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Effect of Raw and Boiled Mung Bean Seeds (*Vigna radiata* L.) on
Flesh Texture of Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1757)

By

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A report submitted in fulfilment of the requirements for the degree of
Bachelor of Applied Science (Animal Husbandry Science) with
Honours.

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled ‘Effect of Raw and Boiled Mung Bean Seeds (*Vigna radiata* L.) On Flesh Texture of Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1757)’ by Muhammad Hafiy Hamizan Bin Md Rife, matric number F14A0150 has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Animal Husbandry Science) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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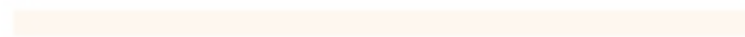
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EFFECT OF RAW AND BOILED MUNG BEAN SEEDS (*Vigna radiata* L.) ON FLESH TEXTURE OF NILE TILAPIA (*Oreochromis niloticus*, Linnaeus 1757)

ABSTRACT

This study was focused on effect of raw and boiled mung bean seeds meal on flesh texture of Nile tilapia (*Oreochromis niloticus*). Flesh texture of fish were divided into two treatments which are Diet 1 contain raw mung bean seeds meal (RwMB) and Diet 2 contain boiled mung bean seeds meal (BoMB) while Control without raw and boiled mung bean seeds meal. Thirty tilapia fingerlings with average weight 4.0 ± 1.0 g were distributed into nine aquarium tanks with 3 replicates for each treatment. Fish were cultured for six weeks and fed at 5 % of fish average body weight. After six weeks of culture, the colour and texture of fish were examined. Texture evaluation in terms of hardness, cohesiveness, gumminess, chewiness and springiness of fillets were measured using Brookfield CT-3 texture analyser. Based on result, value of hardness for flesh of fish tilapia in Control (5871.83 g) was higher compared to Diet 1 (5553.17 g) and Diet 2 (5722.83 g). Thus, there were significantly differences ($p < 0.05$) for hardness value among all treatments. While in terms of colour evaluation, Diet 1 had highest value of lightness which is 36.39 compared to Control (34.65) and Diet 2 (34.17). In this study, Diet 1 was showed the best diet in terms of texture and colour evaluation. In conclusion, different method in preparation ingredients used were effects on flesh texture of Nile tilapia.

Keyword: Tilapia, raw, boiled, mung bean seeds, Brookfield CT-3 texture analyser

KESAN PENGGUNAAN KACANG HIJAU MENTAH AND REBUS (*Vigna radiata* L.) TERHADAP TEKSTUR ISI NIL TILAPIA (*Oreochromis niloticus*, Linnaeus 1757)

ABSTRAK

Kajian ini memfokuskan kesan tepung kacang hijau mentah dan rebus terhadap tekstur isi Nil tilapia (*Oreochromis niloticus*). Tekstur isi ikan telah dibahagikan kepada dua rawatan iaitu Diet 1 mengandungi tepung kacang hijau mentah (RwMB) dan Diet 2 mengandungi tepung kacang hijau rebus (BoMB) sementara Kawalan tanpa tepung kacang hijau mentah dan rebus. Tiga puluh anak tilapia dengan berat purata 4.0 ± 1.0 g telah diagihkan ke dalam sembilan tangki akuarium dengan 3 replika untuk setiap rawatan. Ikan telah dibiak selama enam minggu dan diberi makan 5 % daripada berat badan purata ikan. Selepas enam minggu dibiak, warna dan tekstur ikan diuji. Penilaian tekstur dari segi kekerasan, kepaduan, kelekitan, kekenyalan, keanjalan isi telah diukur menggunakan Brookfield CT-3 penganalisa tekstur. Berdasarkan keputusan, nilai kekerasan isi ikan Kawalan (5871.83 g) lebih tinggi berbanding Diet 1 (5553.17 g) dan Diet 2 (5722.83 g). Justeru, terdapat perbezaan yang ketara ($p < 0.05$) untuk nilai kekerasan di antara semua rawatan. Sementara itu dari segi penilaian warna, Diet 1 mempunyai nilai kecerahan yang paling tinggi iaitu 36.39 berbanding Kawalan (34.65) dan Diet 2 (34.17). Dalam kajian ini, Diet 1 menunjukkan diet yang terbaik dari segi penilaian tekstur dan warna. Kesimpulannya, pelbagai kaedah yang digunakan dalam penyediaan bahan memberi kesan terhadap tekstur isi Nil tilapia.

Kata kunci: Tilapia, mentah, rebus, kacang hijau, Brookfield CT3 penganalisa tekstur

TABLE OF CONTENTS

	PAGE
DECLARATION	ii
ACKNOWLEDGMENT	iii
ABSTRACT	iv
ABSTRAK	v
TABLE OF CONTENTS	vi
LIST OF TABLES	ix
LIST OF FIGURES	xi
LIST OF ABBREVIATION AND SYMBOLS	xii
CHAPTER 1 INTRODUCTION	
1.1 Research Background	1
1.2 Problem Statement	3
1.3 Hypothesis	4
1.4 Objective	4
1.5 Scope of Study	4
1.6 Significance of Study	5
CHAPTER 2 LITERATURE REVIEW	
2.1 Legume Seeds	6
2.2 Nile Tilapia (<i>Oreochromis niloticus</i>)	7
2.3 Microbound diets (MBD)	10
2.4 Use of microbound diets (MBD)	11
2.5 Texture Profile Analysis (TPA)	15
2.6 Texture Evaluation	16
2.6.1 Hardness	19

2.6.2	Springiness	20
2.6.3	Chewiness	20
2.6.4	Gumminess	21
2.7	Sensory Evaluation	21
2.8	Sensory of Colour	22
CHAPTER 3 MATERIALS AND METHODS		
3.1	Proximate Analysis	24
3.1.1	Moisture content	24
3.1.2	Crude Protein	25
3.1.3	Crude Fibre	26
3.1.4	Crude Fat	28
3.1.5	Ash	29
3.2	Nutritional composition	30
3.3	Preparation and formulation of experimental diets	30
3.3.1	Soaking Process	31
3.3.2	Boiling Process	31
3.4	Nile Tilapia (<i>Oreochromis niloticus</i>) Culture	35
3.5	Water quality parameter	36
3.6	Texture analysis	36
3.6.1	Colour Evaluation	36
3.6.2	Texture Evaluation	37
3.7	Statistical analysis	40
CHAPTER 4 RESULT AND DISCUSSIONS		
4.1	Determination of Weight Loss	41
4.2	Texture Evaluation	42

4.3	Colour Evaluation	46
CHAPTER 5 CONCLUSION AND RECOMMENDATION		
5.1	Conclusion	48
5.2	Recommendation	49
REFERENCES		51
APPENDICES		59



LIST OF TABLES

NO.		PAGE
2.1	Composition (% dry weight) of the basal experiment diets used in feeding experiment with <i>Scylla serrata</i> larvae (Holme et al., 2006)	13
2.2	Composition (% dry weight) of experimental MBD in barramundi <i>Lates calcarifer</i> Bloch larvae	14
2.3	Properties of sensory evaluation (Hyldig & Nielsen, 2001)	22
3.1	Nutritional compositions of feedstuffs	30
3.2	Formulation experimental diet of control, raw and boiled mung bean seeds meal (g/100g dry weight)	32
3.3	Water quality parameter based on standard and experimental data from El-Sheriff and El-Feky (2009a) and El-Sheriff and El-Feky (2009b)	36
4.1	Initial, final weight and percentage of weight loss of fish fillets	41
4.2	Hardness values of each sample in different experimental diets	43
4.3	Cohesiveness, springiness, gumminess and chewiness values of each sample in different experimental diets	45
4.4	Lightness (L^*), red chromaticity (a^*), yellow chromaticity (b^*) values of each sample in different experimental diet	44
A.1	One-way ANOVA	59
A.2	Post Hoc Analysis of Duncan Multiple Test for Hardness of Fish Fillets	60
A.3	Post Hoc Analysis of Duncan Multiple Test for Cohesiveness of Fish Fillets	60

A.4	Post Hoc Analysis of Duncan Multiple Test for Springiness of Fish Fillets	60
A.5	Post Hoc Analysis of Duncan Multiple Test for Gumminess of Fish Fillets	61
A.6	Post Hoc Analysis of Duncan Multiple Test for Chewiness of Fish Fillets	61
A.7	Post Hoc Analysis of Duncan Multiple Test for Lightness (L^*) of Fish Fillets	61
A.8	Post Hoc Analysis of Duncan Multiple Test for a^* of Fish Fillets	62
A.9	Post Hoc Analysis of Duncan Multiple Test for b^* of Fish Fillets	62

LIST OF FIGURES

NO.		PAGE
3.1	AMB diet preparation	33
3.2	Mixing all the ingredients on hot plate	34
3.3	Preparation of AMB before oven-dried	34
3.4	Fish killed by pitting method	38
3.5	Filleting of fish process	38
3.6	Fillets weighed process	39
3.7	Brookfield CT-3 texture analyzer was used to analyse	39
A.10	Moisture Analyser MX-50 for moisture analysis	63
A.11	Kjeldahl distillation system Vapodest 30s for crude protein analysis	63
A.12	Samples turn to greyish pink after titration process in crude protein analysis	64
A.13	Foss Soxtec 2055 System for crude fat analysis	64
A.14	<i>Oreochromis niloticus</i> for texture	65

