UNIVERSITI PUTRA MALAYSIA

FORMULATION OF TILAPIA FISH FEED PELLET BY USING BLACK SOLDIER FLY LARVAE

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PROJECT REPORT SUBMITTED IN PARTIALLY FULFILLMENT OF THE REQUIREMENT FOR THE

BACHELOR OF ENGINEERING (PROCESS AND FOOD)

DEPARTMENT OF PROCESS AND FOOD ENGINEERING

FACULTY OF ENGINEERING

UNIVERSITI PUTRA MALAYSIA

2017

APPROVAL SHEET

The project entitled "**FORMULATION OF TILAPIA FISH FEED PELLET BY USING BLACK SOLDIER FLY LARVAE**" prepared by **Nur Hazwani Mhd Yusuf** in partial fulfilment of the requirement for the Bachelor of Engineering (Process and Food) is hereby accepted and approved.

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ACKNOWLEDGEMENT

First of all thank you to Allah S.W.T and I would like to express my gratitude to all those who gave me the possibility to complete this project, especially to my supervisor Assoc. Prof. Dr. Yus Aniza Yusof for her guidance during my research. She was always accessible and willing to help her students with their research. As a result, research life became smooth and rewarding for me.

I want to thank also my PhD senior Mr. Muhammad Tuseef Asghar whose help, stimulating suggestion and encouragement helped me during the duration of research and writing this project. Special thanks to all the assistants engineer at of Process and Food Engineering Department and also Aquaculture Lab, UPM for their patience, cooperation and help.

I would like to extend my thanks and appreciation to Mr. Hisammudin Ab Wahid, a young successful entrepreneur that introduce me Black Soldier Fly Larvae and Mr. Azmi Abdul Manaf, a lecturer from UKM who have provided me with the raw material and also knowledge about it.

I am tempted to individually thank all my friends from my childhood until university, which joined me in the discovery of what life is about and how to make the best of it.

Lastly, thank to my parents and family on whose constant encouragement and love I have relied throughout my time at the university. They have always supported and encouraged me to do my best in all matters of life. To them I dedicate this project.

ABSTRACT

Animal feed manufacturing involves the use of a variety of raw materials to produce compound feeds. The feeds are defined according to certain specifications with regard to nutritive composition based on specified descriptions for nutritional, hygienic and physical quality. The black soldier fly larvae can be the best ingredient to replace fishmeal in the fish feed formulation since the protein content is higher and it is easy to breed and manufacturing cost of the feed can also be reduced.

This study was conducted to formulate Tilapia fish feed pellets by using black soldier fly larvae as an alternative to replace fishmeal ingredients. There were nine extruded diets containing various ratios of black soldier fly larvae and tapioca starch (48:19, 37:29, 27:38) and varying moisture contents of 20%, 30% and 40% were formulated. Floating fish feed pellets were produced by using a single step technique called cooking extrusion by using a single screw extruder. The barrel temperature profiles were set at 80, 100 and 120 °C while the die temperature was set at 160 °C.

The quality properties of extruded diet in terms of expansion ratio, hardness, floatability, water solubility index, water absorption index, and sinking velocity were evaluated. The results showed that increasing moisture level of diet from 20% to 40% increased the value of expansion ratio, water absorption index, floatability, and sinking velocity. The best moisture content level in feed pellet to produce best floating extruded feed was by using black soldier fly larvae with 40% moisture content. Tapioca starch was used as a binder for the formulation of black soldier fly larvae. Floatability of pellets depends on the amount of the tapioca starch added during the extrusion process. Thus this study

provides an opening for further development of Tilapia fish feed pellets for better economic and nutritional advantages.

ABSTRAK

Untuk menghasilkan makanan haiwan yang terbaik, ia melibatkan penggunaan pelbagai bahan yang akan diadun bersama. Makanan haiwan yang terbaik adalah dinilai dari segi kepelbagaian komposisi nutrisi di mana aspek nutrisi, kebersihan dan kualiti fizikal dititik beratkan. Oleh itu, *black soldier fly larvae* adalah bahan mentah yang sesuai untuk menggantikan *fishmeal* di dalam resepi pembuatan makanan ikan. Hal ini kerana, *black soldier fly larvae* dipercayai mengandungi kandungan protein yang tinggi dan ia juga sangat mudah untuk dibiakkan seterusnya dapat mengurangkan kos pembuatan makanan ikan.

Oleh itu, kajian ini dijalankan untuk merangka formulasi terbaru ikan Tilapia dengan menggunakan *black soldier fly larvae* sebagai alternatif untuk menggantikan bahan-bahan makanan ikan. Terdapat sembilan formula baru yang mengandungi nisbah peringkat *black soldier fly larvae* dan kanji ubi kayu (48:19, 37:29, 27:38) dan juga mengikut kandungan kelembapan yang berbeza-beza iaitu pada 20%, 30% dan 40%. Pelet makanan ikan terapung telah dihasilkan dengan menggunakan teknik langkah tunggal yang dipanggil penyemperitan dengan menggunakan penyemperitan skru tunggal. Profil suhu laras penyemperit telah ditetapkan pada 80°C, 100°C, dan 120 °C manakala suhu acuan telah ditetapkan pada 160°C. Sifat-sifat kualiti pelet makanan ikan dari segi nisbah pengembangan, kekerasan, pengapungan, indeks keterlarutan air, indeks penyerapan air, dan halaju tenggelam telah dinilai.

Hasil kajian menunjukkan bahawa tahap kelembapan peningkatan makanan ikan daripada 20% kepada 40% telah meningkatan nilai sifat-sifat fizikal yang diperiksa seperti pengembangan, pengapungan, indeks penyerapan air, dan halaju tenggelam. Oleh itu, tahap terbaik kandungan kelembapan dalam pelet makanan ikan untuk menghasilkan pelet terapung terbaik dengan menggunakan *black soldier fly larvae* adalah pada 40% kelembapan. Kanji ubi kayu dengan kombinasi *black soldier fly larvae* telah dilihat sebagai kombinasi yang baik sebagai pengikat. Tahap pengapungan pelet ikan dalam proses penyemperitan adalah sangat bergantung kepada kuantiti tepung ubi kayu. Kesimpulannya, kajian ini telah telah membuka laluan kepada lebih banyak lagi penambah baikkan yang boleh dilakukan demi menghasilkan pelet ikan Tilapia yang lebih tinggi kadar nutirisinya dan lebih ekonomik.

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LIST OF ABBREVIATION

BSFL	Black Soldier Fly Larvae
WSI	Water solubility index
WAI	Water absorption index
ER	Expansion ratio
BD	Bulk density

CHAPTER 1

INTRODUCTION

1.1.Overview of the Study

Tilapia or *Oreochromis niloticus* is a species of aquatic fish which inhabit in shallow streams, ponds, rivers and lakes, and hardly found living in brackish water. Tilapia has turn to be one of the most important cultured species as the increasing number of it, mainly in the tropics region (De Silva et al., 1989). Hence, the amount of cultured Tilapia was produced tripled between 1984 and 1994 based on world production statistic (El-Sayed, 1998). The evolution of rearing Tilapia gradually expands year by year with the increasing number of world's population. This is due to the need for efficient food source of protein to fulfill consumer requirements.

Since the operating costs of intensive culture of Tilapia shows less than 50% compared to feed cost, special consideration has been given to tilapia nourishment with accentuation on protein necessities. Even though the fish meal (FM) is the most costliest real ingredients in aquaculture feed, but it is generally utilized as the primary source of dietary protein for most economically cultivated fish species. Meanwhile, the lack in world production of fish feed, combined with the expanded request as demand increased and rivalry with earthly domestic animals, has advance increased the fish meal costs. Over the long period, many developing countries will most likely be unable to rely on fish meal as a protein source to their aqua feed. Hence, it is compulsory that other fish meal substitutions ingredients to be found (El-Sayed, 1998).

