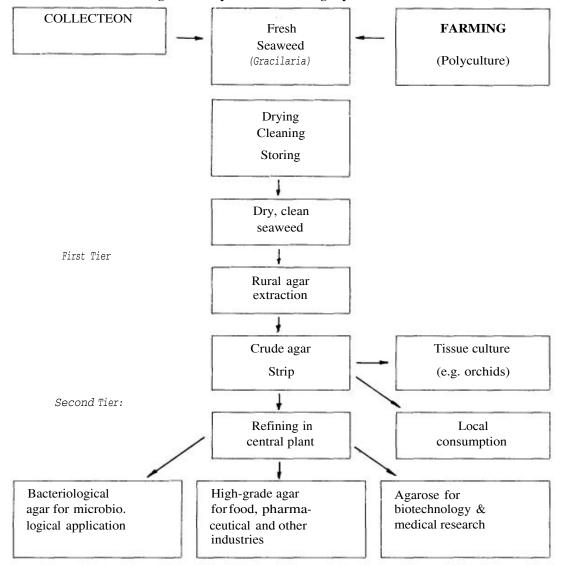


Figure 2: Proposed two-tiered agar production scheme

The planned production of crude agar in the rural areas will allow some of the benefits of seaweed processing to be channelled to the usually impoverished coastal population. By raising their interest in seaweed collection, culture and processing, the raw material requirements of a central commercial agar reprocessing plant will be established.

## References

CHANDRKRACHANG, S. and CHINADIT. U., (1988). Seaweed Production and Processing A New Approach. INFOFISH International No: 4/88. 50628 Kuala Lumpur, Malaysia. pp 22—25.

CHINADIT, U. and CHANDRKRACHANG, S. (1986). Simplified method for agar extraction from some agarophytes in Thailand. Bull Phamacognosy Soc. Thailand, 2, pp 11—13.

EDWARDS. P. (1977). Seaweed Farms: an integral part of rural development in Asia, with special reference to Thailand. Proc. Int. Conf. on Rural Dev. Technol: An Integrated approach, Asian Inst. Technol., Bangkok: 665—680.

# SEMINAR PAPERS: SESSION II SMALL-SCALE AGAROPHYTE PROCESSING

## EXTRACTION OF AGAR FROM *GRACILARIA EDULIS* AS A VILLAGE LEVEL TECHNOLOGY - PRELIMINARY RESULTS

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## ABSTRACT

A simple method of agar extraction, which has been developed in Thailand, may be suitable for adaptation as a village level technology in fishing villages in Tamil Nadu, India. Preliminary results from trials using the method indicated an agar yield of 12.0 - 24.5% depending on the heating time and the method of heating. Suggestions for further research are discussed.

## Introduction

Seaweed collection is an important source of income in fishing villages along the coast of Ramanathapuram district in Tamil Nadu, India. Agarophytes such as Gracilaria spp. and Gelidiella spp. are collected through most of the year by men and women. The seaweed is sold ashore for a few rupees per kilogramme to local agents who, in turn, dry the seaweed before selling it to processing factories for agar extraction. Agar is sold within India and is used mainly in the food industry.

The BOBP's Post-Harvest Fisheries Project is looking into the possibility of agar production being adapted as a village level technology.

By selling agar instead of seaweed, the villagers may be able to increase their income. A simple method of agar extraction has been developed by the Biopolymer Research Unit of the Srinakar-invirot University in Bangkok, Thailand (Chandrkrachang & Chinadit, 1988). This method produces a crude agar which could be upgraded and used in the domestic food manufacturing industry in India. With a few adaptations, this small-scale extraction method might be suitable for use in the villages of Tamil Nadu.

Since June 1989, trials to produce agar from Gracilaria edulis have been conducted in a field laboratory. The main purpose has been to determine the maximum agar yield using this extraction method, and to estimate the amount of seaweed which can be processed per day.

This paper describes the agar extraction technology and the modifications in the technology required to adapt it for use in Indian villages. A summary of preliminary results and suggestions for further research are presented.

## Materials and Methods

Materials: To conduct agar extraction trials, the following materials were used: clean seaweed; fiesh water; tubs to soak and wash the seaweed; a pan (50 1) to boil the seaweed; wooden spoons; a kerosene or wood-fuelled stove; a screw press; two planks and some heavy stones; filter cloths; trays and a platform to dry the agar in the sun.

All materials were purchased locally. The screw press was a larger version of the coconut press used in Thailand.

## Method of extraction:

An outline of the agar extraction procedure is given in Figure 1.

Figure 1: Outline of the agar extraction procedure.

## FRESH SEAWEED



Seaweed collected from natural seaweed grounds, or from a seaweed farm, was cleaned and fully dried in the sun, so that it could be stored for some time. Before processing, the seaweed was washed and then soaked in fresh water for several hours until it felt soft. It was then heated in fresh water, the time depending on the amount and variety of seaweed. After heating, the seaweed was filtered through a cloth with the help of the screw press. The filtration had to be done quickly, and the screw press pre-heated with hot water to prevent the agar solution from setting during the process. The agar formed a gel after cooling. To remove the water from the agar, the gel was enclosed in a thick filter cloth and put under pressure either in the screw press or between two planks weighted with heavy stones for larger quantities. This process takes at least half a day, after which the agar needs drying in the sun for several days.