LIST OF ABBREVIATION AND SYMBOLS

% day ⁻¹	percentage per day
%	percentage
°C	degree Celcius
<i>a</i> *	red chromaticity
am	morning
<i>b</i> *	yellow chromaticity
BL	body length
BoMB	boiled mung bean
BW	body weight
CF	crude fibre
CFM	<i>Cassia fistula</i> meal
CP	crude protein
DO	dissolved oxygen
EFA	Essential Fatty Acid
FCR	Feed Conversion Ratio
FM	fishmeal
g	gram
kg	kilogram
L	litres
<i>L</i> *	Lightness
MBD	microbound diets
mg L ⁻¹	milligram per litre
mJ	milliJoule
mm	millimeter
MPNT	muscle protein of Nile tilapia
P	phosphorus
pH	potential of hydrogen
pm	evening
ppm	part per million

pps	pulse per second
ppt	part per thousand
RLSM	raw linseed meal
ROLSM	roasted linseed meal
RoMB	raw mung bean seeds
SBM	soybean meal
SGR	specific growth rate
TPA	texture profile analysis
w/v	weight/volume
WG	weight gain
WHC	water holding capacity
WVP	water vapour permeability



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CHAPTER 1

INTRODUCTION

1.1 Research Background

Plant-based materials are other sources that could replace the existing protein sources for feed formulation such as fish and soybean meal in. In this study did by Francis, Makkar, and Becker (2001), mung bean seeds had been used to partially replacing soybean meal. Mung bean seeds (*Vigna radiata* L.) is one the legume seeds that contain anti-nutrients such as tannins, phytic acid where may affect digestion of fish and protein digestibility. Mung bean seeds contain 23.7 % crude protein, 1.9 % crude fibre, minerals, vitamins and essential amino acid (Ganzon-naret, 2014). This study was used Nile tilapia as experimental subject because its ability to tolerate against adverse environment and fast growth (El-Sheriff & El-Feky, 2009a).

Texture analysis was used to evaluate texture content in terms of rough, smooth and silky of food. It can be used in many applications such as in remote sensing, automatic examination and texture segmentation (Essex, 1969). Based on Olafsdottir et al. (2004), processing, storage and cooking would influence texture due to reduction of attribute of the connective tissue.

Food quality is an important component for texture analysis. Improvement of manufactured goods, monitoring manufacturing process and assessment on product value are necessary in food industry. Quality of foods is assigned with the sense of feel either by fingers and mouth. Examples of evaluations are hardness, tenderness, and others. Measurements of food texture had related with analysis of food structure (Essex, 1969).

According to Modi, Mahendrakar, Rao, and Sachindre (2003) stated that, cost of production in meat industry can be minimized by substituting extenders, fillers and binders machine while enhanced nutritional value and sensory qualities of final products. For instance, soya flour with high protein content is used as filler in meat products. Shear and sensory evaluation were used to describe the tenderness of texture (Ahmed, Koburger, & Mendenhall, 1972). Previous study by Chu and Sterling (1970), Mao and Sterling (1970a) and Mao and Sterling (1970b) stated that, texture of finfish muscle could be determined by sensory and chemical evaluation.

Sensory methods could provide information and feedback to reflect consumer preference. This information is useful to provide information and help to improved and commercialize the product. Other than that, freshness quality of fish during the storage in terms of assessing aroma, sense and flavour were determined by chemical and physical methods. Mostly, sensory method is remained as satisfactory method in order to evaluate freshness of fish. Sensory evaluation could be conducted to ensure the result showed good agreement with the instrumental tests in assessing freshness of fish (Alasalvar et al., 2001). Criteria of fish were clear observed and were important in order to meet consumer perception in terms of product flavor (Reineccius, 1991).

Finger method was used to evaluate texture of fish. This technique was practiced by pressing finger on the fish fillet and hardness was evaluated (Alasalvar et al., 2001). Based on Papadopoulos, Chouliara, Badeka, Savvaidis, and Kontominas (2003), sensory method was easy, fast and able to provide quality information in time but depends on subjective evaluation for the person who is conducted the measurements. Mechanical food testing equipment was used to evaluate texture of raw fish fillets. According to Alasalvar et al. (2001), main techniques that applied are compression, puncture, shear and tensile stress. Fresh fish texture was recommended to be examined by shearing force and compression techniques. Springiness and hardness were primary components when to measured fish texture.

1.2 Problem Statement

Mung bean seeds contain high protein sources and anti-nutrients which required pre- treatment methods which are soaking, de-hulling and boiling process for eliminate anti nutrients. Presence of carotenoid in legume seeds help to improve colour pigmentation of fish.

Recently, tilapias were becoming more popular among the consumers due to the reasonable price and good quality animal protein sources. Price and demand for tilapia for product had increased due to consumer preference including reasonable price of white flesh that can be replaced other expensive white-fish species. Consumers especially in North American were more preferred flesh with white fillet. Tilapia species such as *Oreochromis niloticus* and *Oreochromis aureus* had flesh with light grey. The study was focused on the effect of raw and boiled mung beans on the flesh texture of Nile tilapia.

1.3 Hypothesis

H_0 = Different preparation of ingredients used in feed does not effect on texture of Nile tilapia

H_A = Different preparation of ingredients used in feed effect on texture of Nile tilapia

1.4 Objective

1. To investigate the flesh texture of Nile tilapia consuming raw and boiled mung bean seeds.

1.5 Scope of Study

Scope of study was focused on commercial feed was used as a control feed. Mung bean seeds as ingredient that was replaced the soybean meal in fish diets. Evaluation of tilapia meat texture was applied after consuming mung bean seeds from different preparation method.



1.6 Significance of Study

Improvement of flesh texture could attract consumer to purchase the products as consumer more preferred white fillets compared to light grey fillets which commonly found in Nile tilapia.

Application of machines is used to determine values of sensory and colour evaluation. Values for each evaluation are necessary in order to differentiate which sample obtained the best result. This study could improve the flesh texture of Nile tilapia by consuming raw and boiled mung bean seeds in their diets instead of increasing protein intake.

CHAPTER 2

LITERATURE REVIEW

2.1 Legume Seeds

In economical diet, legumes act as protein, carbohydrates, soluble and insoluble dietary fibre sources. Hence, it is also better foods which is increased the diet fibre consumption (Anwar, Latif, Przbylski, Sultana, & Ashraf, 2007). The legumes were separated through two categorized. Firstly, by oilseeds like soybeans and peanuts while the other is grain legumes that generally included beans (Venter & Eyssen, 2001).

According to Anwar et al. (2007), legumes are rich in nutrient composition which involved of vegetable protein, dietary fibre, starch, oligosaccharides, minerals and phytochemicals such as isoflavones in soy. Legumes help in minimize risk of osteoporosis and excretion of urinary calcium through replacing animal protein with vegetable. Mineral in legumes was used to reduce the risk of hypertension and most of beans contain low fat.

According to Francis et al. (2001), legumes also contain anti-nutrients such as presence of tannins that can reduce the absorption of vitamin B₁₂. The addition of 2% quebracho tannin powder in carp diet showed positive effect on growth. Inhibitory action of tannins on amylase was removed through interaction between tannins and lectins.

Methods on removal the condensed tannins involved de-hulling the seeds where outer layer of seeds that rich with tannin are removed. Fermentation of lactic acid through autoclaving and alkali treatment helps in reduction of tannin content in seed meal from 20 to 10 kg. Methods such as dry and wet heating, extracting with water and addition of supplements were successfully used in order to decrease anti-nutrients levels in feed. For instance, tilapia species are more tolerant towards increased of anti-nutrients in feed compared to carp. Plant-derived materials had potential to be one of ingredients in the feed even though contain more than one anti-nutrient. Therefore, more than two techniques were required to remove anti-nutrients due to sensitivity of fish towards tannins (Francis et al., 2001).

2.2 Nile Tilapia (*Oreochromis niloticus*)

Species of tilapia were cultured because of fast growth, able to adapt with various environments and easy to breed (El-Sherif & El-Feky, 2009a). The favorable temperature for Nile tilapia at 27±1 °C and pH level at 7 to 8. Stocking density for Nile tilapia species was important as it may affect on fish growth and health.

Previous study by El-Sayed (2004) stated that, in order to obtain maximum performance of tilapia during larval stages, protein requirement is high which between 35 to 50 % and decrease when increasing in fish size. Protein requirement for tilapia juvenile is from 30 to 40 % while adult required 20 to 30 % protein in diets. Tilapia broodstock required 35 to 45 % protein for reproduction, spawning efficiency and larval growth and survival.

Oreochromis niloticus L. juvenile that stocked at 0.5 fish/L had the best average body weight of 14.40 g compared to fish stocked at 2 fish/L had lowest average body weight of 9.15 g due to lowest exchange rate at 5 times per hour (Yousif, 2002). Tilapia fish was able to survive in so low level of dissolved oxygen (DO) and actually it can tolerate dissolved oxygen (DO) levels range between 0.1 to 0.5 mg/L (Magid & Babiker, 1975).

According to Abdel-Tawwab, Hagra, Elbaghdady and Monier (2015), *O. niloticus* (L.) showed that fish under normal DO conditions supplied with four air stones had better growth and had been compared with other treatments due to better feed consumption and digestibility of nutrients. Growth and feed utilization of fish tilapia due to shortage in oxygen availability for fish to growth are caused by the low level of DO level. Good efficiency was shown by tilapia fish when it provide with an adequate dissolved oxygen in water like fish to growth and feed efficiency have effect with DO availability. Improvements of fish performance and health need adequate and should be maintained the dissolved oxygen.