This study will focus on how to formulate an extruded product of fish feed with the adding of black soldier fly larvae by having the same protein content but in the less cost production via extrusion process. Since aquaculture plays an important role in many countries, it is important to make sure that Tilapia gets enough nutrition for growth and reproduce. Thus, the aqua feed industry has move forward in extrusion technology to achieve a highly and digestibility improvement of feed pellet. There are a lot of advantages of extrusion process for aquaculture feed production such as enhanced feed conversion ratio, restrained the pellet density, increase feed stability in water, better production efficiency and versatility (Umar, Kamarudin, & Ramezani-Fard, 2013).

Besides, it improved functional characteristics of protein source without losing its protein quality for instance proteins from other sources such as by-products and waste of traditional food industry which are better in protein quality can be made more acceptable by texturization through extrusion (Shankar et al., 2010). Extrusion is the most dominant heat treatment process to produce fish feed pellet. It helps raised up the economics of production by increasing the feed intake, growth efficient and feed productivity (Abdollahi et al., 2013).

The extrusion is being done by using the equipment known as extruder. It consists of flight screws or worms rotating within a sleeve or barrel. The function of the screw is to convey, compress, melt and plasticized the material and to press it under pressure through small die holes at the end of the barrel. Besides that, heat is allowed to the feed dough as it passes through the screw by one or more of three mechanisms which are viscous dissipation mechanical energy being added to the shaft of screw, heat transfer from steam or electrical heaters surrounding the barrel and direct injection of steam which is mixed with the dough in the screw (Harper, 1981).

Feed that comes out from the die is known as extruded feed product or extruded which have good shapes, full gelatinization with the help of starch and high level of protein that provides energy and builds muscles of Tilapia. The quality of extruded product will be measured at their physical properties like bulk density, floatability, water absorption index (WAI), water solubility index (WSI), sinking velocity, hardness and expansion ratio (ER).

1.2. Problem Statements

The growth of Tilapia is most influence by the dietary protein quality and quantity besides other physiological requirements needed for growth are fulfilled. The major energy source of fish is protein and studies found that the optimal dietary protein level required for maximal growth is considerably higher than that of terrestrial farm animals. Basically, in the ranges between 35% and 55% or an equal to 45%-75% of the gross energy content of the feed must be in the form of protein for the daily taken dietary protein of the fish (De Silva et al., 1989). Currently, in the aqua feed Industry in Malaysia, the protein content in fish feed pellet produce is less than 38%. This situation will somehow effect the growth of Tilapia as Tilapia now becomes increasingly important cultured species as demand for Tilapia is higher too.

Our industry also relies most on imported raw materials such as fishmeal (the protein supplement) which is the most expensive dietary constituent as a main ingredient in their extruded product. In order to reduce the pressure on fishmeal cost in aquaculture sector, a study have been done to find an alternative protein feed ingredient from plant, microbial or other animals sources to replace the fish meal. A decade ago, there was a studies conducted to replace the fishmeal with insects in the diet of fish and the results have brought it to further research. They found that, black soldier flies have a very good potential to use as a fishmeal replacement in the fish diet (Henry et al., 2015). Moreover, there is no expertise in aqua feed industry in Malaysia that able to commercialize this extruded product in large scale at low cost production but still have high stability in water. Therefore, it is important to manufacture new formulation of fish feed pellet with the using of black soldier fly larvae in order to produce high quality of extruded products. The extrusion cooking method have been used in order to produce a floatable fish feed pellets. There were three formulations as shown in Table 3.1 (Appendix I) with the differential of protein content (Black soldier fly larvae) and the starch content have been formulated. Plus, the moisture contents were adjusted for each of replicates.

1.3. Objectives

The specific objectives of this study are:

1. To develop a new formulation of floatable fish feed pellet by using Black Soldier Fly Larvae (BSFL).

2. To determine the physical properties of formulated fish feed pellet in terms bulk density, floatability, water absorption index, water solubility index, sinking velocity, hardness and expansion ratio at different formulations.

1.4. Thesis Structure

This research study consists of five main chapters. In chapter one, the introduction part is about the extrusion method to manufacture the new formulation of fish feed pellet which include the research background, problem statement, objectives, and thesis structures.

Chapter two of this thesis reviewed on the properties of fish feed pellet. The advantage of pelletizing the fish food was also described in this chapter.

Chapter three describes the methodology used for the research study. The sample preparation of ingredients to make the fish feed pellet were explained well in this chapter. Other than that, this chapter will also include the detailed on the experiment work, the material that will be used, the equipment involved and also the unit operation involved to measure the hardness, floatability, bulk density, water stability, and sinking velocity of final extruded product.

Chapter four is a discussion on the results and data obtained from the experimental study. In this part, the moisture level of feed mixture and the physical structural of extruded product will be discussed.

Lastly, chapter five is a summary of all the study finding in previous chapter. The recommendations for future work are also elaborated in this final chapter.

CHAPTER 2

LITERATURE REVIEW

2.1. Overview of Tilapia Fish Feed Pellet

The growth of fish is generally affected by its dietary feed intake. Basically, different types of fish have different quantity daily dietary feed intake. In order to increase the performance growth of Tilapia, the protein content in the dietary feed intake should be correct and accurate according to size of tilapia. De Silva et al., 1989 had been studied that protein intake by fish was then converted into a major sources of energy that helps the growth of fish (De Silva et al., 1989). Based on market survey, the protein content that fish received from the pellet produce by our industry is about 32% (Aquaculture Feed. (2017, June 10). Figure 2.1 below represent the nutritional content inside the feed pellet manufactured by ENGFU Feedmill Sdn. Bhd.

	Crude Protein	Fat	Crude Fibre	Ash	Moisture	Ca	P
9960	32%	6%	4%	10%	10%	1%	0.8%
9961	32%	6%	4%	10%	10%	1%	0.8%
9962	28%	6%	6%	10%	10%	1%	0.8%
9963	25%	6%	6%	10%	10%	1%	0.8%

Figure 2.1: The crude protein in Tilapia Floating Fish Feed at different size of fish

(Source: ENGFU Feedmill Sdn. Bhd., 2016)

Wee, et al., (1988) stated that, there was a progressive increasing in the growth performance with increasing dietary proteins level up to 35% crude protein. As the fish growth, the protein content in the dietary intake also must be increase. Thus, a study done by Henry et al., (2015) explained that insects can be the most suitable substituents for fish meal inside the feed pellet since the price of the fish meal is very expensive. Generally, the most suitable food for fish is in dry food where it mostly found in pellet forms. Nowadays, pellet feed has been introduced to replace traditional feeding process for a successful growth of fish. This is because for fish farm, no matter what system it is, artificial feeding is required. Because feeds represent more than 50% of total production cost in aquaculture, successful operation of a fish farm needs balances, efficient and economical diets.

In order to achieve the economical diets, physical quality of pelleted feeds is important. Firstly, all feeds produce should be resistant to mechanical stress during transportation, handling and in pneumatic feeding devices. Besides, the texture and size of feed pellet also influence the high feed intake and efficient digestion by the fish. Normally, the pellets which are too hard might harm the digestive system of the fish. However, the overfeeding of it will lead to swelling and rupture of the stomach (Sørensen, 2012). Next is the ability of feed pellet to float in the water.

There are a lot of natural binders have been used to manufacture firm pellets with the aim of increasing their water stability without having the nutrient loss. Polysaccharides, such as starch (sago and tapioca) that is available most in Malaysia have been used as promising biopolymers to be employed as binders (Volpe et al., 2012a). Bulk density of the pellet is the most important factor in manufacturing process and can be adjusted according to the level of sinking velocity and buoyancy control. It has to be adjusted based on the eating habits of the fish whether fed at the surface of water, in the water column with use of slow sinking feed or else in the bottom of the water (Sørensen, 2012).

