CODEX ALIMENTARIUS COMMISSION E

Food and Agriculture Organization of the United Nations

World Health Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - Fax: (+39) 06 5705 4593 - E-mail: codex@fao.org - www.codexalimentarius.net

Agenda Item 12 **CX/FFP 11/31/12**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME
CODEX COMMITTEE ON FISH AND FISHERY PRODUCTS
Thirty-first Session
Tromsø, Norway
11 – 15 April 2011

PROPOSED DRAFT AMENDMENT TO THE STANDARD FOR QUICK FROZEN FISH STICKS (NITROGEN FACTORS)

(At Step 3 of the Procedure)

Governments and interested international organizations are invited to submit comments on the attached Proposed Draft Amendment at Step 3 and should do so in writing in conformity with the Uniform Procedure for the Elaboration of Codex Standards and Related Texts (see *Procedural Manual of the Codex Alimentarius Commission*) to: the Secretariat, Codex Alimentarius Commission, Joint WHO/FAO Food Standards Programme, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy, by email codex@fao.org or fax: +39-06-5705-4593**,** with a copy to Codex Contact Point, Norwegian Food Control Authority, P.O.Box 8187 Dep. 0034 Oslo, Norway, Fax: +47.74.11.32.01, email: ccffp@mattilsynet.no **by 15 March 2011.**

Background

1. At the 28th session of the Codex Committee on Fish and Fishery Products (CCFFP), Thailand proposed to the Committee to undertake an experiment to determine Nitrogen factor for tropical fish as the nitrogen factors currently available were for temperate fish species. The nitrogen factor determined and calculated would be used as a basis for determining fish content in frozen breaded and battered fish and other similar products. The study would also aim at determining factors affecting nitrogen content in Tilapia (*Oreochromis nilotica*). Tilapia was selected as it is one of the most popular fish used for producing fish sticks and breaded and battered fish products.

2. The 28th Session agreed to undertake new work on an amendment of the Table: Interim Nitrogen factors to be used for white fish as an ingredient in the Standard for Quick Frozen Fish Sticks (Fish Fingers), Fish Portions and Fish Fillets – Breaded or in Batter (CODEX STAN 166 – 1989).[1] This new work was approved by the 30th Session of the Commission.[2]

3. At the 30th session of the Committee, it was agreed to return the Proposed Draft Amendment to Step 2/3 for redrafting by the Delegation of Thailand, with the assistance of Malaysia, New Zealand and other interested delegations.[3]

4. Malaysia is one of the countries that processes frozen breaded and battered fish products for export. Malaysia expressed its interest in assisting Thailand to determine nitrogen content for tropical fish, particularly Tilapia.

5. Governments and interested international organizations are invited to comment on the proposed draft amendment as proposed in **paragraph 22** of this document.

[1] ALINORM 07/30/18, paras 128 - 129/

[2] ALINORM 07/30/REP, para 96 and Appendix VII

[3] ALINORM 10/33/18, para. 150.

Studies Conducted by Thailand

6. Thailand further assessed the nitrogen content in Tilapia from several major areas of cultivation in order to determine if culture areas had an effect towards nitrogen factor. In this study, 3 provinces (Petchaburi, Samut Prakarn and Nakorn Pathom) were selected as the sources of Tialapia samples. The different types of feeds used in association with farm management systems were thought to have an effect on the nitrogen content in the fish. There are several types of commercially culture practices for Tilapia. The most popular techniques are culturing fish in earthen ponds and cage culture. Tilapia for export is normally obtained from intensive farms which are mostly pond culture. Thailand is a tropical country where temperature change is not drastic throughout the year. Therefore, season is not thought to be an important factor affecting nitrogen content in the fish meat.

7. In this study, Tilapia (approximately 800 gram/fish) were harvested from the 3 provinces and delivered to 2 processing establishments. The fish were subjected to different processing stages to determine the effect of processing towards nitrogen content. The 3 processing stages were 1) control (fish fillets derived from whole fresh fish) 2) fish fillets undergone icing and 3) washing and frozen fish blocks. Appendix 1 shows the processing process to obtain the samples. The same fish samples were distributed to 3 different ISO/IEC 17025 laboratories for proximate analyses (total nitrogen, moisture content, fat and ash).