## **Extraction trials**

Trials were conducted with small (100 g/l) and large (lkg/101 and 2kg/20 I) samples of seaweed. The small (100 g) samples of seaweed (ground or un-ground, bleached or unbleached) were heated in one litre of water for 1, 1 .5, 2, or 2.5 hours in a water bath, or heated directly. The larger samples were heated directly for 2, 3 or 4 hours. All trials were conducted with samples from the same batch of seaweed, which was collected from a seaweed farm and fully dried. Water was added if substantial evaporation occurred during heating, and the water temperature was kept at 90 to 95°C. The seaweed residue was heated for a second time in 500 ml water for 20 minutes (100 g samples), in 4.75 1 for 1 hour (lkg samples) or in 10 l of water for 1.5 hours (2kg samples).

In addition to these experiments, samples from the same batch of seaweed were sent for evaluation of agar yield and gel strength to the Biopolymer Research Unit in Thailand, the Central Salt Marine Chemical Research Institute in India and the Central Marine Fisheries Research Institute in India.

#### Results

Results of the agar extraction trials using 100 g of seaweed heated in | iitre of water are given in Table I. The data have not been analysed statistically, since trials are still continuing. The average agar yield obtained using this extraction method was about 16 per cent. There were no big differences in agar yield between heating the seaweed in a water bath or heating it directly. Grinding the seaweed with an electric mixer half-way through the heating time increased the agar yield by a few per cent. In most trials, the maximum yield was obtained after a heating time of two hours.

Table I: Agar yield (%) from extraction trials using 100g seaweed heated in one litre of water.

	Heating time (hours)			
	1	1.5	2	2.5
Water bath	16.8	14.4	17.6	14.0
	18.0	16.1	19.0	16.8
Water bath mixed	16.5	14.9	20.0	22.9
before heating	14.8	13.5	23.3	24.5
Water bath mixed	15.1	18.6		
during heating	21.3	18.7		
Direct heating	14.3	12.2	17.9	15.3
J	16.8	13.8	18.4	17.4

Increasing the quantity of seaweed being pressed did not greatly affect the agar yield (Table II),, but it should be heated for a longer period. The increase in the heating time, from two to four hours, resulted in an increased agar yield of three and four per cent in the trials with 2 kg samples.

Table II: Agar yield (%) from trials using larger quantities of seaweed.

0 , , ,	0 0 1		
	Heating time (hours)		
	2	3	4
Seaweed/water			
(kg/l) 1/10			
1/10	17.s		17.4
	18.8		
2/20	12.0	14.5	16.4
	14.2	17.4	17.2

Yield (%) and gel strength (g/cm²) of the agar from the seaweed samples sent to the three institutes are presented in Table III. Considering that all the samples came from the same batch of seaweed, the differences in agar yield and gel strength are remarkably high. The differences can be explained only partly by the use of different methods of analysis.

Table III: Agar yield and gel strength from seaweed samples sent to different institutes.

	CMFRI	CSMCRI	BRU	BOBP
	India	India	Thailand	India
Agar yield (%)	42	60	25	16
Gel strength (g/cm²)	24	120	120	

## Discussion

The variation in agar yield within the same trials is most probably caused by two different factors. There will always be some natural difference in agar yield between seaweed plants of the same species caused by the age of the plants and the season of collection. The method of agar extraction and the equipment have purposely been kept simple, hence there may be a consequent reduction in the precision of the experimental technique.

An agar yield of 16 per cent obtained by this simple extraction method is considered to be satisfactory. However, a daily amount of 320 g agar, obtained by processing 2 kg of seaweed, may not give the fisherfolk enough income, and the amount of seaweed processed at any one time should be increased. Larger quantities such as 4 or 5 kg of seaweed were processed, but several problems were encountered.

The screw press was too small to process these larger quantities of seaweed. A bigger press can be made, but it will be more expensive and probably too heavy to operate by hand.

The present method of applying pressure to the gel in a filter cloth does not remove enough water from the agar. Consequently, it takes too long (more than 6 days) for the large quantities of agar to dry and it starts decaying. The pressure (0.016 kg/cm²) exerted on the seaweed by the stones is insufficient. According to Okazaki (1971). a pressure of 100 kg/cm² is needed to remove 50 per cent of the moisture. This can only be achieved by using a hydraulic press. It may be difficult to construct a cheap and simple press which can remove sufficient water It is possible that a solar heat collector will help to improve the drying process, but it too should be cheap and simple to operate.

This simple agar extraction technology is not yet ready to be introduced into the fishing villages of India. A replacement has to be found for the present screw press to enable larger quantities of seaweed to be processed. The technique for removing water from the gel has to be improved. In addition, a market has to be found for the crude agar. Samples have been sent to commercial agar processing factories in India to find out whether it can be upgraded, and if so at what cost.

## References

CHANDRKRACHANG. S. and CHINADIT. U. (1988). Seaweed production and processing: a new approach. INFOFISH Inter 4: 22-25

OKAZAKI. A. (ed). (1971). Seaweed and their uses in Japan. Tokai University Press. Tokyo. Japan. 165 p.