2.2. Fish Feed Formulation

A mixture of selected ingredients that consists all the nutrients necessary for the fish is called complete feeds. They are made in a form which the fish find easy to eat and digest. These feeds are difficult to make on the farm and are usually expensive to buy. Feeds that produced from low quality of raw materials or under adverse processing conditions known to give negative effects on fish growth. Apart from that, feed formulation should be based on nutrient available, reliable data on the digestibility of different ingredients for each species might well be considered as a necessary prerequisite (Gomes, Rema, & Kaushik, 1995). Table 2.1 below shows a recent research data available for some ingredients with a few salmonid species.

Table 2.1: The formulation and chemical composition of the reference diet. Source: (Gomes et al., 1995)

Ingredient	%
Fish meal	40
Meat meal	7
Full-fat soybean	15
Maize gluten	10
Wheat flour	18
Cod liver oil	6
Mineral[a] + vitamin[b] mixture	1
Chromic oxide	1
Binder	2
Chemical composition	
Dry matter (% DM)	89.9
Crude protein (N × 6.25, % DM)	46.7
Crude fat (% DM)	11.2
Gross energy (kJ/g DM)	20.3
Ash (% DM)	10.3

However, due to high demand of aquaculture product and the increase in the prices of fishmeal has prompted the search for sustainable alternatives for aquaculture feeds. Based on research conducted, they found that insects may be a good candidate to replace the fish meal as it has a limited need for arable land. They reviewed that locusts, grasshoppers, termites, yellow mealworms, Asiatic rhinoceros beetles, superworms, domesticated silkworms, common houseflies, common mosquitoes and black soldier flies larva may be potential to be a replacement for fishmeal in the fish diet (Henry et al., 2015) . Thus, a low cost production of feed formulation can be manufactured in order to fulfill the current high demand nowadays.

2.2.1. Overview of Tapioca Starch

Tapioca or *Manihot Esculenta* also is a starch that can be extracted from cassava root. It acts as energy sources for millions of people in the tropics and also plays an important raw material in industrial uses. Most of product produce from Tapioca starch are in pellets formed and then expanded in an oven or fried in oil to become a porous low density product (Sriburi & Hill, 2000). Both of these starch will be used in formulation of Tilapia fish feed pellet to replace maize starch. Besides, sago starch and tapioca starch is more cheaper than maize starch and have some other important characteristics like its ease to gelatinize, have high viscosity, and the ease with which it can be molded (Ahmad et al., 1999).

2.2.2. Overview of Black Soldier Fly Larvae

A review of research found that insects in various developmental stages have been used to feed fish and farm animals. The insects is Soldier fly larvae *(Hermetia illucens L.)* which habit on chicken manure at a commercial caged layer house, were substitute the fishmeal to feed channel catfish and tilapia, also farm animals in combination with high (45%) and low (30%) protein commercial diets (Bondari & Sheppard, 1981). It comes in many sizes up to 250 mg in weight and about 25 mm in length. Based on the study, Bondari & Sheppard, (1981) found that two proximate analyses of dried soldier fly larvae from chicken manure indicated 38-40% crude protein and l6-28%. Larvae of black soldier consist the amount of protein (476 g/kg, dry matter) and fat content about (118 g/kg, dry matter) which are very suitable to replace with fishmeal thus lowering the cost of fish feed production (Kroeckel et al., 2012).

Figure 2.2: Black Soldier Fly Larvae (Retrieved from: http://blacksoldierflyblog.com/)

2.3. Cooking Extrusion Technique

There are three processing techniques that currently dominate the production of pelleted diets in aquaculture and their use often, but not always, facilitate improvement in the raw product which are grinding, steam conditioning and extrusion. The primary technique used for fish feed production over the past three decades ago is extrusion cooking. This is due to extrusion promised the high physical and nutritional quality of the feed (Sørensen, 2012). Extrusion cooking is very common technique used in the food and feed industries because it ensures the versatility in obtaining the required textural properties.

The most important physical properties in aquaculture feed production to be evaluated are floatability and pellet durability. Floatability of the feed is highly depends on the expansion occurred during extrusion process and the pellet durability is achieved when there is various biochemical changes occur inside the barrel including gelatinization of starch (Chevanan & Rosentrater, 2005). The equipment used in cooking extrusion is single-screw extruder that able to chop and mincing soft food by forcing them through die plates.

2.3.1. Advantages of Cooking Extrusion

Generally, extrusion cooking works with a tool used to introduce thermal and mechanical energy to food and feed ingredients, forcing the basic components of the ingredients, such as starch and protein, to undergo chemical and physical changes and forming a predetermined shape. Besides, it offers a lot of advantages to the mixture of ingredients such as modifying the functional properties of food ingredients, texturing the feed ingredients, can be produced in high volume productions, and it is low cost production (Cheftel, 1986). Apart from that, recent studies found that cooking extrusion gives advantages as follows:

1. Versatility – there are various types of extruded product can be made using the same extruder by adjusting the ingredients and extrusion conditions (Cheftel, 1986).
2. High productivity level – it is a continuous cooking system whereby the raw materials keep on be extruded until the raw materials were finished. Thus, the cooking time will be reduced (Cheftel, 1986).
3. No effluent – No losses or waste could happen in cooking extrusion since all the raw materials that comes in through the feeder will totally be extruded to become extruded product (Cheftel, 1986).

2.3.2. Unit Operations

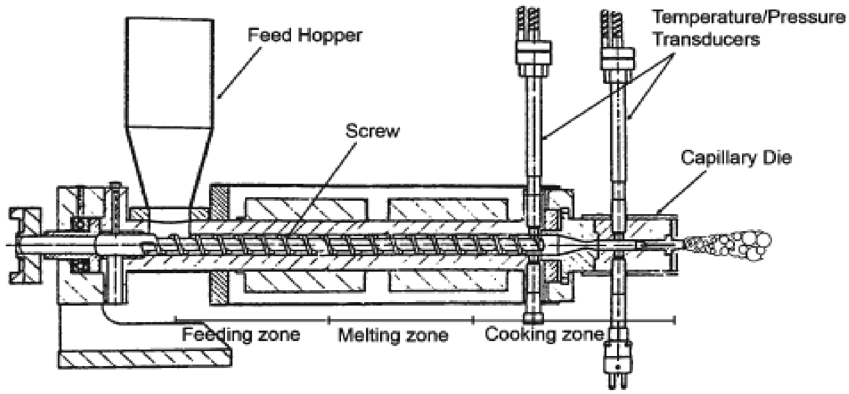

Figure 2.3.: The single-screw extruder (Singh & Muthukumarappan, 2016)

Figure 2.3 shows the components of feed extruder. Feed extruder consists of screw, barrel, heater, hopper, die, and temperature transducers. Basically, there are five main components of an extruder which are screw, extruder drive, barrel, feed hopper, and die. The most important part of extruder is helical structured extruder screw because it includes transport, heating, melting and mixing functions for extruded product. The speed of screw give effect to the physical properties of extruded feed since it developed the pressure inside the barrel (Chevanan & Rosentrater, 2005). The electrical motor will supply the power in order the screw to rotate. Physical characteristics of products like stability and quality of products is highly dependent to the design of the screw. The function of the extruder barrel located at the outside of the screw is providing heating and cooling capabilities.

The barrel housing with one or two rotating screws (single – or twin screw extruder) was present in extrusion system. It also equipped with a pre-conditioner as well as an accompanying machine control system. The function of pre-conditioner is to mix water and steam into the blend of dry ingredients with help of high speed mixing unit. (Sørensen, 2012) claimed that the overall goal with preconditioning is to supply the extruder barrel with an evenly moistened and preheated mix. There is a feed throat connect to the feed hopper and barrel. The feed hopper was designed to hold the mixing ingredients, and allows its flow into the barrel steadily.

