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Influence of Torula yeast *(Candida utilis)* as the Fishmeal replacer on Rheological and Physical qualities of Pelleted shrimp feed.

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Abstract

Global shrimp farming is increasing and it has become the potential in many countries. Fishmeal (FM) has been the major protein sources due to its nutritional qualities as well its reasonable price. The constant FM supply with the increasing demand lead to price elevation. Due to high demand and limited FM supply, many studies to identify the alternative protein sources have been conducted. The acceptable alternative source must have good nutrient qualities, reasonable price, environmental friendly and that cannot compete with human resources such as water, land and food. In this study Torula yeast (TY) *Candida utilis* seems to meet some of mentioned criteria among the other alternative sources of protein.

The objective of this paper was to analyze the influence of TY as a FM replacer on rheological and physical qualities of pelleted shrimp feed. The rheological and physical qualities analyzed were moisture content, tensile strength, water activity (a_w), surface water/ oil contact angle (CA) and under water pellet swell (UPS).

Six-pelleted treatments were analyzed. In the first five treatments (Tr1, Tr2,Tr3, Tr4 and Tr5). FM were replaced by TY on the amount of 0%, 2.5%, 5%, 10% and 20% respectively whereby the last treatment (Tr6) was 100% of TY as an ingredient. The rheological and quality characteristics results for all parameters were promising. Among all treatments, the replacement of FM with torula yeast (20%) in the pelleted shrimp feed has more promising rheological and physical qualities to all parameters analyzed. No negative influence on analyzed rheological and physical parameters. Perhaps TY can replace FM in the future.

Abbreviations

A	- Area
ANOVA	- Analysis of Variance
CA	- Contact angle
CA_{oil}	– Surface oil contact angle
CA_{water}	– Surface water contact angle
FM	- Fishmeal
g	- Gram
MCAP	- Moisture content after pelleting
MCBP	- Moisture content before pelleting
MCP	-Mono Calcium Phosphate
MgO	-Magnesium Oxide
m	-minutes
mm	-millimeter
MnO	- Manganese Oxide
MPa	- Mega Pascal
ms	- millisecond
MSP	- Mono Sodium Phosphate
MT	– Metric Tones
Mt	- Million tones
N	-Nitrogen
^oC	- Degree centigrade
p	- Probability of variance
P_{max}	- Maximum pressure for pellet discharge
r	- radius
SBM	- Soya bean meal
SPC	- Soya Protein Concentrate
s	- second
Tr	- Treatment
Tr1	-0% yeast
Tr2	- 2.5% yeast

Tr3	-5% yeast
Tr4	-10% yeast
Tr5	-20% yeast
Tr6	- 100% yeast
TS	-Tensile strength
TY	- Torula yeast / *Candida utilis*
UPS	- Under water pellet swelling

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Chapter I: Introduction

Higher rise in aquaculture production lead to increased demand of aqua feed (Gamboa - Delgado et al., 2013). Technological progress, favorable economics of large-scale intensive farming, productivity growth and globalizing trade are factors that drive the rapid growth of aquaculture (Bastock et al., 2010). Fishmeal (FM) is the primary source of protein in aquaculture (Olvera-Novoa, 2002). FM was chosen as the important protein ingredient for both aquatic and terrestrial animal due to nutritional qualities and relatively cheap price (Olsen & Hasan 2012). The most protein source used in aquaculture is FM (Tacon, 1995). Swick et al. (1995) and Samocha et al.,(2004) reported that usefulness of FM in aqua feed is due to essential nutrients it have. Nutrients such as vital amino acids, protein, cholesterol, minerals, vitamins, undefined growth factors, and attractants are available in FM as well as its palatability property they are highly required in aquaculture industry.

FM supply varies and its availability is limited due to reduction in fish stocks caused by the decline of ocean fisheries stock, over exploitation, and some climatic phenomenon such as El-Nino (Olvera-Novoa, 2002; Tacon & Metian 2008). The mentioned challenges can seriously affect the aquaculture profitability and sustainability.