Previous study by El-Sayed (2004) reported a maximum performance of tilapia fish during larval stages, the proteins needed by fish in between 35 until 50 % and was decreased if the size of fish become raised on its body. For juvenile of tilapia, the protein requirement was from 30 until 40 % but in adult it needs 20 until 30 % of protein compositions diet. According to the Ahmad, Abdel-Tawwab and Khattab (2004), in range 5 to 25 g of tilapia fish required 25 to 35 % protein but in range of 1 to 5 g of tilapia needs 30 to 40 % of protein. Highest growth rate of *O. niloticus* had shown with initial weight from 4 to 5 g for dietary protein content which content 33.32 % of dry matter (Ogunji & Wirth, 2000).

In Yousif (2002) proved that, juvenile *O. niloticus* that was stocked at 0.5 fish/L had the best average of body weight with 14.40 g compared to fish which was stocked at 2 fish/L was had the less average body weight with 9.15 g. Based on the El-Sayed (2004), maximum growth performance can be achieved when fish dietary lipid at range between 10 to 15 %. The fish oil contain diet rich in n-3 essential fatty acid (EFA) that had been fed on was decreased compared to other fish that utilized with fed on soybean oil or maize oil was called as vegetable oil which contain rich in n-6 EFA. Tilapia fish known as herbivores and able to consume dietary carbohydrates in high efficient compared to carnivorous fish. There were the ingredients which used in carbohydrates like wheat bran, maize and rice bran. Tilapia also able to utilize floating and sinking pellets in efficient way (El-Sayed, 2004). In daily feeding frequency of tilapia was affected by carbohydrate that consume by tilapia. Based on the Tung and Shiau (1991), increasing in feeding frequency was able helping in improvements of carbohydrate utilization, growth rate and protein requirements.

2.3 Microbound diet (MBD)

Artificial diet for barramundi larvae from research development was focused on microbound diet (MBD) which contained dietary ingredients that held within the binder. Artificial diets of fish larvae are suitable in form of MBD as it can be made within acute size range. It also flexible in the nature of their dietary composition and prepared with easy techniques. Microbound diet also lack of capsule wall which help in improving larvae digestion (Partridge & Southgate, 1999).

The formulation of aquaculture feeds adopted in different types of binder. Types of binder and strength of binder were acts as vital roles in identifying the attractiveness and digestibility of MBD. For instance, it can be limited from the tightly bound diets in leaching of attractant molecules. Attractiveness and digestibility of MBD towards aquatic were depends on edibility of binder as high concentration of binder would reduced in diet digestibility. Hence, diets that contained less binder might be dissolved in water and thus, resulted in poor water quality and important dietary nutrients were lost (Meyers et al., 1972).

Partridge and Southgate (1999) did a study on the effects of binder type, combination of different binder and binder concentration on the rates of ingestion and assimilation of MBD by barramundi larvae. It also study on the effect of binder concentration towards nutrient leaching from MBD. Ingestion, assimilation and discharge were losses from MBD that contain ¹⁴C-labelled rotifers. Microbound die that used alginate and zein as binder had high ingestion with low assimilation efficiency (AE) whereas gelatine- and carragenan-bound MBDs were well assimilated but ingested to a lesser degree (Partridge & Southgate, 1999).

According to the Genodepa, Zeng and Southgate (2007), various binder showed different leaching rate in the *Scylla serrata* larvae feeding. Therefore, dietary nutrients that discharged from microbound diets would impact on water quality. It was suggested to use binder with low leaching rates such as zein that commonly used and available binder for MBD developed for *S. serrata* larvae. Water stability, nutrient leaching and settlement rate of MBD particles are factor that are caused by binders. MBD particles was successfully used in study the nutritional requirements of larvae. Fish larvae that used in study was proved and shown ingestion of MBD particles significantly caused by preparation and type of binder used (Partridge & Southgate, 1999). The effect from nutrient leaching of MBD particles of various binders has been combined the information that correlated in ingestion from same micro bound diets are useful in future development of MBD.

The palatability of the diet had been expressed in ingestion process and was determined by nutrients are suitable for digestion (Lee & Meyers, 1996). The results in based on research by Genodepa, Southgate and Zeng (2004) shown the alginate-bound MBD was well accepted by the whole stages of *S. serrata* larvae as ingestion of MBD had compare with dry weight of live *Artemia* that ingested by larvae.

2.4 Use of Microbound diets

The formulation of microparticulate diets using binder was made from gelling mixture of dietary ingredients with binder. Then followed by drying, grinding and sieving the mixture in order to obtain desired size (Langdon, 2003). According to Holme, Zeng and Southgate (2006) study on the use of microbound diets for larval culture of the mud crab *Scylla serrata* and microbound diets was prepared and

handling by combine all the dry and moist ingredients in separate containers. After that, dry and moist ingredients were mixed well before binder (zein) was dissolved in 70 % alcohol and homogenized with diet mixture. Homogenous paste were spread thinly and uniformly in aluminium dishes and then dried at 50 °C for 48 hours.

Next, the dry diets were ground by using mortar and pestle and sieve in order to obtain desirable size (Holme et al., 2006). The composition (% dry weight) of the basal experiment diets used in feeding experiments with *Scylla serrata* larvae were presented in Table 2.1.

Table 2.1: Composition (% dry weight) of the basal experiment diets used in feeding experiment with *Scylla serrata* larvae (Holme et al., 2006)

Ingredients	Dry Weight (%)
Squid meal	39.7
Dried rotifer	39.7
Fish oil	5
Corn oil	1
Lecithin	3
Cholestrol	1
Dibasic calcium phosphate (DCP)	0.6
Vitamin mix	4
Mineral mix	3
Zein (Binder)	3
Total	100

According to the Partridge and Southgate (1999), the microbound diet that prepared was adding dry ingredients and mixing with oil and lecithin. All binders such as agar (Swallow Globe Brand), carrageenan, alginate and gelatin except zein were dissolved in hot water before mix with other ingredients. Diets were dried in the oven at 45 °C for 72 hours and ground in order to obtain desirable particle size. Composition (% dry weight) of experimental MBD in barramundi *Lates calcarifer* Bloch larvae was presented in Table 2.2.

Table 2.2: Composition (% dry weight) of experimental MBD in barramundi *Lates calcarifer* Bloch larvae

Ingredient	Radioactive diet	Non-radioactive diet
¹⁴ C-labelled rotifers	50	-
Squid powder	36.25	86.25
Fish oil	4	4
Lechitin	2	2
Pancreatin	0.05	0.05
Choline chloride	1	1
CaHPO4.2H2O	0.6	0.6
Mineral mix	1	1
Vitamin mix	1	1
Vitamin C	1	1
Vitamin E	0.1	0.1
Binder	3	3
Total	100	100

Other than that, method in preparation of microbound diets by Gallardo et al. (2002), dry ingredients consist of anchovy (*Engraulis mordax*) (*Loligo spp*), squid and shrimp powder were mixed for 15 minutes. Next, oil and water was added into mixed ingredients and continuous mixing for another 15 minutes until paste is formed before dried at 60 °C. Based on Kolkovski, Curnow, and King (2010), ingredients were blended using a mechanical mixer. Then, binder (gelatine) was dissolved in hot water

at (80 °C) and added into mixed ingredients. Additional cold water was added in order to obtain uniform paste. Next, paste was extruded and dried in the oven at 45 °C for 48 hours.

2.5 Texture Profile Analysis (TPA)

In popularizing food product, the composition and texture quality play a vital role. Based on Mehinagic et al. (2003), texture and flavour were appeared to one of the most important characteristics for consumer. TPA machine was including application of controlled force to the product and recording its response with time. It was widely used to evaluate texture of various protein gels and food products. Texture Profile Analysis (TPA) also useful in gel texture analysis because parameters of textural were obtained from TPA curves that connected with sensory evaluation of textural parameters (Sanderson, 1990).

Based on Morkore and Einen (2003), TPA was related with near simulated condition of oral mastication. Force was applied towards food while oral mastication was obtained from TPA in force-time curves. Different parameters were obtained after conducted TPA such as fracturability, chewiness, cohesiveness, springiness, resilience, gumminess, hardness and adhesiveness. Instrumental analysis did not fully imitate the overall experience of texture as it did not measures specific textural properties. So, it is critical to identify measurements which related with sensory attributes in order to attract processing industry and consumers (Morkore & Einen, 2003).

Based on Cardoso, Mendes, and Nunes (2009) and Vácha, Stejskal, Vejsada, Kouřil, and Hlaváč (2013), force generated time curved was used from TPA in order to quantify a number of textural properties that correlate well with data from sensory evaluation. Vácha et al. (2013), did evaluate the differences in texture profile of tench flesh. TPA was focused on hardness, cohesiveness, springiness, gumminess and chewiness performed with texture analyser. In comparison, the flesh male tench in both groups was harder, gummier and chewy compared with females. Cohesiveness was lower from both groups of females. Based on results, it proven that the flesh fish raised intensively was springier, firm, more cohesive and gummier. Thus, it is able to attract consumers.

In texture measurements, suitable compression plate was used to analyse fish fillet. Tension, compression cycle and texture tests were provided in order to generate true 3-4 dimensional product analysis such as force, time and distance (Vácha et al., 2013).

2.6 Texture Evaluation

Hyldig and Nielsen (2001) stated that, texture is indicated that sensory evaluation is complex because all impression about the food is involved with human sense likes finger, teeth and tongue. Texture evaluation is by touch, mouth and other parts of body will be involved but chemical senses of taste and smell are not be related. Hence, the challenges in sensory perception of texture remain because the result of the perception that breakdown of food structure while eating processing. Therefore, the sensory method is always a challenge and poor result such as high variability that lead to the inappropriate conclusion when inappropriate methods or

approaches are used. This study also state other scientific paper said cooked samples are performed by sensory evaluation but raw muscle are performed by instrumental measurements.