The die is located at the end of the extruder, which determined the shape of the product. The size and shape of the extruded products will not be exactly the same as the size and shape of the die due to some reasons for example draw down, cooling, swelling and relaxation (Vijayagopal, 2004). Cooking extrusion technique allows the process material to undergo significant physical and chemical changes. The suitable selection of parameters such as temperature, moisture content, and processing time, the wet ingredients becomes fully gelatinized and cooked (Vijayagopal, 2004). The source of heat which is steam is directly fed to the mixture, heating units of the barrel and the transition of mechanical energy into heat.

2.4. Post Drying Technique

2.4.1. Oven Drying

Drying may cause unwanted changes in physical appearance, texture, colour and flavour that are absolutely cause problem with the increasing demand of consumer for the highest quality of finished product (Arslan et al., 2010). As many other food process, drying needs to be optimized with respect to process economics and product quality which has led to the development of new drying techniques. The amount of water present in the feed ingredient mix plays an important role in determining the expansion as well as strength of the extruded feed.

There are a lot of drying technique introduced for example microwave drying, sun drying, air drying and also oven drying. Based on (Arslan & Musa Özcan, 2008) studies, oven drying gave higher mineral content than the sun and microwave drying. The method of drying with oven drying is time dependent as duration of drying can be set according to variation temperatures used such as 70–105°C plus, it is much easier than other method. Besides, it is the most suitable and widely used for moisture content estimation of dry or semi-wet agricultural products (Karathanos, 1999).

Oven drying have a principle of operation based on fine gravity air convection in an electrically heated chamber. Oven chamber which are jacketed will ensuring a homogenous temperature profile in the chamber. Hot air inside the chamber is moving by the force of circulation. The force air makes hot air reached the top of chamber to circulate back to bottom of the chamber.

CHAPTER 3

METHODOLOGY

3.1. Materials

The raw materials (tapioca starch, soy bean, and vegetable oil) were purchased from a local supermarket. While the black soldier fly larvae was contributed from a breeder of flies Black Soldier Fly Larvae Malaysia. The experiments were carried out at Biomaterials Engineering Properties and Nutraceuticals Laboratory, UPM.

3.2. Sample Formulation

This feed was formulated using the following ingredients: tapioca starch, black soldier fly larvae, soy bean, vitamin premix, mineral premix, and vegetable oil. The formulation for the pellet is as shown in Table 3.1. According to Table 3.1, there were three different formulations in terms of black soldier fly larvae and tapioca starch percentage. Each of formulation were maintained to have more than 40% crude protein content (Olvera-Novoa et al., 1990) as source of energy for fish growth. For formulation 1, there were 49% of black soldier fly larvae, 19% of tapioca starch, 29% of soy bean, 2% of vitamin premix, 1% of mineral premix and 2% of vegetable oil. Formulation 2 consists of 37% of black soldier fly larvae, 29% of tapioca starch, the rest ingredients were constant as Formulation 1, while for Formulation 3, there were mixing of 27% of black soldier fly larvae, 38% of tapioca starch and the rest ingredients were same as well as Formulation 1.

Table 3.1: The formulation of fish feed by using black soldier fly larvae

Ingredient	Diets (Black Larvae : Tapioca starch) (%)		
	Formulation (1) 46:19	Formulation (2) 37:29	Formulation (3) 27:38
Black larvae	46%	37%	27%
Tapioca starch	19%	29%	38%
Soy bean	29%	29%	29%
Vitamin premix	2%	2%	2%
Mineral premix	1%	1%	1%
Vegetable oil	2%	2%	2%

Figure 3.1 show the flowchart process of tilapia fish feed pellet. Firstly, the black soldier fly larvae and soy bean were finely ground by using a laboratory grinder. All ingredients which are tapioca starch, vitamin, mineral, black soldier fly larvae, and soy bean (Table 3.1) were then mixed well by using a stand mixer (KitchenAid Professional 600 Series, USA) with consistently added water to obtain targeted feed moisture content which are 20%, 30% and 40% total moisture for each replicate (Umar et al., 2013). All replicate diets were extruded by using a laboratory scale stand-alone single-screw extruder with a throughput of 5kg/h (Brabender KE19: Brabender Gmbh,

Germany) located at Aquaculture lab Faculty of Agriculture, UPM. The experiments were carried out for nine runs by manipulating the total moisture of each three formulation feed diet. The barrel length and barrel diameter of extruder were set at 420 mm and 19mm, while the length to diameter ratio of 22:1.

A uniform pitch screw with a length to diameter ratio of 25 was used in this experiment. The barrel temperature profile was set at 80°C (feeding zone), 100°C (compression zone), and 120°C (metering zone) while the temperature of the die was set to 160°C (forming zone) (Umar et al., 2013). The other extrusion parameters were set as follows: compression ratio speed 3:1 (Shankar et al., 2010) to prevented leakage flow along the screw and kept the extruder output steady; 3 mm die outlet; screw speed: 40,120, and 300 rpm; pressure: 8.0-10.0 Mpa (Umar et al., 2013). After the extrusion, pellets were oven dried at 45°C for 10 hour (Umar et al., 2013). The vegetable oil was then sprayed to the pellets. For further analysis, dried extruded product was kept in polyethylene bags at room temperature. An airtight container was used to store the polyethylene bag of pellets for further analysis.

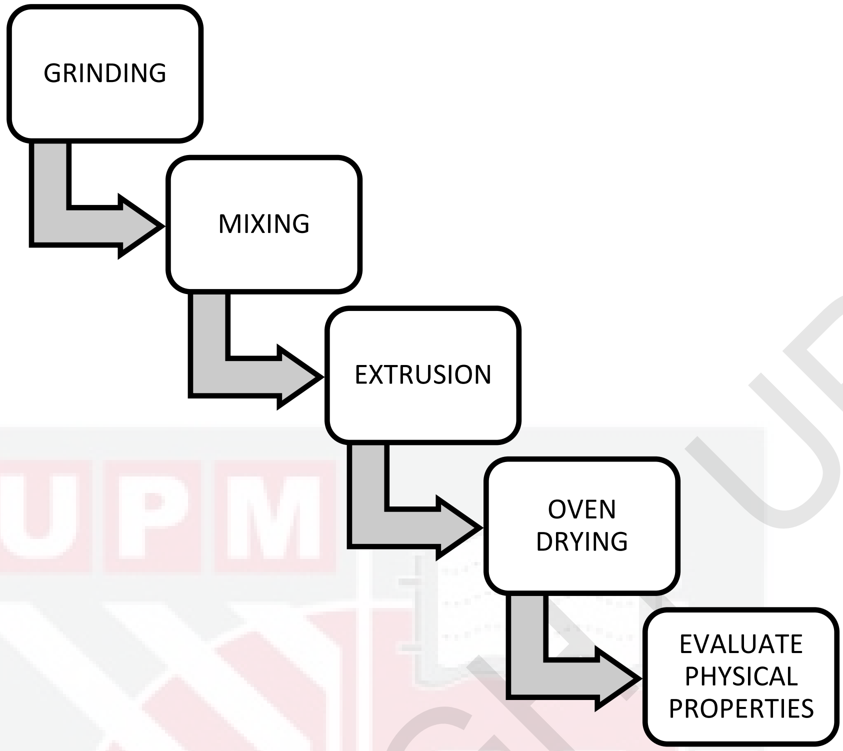

Figure 3.1: Flow chart process of Tilapia fish feed formulation (Umar et al., 2013)

3.3. Determination of Extruded Product Properties

3.3.1. Expansion Ratio

Expansion ratio of an extruded was calculated using the below equation after the diameters of 10 pellets (D_f) of each replicate was measured via digital caliper (Umar et al., 2013).

$$ER = \left[\frac{Df}{Di}\right] \times 100 \quad \textbf{(3.1)}$$

Where, D_i = initial die diameter (3 mm)

3.3.2. Bulk Density (BD)

The bulk density was calculated by poured each replicate into a 1000 ml cylinder in order to measure the weight of 1000 ml diet. Then, the poured density was calculated from the mass and volume of the pellets (Berggren & Alderborn, 2001). The steps were repeated by taken five measurements.