Commercial feed demand to the limited FM available has been enhanced by the increase in the carnivore species production such as shrimps, salmonids and other, hence the sufficient supply of the sustainable feed sources with high quality will determine further expansion in production (Øverland & Skrede 2016). Gamboa- Delgado et al. (2016) reported the improvement or manufacturing of the highly demanded FM by the alternative feed ingredients.

By products from plants and animals, and microbial sources have been tested as the alternative ingredients to replace FM or tested to be used as feed ingredients (Gamboa-Delgado et al. 2016; Tantikitti 2014). The use of plant and microbial sources as protein substitution in shrimp feeds around the world is due to their suitable content of amino acids, acceptable level of protein, consistent quality and economic opportunity (Watanabe, 2002; Sookying et al., 2013). Considering human health environmental implication, use of

pesticides, substituting marine feed ingredient with products from plants in aquatic feed in a sustainable way is questionable due to their demand on arable land and water, their use of pesticides (Fry et al., 2016). Also, anti-nutrients (Nigam, 1998) insecticides (Schlechtriem et al., 2016) and potential mycotoxins contents (Anater et al., 2016) are the challenges associated with further increase in plant protein sources. Genetically modified plants are increasing (Flachowsky et al., 2005) and this limits its acceptability among consumers in some markets, hence the change to microbial sources is the attractive research area in recent years (Somerville et al. 2010; Balan, 2014). Identification of protein sources that are novel non-conventional that can be converted into the feed ingredient of high quality in aquaculture, and that is locally available is urgent due to challenges existing in plant and animal protein sources (Øverland and Skrede 2016, Øverland et al. 2013, Samocha et al., 2004). There is a requirement of the environmentally friendly, economically competitive, sustainable novel non-food ingredients for further expansion of production of carnivores' species (Øverland and Skrede 2016, Øverland et al. 2013). Among bacteria, algae, yeast is the mostly used one (Tacon, 1994).

Rheological characteristics are very important in pellet formation (MacRitchie et al., 2002). Rheology is applied all along the production line in pelleting process and (MacRitchie et al., 2002). The study of the flow and deformation of matter is what is known as rheology (Maali et al., 2011). Faith & Morrison, (2004) reported that the materials some flow in un-usual manner (example dough) and some flow in a usual (example water and oil) manners. Some of the rheological and physical quality parameters mechanisms are described in chapter II of this paper. Viscosity is applied to any study dealing with the property of materials (Maali et al., 2011). According to Barnes et al. (1989), Viscosity of the materials depends on pressure, shear time and rate, and temperature with variations rheological property of material as:

- Shear rate variation is estimated by the ratio of the flowing liquid velocity and the flowing geometry in which it is flowing example radius, and shear layer thickness.
- Temperature variation, the interest is concerned from 0^{o} to 100^{o}C though high temperature reported to have better output.

- Variation with pressure, liquid viscosity increases with pressure. Viscosity of oil requires more elevated pressure than that of water. The viscosity of water and glycerol at room temperature is reported to be 10^{-3} and 10^{0} respectively.

According to Barnes et al. (1989), Solid is defined as the material that when subjected to a given stress will not continuously change there will be final fixed change for a given stress. While liquid was defined as a material that change its shape (i.e. will flow) continuously when a given stress is applied, regardless of how small the stress is.