8. In conclusion of this study, nitrogen content of Tilapia was found to significantly vary depending on farm locations where different farm management systems and various types of feed were used. These also significantly affected other chemical components in the fish flesh which were moisture content, fat and ash. However, the processing stages in the production of fish fillets and frozen block fish did not have an effect on nitrogen content of the fish. Upon determination of 108 Tilapia samples (216 observations) by 3 different ISO/IEC 17025 accredited laboratories, the nitrogen factor of 3.00 was proposed at the 30th session of CCFFP for Tilapia.

Studies Conducted by Malaysia

9. Malaysia conducted similar studies on fish samples collected from earthen ponds and cage cultures throughout the country. At the 30th session of CCFFP, Malaysia proposed the nitrogen factor of 2.62 for Tilapia.

Recommendations from the 30th Session of CCFFP

10. **Thailand and Malaysia were recommended to conduct a ring trial to determine any variation in laboratory procedu**res as there was a wide range of variation in the data on nitrogen content of the same fish (Tilapia) presented by Thailand and Malaysia.

Results of the Ring Trial and Additional Study on a Larger Number of Fish

Thailand

11. Thailand conducted a ring trial for the participating laboratories using 2 sets of standard reference materials (canned fish paste, 150 g/can, No. T2548 and T2570) were purchased from FAPAS, United Kingdom. The standard reference materials were distributed to a total of 5 ISO/IEC 17025 accredited laboratories. There were 3 laboratories participating in the previous experiment. Each laboratory received 2 standard reference materials for determination of total nitrogen, moisture content, fat and ash. Appendix 2 shows the references of analytical methods used by the participating laboratories.

12. The analytical results were evaluated if they were within the acceptable ranges assigned by the material provider (FAPAS). It was found that 4 out of the 5 laboratories were able to provide satisfactory results. The unsatisfactory results derived from 1 of the 3 laboratories participating in the first study. Therefore, the analytical results particularly the nitrogen content obtained from this laboratory were not incorporated into the studies on determination of nitrogen factor in Tilapia.

Malaysia

13. The ring trial was conducted in 2010 using 2 reference samples purchased from FAPAS, United Kingdom. The reference samples were T2541 (high nitrogen, low fat) and T2548 (high fat, low nitrogen) fish pastes. The participating laboratories were 1) an accredited private laboratory (Lab 1); 2) and accredited government food analysis laboratory (Lab 2); and 3) MARDI's (Malaysian Agricultural Research and Development Institute's) R&D laboratory (Lab 3). The methods used for moisture, nitrogen and fat analysis were AOAC methods or in-house methods based on AOAC (Appendix 3). The data of the ring trial study

were subjected to the Analysis of Variance (ANOVA) procedure and the differences among means were compared by t-test ($P<0.05$).

14. The results of the reference samples produced by the 3 laboratories were within the given range except the fat content in the reference material T2548 analysed by Laboratory No. 2. This laboratory is using acid hydrolysis method instead of direct extraction method. The mean values for moisture, nitrogen and fat content produced by the 3 different laboratories are shown in Appendix 4.

Additional Studies to Determine Nitrogen Content in Tilapia

Thailand

15. Thailand decided to conduct additional study by expanding the number of fish samples obtained from throughout Thailand, covering both earthen ponds and cage culture techniques. The results were thought to better reflect the intrinsic nitrogen contents of Tilapia from both culture techniques and to better represent national figures. In this second study, Tilapia was obtained from additional 80 locations including 3 wholesale fish markets in 10 different provinces. The 10 provinces included the 3 provinces previously selected for the first study.

16. Taking into account the satisfactory results of the ring trial, the research team decided to select 2 new laboratories to participate in the second round of the study. This was due to the fact that both laboratories were capable of providing faster analytical services and able to handle a much larger number of fish samples in limited time of this second experiment.

17. In the first study, it was found that different processing stages had no effect on the nitrogen content. Therefore, in this second study, the fish samples used were obtained directly from whole fish. Tilapia sized 500-700 gram/fish from earthen ponds and cage culture techniques were harvested from 10 provinces which are the major source of Tilapia culture in Thailand. The 10 provinces were Chachoengsao, Prachin Buri, Suphan Buri, Kanchanaburi, Nakhon Pathom, Ratchaburi, Phetchaburi, Nakhon Nayok, Samut Prakan and Samut Sakhon. Fish were harvested from 4 to 8 farms in each provide during April - September 2010. In addition, this study also assessed nitrogen content of Tilapia fillets from 3 central wholesale markets which were Thai Market, Khong3 Market and Talay Thai Market. Samples were collected from a total of 8 vendors per place. Such samples represented the Tilapia cultivated from all over the place in Thailand. Fish samples were then prepared as frozen fish fillet by using cryogenic freezing and stored at -18^{o}C before sending out to 2 participating laboratories. Appendix 5 shows the sample preparation method. The analyses on total nitrogen were conducted in duplicate. Appendix 6 shows the average nitrogen content obtained from the first and second studies.