3.3.3 Floatation Test

The floatation tests were carried out by testing each replicate using 100 ml beakers. Ten replicate diets were dropped into the beakers and observed for 20 minutes. The number of floating pellet (F_f) floated or sunk were observed after 20 minutes and F were calculated using equation below (Solomon et al., 2011). Data are reported as the average of five measurements per sample.

$$F = \frac{Ff}{Fi} \times 100 \quad \textbf{(3.2)}$$

Where F_f is the final number of floating pellet and F_i is initial number of floating pellet.

3.3.4. Sinking Velocity (SV)

In order to measure the sinking velocity (SV), ten pellets from each replicate diet were immersed into a 2000 ml graduated cylinder filled with distilled water, and the time taken to reach the bottom (T) for each replicate were recorded. Data are reported as the average of five measurements per sample. The height of the water column (H) was firstly determined and the sinking velocity was evaluated as equation follows:

$$\textbf{SV} = \frac{\textbf{Height of water column}}{\textbf{Time taken to reach the bottom}} \qquad \textbf{(3.3)}$$

3.3.5. Water Absorption Index (WAI)

The procedure is according de Cruz et al., (2015). A kitchen blender was used to grind extruded product. In a weighed centrifuge tube, 2.5g of ground sample was added with 30 ml of distilled water. The centrifuge tube then agitated for 2 min in at room temperature. The mixture of ground extruded product and distilled water then centrifuge in a Universal 320 Hettich centrifuge (Andreas Hettich GmbH & Co., Tuttlingen, Germany) for 15 min at 3000 rpm. Sediment left in the centrifuge was weighed and the water absorption index was calculated as:

$$\textbf{WAI} = \frac{\textbf{weight of sediment formed (g)}}{\textbf{weight of sample (g)}} \qquad \textbf{(3.4)}$$

Data are reported as the average of five measurements per sample.

3.3.6. Water Solubility Index (WSI)

The supernatant liquid form was poured into dry evaporating dish and dried at 110°C for 24 hours (de Cruz et al., 2015). The weight of dried solids supernatant recovered in evaporating dish was calculated. Lastly, the weight of the gel remained in the centrifuge tube was also evaluated by using the following equations:

$$\mathbf{WSI} = \frac{\textbf{weight of dried solid from the supernatant (g)x 100}}{\textbf{weight of sample (g)}} \qquad \textbf{(3.5)}$$

Data are reported as the average of three measurements per sample.

3.3.7. Hardness

A texture analyzer (Stable Micro System, TA.XT plus, United Kingdom) has been used to test the hardness of extruded product. The texture analyzer model is equipped with a 5 kg load cell. To test the mechanical properties (hardness), extruded product was placed on heavy-duty platform and compressed with a 2 mm stainless steel cylinder probe at a speed of 10 mm/s (Jimmenez et al., 2015). The compression test was also set at 75% of extruded product deformation. The hardness value was given in force (N) at breakage point. The measurements were done in triplicate where each measurement was an average of 5 pellets. A force time curve will be recorded from the analysis. Result of this analysis will show the maximum peak force.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Overview of Result

The nine replicates formulations were formulated to find the best floatable pellets by using extrusion method. The result of the physical properties tested was tabulated in Table 4.1 (Appendix I). There were seven properties that have been measured which are hardness, water absorption index, water solubility index, floatability, sinking velocity, bulk density and expansion ratio. The details of the explanation were discussed below.

4.2. Effect of Water Absorption Index, WAI

The variation of moisture content and different ratio of black soldier fly larvae and starch for formulation on water absorption index of fish feed pellet is shown in Figure 4.1. Water absorption index measure an index of starch gelatinization was in the range of 294.60% and 322.25% in Table 4.1 (Appendix I). The highest water absorption index for pellets is 322.25% at 40% moisture content, while the lowest value for water absorption index for pellets is 294.60% at 20% moisture feed content for Formulation 3 (Table 3.1). It indicated that water absorption index value increasing with the increasing of moisture content. At higher temperature, starch granule was disrupted and more water was bound to the starch molecule resulted in high value of water absorption index (Seth et al., 2015). In addition, at higher moisture content, the viscosity of starch would be low and it was allowing the starch to move freely and thereby enhancing the penetration of heat as a result of greater gelatinization. However, at lower moisture content, it can be interpreted that more shearing action in the barrel causing more mechanical damage to starch, thus lowering the water absorption value (Seth et al., 2015).

Theoretically, the water absorption index will be high if the starch gelatinization is high. Water absorption index can be well correlated with the degree of starch gelatinization occurs during extrusion (Volpe et al., 2012a). It is followed by degradation and dextrinization which are mainly responsible by water absorption. Unfortunately, the high value of water absorption index is not sufficient for storage of extruded (Singh & Muthukumarappan, 2016). If extruded absorbs more water, it will be susceptible to microbial attack. From our result (Figure 4.1), it can be seen that WAI increase as the moisture content increase which is in agreement with studies conducted by Singh & Muthukumarappan, (2016).

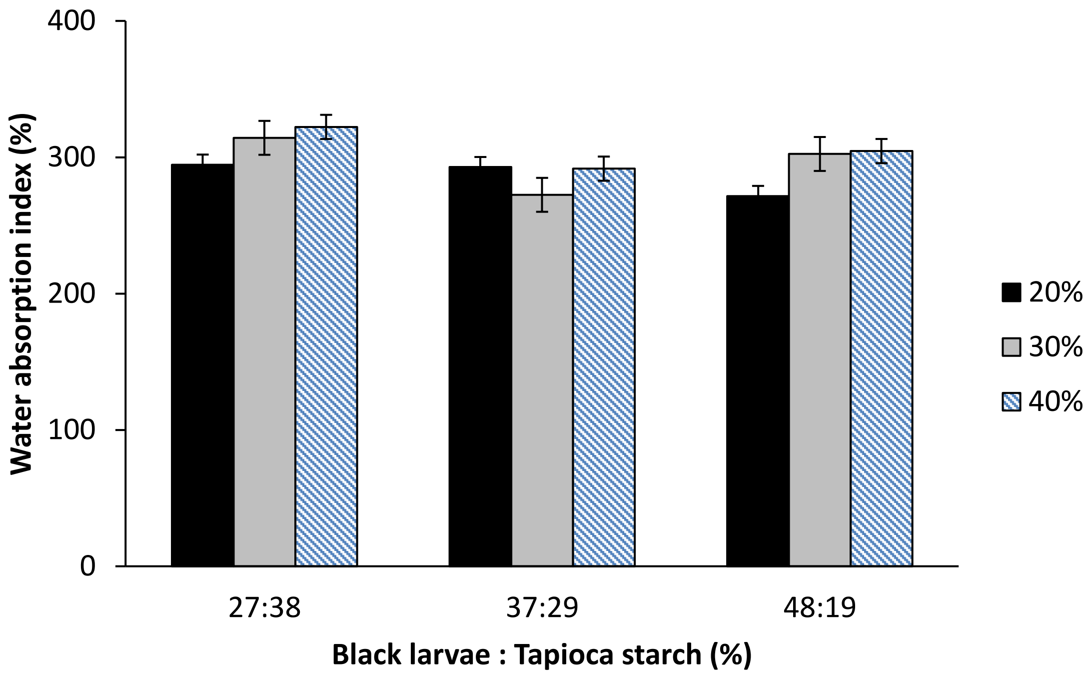

Figure 4.1 : Effect of moisture content (%) on water absorption index (%) of extruded product.