1.1 Literature Review

1.1.1 Shrimp production

Two major species of shrimp were restricted in 2010, that is the black tiger prawn (*Penaeus monodon*; 781 582 tonnes or 21 percent) and white leg shrimp (*L. vannamei*; 2 720 929 tonnes or 72 percent of total production by weight) (Tacon et al., 2013). Other species are cultivated also as a minor species such as banana prawn (*Fenneropenaeus merguiensis*; 19 821 tonnes or 0.5 percent), the Indian white prawn (*P. indicus*; 27 325 tonnes or 0.7 percent), the fleshy prawn (*P. chinensis*; 45 339 tonnes or 1.2 percent) and the kuruma prawn (*P. japonicus*; 56 739 tonnes or 1.5 percent) (FAO, 2012: Tacon et al., 2013). Shrimp is one among the most potential seafood commodities and most of them are exported from developing countries (Sani et al., 2013). Shrimp farming has begun developing in many countries and it is rapidly growing now. In 2001 to 2010 shrimp production grew from 1 million tones (mt) to 4.02 mt due to shrimp vibrant production potentials. Shrimp production is reported by 59 to the Food and Agriculture Organization of the United Nations (FAO) (Tacon et al., 2013), Countries such as China 38.4 percent by weight of total production of the global, Thailand (15.0 percent), Indonesia (10.1 percent), Ecuador, India, Mexico, Malaysia, Brazil and Philippines in (5.9, 3.0, 2.8, 2.3, 1.8, 1.5 percent respectively) has been reported as the major producing countries in 2010 (FAO, 2012 : Tacon et al., 2013). The growth of shrimp production is expected to reach 50% in 2010-2030 globally (Larkin, 2012). Josupeit, (2004) also reported the increasing production of commercial shrimp farming. The increase in shrimp industry led to the increase in feed production too. The increase in shrimp feed production made its industry to have paralleled

growth (Sookying et al., 2013). Cheng et al. (2002) reported lipid and protein sources used in shrimp feeds are from fish oil and fishmeal.

1.1.2 Shrimp nutrient requirements

Formulated ingredient mixture of shrimps are composed of proteins and amino acids, carbohydrates and sugars, fats and fatty acids, minerals and trace elements, and vitamins (Tacon et al., 2013). Example Table 1 present the minimal requirement of *L. vannamei* maximum with Vitamin, minerals and fatty acids and amino acids limited values. The values represent nearly 100% bioavailability. Reported by the National Research council (NRC 2011) (Sookying et al., 2013). More balanced nutrient profiles can be obtained through use of complementary diets in the feeds (that is minerals, fatty acids, essential amino acids) and facilitate processing of feed and increase utilization of nutrients (Amaya et al. 2007a). Reasonable palatability, favourable profile of amino acid and high digestibility of nutrients are essentials in the formulated shrimp diet (Gatlin et al., 2007; Naylor et al., 2009). Due to shrimp feeding behavior organoleptically and nutritionally adequate diet is important for satisfactory feed intake and good growth (Tantikitti, 2014). Histidine, lysine, threonine, isoleucine, methionine, tryptophan, valine, leucine, phenylalanine and arginine are ten indispensable amino acids that are necessary for shrimp growth and maintenance (Kanazawa, 1989; Guillaume, 1997).

Shrimps require lipids that constitute the major macronutrients for cellular building blocks and energy provision, growth maintenance, health, reproduction and welfare (Lim et al., 1997, Sookying et al., 2013). We must ensure that EFAs required by shrimps are met once replacing fishmeal with the alternative ingredients (Sookying et al., 2013). Crustaceans and shrimps require lipid at the level of 5 and 10 g/ kg (Cheng et al., 2002).

Cholesterol is the important component of the cell membrane and it has been reported to be the most essential component for growth and survival of shrimps (Kanazawa et al., 1971; Gong et al., 2000; Morris et al., 2011). Cheng & Hardy, (2004) reported cholesterol to be the precursor of mouIting hormones, bile acids and steroids. Interaction of the dietary phospholipid and cholesterol lead to the improvement of growth as well as triglycerides

and total lipid retention in hepatopancreas and cholesterol of *L. vannamei* juveniles muscle.

Minerals and vitamins can be directly assimilated by shrimp from the aquatic environment (Montoya et al., 2000). Some soluble minerals can be utilized by shrimps such as iron, phosphorus, calcium, sodium, selenium, potassium, copper, zinc and magnesium though epidermis, gills or both from the water (Sookying et al., 2013). Though phytoplankton require much level of phosphorus concentration that available in natural water (Boyd, 2007). Thus, when replacing FM, phosphorus supplementation is very crucial for the optimal growth of shrimps. Davis et al. (1993) reported that the phosphorus range of about 3.4-20 g/ kg required by juvenile *L. vannamei* while post larval *L. vannamei* require the range of about 20.9 – 22.0 g/kg (Niu et al., 2008). (Davis et al., 1993; Cheng et al., 2006) explained that, the calcium contents in the diet can determine the level of phosphorus required in the diet of shrimp, although there is no need to supplement calcium in the shrimp diet.