# SEMINAR PAPERS: SESSION II SMALL-SCALE AGAROPHYTE PROCESSING

## SMALL-SCALE GRACILARIA CULTURE AND AGAR PROCESSING — SOME ECONOMIC ISSUES

## by Bjorn Liudeblad

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## **ABSTRACT**

An economic model has been established for seaweed farming and agar extraction at the village level. The experience gained by the BOBP pilot farms near Mandapam, Tamil Nadu, India, provides the quantitative data. At a production level of 800 kg dry weight of *Gracilaria edulis* per plot, it is concluded that an agar-selling price of Rs.125 per kg is required, assuming a 1.5% yield of agar, for the project to he profitable. If seaweed production falls to 500 kg. an agar price of Rs.150 per kg is needed, together with an agar yield of 16%. The model shows that the project may still be profitable after fencing each plot to prevent grazing by rabbit fish.

## **Background**

During the last two years BOBP has operated small-scale pilot farms for *Gracilaria edulis* near Mandapam on the coast of Tamil Nadu, India. Recently, small-scale extraction of agar has been evaluated. A model for assessing the economic viability of the activities has been established. This paper describes and assesses this model, and then illustrates some of the economic relationships between key technical and economic parameters. It should be stressed that the model is an example, and that there are several alternatives that could be equally or more appropriate.

## DESCRIPTION OF THE MODEL

## Structure

The economic model is intended to be dynamic. permitting simulation of different scenarios and their impact on the overall profitability of the project. The main profitability criterion utilised is internal rate of return, but to avoid academic discussions on the pros and cons of different criteria, net present value and pay back period have also been included. The model focuses on actual annual cash flows, rather than any accounting-related measurements of returns.

To simulate different scenarios of possible outcomes of the project, a point of departure, here called "base-case", has been established. This includes the most likely values of all variables involved at the time the model was created. This base-case version is described in Appendix 1.

## Assumptions

The model is intended for a 0.1 hectare plot of G, edulis culture. All equipment for culture and drying of the seaweed, as well as extraction of agar, is assumed to be used exclusively for this plot. Because of grazing problems encountered in Mandapam, a major investment in fencing the plot has been included. The option of using natural seaweed stocks for agar production has not been considered, since the natural resources in the area have been severely depleted by harmful harvesting methods. Natural resources are, however, still harvested and sold as wet seaweed. Cultured seaweed, sold directly as seaweed, is obviously not cost competitive with that from natural resources, so this option has not been examined.

The opportunity cost of labour has been fixed at Rs.20 per man day, the salary presently paid to fisherfolk participating in the pilot culture. The amount of labour required for planting and maintaining the seaweed farms has been considered as fixed, i.e. it does not vary with the amount of seaweed produced. The labour utilised to harvest and dry the seaweeds is expected to vary with

the amount of seaweed produced. For extracting the agar, it is estimated that one man day will be needed for every 4 kg of dry seaweed processed.

A detailed list of necessary investments is included in Appendix 1.1. The economic lifespan of individual items has been estimated to equal their expected technical lifespan. All replacement investments and future cash flows are expressed in fixed year one rupees

The income statement contains a specification of sales revenue, all operating costs, and the annual depreciation of investments.

The final table of the model, containing the components of net cash flow per year, is the basis for measuring the profitability of the project. Using net present value, the annual net cash flows have been discounted at an annual discount factor of 15% and 25%. The internal rate of return expresses the discount factor at which the net present value of the project is equal to zero. Finally, the payback period, though crude, informs the prospective investor how much time it will take to recover his initial investment.

#### A critical assessment

Among the assumptions with a high level of uncertainty is the projected seaweed production figure. Pilot farms in Mandapam have not been able to produce anywhere near 800 kg dry weight per plot per year. This seems to be due mainly to grazing and to wind conditions in the open sea culture. If these problems can be overcome, however, it is still felt that an annual production of 800 kg dry weight of G edulis, representing around 2.7 kg wet weight per metre of line per year, is technically feasible.

The technical parameters for agar extraction have been established during four months of practical trials. and consequently should be considered to be fairly reliable. The agar selling price is definitely on the high side. At present, local food grade agar is sold for between Rs.150 and Rs.250 per kg, the price varying because of festive seasons.

It can be argued that the opportunity cost of labour should be adjusted downwards during the lean fishing and seaweed collecting periods. The quantity of labour needed is based on two years of experience, and should therefore be fairly accurate.

The expected lifespans of the various investments are naturally hard to predict, given the long planning horizons involved. Residual values of the assets at the end of the period have not been included, since they can be expected to be very small for the items not fully depreciated.

Interest costs and interest earnings have been omitted from the model. In "base-case", the initial financing required could be repaid within three years, and interest earnings could be generated on the positive cash flows of subsequent years. It could, however, be argued that the positive cash flows from year four onwards would most likely be used to increase standards of living, in which case little or no interest earnings would be generated. There is also a high variability in the cost of financing, depending on the credit source. A subsidised bank loan at 45%, per annum would naturally result in a significantly more profitable project than a long term credit from the informal sector at considerably higher interest rates.

In summary, the optimistic predictions on primary seaweed production and agar price are only partly offset by over estimating the opportunity cost of labour, not including residual values, and a probably unnecessarily expensive fencing method.