4.3. Effect of Bulk Density, BD

The targeted fish (Tilapia) is a surface feeder and a lower bulk density is required to obtain floating feed. By changing the moisture content of ingredient mixture had a significant effect on the bulk density. Based on Figure 4.2, the highest value of bulk density for this formulation was found at 89.28 kg/m^3 while the lowest value of bulk density was found at 81.90 kg/m^3. The results show that the bulk density of the fish feed pellets decrease with increasing of moisture content. The different ratio of starch also represents the different value of bulk density. Different in pellets sizes also give different in bulk density.

The inclusion of tapioca starch in the formulation has helps the pellets to expand more. Bulk density of extruded product is usually related to the gelatinization characteristic of starch. At higher temperature, starch is likely to gelatinize and cause the volume of the extruded product increased (Case et al., 1992). Normally, the higher the percentage of starch in the formula, the easier it is to produce an expanded pellet that will float. In practice, around 20% or more starch is normally required to produce floating pellets (Vijayagopal, 2004). .

It was observe that generally bulk density of the fish feed pellets decrease with increasing of moisture content and also the volume of extruded that related to gelatinize of starch (Ojo et al, 2014). The degree of superheating of water within the extruder was increased with the high temperature used in barrel lead to encourage of bubble formation and also a decrease in melt viscosity leading to reduce density which was observed in this work (Singh & Muthukumarappan, 2016). Thus, Formulation 1 (Table 3.1) was suitable to produce floatable pellet since the starch content was highest and can cause better gelatinization in order to expand the pellets for floatation purpose.

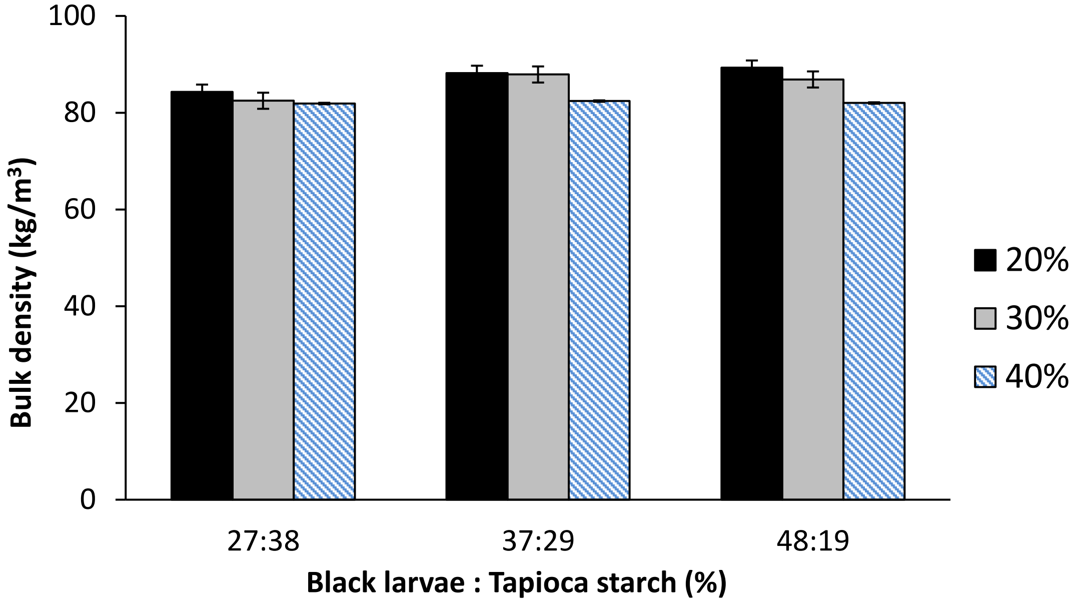

Figure 4.2 : Effect of moisture content (%) on bulk density (kg/m^3) of extruded product.

4.4. Effect of Floatability

Figure 4.3 show the different value for floatability of the fish feed pellets. The results indicated that the floatability value increased with the increasing of starch percentage. The floatability value shows an increasing from 25.83% to 99.87% as the starch content increase too. The highest value for floatability was found for 38% starch ratio at 40% moisture content. While the lowest value of floatability at 19% starch content is at 14.08%. The acceptable high floatability ratio was only achieved when the extrusion mixture moisture was maintained at 40%. The moisture content of extruded also affects the floatability value.

The factor that made the pellet to float was by inclusion value of starch. Including starch in the formula can only produce an expanded pellet and varying the degree of expansion for the pellet (Vijayagopal, 2004). According to recent studies, they found that around 20% or more starch inclusion was normally required to produce floatable pellets (Vijayagopal, 2004). Fine grinding of the starch assists gelatinization and expansion (Volpe et al., 2012). Fat level also may help the pellets to float on the water due to the vegetable oil spraying after the extrusion.

Fat level in the extruded mixture must be kept at 3% or less in the production of floating diet (Umar et al., 2013). The floatability was probably related to the diet expansion rather than bulk density as the expansion increased with the increase of mixture moisture (Umar et al., 2013). Vijayagopal, (2004) stated that fat levels in the pellets should be kept or 6% or less if a floatable pellet is the desired product. This is because fat in the extruded mixture tends to retard the expansion and it complex with the starch inhibiting the gel forming characteristics of this material. As conclusion, the Formulation 3 shows the most optimum formulation for producing floatable pellets since it contains the highest amount of starch content.

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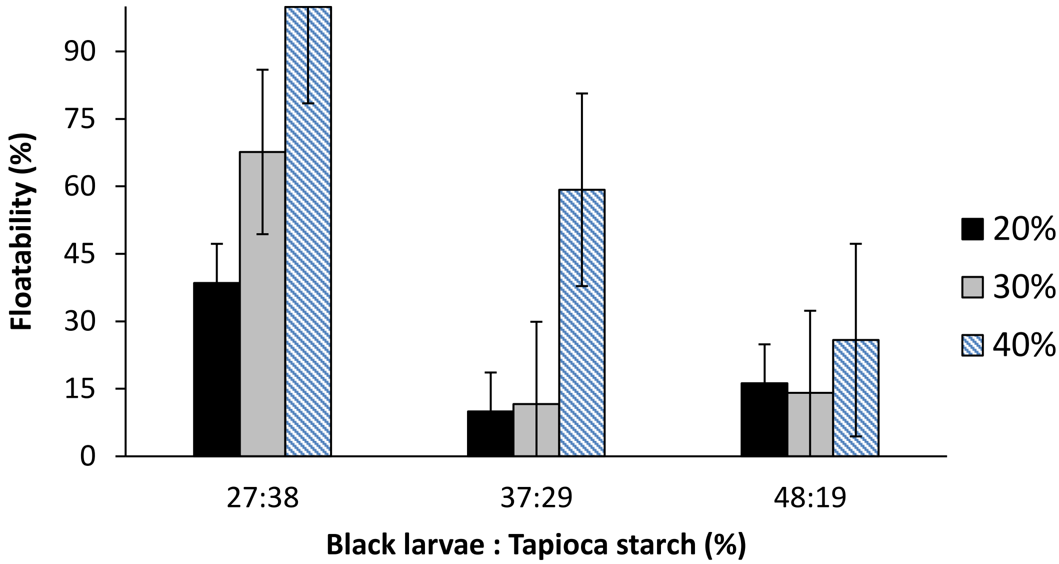

Figure 4.3: Effect of moisture content (%) on floatability (%) of extruded product.

4.5. Effect Water Solubility Index, WSI

The effect of extrusion using different formulation on water solubility index of fish feed pellet is shown in Figure 4.4. Several studies found that increasing the feed moisture content will increases the bulk density, water solubility index, water absorption index and hardness and decreases the expansion ratio (Seth et al., 2015). From the Figure 4.4 it can be seen that, the water solubility index decrease with increase in feed moisture from 20% to 40 % whereby the value at 20% moisture is 81% followed by 30% moisture content at 75.40% and at 40% moisture content the value would be 68.09% water solubility index for 19% starch content. It shows similar findings with Vijayagopal, (2004) whereby the water solubility index decrease as the feed moisture increase. However, the value of water solubility index shows an increasing as the moisture content increase at 29% starch content.