Borderias, Lamua and Tejada (1983) stated that, subsequent investigations were necessary in order to create relationship between instrumental parameter and sensory criteria. Sensory method used to determine various sensory properties of the fish correlated with instrumental. Based on Jiang, Wang, Santen and Chappell (2008), thickness and shape of fillets were analysed using sampling techniques. Identification force (g) and shear force (g) of teeth method were measured at different shape levels of four myomere cone bands on the fillets. It also can be used to measure texture properties of catfish fillet. These sampling techniques also used in irregular fillet shapes. Hence, the different texture characteristic also has different fish species. In previous survey, seventeen species of North Atlantic fish were used to evaluate on hardness, flakiness, fibrousness, moistness and oily mouth coating and appearance (Hyldig & Nielsen, 2001).

According to Jiang et al. (2008), freshness of sample is critical point as to ensure good quality. Evaluation of catfish freshness was determined by chemical and physical measurements technique. Smoked catfish in odour and appearance were influenced by freshness of samples. The panellists scored are odour, flavor and acceptance of consumers decreased as increased of storage period. This indicate that time of storage affect on texture of fish (Jiang et al., 2008). Other study by Papadopoulos, Chouliara, Badeka, Savvaidis, and Kontominas (2003) said that, sea bream (*Sparus aurata*) and channel catfish fillets showed similar result which show indentation force of the fillets decreased as increased storage time after harvesting.

The best fitting structure on raw and smoked fillet were performed by finger method. Smoked fillets was evaluated by finger method acts as indication by non-significant interaction. Finger method were increased gradually when indentation force on raw catfish fillet along the thickness. It is not suitable to used finger and tooth method due to small contact area between ball tip and fillet tip muscle (Jiang et al., 2008).

Previous study by Borderias et al. (1983) stated that, main characteristics, response towards properties of samples was the main characteristics that should be evaluated for texture analysis are firmness, wateriness and others. In firmness, sample was compress between the molars or between the tongue and palate, thus force was required. Based on scale 1: soft and fewer consistent from fillet trout while scale 7 is more firm and consistent. In term of wateriness, the water was released after compression that is the initial response and juiciness was distinguished. The evaluation of wateriness is based on scale 1 is less water release while scale 7 which more water is released from the fillet.

Elasticity is the ability of fillet to be chewing by consumer that was determined by compressing slightly the substance between the molars or between the tongue and palate. This scale 1 is less elastic compared with scale 7 which more elastic to fillet. The cohesiveness is the amount of sample that can be deformed before it ruptures with scale 1 is less cohesive compared with scale 7 is more cohesive to fillet. So, for the secondary characteristics, the response after chewing fillet for a few minute. The ability of the fillet to be breakdown indicate as hardness. In scale 1, the fillet is tenderer and scale 7, the trout of fillet is tougher. For A instron model 1140 texturometer at room temperature (20 ± 2 °C) was used in order to evaluate and

determine firmness, elasticity and cohesiveness. Puncture analysis was defined as maximum force that applied when driving a flat-ended puncture test. This instrumental test were applied for raw and cooked sample in order to determine texture quality (Borderias et al., 1983). Based on Wang, Zhang, and Mujumdar (2011) stated that, each texture analysis were performed by two compression cycles and was used texture analyser to test texture.

Hyldig and Nielsen (2001) stated that, composition of fish muscle was influenced by diet given to fish. Firmness of texture did not influenced by fat content in feed. Farmed salmon, rainbow trout and sea bream that feed with high percentage of fat had high fat content in body were compared with same species feed with low percentage of fat showed slightly similar firmness values.

2.6.1 Hardness

According to Szczesniak (1963) and Szczesniak, Brandt, and Friedman (1963), hardness scale also was judge as organoleptically which is the force that required penetrating the sample with the molar teeth, milestone method in laboratory was called by sensory evaluation system. It was device followed using the results preliminary sensory evaluation and a modification of the standard scale. Hardness also was defined that the maximum compression of resistance during the first compression and rated on a nine point scale. The evaluation was restricted for solid and semisolids because human perception is limited for sample as it can be confined between the teeth.

2.6.2 Springiness

Springiness is a bouncing property of sample through several consecutive bites. Springiness also known as the degree which the sample rapidly back to its original form after partial deformation between the molar teeth (Lee, Rha, & Imoto, 1978). Study by Wang et al. (2011) stated that, the results of springiness had significantly reduced ($p < 0.05$) when the hardness significantly rose in increasing temperature. Values of springiness and hardness of fresh samples that were rehydrated changed noticeably ($p < 0.05$). It was affected dehydration caused by tenderness loss and characteristic of texture. The decreased in springiness due to the conformational in myofibrillar proteins was changed caused by heat. Springiness and hardness in samples had significantly effect by drying temperature.

2.6.3 Chewiness

The definition of chewiness was force that required chewing solid food product before it was prepared for swallowing. It was correlated with primary parameters such as hardness, cohesiveness and elasticity (Szczesniak et al., 1963).

Chewiness was a number of chews required to swallow a certain amount of sample. It is also was rated the scale by the length of the time in second which required to masticate the sample at a constant rate of force application and at the rate of one chew per second in order to minimize it for a consistency satisfied and suitable in swallowing. Hardness was rated chewiness on a seven point scale and was surround the primary parameters as cohesiveness, elasticity and hardness

(Szczesniak et al., 1963). Based on Lee, Rha and Imoto (1978) stated that, chewiness scale was identified by the number of chews counted required to swallow the sample.

2.6.4 Gumminess

Gumminess scale was secondary factor and product with low level of hardness and high level of cohesiveness. It was refer to semisolid materials and organoleptically and explained as denseness which persist throughout mastication. It was indicated on gumminess that are rated on five point scale was used (Szczesniak et al., 1963).

2.7 Sensory evaluation

The properties of sensory evaluation can be classified into firmness, hardness, springiness and adhesiveness. Sensory evaluation can be evaluated as presented in Table 2.3.

Table 2.3: Properties of sensory evaluation (Hyldig & Nielsen, 2001)

Property	Definition
Firmness	The ability to bite of the samples by the front teeth.
Hardness	Resistance to breakdown or chewing the samples before swallowing
Elasticity/Springiness	The ability for the samples to return into its original shape
Adhesiveness	The rates of fish sample stick in the mouth surface.

2.8 Sensory of colour

According to Ginés, Valdimarsdottir, Sveinsdottir, and Thorarensen (2004), raw texture measurements of some fillets were used for calorimeter determinations. Minolta Chroma Meter CR-300 (Minolta Osaka Japan) have been used of the same fillets, which followed by system of CIE (1976). For example, L^* describing lightness ($L^* = 0$ for black, $L^* = 100$ as white), a^* describing as intensity in yellow ($b^* > 0$) and the Chroma $(a^{*2} + b^{*2})^{1/2}$ being the position in colour space. Above the lateral line, the colour was measured at three positions that closed to head, in the middle of the fillet and close to tail. In the illumination system, rotation of (90°) between triplicate measurements per position were applied.

Based on Rahman et al. (2012), halwa samples were used to evaluate colour by using colour meter at room temperature (Minolta CR-310, Minolta, Japan). It was calibrated that equipped with a white standard calibration plate provided by the manufacturer. Halwa samples were placed on flat plate and tip of the measuring head was pointed at the sample for measurement. Each value was the mean of six measurements. The results were expressed in Hunter L, a, b values, in which L is the lightness or darkness (black, L=0; white, L=100), +a is the redness, -a is the greenness, +b is the yellowness, and -b is the blueness. The color (CL) was also calculated as $CL = (L \cdot b) / a$.

CHAPTER 3

MATERIALS AND METHODS

3.1 Proximate analysis

Proximate analysis for feedstuffs (soybean meal, fish meal, rice bran, tapioca, raw and boiled mung bean seeds meal) was conducted at Animal Nutrition Laboratory, Faculty of Veterinary Medicine in University Malaysia Kelantan (UMK), Pengkalan Chepa Campus, Kota Bharu, Kelantan.

3.1.1 Moisture content

Moisture Analyser MX-50 (A&D Company, Limited) was used to determine moisture content. It was used heating and drying method in which able to compare weight before and after heating and drying of sample. Five grams of samples was weighed and placed on the sample pan. Then, button start was pressed in order to run the analysis. During heating process, sample was heated for a period of time at 160 °C in order to dry the sample and evaporate its water. After few minutes, value of moisture content of sample could appear on the screen of machine analyser. The data was recorded.

3.1.2 Crude protein

Kjeldahl method was used in determining crude protein. It was determined that by Kjeldahl nitrogen was multiplied by 6.25 which developed by Johan Kjeldahl in 1883. In this method was divided into three stages which are digestion, distillation and titration.

Therefore, the sample with one gram was weighed into a Kjeldahl flask. Then, two pieces of Kjeldahl tablet and 12 mL of sulphuric acid were added into the flask. Kjeldahl flask was then digested in the digester KJELDATHERM Block Heating System in 1 hour 30 minutes. After digestion process, the sample has been cooled in the fume hood for one hour before proceed with the next process which is distillation process. Next, Kjeldahl distillation system Vapodest 30s was warm up for ten minutes. 30 mL of receiver in conical flask containing 4 % of boric acid, 1 mL of bromocressol green, 0.7 mL of methyl red and 100 mL of distilled water was correlated with distillation unit. After distillation process has been completed, sample in conical flask was titrated with 0.1 mL of hydrochloric acid (HCl) until the sample turns greyish pink.