Table 1: Nutrient requirements of *L. vannamei* (DM basis) (NRC, 2011: Sookying et al., 2013)

Item	Minimum requirement for *L. vannamei*
Typical energy and protein concentrations	
Digestible energy (kcal kg^{-1} diet)	3000
Digestible protein (g kg^{-1})	300
Amino acid (g kg^{-1})	
Lysine	16
Fatty acids (g kg^{-1})	
n-3 LC-PUFA	2.5–5.0 g kg^{-1}
Cholesterol (g kg^{-1})	1.3
Macrominerals (g kg^{-1})	
Magnesium	2.6–3.5
Phosphorus	3–7
Microminerals (mg kg^{-1})	
Copper	16–32
Selenium	0.2–0.4
Zinc	15
Fat-soluble vitamins	
A (mg kg^{-1})	1.4
E (mg kg^{-1})	100
Water-soluble vitamins (mg kg^{-1})	
Vitamin B6	80–100
Vitamin C	50–100

1.2 Challenges within the pelleted shrimp feed

1.2.1 FM production and demand

Hardy & Tacon, (2002) reported that the fishmeal production has been constant over the past 15 years for about 6,200,000 MT. Cabral et al. (2011) reported the finite fishmeal and fish oil supply. Increase in cost of FM lead to the increase in price of shrimps (Shiu et al., 2015).

Tacon & Barg (1998) reported that, about 25% of the fishmeal is fed to the farmed shrimps. In the year 2000, the amount of fishmeal used in shrimp farming was about 372 000 MT. And about 17.6% of the FM was used for production of feeds for general aquaculture worldwide (Barlow, 2000). About 25% to 50% of FM is used in the commercial feed production for shrimps (Gonzalez-Rodriguez and Abdo de la Parra, 2004; Tacon and Barg, 1998). Different studies also reported the high demand of FM of about 200-300g per kg of feed in shrimp formulation (Tacon and Metian, 2008). FM has also been used in compounded feed for herbivores/ omnivores species such as crucian carp (*Carassius carassius*), common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idellus*.This high demand of FM requires sustainable alternative source of protein to cover this the demand (Piedad-Pascual et al. 1990; Sudaryono et al. 1995; Cruz-Suarez et al., 2001; Smith et al., 2001; Samocha et al., 2004; Amaya et al., 2007a, b; Roy et al., 2009).

1.2.2 Physical quality of the pellet

Small particles agglomeration into large particles by the mechanical processes in the combination of pressure, heat and moisture is known as pelleting (Falk, 1985).Behind the pelleting process several factors are considered as motivations such as:

- Increasing the feed intake of the animal that lead to the improvement in the body weight gain of the animal (Abdollahi et al., 2013).
- More nutrients are obtained per every single unit fed by the animal, which is not possible when feed the mash (Jones et al., 1995:Abdollahi et al., 2013).
- Wastage of the feed is reduced (Jensen, 2000).
- Complete diet is obtained by the animal and sorting of nutrients is prevented
- Ingredients are fixed within the pellet even those that have segregation behavior

such as limestone (Greenwood & Beyer, 2003).

- Transportation and flow property of the feed is improved though pelleting due to increasing of bulk density of the mash feed (Abdollahi et al., 2013).

In manufacturing of the aquatic feed the water stability is an important quality parameter (Obaldo et al., 2002). Pellet water stability is referred to as the pellet's ability to undergo minimal disintegration and leaching of nutrients (withstanding integrity) while in water until consumed by animals (Obaldo et al., 2002). Benthic and slow feeding habit of shrimps lead to their suitability to high water stable pellets (Obaldo et al., 2002). The benefits of high water stable pellets to shrimps are such as maximum nutrient utilization and hence reduction of feed costs as well as improvement of water quality and survival rates (Tacon et al., 2013). Integrity of the pellet can be determined though obtaining the knowledge of the binding property of ingredients, the use liquids (molasses) or binders (Thomas & Van der Poel 1996).However, if the diet has feed attractants that enhance the feed consumption, then the duration degree required for the pellet to stay stable in water is minimized (Lim & Cuzon, 1994).