## Some economic relationships

The intention of the model has been to assist in quantifying the project in economic terms. It was felt that there was a need to assess the impact of alterations in cost, revenue, and output levels on profitability. The parameters identified as crucial for economic viability were seaweed production level, agar yield, agar selling price and fencing costs.

In the figures presented below, internal rate of return has been used throughout to measure profitability. The project should be considered economically viable when the internal rate of return exceeds the opportunity cost of capital. The opportunity cost of capital equals the return forgone by investing in the project rather than the best alternative project of equivalent risk.

It can be argued that very few alternatives are available to the fisherfolk around Mandapam. But they will definitely require a project return that covers the interest rates offered by banks, plus a healthy risk premium. The local hank deposit rates are around 5% per annum, and the fisherfolk would probably demand a risk premium of at least 20—25%. This adds up to a required rate of return somewhere in the 25--30% range.

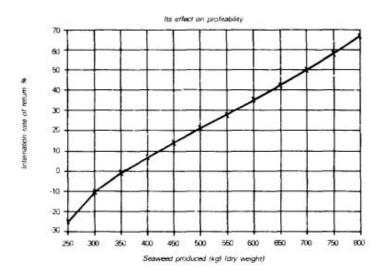


Figure 1 SEAWEED PRODUCTION VOLUME

Let us start by looking at the effects of different seaweed production volumes on project profitability, keeping all other variables constant at their "base-case" levels. We can see that an annual production of at least 550 kg (dry weight) of G. *edulis* is required to reach a satisfactory level of profitability.

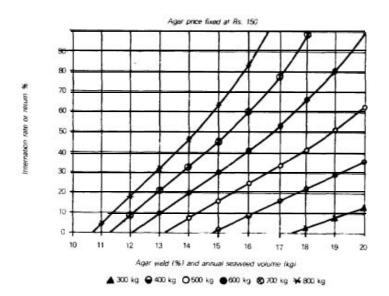


Figure 2 AGAR YIELD & SEAWEED PRODUCTION VOLUME

We may then introduce variations in the agar yield, under different seaweed production volumes. In the upper range of seaweed production levels, between 600 kg and 800 kg, there seems to be a trade-off between agar yield and seaweed production, such that a decrease in seaweed production of 100 kg, may roughly be compensated for by a 1% increase in agar yield. This relation holds valid only for agar yields up to around 16%.

It can be observed that, since yields above 17—18% are unlikely, seaweed production levels of 300—400 kg will not be viable.

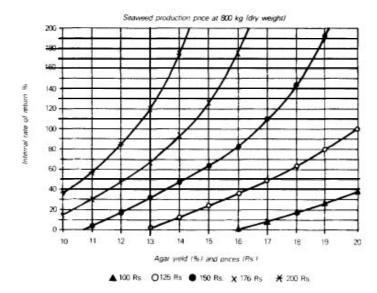


Figure 3 THE IMPACT OF AGAR YIELD & AGAR PRICE

This graph introduces variations in agar prices, keeping agar yields variable, but seaweed production volume fixed at 800 kg (dry weight) per year.

The most valuable information from this graph is that if the agar selling price falls below Rs. 125, it will be very difficult to make the seaweed farm a profitable venture.

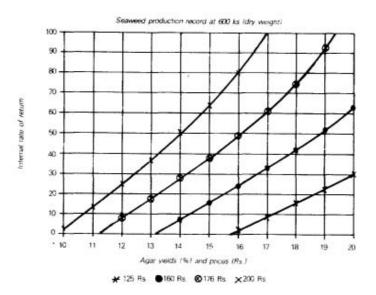
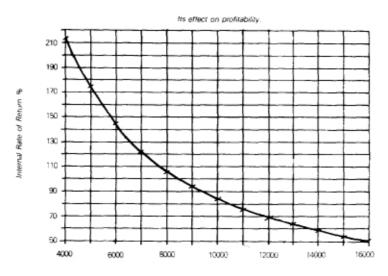


Figure 4 THE IMPACT OF AGAR YIELD & AGAR PRICE

If the seaweed production level falls to 500 kg (dry weight) per year, any agar price below Rs. 150 will be too low to be viable, unless drastic technological progress is made, and agar yields above 20% can be achieved.

Figure 5 FENCING COST



Fencing cost per plot (rupees)

This graph illustrates the effect on overall project profitability of changes in the fencing cost. All other variables are kept constant at their "base-case" level. The project will, in its "base-case" version, continue to he profitable even with quite a substantial investment in fencing.

## APPENDIX 1.1

Economic analysis of seaweed and agw prothiction in Mandapam- Tamil Nadu

This is a model to assess the economic viability of the above activities. Any input data that is high-lighted like this line, can be modified. Please make the directory containing this file the current directory. To access the main menu, just press "Alt" and "M" simultaneously.