The water solubility index measures the level of starch conversion and indicates degradation of molecular compound during extrusion whereby at lower water solubility index there is minor degradation of starch and this leads to fewer soluble molecules in the extrudates (Shrestha et al., 2010). In addition, it also measures the amount of soluble components released from starch after extrusion cooking. Generally, as the moisture content was lowering so it tends to have increased the drag force at the die making the starch to gelatinize. Plus, the higher moisture content above 18 % might have helped the polysaccharides to dissolve easily to the food matrix, as a result increased the water solubility index.

Besides moisture content, barrel temperature also give effect to water solubility index. It was due to the degradation of starch at higher temperature (160°C) expose to product inside the barrel and greater shearing action of the blend (Seth et al., 2015). When the value of water solubility index is high, it will help in digestion of fish. For aqua feed production it is desirable to have more water solubility index value.

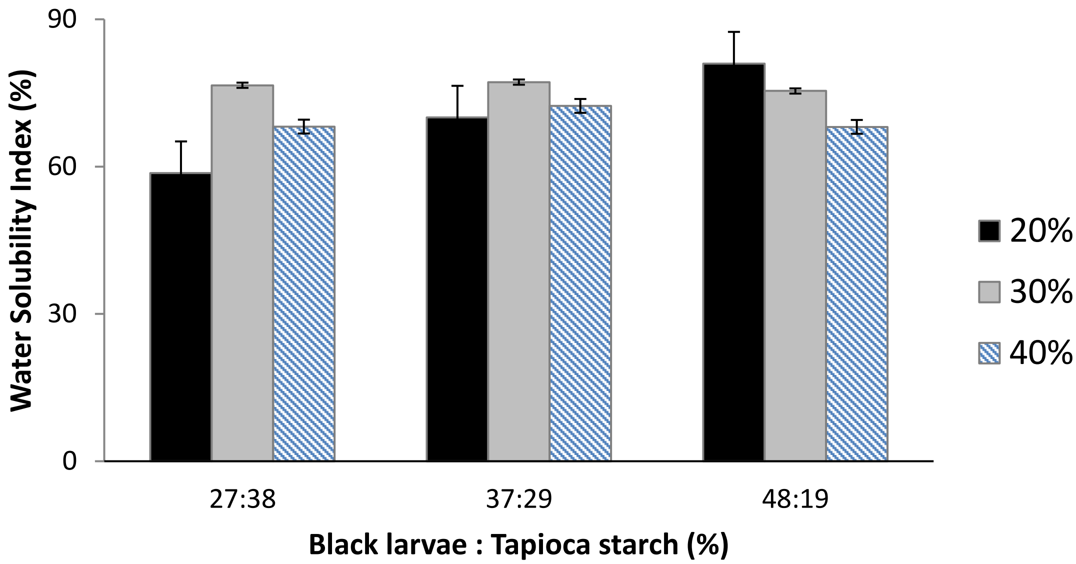

Figure 4.4: Effect of moisture content (%) on water solubility index (%) of extruded product.

4.6. Effect of Expansion Ratio

Figure 4.5 shows the effect of moisture content and starch content on expansion ratio of fish feed pellets. The highest expansion value (1.17) was determined at 40% feed moisture and 29% and 38% starch content, whereas the lowest value expansion ratio (0.45) at 20% moisture content and 29% starch content. Expansion of extruded product is caused by the formation of starch matrix that entraps water vapour. Water vapour that entrapped in the starch will form bubbles during cooking extrusion (Ali et al., 1996).

There are two factors that have been found to affect expansion ratio of extruded product which are elastic force and bubble growth force. Extruded products expand at the die exit, where at this point the moisture of mixture removed off as steam. Based on recent studies, they had been reported that expansion ratio increased as well as feed moisture increase (Singh & Muthukumarappan, 2016). Similar trend was observed in this study. It is probably happen because black soldier fly larvae competed with starch and soybean for moisture that stimulates the starch gelatinization and increased the air cell size during extrusion which makes pellet more expended in diameter and floats.

This condition surely affected the degree of gelatinization, degree of expansion and bulk density of extruded. Gelatinization is crucial because it affects feed digestibility, expansion, and contributes to water solubility and particle binding (Foley & Rosentrater, 2013). It concludes that the increased in moisture content will increased the drag force that exerts more pressure at the die that resulting in greater expansion of extruded at the exit. Furthermore, with the higher temperature at barrel temperature of the extruder can attribute to the starch gelatinization and strengthening of structure of the pellet (Ali et al., 1996). In conclusion, Formulation 3 (Table 3.1) will be chosen as the best formulation to produce floatable pellet.

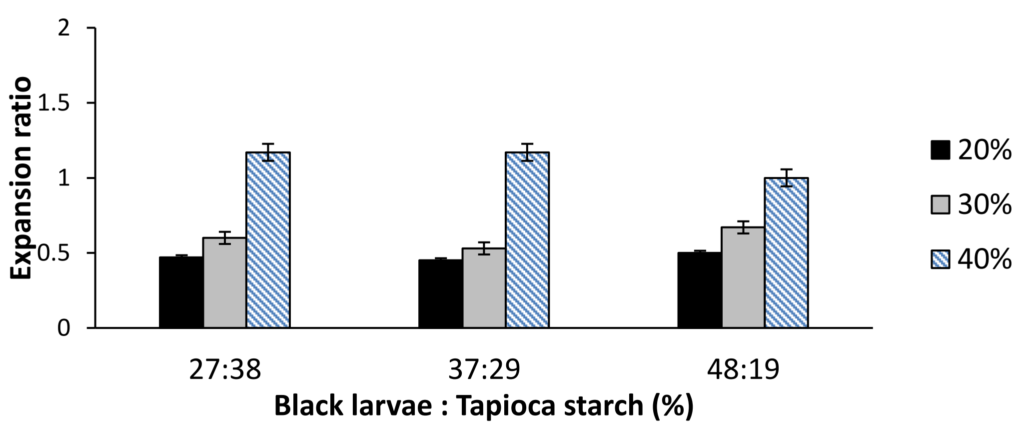

Figure 4.5: Effect of moisture content (%) and starch content (%) on expansion ratio of fish feed pellets.

4.7. Effect of Sinking Velocity

The determination of sinking velocity is related to the absorption of water when the feeds float on the water surface (Umar et al., 2013). Based on the Figure 4.6, the floating extruded started to sink within 4-6 minutes. The sinking velocity was 33.33 m/s when moisture content is 20%, 14.2 m/s when moisture content is 30% and 11.83 m/s when moisture feed is 40%. The trend was quite similar for other formulation even the starch ratio is different.

However, the pellets were sinking faster with the increasing of feed moisture in the formulation. This is due to the greater the gravitational force acting downward on it. The force of buoyancy on the object is how much the water pushes it upward. The strength of this buoyancy force is proportional to the volume of the object. If the particle is denser than water, its gravitational force exceeds its buoyancy force and the net force on the particle is downward. If the particle is less dense than water, its buoyancy force is stronger than the gravitational force and the net force on the particle is upward. So with the increasing of moisture content, it also increases the volume of the pellet hence made the pellets denser than water.

Basically, the velocity determines the stability of extruded diets in water. Studies from Umar et al., 2013 stated that, the increasing of expansion of an extruded diet correspond to the increasing of feed moisture content which cause increasing in bulk density and sinking velocity. In contrast, the present study indicated that an increase in diet expansion with the increase in diet mixture moisture lowering the bulk density and increasing in sinking velocity. It shows that Formulation 1 (Table 3.1) was good to use in producing floatable pellet since it took more time to float on the water.