According to Tacon et al. (2013), successful nutritional performance and economic success of the shrimp feeds, partnership and collaboration between the food producers and the farmers is important through five interconnected factors such as:

- Water stability and physical properties of the diet,
- Composition and content of nutrients in the diet,
- Storage and transportation of the diet prior to feeding,
- The method of feeding used and applied in the farm,
- System of farming, water management, stocking density and natural foods availability,
- Whereby the first two factors can be directly controlled by shrimp feed manufacturers while the last three factors can be controlled by farmers and their stuff.

Advantages of good physical quality of the pellets are such as reduction of fines that may

happen during handling on the farm, in the feed factory as well as during transportation (Skoch et al., 1983; Stevens, 1987; Koopmans et al., 1989a, Koopmans et al., 1989: Thomas & Van der Poel, 1996).

1.2.3 Pond water quality

Water stable pellets are an important quality parameter in formulation of aquatic feed, in this case shrimp (Obaldo et al., 2002). This would reduce the feed loss and nutrient leaching that lead to pond pollution due to overloaded nutrients (Tantikitti, 2014). Shrimps assimilate about 16% of phosphorus (P) and 29% of nitrogen (N) that are added to the ponds as fertilizer input and as food during production cycle (Avnimelech & Ritvo, 2003). The added nitrogen and Phosphorus released to the environment are effluents rich in particulates for example feces, phytoplankton and uneaten food) and dissolved inorganic and organic species (Thomas et al., 2010; Jackson et al., 2003). Famers have incurred massive financial losses due to water quality deterioration and that led to the production breakage and bottleneck of the output (Ferreira et al., 2011; Gertjan & Mark, 2005).

Survival, growth and reproduction of microorganisms are affected by poor water quality (Cabajal-Hernandez et al., 2013). Shrimp cultured in poor water quality are likely to face many stresses such as diseases susceptibility and poor production (Ferreira et al., 2011). Shrimps can be stressed by high concentration of nitrate although ammonia and nitrate are converted into nontoxic nitrate (NO3). Effluents have been reported to influence production of bacteria (McKinnon et al., 2002). Effluents have been also reported to promote water column or anoxia as well as increase in demand of biological oxygen (Trott & Alongi, 2000; Thomas et al., 2010). Less information is known concerning the effects of effluents from shrimp farm within the benthic compartment. Despite of the water stable feeds and palatable feeds, water quality parameters also must be monitored to avoid the crisis on the entire ecosystem (Table 3 in the annex) (Carbajal-Hernández et al, 2013). For more information about water quality assessment, potentials and impacts of quality control of water in shrimps read Carbajal-Hernández et al. (2013).

1.2.4 Economic issues

Profit is the underlying aim of aquaculture, hence it is important to estimate cost and analysis of return of all costs such as cost of feed, cost from sale of production, electricity, labour, stocks, interest of money borrowed and other (Olsen et al., 2012). To produce successful shrimp culture, management of feeding and feeds are the key factors in terms of cost and promotion of growth as shown in Figure 2.