Assumptions	BASECASE	Year	Year 2-10	
Opportunity co	ost of labour:	20	20	Rs. per man-day
Seaweed produ	iction:	800	800	Kg of dried seaweed/year
Seaweed used	for agar production:	800	800	Kg of dried seaweed/year
Seaweed proce	ssing capacity:	4	4	Kg of dried seaweed/man-day
Average agar y	ield:	14.0%	16.0%	Agar produced as % dried seaweed used
Fuel consumpt	ion (kerosene = =	0.17	0.17	Fuel units per hour of boiling
Average boilin	g time:	2	2	Hours
Average boilin	g volume:	0.25	0.25	Kg of dried seaweed
Fuel cost (kero	sene = =	3.5	3.5	Rs. per fuel unit
Cost of filter cl	oth:	0.25	0.25	Rs. per processed Kg of dried seaweed
Seaweed sellin	g price:	5	5	Rs. per Kg
Agar selling pr	ice:	150	150	Rs. per Kg

Lahour requirements

	Rs. per man-day	Man-days per ye Year 1Year 2	
Vegetative propagation (F)	20	30	10
Outplanting (F)	20	4	4
Maintenance (F)	20	75	75
Harvest(V)	20	15	15
Drying(V)	20	9	9
Agar production (V)	20	200	200
TOTAL		333	313

(F): Fixed labour cost(V): Variable labour cost

APPENDIX 1.2

## Income statement and cash flow

Version: BASECASE

Income statement

	Quant	ity	Value	e
	Year 1	Year 2-10	Year	Year 2-10
Sales seaweed	0	0	0	0
Sales - agar	112	128	16,800	19,200
Labour - seaweed	133	113	2,660	2,260
Labour - agar	200	200	4,000	4,000
Filter-cloth	N.A	N.A	200	200
Fuel	I ,067	1,067	3,733	3,733
Other:	0	0	0	0
Total operating cost			10,593	10,193
Gross margin			6,207	9,007
Depreciation - seaweed			1.800	1,800
Depreciation - agar			740	740
Income before financial expenses			3.666	6.466

## Cash flow Year 1 Year-3 Year 3 Year4 Years5 Year6 Year7 Year8 Year9 Year 10

Sales (+)	16.800	19,200	19,200	19,200	19,200	19.200	19,200	19,200	19,200	19,200
Investment (-)	18,465	215	891	1,025	991	475	1,701	215	991	1,025
Operating costs (-)	10.593	10,193	10,193	10,193	10,193	10,193	10,193	10,193	10,193	10,193
Net Cash flow (=)	(12,258)	8,792	8,116	7,982	8,016	8.532	7,306	8,792	8.016	7.982

Net Present Value at 25% discount rate: 13,048 Rupees
Net Present Value at 15% discount rate: 23,434 Rupees

Internal Rate of Return: 67:6%

Payback Period

(investments year 2-10 = costs): 2.3 Years

## APPENDIX 1.3

Total

Seaweed culture - an investment summary

Version: BASECASE

	Number	Unit	Total	Economic	Annual							1			
Item	of units	price (Rs.)	investment (Rs.)	lifespan (Years)	depreciation	Year 1	Year2	Year 3	Year4	Year 5	Year6	Year7	Year8	Year 9Y	ear 10
Seaweed (kgs wet)	225	1.07	240	2	120	240	0	240	0	240	0	240	0	240	0
Stone post	30	21.7	650	10	65	650	0	0	0	0	0	0	0	0	0
HDPE rope 5 mm (M)	300	0.33	loo	2	50	100	0	100	0	100	0	100	0	100	0
HDPE roße mm (M)	3000	0.25	750	3	250	750	0	0	750	0	0	750	0	0	750
Shed			500	10	50	500	0	0	0	0	0	0	0	0	0
Drying frame			200	5	40	200	0	0	0	0	200	0	0	0	0
Fencing	2 10	58.4	12,254	10	1,225	12.254	0	0	0	0	0	0	0	0	0
Other:			0		0	0	0	0	0	0	0	0	0	0	0

14,694

1.800

Agar processing - an investment summary

14,694

Version: BASECASE

Annual investment - Agar processing

1,090

200

340

0

750

340

340

0

7.50

Annual investment - Seaweed production

Item	Number of units	Unit price (Rs.)	Total investment (Rs.)	Economic lifespan (Years)	Annual depreciation	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7	Year 8	Year 9 Y	ear 10
Coconut presser	1	3,000	3,000	10	300	3,000	0	0	0	0	0	0	0	0	0
Stove	1	100	100	4	25	100	0	0	0	100	0	0	0	100	0
Manual grinder	1	60	60	5	12	60	0	0	0	0	60	0	0	0	0
Pressing planks	4	25	100	2	S 0	100	0	100	0	100	0	100	0	100	0
Manual chopper	1	60	60	3	20	60	0	0	60	0	0	60	0	0	60
Aluminium vessels (s)	6	30	180	1	180	180	180	180	180	180	180	180	180	180	180
Aiuminium vessels (I)	1	35	35	1	35	35	35	35	35	35	35	35	35	35	35
Plastic vessels (s)	2	38	76	2	38	76	0	76	0	76	0	76	0	76	0
Plastic vessels (I)	2	80	160	2	80	160	0	160	0	160	0	160	0	160	0
Other 1:			0		0	0	0	0	0	0	0	0	0	0	0
Other 2:			0		0	0	0	0	0	0	0	0	0	0	0
Total			3,771		740	3.771	215	551	27.5	651	275	611	215	6.51	275

SEMINAR PAPERS: SESSION II

SMALL-SCALE AGAROPHYTE PROCESSING

## A SIMPLE PROCESS FOR EXTRACTING AGAR FROM POLYCAVERNOSA CHANGII

## by Ramli Bin Saad

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## **ABSTRACT**

Polycavernoso changii from the Middle Bank of Penang. Malaysia was sun-dried. Extraction of agar was achieved with hot water. After filtration, the extract was set aside to cool and gel. Removal of water from the gel was by syneresis. Final drying was by oven and sun. P. changii was found to yield 33-48% agar.