18. The location of earthen ponds and central wholesale markets had an effect on nitrogen content of the fish because of different feeds composition used and farming management system. However, cage culture at different locations did not statistically have any effect on nitrogen content. Differences caused by various sources of raw materials from each culture technique were determined using ANOVA and Least Significant Difference (LSD) at 0.05 significance level. In comparing nitrogen content from two culture techniques and central wholesale markets, it was found that the average nitrogen content obtained from cage culture was of the highest value at 3.079 while the values obtained from earthen ponds and central wholesale markets were 2.921 and 2.918 respectively. Both average nitrogen contents from earthen pond culture and wholesale market were not statistically different because Tilapia sold at the markets mostly come from earthen ponds culture.

Malaysia

19. Malaysia conducted a new study on determination of nitrogen content in Tilapia. A total of 23 samples had been collected from selected farms in the 10 States in the Peninsular of Malaysia. Each sample constitutes of about 7-10 kg fish or 8-10 fish. The collected fish samples were produced under different culture systems i.e. pond, cage, tank and wild fish from ex-mining pools and lakes. Samples were brought in ice on the same day to Fish Processing Laboratory, MARDI Research Station, Kuala Tarengganu, where the samples were recorded and immediately processed into fillet. Otherwise, samples were blast-frozen and brought to the station within 48 hours after harvest. Samples were prepared as in the flow-chart below;

Tilapia
↓
Manually Filleted/pin bones removed
↓
Cleaned and rinsed in portable water
↓
Machine skinned
↓
Divided into 3 sub-samples of not less than 500g each and coded
↓
Vacuum packed
↓
Blast-freezing at -40 °C to a core temperature of -18 °C
↓
Transported frozen to 3 designated laboratories for analysis

20. The fillet of each sample was divided into 3 sub-samples and placed in sample bags, properly labeled, vacuum packed and blast-frozen at -40^{o}C to reach the core temperature of -18^{o}C and sent to the 3 separate laboratories, participating in the ring trial, for the analyses of nitrogen, fat and moisture.

21. The data had been analysed, omitting the samples of wild fish obtained from lakes and old mining ponds. Thus the results showed that the average nitrogen, fat and moisture contents in Tilapia were 2.76%$\pm$0.33, 2.64$\pm$0.45 and 80.17$\pm$2.36 respectively (Appendix 7).

Newly Proposed Nitrogen Factor for Tilapia

22. Based on the results from the first and second studies by Thailand, including consideration on the supply chain information, more than 80% of Tilapia supplied to the fish processing industry for export was from earthen pond culture. Therefore, the data on nitrogen content from Thailand used for statistical analysis were from earthen pond culture only while the data from Malaysia derived from both ponds and cage culture, as cage culture is more popular in Malaysia.