Crude protein was calculated as below (Kwikiriza, Tibenda, Wadunde, Abaho, & Ondhoro, 2016):

$$\text{Kjeldahl Nitrogen, \%} = \frac{(V_s - V_B) \times M \times 14.01}{W \times 10}$$

$$\text{Crude protein, \%} = \% \text{ Kjeldahl Nitrogen} \times F (6.25)$$

Where,

V_s = volume (mL) of standardized acid used to titrate a test

V_B = volume (mL) of standardized acid used to titrate reagent blank

M = molarity of standard HCl

14.01 = atomic weight of N

W = weight (g) of sample

10 = factor to convert mg/g to percent

F = factor to convert N to protein; 6.25

3.1.3 Crude fibre

Crude fibre was analysed using Manual Fiberbag System (Gerhardt Analytical Systems). Before analyse, it is need to start with drying fibre bag for one hour at 105 °C. Then, let it cool in the desiccator for 30 minutes. The weight of fibre bag named as M_1 . One gram of sample was weighed into the fibre bag and named as M_2 . Glass spacer was inserted into the fibre bag in order to make sure good flow through of the reagents. Next, the fibre bag the sample containing sample was washed in petroleum ether 40/60 (cold) in three times and dried for two minutes. Only six fibre bags can be put into a sample carousel. Then, the beaker was place on the hot plate.

The fibre bag should undergoes two washing phase which for the first phase by boiling in 360 mL sulphuric acid for 30 minutes after the acid has been starting to boiled then, three times washing with hot water in order to remove acids and for the second phase is by boiling with 360 mL sodium hydroxide solution for 30 minutes after alkali was starting to boil. Then, it needs three times washing with hot water in order to remove alkali. The fibre bag was removed from carousel and dried for 4 hours at 105 °C before it has been placed in the desiccator for 30 minutes.

Next step is preparation of crucible for incineration has been heated in the oven at 600 °C for 30 minutes then placed in the drying chamber at 105 °C for 30 minutes until to cool it off. Next, crucible was placed in the desiccator for 30 minutes and weighed of crucible with fibre bag was recorded and named as M_3 .

In the last step, fibre bag had been incinerated for 4 hours at 600 °C then placed in the drying chamber at 105 °C in 30 minutes. Next, fibre bag was placed in the desiccator for 30 minutes and weighed of crucible containing ash was recorded and named as M_4 . The formula in determining crude fibre as below and calculated by using the software:

$$\text{Crude fibre, \%} = \frac{(M_3 - M_1 - M_4) - (B_3 - B_1 - B_4)}{M_2} \times 100$$

Where,

M_1 = Weight of fibre bag (g)

M_2 = Initial sample weight (g)

M_3 = Incinerating crucible and dried fibre bag after digestion (g)

M_4 = Incinerating crucible and ash (g)

B_1 = Blank value of empty fibre bag (g)

B_2 = Incinerating crucible and dried fibre bag blank value after digestion (g)

B_3 = Incinerating crucible and ash blank value (g)

3.1.5 Crude fat

The initial weights for aluminium cups were recorded. Then, the sample with one gram was weighed in a fine powder into thimble. The thimble and sample was placed into the extraction units by attached them to the magnets. Eighty millilitre of petroleum ether as extracting solvent was added in the aluminium cups and placed into the extraction unit with the cup holder. This was fit together in the Foss Soxtec 2055 system. The sample contained in the thimble was extracted in the extracting solvent by boiling at 135 °C for 15 minutes and rising, recovery and pre-drying for 20, 10 and 2 minutes respectively. Then, aluminium cups were dried in oven at 105 °C for 30 minutes and then, cool in the desiccator at room temperature for 20 minutes. The final weights of aluminium cups were recorded. Fat content was calculated as below (Kwikiriza et al., 2016):

Percentage of fat =

$$\frac{\text{Final Weight of Aluminium Cup (g)} - \text{Initial Weight of Aluminium Cup (g)}}{\text{Sample weight (g)}} \times 100$$

3.1.6 Ash

Based on the method did by the Thiex, Novotny and Crawford (2012), 2 g of sample was weighed and placed it into porcelain crucible. All weights of sample were recorded and tabulated. Then, the porcelain crucible which containing sample was placed into temperature-controlled furnace. Furnace need for more than one hour in order to rise the temperature until 550 °C. Temperature at 550±10 °C was held or stay in range three hours. Next, furnace was allowed to cool to below than 200 °C after the three hours. Then, porcelain crucible were transferred into desiccator, cool and weight within one hour. The final weights of porcelain crucibles were recorded and tabulated. Ash content was calculated as below:

Percentage of ash, % =

$$\frac{\text{Weight of Crucible with sample after ashing (g)} - \text{Initial Weight of Crucible (g)}}{\text{Weight of Sample (g)}} \times 100$$

3.2 Nutritional composition

Nutritional composition of feedstuffs was presented in Table 3.1.

Table 3.1: Nutritional composition of feedstuffs

Components	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash (%)	Moisture (%)
Fish meal	27.35	2.72	0.28	64.52	7.07
Soybean meal	52.55	0.16	3.95	9.95	14.60
Tapioca	2.31	5.49	0.24	0.24	13.36
Rice bran	19.48	10.70	-	11.85	15.03
Raw mung bean meal	28.20	0.72	8.07	7.65	8.55
Boiled mung bean meal	26.14	1.30	5.87	11.87	8.77

3.3 Preparation and formulation of experimental diets

The material used in this study was mung bean seeds (*Vigna radiata* L.) obtained from the local market in Kelantan. The experiment was conducted at Animal Laboratory, Faculty of Agro Based Industry, University Malaysia Kelantan Campus

Jeli, Kelantan. Referring to Ganzon-naret (2014) and Mubarak (2005), raw mung beans were washed in order to remove dirt and other impurities.

3.3.1 Soaking process

Mung bean seeds were soaked in distilled water for 12 h at room temperature (~ 25 °C). Then, mung bean seeds was drained and rinsed three times with distilled water. After 20 hours of drying, rinsed 1/2 of mung bean seeds were mashed, dried in the oven and ground to fine powder passing through sieve.

3.3.2 Boiling process

Another 1/2 of rinsed mung bean seeds were boiled in tap water (100 °C) at ratio of 1:10 (w/v) on portable stove until they become soft approximately 60 to 90 minutes. Boiled mung bean seeds also were mashed, dried in the oven and ground to fine powder passing through sieve.

All the powdered of mung bean meal from raw and boiled process were stored in the closed container for further analysis.

There were three experimental diets which are controls as Diet 1 without raw and boiled mung bean seeds meals, Diet 2 and Diet 3 contained raw and boiled mung bean seed meals respectively. The formulation of experiment diets was presented in Table 3.2.

Table 3.2: Formulation experimental diet of control, raw and boiled mung bean seeds meal (g/100g dry weight)

Ingredients	Diets		
	Control	Diet 1	Diet 2
Fish meal	45.97	29.9	29.91
Soybean meal	30.00	21.00	21.00
Raw mung bean meal	-	25.00	-
Boiled mung bean meal	-	-	22.50
Rice bran	20.00	20.00	20.00
Tapioca	0.03	0.10	0.09
Vegetable oil	1.00	1.00	1.00
Vitamin C	1.00	1.00	1.00
Vitamin premix	2.00	2.00	2.00
Mineral premix	2.00	2.00	2.00

¹Control: control without raw or boiled mung bean seeds meal; Diet 1; raw mung bean seeds meal; Diet 2: boiled mung bean seeds meal

In preparation of control diets prior addition of agar powder (Swallow Globe Brand), vitamin and mineral premix, rice bran, tapioca, soybean meal and fishmeal were homogeneously mixed in the heavy duty mixer. Therefore, vegetable oil was added slowly into the mixture. The mixture was stored in the tight container or closed container. Then, preparations of experimental diets also same with the preparation of control diets but with addition of raw and boiled mung bean seeds meal for Diet 1 and Diet 2 respectively.

Preparation of microbound diet had been prepared according to Partridge and Southgate (1999), Gallardo et al. (2002) and Kolkovski et al. (2010). The method had been modified by using the agar powder and diet mixture. Three grams of agar

powder (Swallow Globe Brand) was added with 97 grams of diet mixture. The mixture was homogenously mixed on hot plate at 100°C with addition of enough amount of distilled water. The mixed ingredients were continuous mixed until agar was melt and distilled water was completely blended with it. Then, the complete diet mixture was spread into an aluminium foil tray. The mixture was formed in rectangular shape before cut into cube. Cubes were placed in the oven dried for 12 hours at 40 to 50 °C. Next, dry microbound diets (MBD) were ground by using mortar and pestle. The feed was ready to fed fish.