#### Introduction

Polycavernosa changii is a Gracilaria-like seaweed commonly found in Penang, and other parts of Malaysia. The Fisheries Research Institute studied methods of growing this seaweed and possible commercial-scale culture (Doty, 1977; Faazaz, 1986).

The present study was carried out with the aim of developing a method that can be performed **by** traditional fisherfolk for extracting agar from this seaweed. There is no agar manufacturing industry in Malaysia at present. All agar is imported in particular from Korea, China and Japan.

## Materials and Methods

The extraction process was based on a report by Coppen (1988) and Trono et **al** (1988). There were four stages, namely harvesting and drying, gel extraction, dewatering of the gel, and chemical treatment.

## a. Harvesting and drying

Polycavernosa changii was collected by hand from the Middle Bank of Penang, and sun-dried on netlon attached to an iron rack. It was then soaked in fresh water for 20 minutes for pre-bleaching treatment and then sun-dried again. When it was totally dried, the seaweed was kept in a plastic bag until required for agar extraction.

## b. Gel extraction

Extraction was carried out with hot water. A 100 g sample of the dried seaweed was soaked in fresh water for 30 minutes to soften it and to remove impurities. It was then cooked with 2 litres of fresh water in a stainless steel pot for 30 to 60 minutes. The mixture was filtered using double layers of soft cotton cloth and pressed. The extract was collected in an aluminium tray and set aside to cool and gel.

## c. Dewatering of the gel

Water was removed from the gel by syneresis, a process by which frozen agar liberates water when it thaws. In the first three experiments, the gel was cut into 1.5 cm bars and kept in the freezer overnight. In the fourth and fifth experiments, the gel was put into the freezer uncut. In all the experiments, the frozen gel was removed from the freezer the next morning and put in the sun for about three hours to thaw. The gel was then put back in the freezer for another four hours, thawed again for one hour and then kept in the freezer overnight. The next day, the gel was thawed again

for 3 hours. For the fourth and fifth experiments, the gel was cut into small strips and then dried with the gel from the other three experiments alternately in an oven and in the sun.

## d. Chemical treatment

Sodium hypochlorite (2-5% solution) was used to bleach the agar in experiments one, two and three. It was also used to bleach the seaweed before extraction in experiments four and five. Sodium hydroxide was used to treat the seaweed before extraction in experiment three.

## Results and discussion

It was observed that the quality of the agar produced was largely dependent on the period of extraction and on the bleaching agent used. When the period of extraction was 60 minutes, the seaweed was crushed and the agar produced was blackish brown in colour. This was largely due to the pigments and particles from crushed seaweed which appeared in the extract through excessive heating. The shorter extraction period. 30 minutes, was found to produce agar that was bright and clear.

Treatment with sodium hypochlorite to clear the dry agar resulted in the agar being crushed and some chlorine residue could also be detected. However, treatment with sodium hypochlorite prior to extraction resulted in agar that was clear and bright. Treatment with sodium hydroxide also resulted in an agar which was clear.

The effect on the gel strength was not measured because the apparatus was not available In general. pretreatment with sodium hypochlorite and sodium hydroxide decreased the quantity of agar produced. The results are tabulated below.

Experiment No:	Pre-extraction treatment	Cooking duration in 21 fresh water (minutes)	Agar Yield (gm)	Remarks
1.	None	60	45.8	Post bleaching treatment-crushing the agar
2	None	30	47.8	_ "-
3	200 ml NaOH (0.1 m) in 2 litres of water for 10 minutes	30	37.5	_'''_
4.	5% NaOCL for 30 mins.	30	33.0	No post-bleaching treatment
5	2% NaOCL for 20 mins.	30	34.x	_ " _

Further studies will be carried out using different concentrations and durations of soaking in NaOCl, NaOH and other chemicals, to determine the maximum yield of agar with high gel strength that can be produced.

## Acknowledgement

I would like to thank the Director of the Fisheries Research Institute, Mr Ong Kah Sin, for his encouragement and constructive criticism on this paper. Lastly, I would like to thank the Director-General of Fisheries, Malaysia, Dato Shahrom bin Hj. Abdul Majid, for permission to present this paper.

## References

COPPEN. J.J.W. (1988) The processing of agar and alginate-producing seaweeds: A desk study. Unpublished report of Bay of Bengal Programme. Madras, India.

DOTY, M.S. (1977). Seaweed resources and their culture in the South China Sea region. South China Sea Fisheries Development and Coordinating. Programme, working paper.

FAAZAZ ABDUL LATIFF (1986). Preliminary results of experimental culture of the red seaweed Gracilaria sp. in Malaysia. Buletin Perikanan No: 40. Department of Fisheries, Ministry of Agriculture, Malaysia.

TRONO. G.C. Jr., GANZON, E.T. and FORTES. M.D. (1988). Philippines Seaweeds. National Book Store, INC. Metro Manila, Philippines.