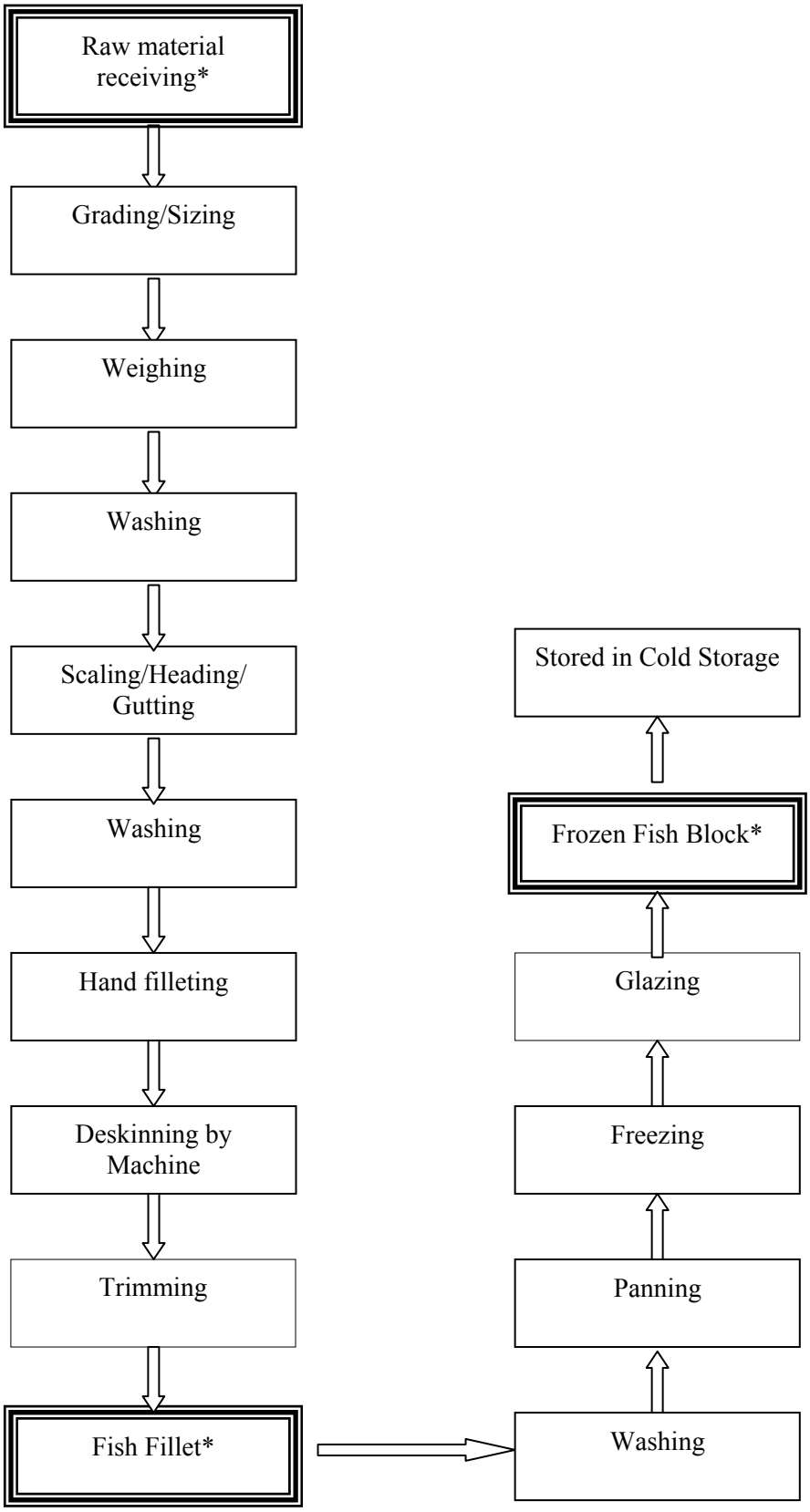
23. Appendix 8 shows the comparison of the nitrogen content in Tilapia fillet from Thailand and Malaysia which were 2.921$\pm$0.119 and 2.76$\pm$0.3 respectively. The value proposed by Thailand derived from incorporating the results from the first experiment and the second experiment with encompassed a much larger sample sizes obtained from throughout Thailand. The actual observations or raw data of nitrogen content of both Thailand and Malaysia were combined and statistically analysed. **As a result, Thailand and Malaysia would like to propose the new value of 2.88 as the nitrogen factor for Tilapia**.

Remarks

24. The nitrogen content from aquaculture fish significantly varies upon feed compositions, water quality and farm management techniques. The nitrogen factor estimated based on such conditions was of a dynamical challenge. The nitrogen factor proposed should be regularly updated particularly due to improving culture techniques.

Appendix 1

Processing of Block Frozen Tilapia Samples (Thailand)



Remarks: * Samples collected for proximate analyses

Appendix 2

Analytical Methods Used by the Participating Laboratories (Thailand)

Laboratory	Proximate analyses			
	Total Nitrogen	**Moisture**	**Fat**	**Ash**
1	Kjeldahl based on AOAC(2000) 928.08	Based on AOAC (2005) 950.46 (LBAG-00124)	In house method LBAG-00110 based on AOAC(2005) 954.02, 922.06, 925.12, 948.15	Based on AOAC (2005) 920.153 (LBAG-00124)
2	Modified Method based on AOAC (2000) 981.10	AOAC(2005), 950.46(B)	AOAC(2005), 948.15	AOAC(2005), 920.153
3	In-house method based on AOAC 2005: 991.20 (NFI T 927)	AOAC 2005: 950.46 (NFI T 923)	AOAC 2005: 948.15 (NFI T 966)	AOAC 2005: 920.153 (NFI T 924)
4	In- house method based on AOAC (2005) 940.25	In- house method based on AOAC (2005) 952.08	In- house method based on AOAC (2005) 948.15	In- house method based on AOAC (2005)938.08
5	In-house method based on AOAC 2005:991.20	AOAC 2005: 952.08 A	AOAC 2005:948.15	AOAC 2005: 938.08