Figure 3.1: AMB diet preparation

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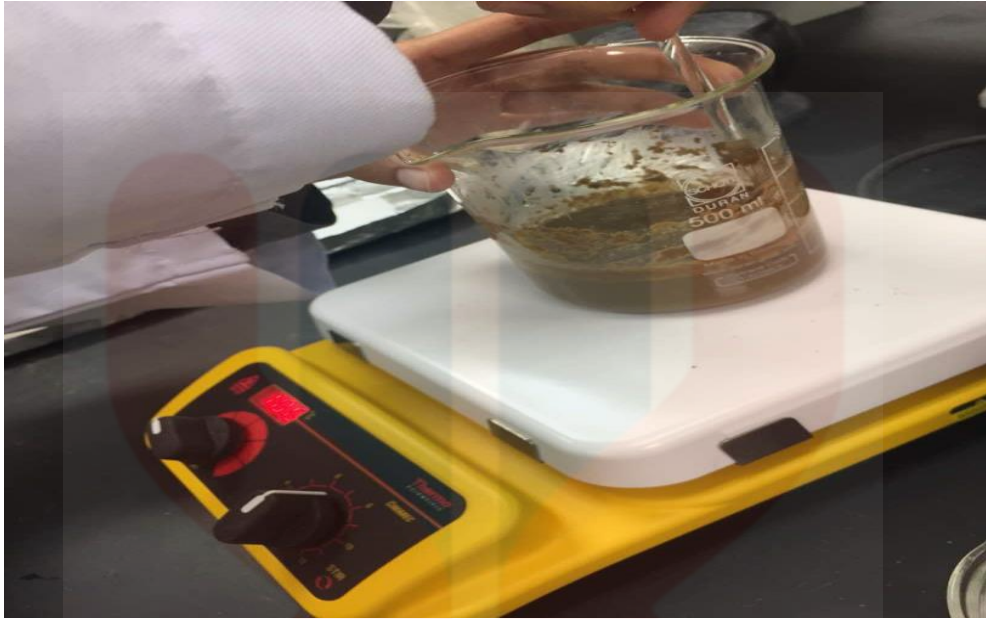


Figure 3.2: Mixing all the ingredients on hot plate



Figure 3.3: Preparation of AMB before oven-dried

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3.4 Nile Tilapia (*Oreochromis niloticus*) Culture

The experiment was conducted in an open system at Aquaculture Laboratory, Faculty of Agro Based Industry, University Malaysia Kelantan (UMK), Jeli Campus, Jeli, Kelantan. Tilapia fingerlings were obtained from local farmers in Gual Ipoh, Kelantan with weight of 3.0 ± 1.0 g were acclimatized in order to adapt and give suitable with the environment in the cement tank for seven days. Then, tilapia were being fed with commercial diet (Star feed from CP). After one week of acclimation, 30 tilapia fingerlings of similar sizes with the initial weight 4.0 ± 1.0 g were randomly distributed into nine aquariums filled with 32 litres of water and aeration. Each tank was aerated with two air stones. Each of the experimental diets was assigned to each tank at three replicates.

Culture water was changed twice daily. Tilapia fingerlings were fed with 5 % of their total body weight in three portions per day with experimental diet in three times. *O. niloticus* were fed 3 times daily at 8.00 a.m., 12.00 a.m. and 5.00 p.m. (El-Sayed & Kawanna, 2004). The amount of feed given was recorded. Five fish were weighed for every seven days. The average body weights of each sample of fish in each tank were recorded and mortality was monitored and recorded. Hence, the amount of daily diet was adjusted accordingly of their changing in body weight. Feeding trial was run for six weeks.

3.5 Water Quality Parameter

Fresh water condition was monitored every day using YSI Pro Plus Water Quality Meter in order to maintain the condition at temperature 27 ± 1 °C, pH 7 to 8 and dissolved oxygen of 7.1 to 8.1 mg L⁻¹ (El-Sherif & El-Feky, 2009a; El-Sherif & El-Feky, 2009b). Water quality of the experimental tanks was measured at 9.00a.m every day. Water quality parameter was presented in Table 3.3.

Table 3.3: Water quality parameter based on standard and experimental data from El-Sherif and El-Feky (2009a) and El-Sherif and El-Feky (2009b).

Water quality parameter	Standard
Temperature (°C)	27 ± 1
pH	7 to 8
Dissolved oxygen (DO) (mg L ⁻¹)	7.1 to 8.1

3.6 Texture Analysis

3.6.1 Colour evaluation

Method in colour evaluation was based on Ginés et al. (2004), colour meter named Minolta CR-300 (Minolta, Osaka, Japan) was used to evaluate 18 samples fillets of fish. L^* describe as lightness ($L^* = 0$ for black, $L^* = 100$ for white), a^* describe as intensity in yellow ($b^* > 0$). The colour was measured in the middle of the fillet. The data was recorded after appeared on the screen of colour meter.

3.6.2 Texture evaluation

Fish texture was analyzed using Brookfield CT-3 Texture Analyser in the Physics and Chemistry Lab of University Malaysia Kelantan (UMK), Jeli Campus, Jeli, Kelantan.

Before fillets were analyzed, initial and final weight of every samples were calculated by using formulae:

Weight loss (%) =

$(\text{Initial weight of sample before chilling} - \text{Final weight of sample after chilling}) \times 100$

According to the Rahman et al. (2012), instrumental TPA were measured by using a TA.XT2 Texture Profile Analyzer (Stable Microsystems, Godalming, Surrey, UK) with two cycles of compression-decompression and had been modified using the Brookfield CT3 texture analyzer. Double compression help to calculate the TPA values from a plot of a force-times curves (Sigurgisladdottir et al., 1997). The texture analyzer was connected with computer software.

In this experiment, load cell was at 5 kg weight. The samples (fillets of fish) were compressed by flat-ended cylinder. About 2.5 mm of constant penetration depth were applied on the fillets around 10 mm of thickness. 2.5 mm penetration depth was maximum distances was applied without the breaking muscle fibre and affect on muscle structure. According to the Alasalvar et al. (2001), three sampling point were selected in each fillet which are dorsal, tail at 10 mm from the edge of tail and between dorsal and tail and was modified by the position of sampling point was selected only in the middle of fillet. The tilapia samples were placed on stage of texture analyzer and

compressed with flat- ended cylinder. The double compressions were applied to conduct the texture profile analyses (TPA) parameters. The speed was used to compress the fish sample is 2 mm per second (2mm/s). At least six replicates were analyzed for each treatment.



Figure 3.4: Fish killed by pitting method



Figure 3.5: Fillet of fish process

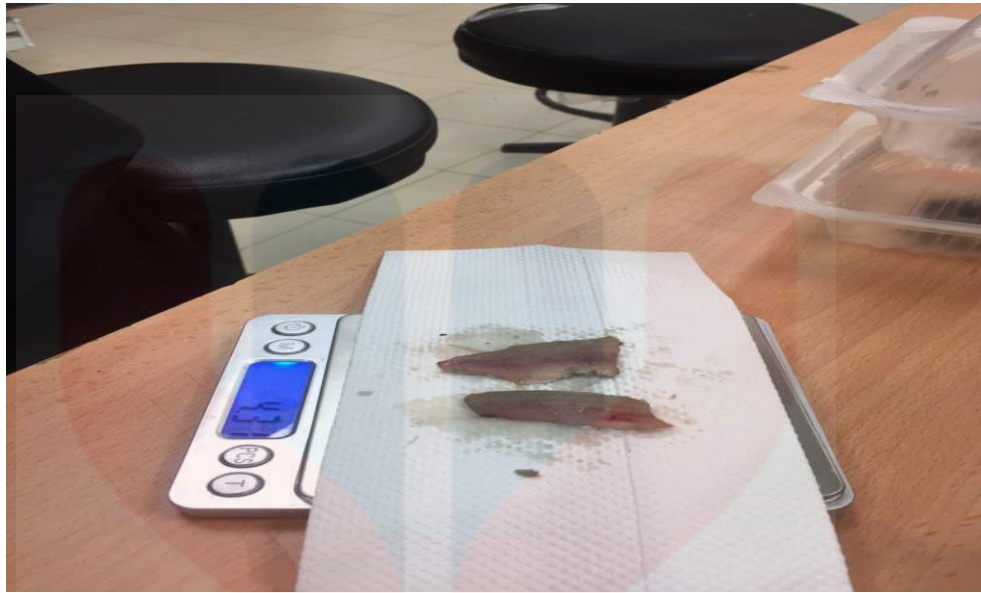


Figure 3.6: Fillets weighed process

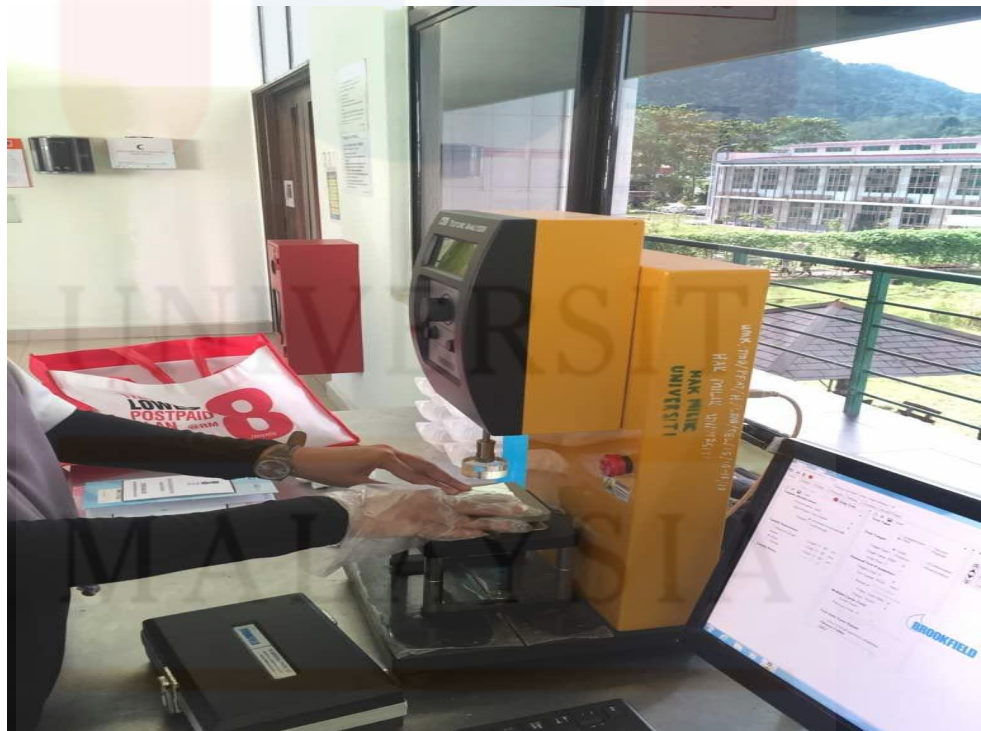


Figure 3.7: Brookfield CT-3 texture analyzer to analyse

3.7 Statistical analysis

All the data were subjected to One-way Analysis of variance (ANOVA) subjected by Duncan multiple range test with significance ($p < 0.05$) using as SPSS Software Programs for Windows, Version 22.0.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Determination of Weight Loss

Final weight of fish fillets in all treatments were decreased after stored in refrigerator for 12 hours at 4 °C. Initial and final weight of fish fillets after stored in refrigerator was presented in Table 4.1.