# SEMINAR PAPERS: SESSION II SMALL-SCALE AGAROPHYTE PROCESSING

# GRACILARIA CORTZCATA: A POTENTIAL SOURCE FOR BIOACTIVE SUBSTANCES by Kalpana H Mody\*

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## **ABSTRACT**

Crude extracts of *Gracilaria corticata* exhibited anti-bacterial activity only against gram + bacteria but after fractionation, samples were active against gram- bacteria and *Mycobucterium tuberculosis*. These fractions have been found to be more effective than penicillin, chloramphenicol and sulphadiazine against a number of test organisms.

## Introduction

During the last few years, interest has been awakened in marine biodynamic substances. Anti-bacterial potential has been detected in a number of macroscopic marine algae from all over the world. Distribution of these compounds in Rhodophyceae has been reported by Gerber *et al.* (1958), Glombitza (1970), Glombitza and Heyser (1971), Glombitza and Stoffelen (1972), Glombitza *et al.* (1973), Glombitza *et al.* (1974), and Hornsey and Hide (1974). Agar extracted from *Gelidium* and *Gracilaria* exhibited a marked inhibitory effect on the growth of the influenza B and mumps viruses (Gerber *et al.* 1958 and Takemoto and Spicer, 1965). This has been attributed to the agar galactan units (Nigrelli et al. 1967). In the late 1970s a study was undertaken on the antibacterial activity of Indian marine algae. This paper describes a part of that study, in which *Gracilariu corticutu* was identified as a potential source of bioactive substances.

## Materials and methods

G. corticuta, collected from Diu on the coast of Gujarat, was thoroughly washed to remove extraneous matter and epiphytes and then frozen until required.

A method has been standardised for extracting plants using different solvents (organic and aqueous) and temperatures (20°C and 50°C). Staphylococcus aureus, Bacillus meguterium (gram + ), Escherichia coli, Proteus vulgaris, Shigella sonnei and Salmonella typhosa para A (gram - ) were used as test bacteria. Bioassay was by the agar plate diffusion test. The degree of sensitivity of the test organisms was determined by measuring the zone of growth inhibition.

Four different fractions i.e. fraction A, fraction B, phenols and pigments were prepared from *G. corticutu* using the method reported by Parekh (1978). An in vitro comparative study of these fractions was conducted with known antibiotics (supplied by Bharat Laboratories, Bombay) against all the test organisms.

The antitubercular activity of different concentrations of fractions A and B was evaluated at Shri K. J. Mehta T.B. Hospital, Amargadj, Madhya Pradesh.

## Results & Discussion

The antibacterial activity of crude extracts of G. corticutu against gram + bacteria is shown in Table I. The diethyl ether, acetone and ethanol extracts exhibited better activity than the chloroform extract. Hot and cold aqueous extracts and ammonium sulphate extract were not effective against any of the test organisms. The heat stability of the active principle was demonstrated, there being no difference in activity by acetone extracts prepared at 20°C and 50°C.

<sup>\*</sup> Paper presented at the seminar by Dr. V.D. Chauhan, CSMCRI.

Table I: Antibacterial activity of crude extracts of G. corticata

Test Organisms	<b>Zone of inhibition (mm)</b> Solvents used for extraction									
	Diethyl ether	Ace- tone	Alco- hol	CHCI3	Hot Water	<b>Cold</b> Water	Ammonia SO, Soln.			
B. megaterium S. aureus	1 6 13	<b>15</b> 11	14 23	T -	-	-	-			

<sup>- =</sup> No activity

## E. colodi P. vulgaris, P. aeruginosa, S. sonnei and S. typhosa para A were resistant

Seaweeds are highly susceptible to microbial decomposition as soon as they are removed from their natural habitat. Attempts have been made to find a suitable method for preserving the plants along with their bioactive properties. Acetone extract prepared from *G. corticata* dried at 37°C was almost as active as that from deep frozen undried material. Acetone extract from plants dried at 60°C showed no activity against any of the test organisms.

Marked seasonal fluctuations in quantitative antibiosis have been reported, with the best antibiotic production occurring at the time of most rapid growth and proliferation (Baslow. 1969). In the present study, G. *corticata* exhibited antibacterial activity throughout most of the year; there was a period of high activity, between the months of September and January (Table II).

Table II: Seasonal variation in the antibacterial activity of G. corticatu against S. aureus and B. megaterium

Month of	Zone of in	Zone of inhibition (mm)				
collection	S. aureus	B. megaterium				
March	T	T				
April	T	T				
M a y	8	9				
June	10	8				
July	10	9				
August	10	8				
September	12	10				
October	12	10				
November	15	12				
December	12	12				
January	12	12				

Extracts were prepared in acetone

Crude extracts of G. corticata were not active against gram - bacteria. It may be that the active principle is masked or inhibited in crude extracts. Hence, fractionation into fraction A, fraction B, phenols and pigments was carried out and each was tested separately for its antibacterial activity. Fraction A was active against all the test organisms. whereas fraction B was active against all bacteria except S. **typhosa** Para A. Phenols showed activity against both gram + and gram - bacteria.