Appendix 3

Analytical Methods Used by the Participating Laboratories (Malaysia)

Laboratory codes	Moisture	Total Nitrogen	Fat
Lab 1	AOAC 950.46 (2002)	AOAC 984.13 (2002)	AOAC 963.15 (2002)
Lab 2	AOAC 950.46 (2002)	TECATOR MANUAL (Kjeltec Auto 2300 Analyzer, 10007729, Rev. 1.2 (2002)	MODIFIED based on AOAC 948.15
Lab 3	AOAC 950.46 (2002)	AOAC 981.10 (2000)	AOAC 948.15

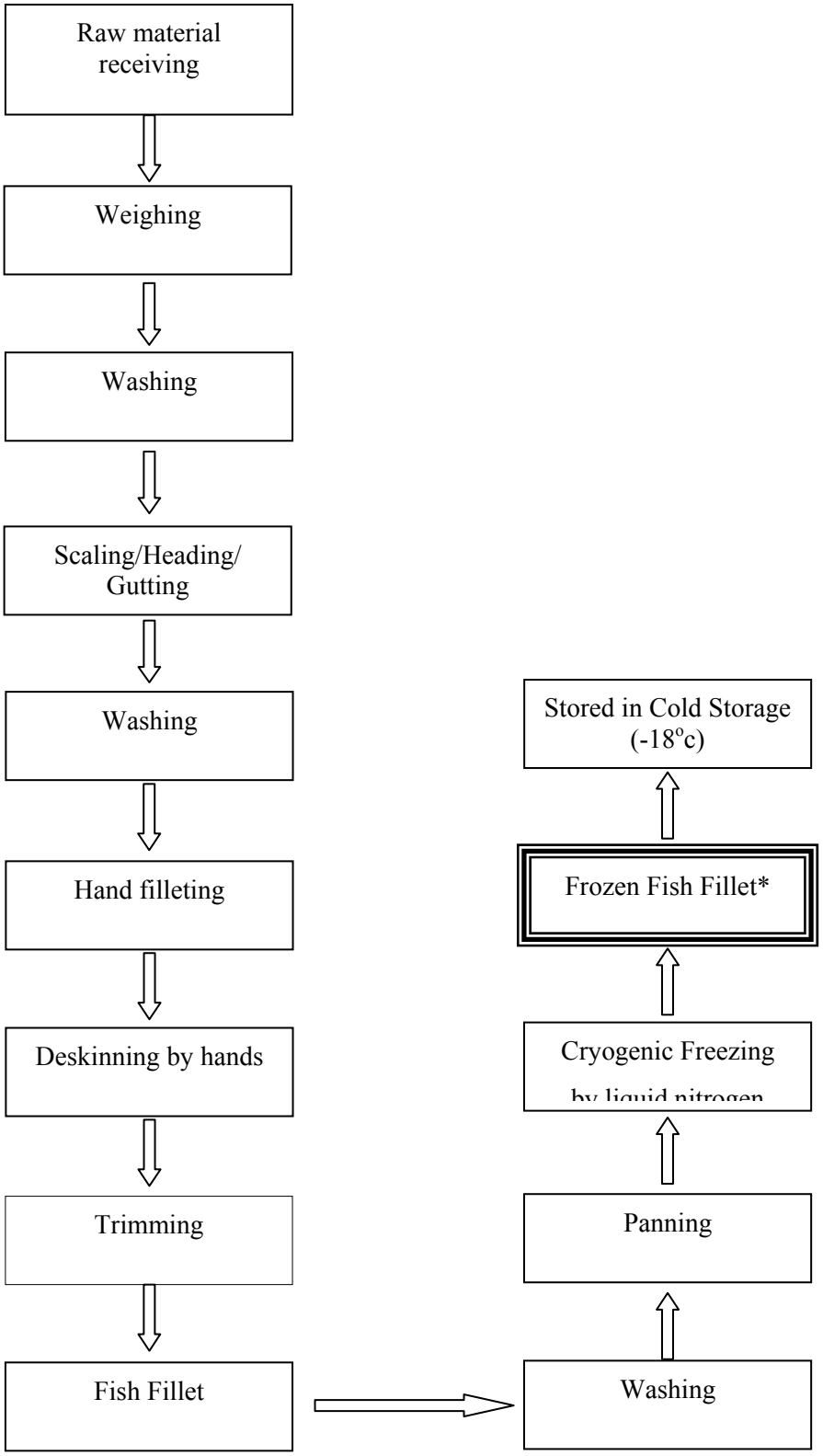
Appendix 4

Analysis of the FAPAS Reference Material (Fish Meat) by 3 Laboratories Used in the Study (Malaysia)