Table 4.1: Initial, final weight and percentage of weight loss of fish fillets

Treatments / Parameters	Initial weight (g)	Final weight (g)	Percentage of weight loss (%)
Control	3.19	3.09	-10
Diet 1	4.21	4.10	-11
Diet 2	4.76	4.71	-5

*Control: control without raw and boiled mung bean seeds meal; Diet 1: raw mung bean seeds meal; Diet 2: boiled mung bean seeds meal

Arannilewa et al. (2006) said that, there were disadvantages during frozen storage such as product dehydration, drip loss, product bleaching which could influence quality of frozen foods. During storage resulted in slightly changes in colour and taste as storage days increases and encourage bacteria growth.

Frozen storage caused changes, denaturation and aggregation in the muscle integrity (Makri, 2009). Gang (2014), said that longer and storage time would cause more drip loss when temperature increased gradually. This indicated that free water within the muscle was lost due to environment changes during processing. The increased the drip loss also due to longer storage time and drip loss of small size fillet was increased faster than large size fillets.

Based on Humaid and Jamal (2014) said, Indian mackerel stored at 4°C had highest quality scores which indicates higher quality and slower spoilage rate as low temperature reduced enzymatic and microbial activities. This was due to unfavourable environmental conditions for bacteria to growth at low temperature.

4.2 Texture Evaluation

Value of hardness in Control was highest which is 5871.83 g compared than Diet 1 and Diet 2 which are 5553.17 g and 5722.83 g respectively. However, there were significant differences ($p < 0.05$) in hardness in all experimental diets. Hardness values of each different experimental diet were presented in Table 4.2.

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Table 4.2: Hardness values of each sample in different experimental diet

Treatment / Parameter	Hardness (g)
Control	5871.83±115.50 ^a
Diet 1	5553.17±28.56 ^b
Diet 2	5722.83±94.68 ^{ab}

¹Values are means±SE. Values in the same column with different superscripts are significantly different (p<0.05).

²Control: control without raw and boiled mung bean seeds meal; Diet 1: raw mung bean seeds meal; Diet 2: boiled mung bean seeds meal

According to Probola and Zander (2007), hardness is important to the consumers as it evaluate commercial value of meats. Fish muscle had higher levels of indigenous protease which cause breakdown of proteins after harvesting, during processing, cooking and improper handling storage (Toyohara, Sakata, Yamashita, Kinoshita, & Shimizu, 1990). Based on Jiang et al. (2008), said the muscle of fish was more fragile and softer when the fillet become thicker. This was supported by Wiles et al. (2004), the addition of binding agents such as tapioca, locust bean gum and rice flour lowered the values of hardness and chewiness of product. It was supported by Haard (1992) said, cultured fish had softer texture, less preferable than free living sources.

During frozen storage, myosin undergoes aggregation reaction which leads to development of toughness, firmness and loss in water-holding-capacity of muscles due to denaturation and myofibrillar proteins (Sigurgisladottir, Ingvarsdottir, Torrissen, Cardinal, & Hafsteinsson, 2000; Makri, 2009). Water-holding-capacity (WHC) is ability of meat to maintain moisture in itself. Based on Taylor, Fjaera, and Skjervold (2002)

said, main cause of loss fillets hardness within the first 24 hours post mortem was detachment of myofibre to myofibre likewise the cause of breaks in the myofibre to myocommata at detachment was rigor stiffness loss was a slower process. Stien et al. (2005) supported that, rapid loss of hardness post mortem was brought the resolution of rigor stiffness in fish. Actin degradation resulted in changes of structural and softening of fish flesh (Godiksen, Marzel, Hyldig & Jessen, 2009).

Emire and Gebremariam (2010) stated that, huge amount of polyunsaturated fatty acid moieties proved fish lipids that high susceptible to oxidation through autocatalytic mechanism. According to Aubourg and Medina (1999), said the lipid deterioration was limit the prolonged of life fillets and influenced the loss of flavour and nutrition while creates toughening and effect on texture and result in unpleasant odour and flavour as rancidity.

Value of cohesiveness in Control (0.54) and Diet 2 (0.54) were slightly similar. While, Diet 1 (0.50) had lowest value of cohesiveness. However, there were no significant difference ($p>0.05$) in cohesiveness between Control, Diet 1 and Diet 2. Cohesiveness, springiness, gumminess and chewiness values of each sample with different experimental diets were presented in Table 4.3.

Table 4.3: Cohesiveness, springiness, gumminess and chewiness values of each sample in different experimental diets

Treatments/ Parameters	Cohesiveness	Springiness (mm)	Gumminess (g)	Chewiness (mJ)
Control	0.54±0.02 ^a	42.54±0.20 ^a	3165.17±139.93 ^a	1471.02±155.63 ^b
Diet 1	0.50±0.02 ^a	42.13±0.14 ^{ab}	2777.17±103.24 ^a	1147.18±41.82 ^a
Diet 2	0.54±0.03 ^a	42.03±0.07 ^b	3091.17±132.79 ^a	1274.07±54.69 ^{ab}

¹Values are means±SE. Values in the same column with different superscripts are significantly different (p<0.05).

²Control: control without raw and boiled mung bean seeds meal; Diet 1: raw mung bean seeds meal; Diet 2: boiled mung bean seeds meal

Results from this study was in agreement with Szczepanik, Kryża and Ostrowska (2010). They reported that, cohesiveness were significantly related with resilience, moisture content and sublimation-derived weight loss. The lower moisture content and sublimation-derived weight loss were observed, the higher value of cohesiveness was measured. This situation can be observed in Table 4.1 and 4.3 where, Control and Diet 2 had low percentage weight loss and high mean value of cohesiveness.

The means value of springiness between Control was highest which is 42.54 mm compared to Diet 1 and Diet 2 which are 42.13 mm and 42.03 mm. However, there were significant differences (p<0.05) between all experimental diets. The means value of gumminess in Control was the highest which is 3165.17 g compared to Diet 2 and Diet 1 which are 3091.17 g and 2777.17 g respectively. However, there were no significant differences (p>0.05) between Control, Diet 1 and Diet 2.

According to study from Szczepanik, Kryża and Ostrowka (2010), gumminess and chewiness of Atlantic herring decreased throughout the whole period of frozen storage within 1, 2, 3 and 4 months. The means value of chewiness in Control was the highest which is 1471.02 mJ compared to Diet 2 (1274.07 mJ) and Diet 1 (1147.18 mJ). There were significant differences ($p < 0.05$) between all experimental diets.

4.3 Colour Evaluation

Lightness (L^*), a^* and b^* values of each different experimental diet were presented in Table 4.4.

Table 4.4: Lightness (L^*), red chromaticity (a^*) and yellow chromaticity (b^*) values of each sample in different experimental diets

Treatments/Parameters	L^*	a^*	b^*
Control	34.65±1.18 ^a	6.50±0.80 ^a	10.84±0.72 ^a
Diet 1	36.39±1.41 ^a	7.01±0.55 ^a	10.54±0.40 ^a
Diet 2	34.17±0.67 ^a	7.04±0.33 ^a	10.47±0.26 ^a

¹Values are means±SE Values in the same column with different superscripts are significantly different ($p < 0.05$).

²Control: control without raw and boiled mung bean seeds meal; Diet 1: raw mung bean seeds meal; Diet 2: boiled mung bean seeds meal

Based on Table 4.4, redness (a^*) was higher in Diet 2 compared to Control and Diet 1. There was no significantly differences ($p > 0.05$) in redness between Control, Diet 1 and Diet 2. This might be due to removal of some anti-nutrients during

soaking and boiling process. Thus, mung bean seeds contain carotenoid pigments that was helps in improve skin colour of fish as it depends on diverse array of pigments and chromatphore cell. Mostly, fish unable to produce carotenoid pigments. It was agreement by Haard (1992) said that, carotenoid pigment need to be included in the diets of cultured fish and shellfish as helps in red coloration for market and consumer acceptability. The factors of insufficient amount of carotenoids, the skin appearance of cultured striped jack and sea bream were inferior to that wild fish and also influenced both appearance and stress susceptibility of live animal (Howell & Matthews, 1991).

Lightness (L^*) value of Diet 1 was higher which is 36.39 than Control and Diet 2 were 34.65 and 34.17 respectively. Based on Diet 1 had the highest lightness (L^*) value as it diet contain raw mung bean seeds meals. Raw mung bean was undergoes soaking technique that only removed low amount of anti-nutrients and other beneficial nutrients such carotenoid. While Diet 2 contained boiled mung bean seeds meal that undergoes soaking and boiling process which high amount of anti-nutrient and beneficial anti-nutrients were removed. So, less amount of carotenoid presence in Diet 2 result in dark colour of fish fillets. Other study by Gang (2014) said that, low lightness (L^*) value result in darker colour of fish fillets.