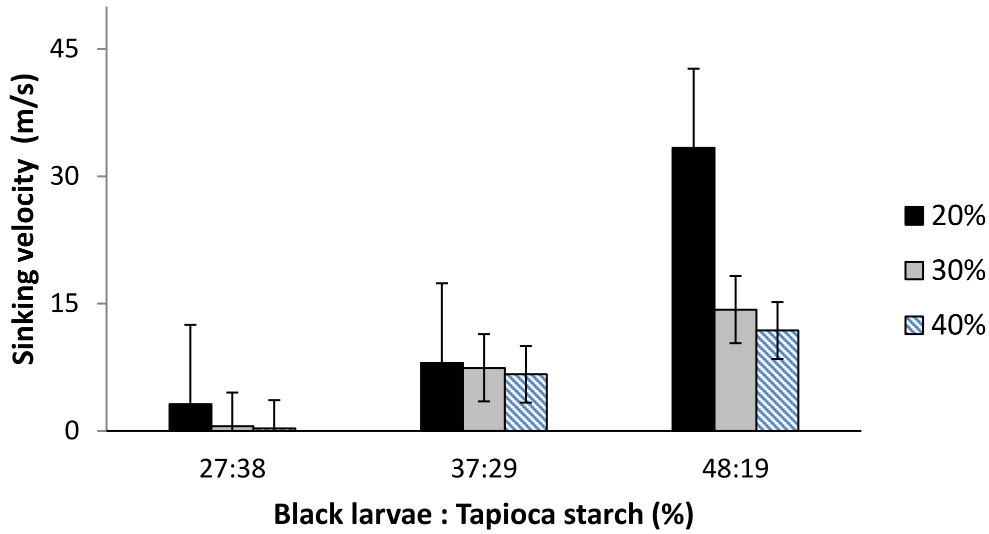

Figure 4.6: Effect of moisture content (%) on sinking velocity (m/s) of extruded product.

4.8. Effect of Hardness

Hardness can be defined as the maximum force required for a probe to penetrate the extruded product (Ding, 2006). The highest maximum force 271 N was measured on the extruded product formed at moisture content 40% (Figure 4.7) while the lowest maximum force is 25 N. When the moisture content decreased to 20%, the force decreased to 199N. Supposedly, as the moisture content of extruded decrease so the hardness of the extruded will be higher. However this was occurred due to the lowest starch level among the protein sources that have been evaluated.

There were significant differences in extruded hardness for the different protein ratio. The hardness range is from 82 N to 271 N. It was occurred within 40% where the feed moisture was the highest. In general, the hardness of extruded are essentially referring to the physical integrity of the finished product in terms of handling and transport plus also relates to generation of broken extruded (Draganovic et al., 2011). Our results show that the extruded hardness was hardly correlated to feed moisture and also protein source ratio together with starch content. The lowest hardness value may be due to the particle size range of mixing ingredients as the size of grinding feed ingredients is not uniform whereby the size of black larvae was 200µm and soy bean ground were 500µm while the tapioca starch was at the very finest particles size in powder. There is a major effect in mixing when the particles of sizes were not uniform.

The pulverization fineness of feed ingredients plays the decisive role in feed pellets hardness. Generally, the finer the particle size the better the starch gelatinization. Fully gelatinizing the starch can ensure the good bonding effect of feed ingredients which makes the feed pellets not easily broke and have good hardness. The greater the gelatinization of starch is, the harder the pellet would be. Other ways could be used to reduce pellet hardness. However, this needs further investigation.

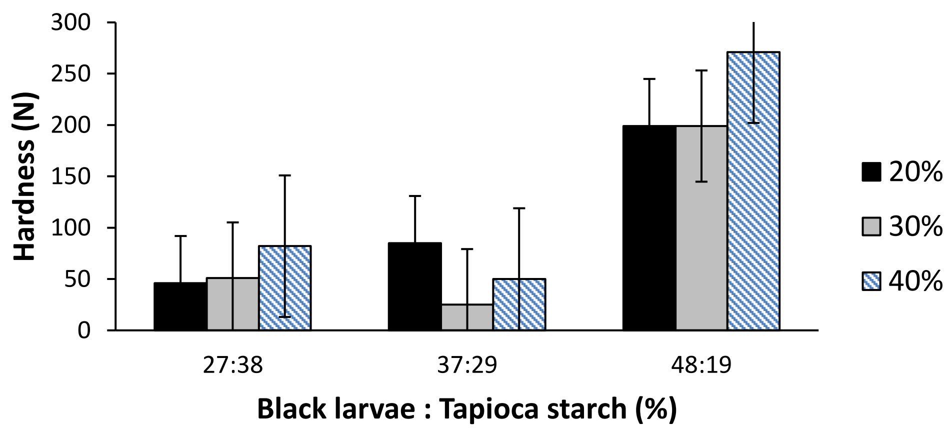

Figure 4.7: Effect of moisture content (%) on hardness (N) of extruded product.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusions

This project was designed to develop a new formulation of fish feed pellet by using Black Soldier Fly Larvae (BSFL). By varying the formulation of moisture content in each replicate with different ratio of BSFL and tapioca starch, the floatable pellets are produced via extrusion process. The physical characteristics of the pellet like bulk density, water solubility index, water absorption index, expansion ratio, floatability, hardness and sinking velocity of each extruded were finally determined.

Theoretically, the pellet produced in this study should be able to float as their bulk density were less than 550g/l according to study conducted by Vijayagopal, (2004). The vegetable oil was sprayed after the extrusion process. In this study, an acceptable high floatability ratio was only achieved when the extrusion mixture moisture was maintained at 40%. By increasing the moisture content from 20% - 40%, a floatable pellet can be achieved due to the lowest the bulk density due to the gelatinization of starch and expanded the size of pellet and make the pellet to float.

The floatability was probably related to the diet expansion rather than bulk density. This is due to the increasing of feed moisture directly increased the expansion ratio of pellet. Water absorption also increased as the feed moisture increase and it shows that the pellets are good for storage. It was found that the particle size of feed ingredients did affect the hardness. The hardness of pellet was increased as the finer the particle was. In conclusion, the black soldier fly larvae can be the best ingredients to replace fishmeal in the fish feed by using Formulation 3 (Table 3.1) for 40% feed moisture mixture since the protein content is higher and it is easy to breed also will reduce the cost to manufacture the pellets.

5.2. Recommendations

Findings from this project can be extended for other new study that related to cooking extrusion which can improve the quality and acceptability of this product in the future. It is possible to commercialize this product to a larger scale since Malaysia have great source of tapioca starch and also the suitable condition to breed black soldier fly larvae. The following recommendations could be done:

1. Study the effect of barrel temperature and the range of screw speed during the extrusion cooking.
2. Study the effect of oven drying method on physical properties of extruded because the more time taken take to dry the pellets it will cause the sinking velocity to decrease.

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APPENDIX I

Table 4.1: Determination of physical properties of fish feed pellet

Black larvae : Tapioca starch (%)	Moisture content (%)	DM (g/kg)	BD (kg/m^3)	F(%)	WAI (%)	WSI (%)	SV(m/s)	ER	Hardness (N)
48:19	20	166.20	89.28	16.21	271.62	81.00	33.33	0.50	199.00
	30	190.00	86.87	14.08	302.42	75.40	14.28	0.67	197.00
	40	198.90	82.00	25.83	304.56	68.09	11.83	1.00	271.00
37:29	20	174.80	88.20	9.95	292.82	70.01	8.03	0.45	85.00
	30	195.10	87.90	11.63	272.44	77.21	7.43	0.53	25.00
	40	198.00	82.40	59.25	291.65	72.36	6.67	1.17	50.00
27:38	20	179.70	84.30	38.55	294.60	58.69	3.16	0.47	46.00
	30	196.70	82.47	67.65	314.24	76.57	0.55	0.60	51.00
	40	199.80	81.90	99.87	322.25	68.15	0.28	1.17	82.00