Types of sample and laboratory	FAPAS ref material T 2548			FAPAS ref material T 2541		
	Moisture (g/100g)	Total fat (g/100g)	Nitrogen (g/100g)	Moisture (g/100g)	Total fat (g/100g)	Nitrogen (g/100g)
Given range value	64.57-66.41	11.83-13.20	1.80-1.94	58.12-59.77	3.38-4.80	3.39-3.70
Lab 1	65.3	11.7	1.8	58.7	3.5	3.5
Lab 2	65.90	8.83*	1.84	58.64	3.39	3.46
Lab 3	65.4	11.9	1.91	58.49	3.12	3.7

Appendix 5

Sample preparation of frozen Tilapia Fillet (Thailand)



Remarks: * Samples collected for nitrogen content analyses

Appendix 6

Nitrogen Content Analytical Results (First and Second Studies Combined) (Thailand)

Table 1. Nitrogen content in Tilapia Fillet from earthen ponds culture technique.

Culture Technique	Province	Nitrogen Content
Earthen ponds	Chachoengsao	2.964 [a]
	Prachin Buri	2.927 [a]
	Suphan Buri	2.975 [a]
	Kanchanaburi	2.875 [b]
	Nakhon Pathom	3.012 [a]
	Ratchaburi	2.936 [a]
	Phetchaburi	2.811 [b]
	Nakhon Nayok	2.942 [a]
	Samut Prakan	2.910 [ab]
	Samut Sakhon	2.860 [b]
	Nitrogen (Agv.)	**2.921**

Approx. LSD = 0.05 (smallest difference that is significant at the 5% level)

Table 2. Nitrogen content in Tilapia Fillet from cage culture technique

Culture Technique	Province	Nitrogen Content
Cage culture	Suphan Buri	3.096 [a]
	Kanchanaburi	3.062 [a]
	Nitrogen (Agv.)	**3.079**

Approx. LSD = 0.05 (smallest difference that is significant at the 5% level

Table 3. Nitrogen content in Tilapia Fillet from three central wholesale markets

Central Wholesale Market	Nitrogen Content
Thai Market	2.837 [b]
Khong3 Market	2.976 [a]
Talay Thai Market	2.942 [a]
Nitrogen (Avg.)	**2.918**

Approx. LSD = 0.05 (smallest difference that is significant at the 5% level)

Table 4. Comparison difference of nitrogen content in Tilapia Fillet from two culture technique and the central wholesale market.

Culture techniques	Nitrogen Content
Earthen ponds	2.921[b]
Cage culture	3.079[a]
Central Wholesale Market	2.918[b]

Approx. LSD = 0.05 (smallest difference that is significant at the 5% level)

Appendix 7

Summary of the T-test for the Moisture, Fat and Total Nitrogen in Tilapia from Different Culturing Systems (Malaysia)

Table 1. Mean values for samples from pond, tank, cage culture **including wild fish**

Variable Name	N	Mean	Std dev.
Moisture, g/100g sample (**)	23	81.27	2.34
Crude Fat, g/100g sample (*)	23	2.83	0.55
Nitrogen, g/100g sample (**)	23	**2.52**	0.41
Protein, g/100g (**)	23	15.71	2.53

Table 2. Mean values for samples from pond, tank and cage culture systems

Variable Name	N	Mean	Std dev.
Moisture (**)	14	80.17	2.36
Fat (ns)	14	2.64	0.45
Total Nitrogen (**)	14	**2.76**	0.33
Protein (**) (Nx6.25)	14	17.23	2.06

Remarks: ** = Statistically significant, ** = Highly significant, ns = Not significant*

Appendix 8

Comparison of Nitrogen Content in Tilapia Fillet from Thailand and Malaysia

Country	Sample size	Average Nitrogen Content	Standard deviation
Malaysia	14	2.76	0.33
Thailand	40	2.921	0.119
	Average	**2.879**	**0.174**

-4, II-5 and II-6 are met.