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CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In this study, Diet 1 had lowest value of hardness (5553.17 g) and then followed by Diet 2 and Control. There were significant differences ($p>0.05$) in hardness between all experimental diets. While in colour evaluation, Diet 1 had highest value of lightness at 36.39. This was due to soaking method that only remove certain nutrients either anti-nutrients or beneficial nutrients such as carotenoid that helps in pigmentation of fish. There were no significant differences ($p>0.05$) in lightning between Control, Diet 1 and Diet 2.

In conclusion, it was recommended further study in investigating texture of fish by using similar preparation methods of other legumes with further evaluation such as sensory evaluation to evaluate the texture of fish fillets in coming soon. In this study, Diet 1 was the best diet in terms of texture and colour evaluation. There were significant differences ($p<0.05$) on texture evaluation in terms of hardness, springiness and chewiness. Based on this study, different preparation of ingredients effect on flesh texture of Nile tilapia thus, H_A was accepted.

5.2 Recommendation

In the future, it was suggested that fish fillet should be undergoes human sensory evaluation in order to obtain positive results and feedback to test the consumer preference. In terms of texture and taste, it is necessary to conduct human sensory evaluation in order to determine consumer preferences towards quality and acceptance of food products. Consumers are more prefer white flesh texture as it is more attractive.

Other recommendation, plant protein sources such as legume seeds can be used in fish diets in order to replace main protein sources such as soybean meal and fish meal. Plant protein sources able to minimize feed cost as legume seeds are cheap and widely available in local market. It is also able to improve flesh colour as plant protein sources contain carotenoid that help in colour pigmentation.

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APPENDICES

A.1 One-way ANOVA

		ANOVA				
		Sum of Squares	df	Mean Square	F	Sig.
Initial Weight	Between Groups	7.628	2	3.814	4.104	.038
	Within Groups	13.940	15	.929		
	Total	21.569	17			
Final Weight	Between Groups	8.106	2	4.053	4.318	.033
	Within Groups	14.078	15	.939		
	Total	22.185	17			
Hardness	Between Groups	305072.444	2	152536.222	2.678	.101
	Within Groups	854296.500	15	56953.100		
	Total	1159368.944	17			
Cohesiveness	Between Groups	.006	2	.003	.988	.395
	Within Groups	.043	15	.003		
	Total	.048	17			
Springiness	Between Groups	.876	2	.438	3.353	.063
	Within Groups	1.960	15	.131		
	Total	2.837	17			
Gumminess	Between Groups	509232.000	2	254616.000	2.659	.103
	Within Groups	1436122.500	15	95741.500		
	Total	1945354.500	17			
Chewiness	Between Groups	319512.954	2	159756.477	2.758	.095
	Within Groups	868853.071	15	57923.538		
	Total	1188366.025	17			
Lightness (L*)	Between Groups	16.416	2	8.208	1.075	.366
	Within Groups	114.556	15	7.637		
	Total	130.972	17			
a*	Between Groups	1.108	2	.554	.263	.772
	Within Groups	31.584	15	2.106		
	Total	32.692	17			
b*	Between Groups	.461	2	.230	.155	.858
	Within Groups	22.268	15	1.485		
	Total	22.728	17			

A.2: Post Hoc Analysis of Duncan Multiple Test for Hardness of Fish Fillets

Hardness

Duncan^a

Treatment	N	Subset for alpha = 0.05	
		1	2
Diet 2	6	5553.1667	
Diet 3	6	5722.8333	5722.8333
Diet 1	6		5871.8333
Sig.		.237	.297

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

A.3 Post Hoc Analysis of Duncan Multiple Test for Cohesiveness of Fish Fillets

Cohesiveness

Duncan^a

Treatment	N	Subset for alpha = 0.05	
		1	
Diet 2	6		.5017
Diet 1	6		.5383
Diet 3	6		.5400
Sig.			.256

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

A.4 Post Hoc Analysis of Duncan Multiple Test for Springiness of Fish Fillets

Springiness

Duncan^a

Treatment	N	Subset for alpha = 0.05	
		1	2
Diet 3	6	42.0300	
Diet 2	6	42.1300	42.1300
Diet 1	6		42.5400
Sig.		.639	.068

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

A.5 Post Hoc Analysis of Duncan Multiple Test for Gumminess of Fish Fillets

Gumminess

Duncan^a

Treatment	N	Subset for alpha = 0.05	
		1	
Diet 2	6	2777.1667	
Diet 3	6	3091.1667	
Diet 1	6	3165.1667	
Sig.			.056

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

A.6 Post Hoc Analysis of Duncan Multiple Test for Chewiness of Fish Fillets

Chewiness

Duncan^a

Treatment	N	Subset for alpha = 0.05	
		1	2
Diet 2	6	1147.1833	
Diet 3	6	1274.0683	1274.0683
Diet 1	6		1471.0167
Sig.		.376	.177

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

A.7 Post Hoc Analysis of Duncan Multiple Test for Lightness (L^*) of Fish Fillets

Lightness (L^*)

Duncan^a

Treatment	N	Subset for alpha = 0.05	
		1	
Diet 3	6	34.1700	
Diet 1	6	34.6467	
Diet 2	6	36.3917	
Sig.			.206

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

A.8 Post Hoc Analysis of Duncan Multiple Test for a^* of Fish Fillets

a^*

Duncan^a

Treatment	N	Subset for alpha = 0.05	
		1	
Diet 1	6		6.4983
Diet 2	6		7.0117
Diet 3	6		7.0367
Sig.			.552

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.

A.9 Post Hoc Analysis of Duncan Multiple Test for b^* of Fish Fillets

b^*

Duncan^a

Treatment	N	Subset for alpha = 0.05	
		1	
Diet 3	6		10.4733
Diet 2	6		10.5417
Diet 1	6		10.8417
Sig.			.627

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.000.



Figure A.10: Moisture Analyzer MX-50 for moisture analysis



Figure A.11: Kjeldahl distillation system Vapodest 30s for crude protein analysis

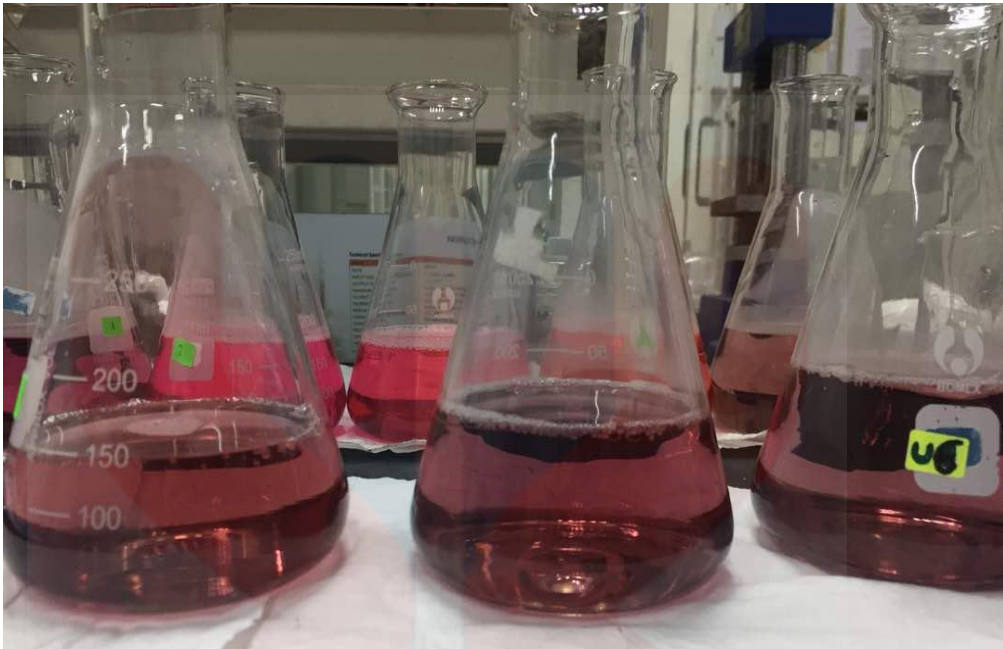


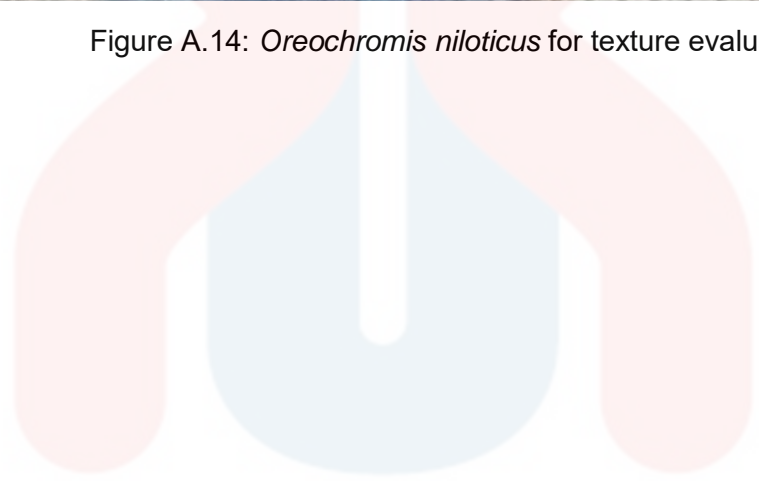
Figure A.12: Samples turn to greyish pink after titration process in crude protein analysis



Figure A.13: Foss Soxtec 2055 system for crude fat analysis



Figure A.14: *Oreochromis niloticus* for texture evaluation



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