Water-soluble derivatives of chlorophyll have been reported to have antibacterial activity (Smith, 1964). Pigments extracted from G. **corticata** did not show antibacterial activity, thus excluding the possibility that the antibacterial activity of the crude extract was due to pigments (Table III).

Table III: Antibacterial activity of different fractions obtained from G. corticata

Zone of inhibition (mm)											
Fractions	S. aureus	B. megaterium	E. coli P.	vulgaris	P. aeruginosa	S. sonnei	S. typhosa				
Fraction A	12	16	18	10	9	15	12				
Fraction B	10	15	14	12	8	7	NT				
Phenols	12	13	11	10	T	NT	NT				
Pigments		-			-	-					

<sup>- =</sup> No activity

T = Trace activity

T = Trace activity

NT = Not tested

In vitro comparative study of these fractions with commercially available antibiotics indicated that the activity of fractions A and B was greater than that of penicillin, chloramphenicol and sulphadiazine, and comparable to that of tetracycline, streptomycin. kanamycin, garamycin, polymyxin and erythromycin (Table IV).

Table IV: In vitro comparative study of antibiotic activity of G. corticata with known antibiotics

		Zone	of inhibi	tion (mm)			
Antibiotic							
(Conc/disc)	S. aureus	B. megateriun	n E. coli	P. vulgaris	P. aeruginos	a S. sonnei S	. typhosa
Penicillin		8				-	
(5 units)							
Streptomycin	25	15	9	18	21	T	23
(25 μg							
Tetracycline	20	15	10		T	20	11
$(10 \mu g)$							
Erythromycin	14	16	11	11	T	T	10
(10 µg)							
Chloramphenicol	13		-	-	-	-	-
(50 μg)							
Sulphadiazine	13		-	-		_	
(200 µg)							
Kanamycin	25	17	19	21	10	T	21
(30 μg)	• •					_	• •
Garamycin	28	23	18	18	22	T	28
(10 µg)						_	
Polymyxin	18	17	16	15	18	T	15
(250 µg)					_		
Fraction A	12	20	20	13	T	13	12
(50 μg)	10	1.5	15	1.0	<b>T</b>		
Fraction B	12	15	17	12	T	21	15
(50  mg)							

<sup>- =</sup> No activity

Antimycobacterial activity of seaweeds has been reported by Burkholder (1960). Sarganin and Chonalgin, isolated from brown and red algae, also showed inhibitory activity against Mycobacterium **smegmatis**(Martinez-Nadal et al. 1966). In the present study, fractions A and B of G. corticata were tested against isolates of Mycobacterium tuberculosis for antitubercular activity. The results revealed the complete inhibition of growth of strains 111, 129, 167, 207,442, 361A, and 362A in culture of 64 µg/ml of L.J. medium, indicating high effectiveness towards mycobacteria.

From the studies, it is concluded that G. corticata can be explored as a good source of biomedicals.

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## References

BASLOW M.H. (1969). Marine Pharmacology. Williams & Wilkins Co.. Baltimore. p.56-85.

BURKHOLDER. P.R. (1960). Drugs from the sea. Freudenthal, H.D.. Ed. Marine Technology Society. Washington D.C. p.87-112

GERBER, P., DUTCHER, J.D., ADAMS, E.V. AND SHERMAN, J.H. (19%), Proc. Soc. Exp. Biol. Med., 99, 590-93

GLOMBITZA, K.W. (1970). Planta Medica, 18(3): 210-221.

GLOMBITZA. K.W. AND HEYSER, R. (1971). Halgolander wiss Mecrss Unten. 22: 442.

T = Trace activity

GLOMBITZA, K.W. AND STOFFELEN. H. (1972). Planta Medica. 22: 391-395.

GLOMBITZA. K.W., RESENER. H.V., VITTER, H. AND RAUWALD, W. (1973). Planta Medica. 24: 301-303

GLOMBITZA, K.W.. STOFFELEN. H., MURAWSKI. V.. BIELAEZEK. J. AND EGGE. H. (1974). Planta Medica 25: 105-1 13.

HORNSEY. I.S. AND HIDE, D. (1974). Br. Phycol, J.. 9(1): 353-361.

JORGENSEN. E. G. (1972) Phytochem 11 (4): 1423-32.

MAHACHALAN. J AND CRAIGIE. J. S. (1966). J. Phycol.. 2: 133-35

MARTINEZ-NADAL. N.G., CASILLAS, C., RODRIGUEZ-PERAZZA. J.R. ANDTORRA-ERA, L. (1966). Bot. Mar. 9: 21-26

NIGRELLI, R.F., STEMPLEN, M.F., RUGGIERI, G.D., LIGUORI, U.R. AND CEDL, J.T. (1967), Fed. Proc. 26: 1197-1205.

PAREKH. K.S. (197X). Antibacterially and pharmaceutically active substances from marine algae. Ph.D thesis submitted to Saurashtra Univ., Rajkot. India.

SIEBURTH. J. MCN (1968). Advances in microbiology of the sea Vol. I. Droop. M. R. and Wood. E. J. P: Eds. Academic Press. New York. p. 63-94.

SMITH, N.L. (1964). Am. J. Med. Sci. 207: 647-654.

TAKEMOTO. K.K. AND SPICER. S.S. (1965). Ann. N.Y. Acad. Sci.. 130: 365-375.