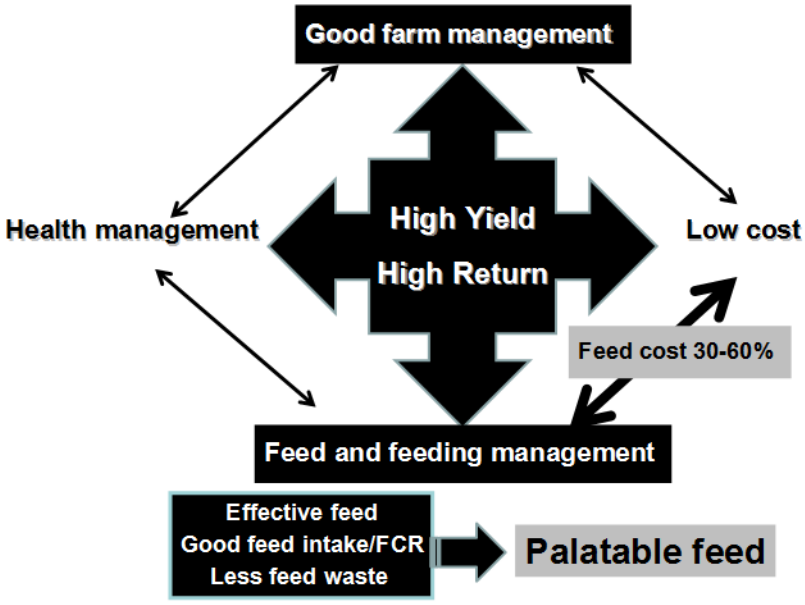


Fig 2: Important factors in aquatic animal production (Tantikitti 2014).

In culture of shrimp about 30 -60 % of variable costs are from feeds (Tantikitti, 2014). High demand of FM led to increase in price and hence increase in cost of feed production (Figure 3). Other researchers also reported the price of FM that was highly increased from 600 USD/ tonne in 2002 to 1200 USD/ tonne in 2009 (Olsen et al., 2012). Over hunting of FM, elevation of prices of FM, diminishing of FM stock and it's volatile in markets lead to dwindling in FM supply. The dwindling on FM supply and price elevation lead to the search of the suitable cheaper FM meal substitutes as the protein source (Tantikitti, 2014).

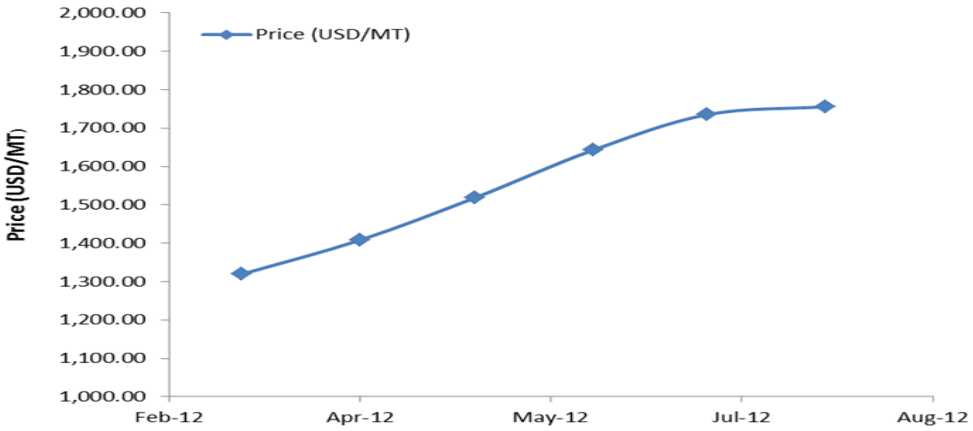


Figure 3: Fishmeal, Peru Fish meal/pellets 65% protein, US Dollars per Metric Ton (World Bank Commodity Price Data, 2012) (Tantikitti, 2014).

1.3 Protein sources to replace FM in shrimp feed

Research institutions and aquaculture feed industry has been conducting a lot of studies to reduce FM dependency because of its limited availability and expensiveness (Olsen & Hasan, 2012). The reduction in the average inclusion level of FM has been attained by many of these studies from 1995 to 2010 (Table 4 at the annex). The projections from 2010 to 2020 are 16% to 8% for shrimps, 22 to 12% for salmon, 26 to 12% for marine fish and 3 to 1% for carps and tilapias (Olsen & Hasan, 2012). Different sources of protein are available in animal feed production such as animal, plant, and microbial sources (Tantikitti 2014).

Animal by products such as bone meal, meat meal, poultry by product meal, and blood meal has been considered as good FM replacer (Olsen & Hasan, 2012; Tacon et al., 2006). Low digestibility and amino acid profile in the blood meal and hydrolyzed feathers has been reported (Olvera-Novoa et al., 2002). Health related problems of animal – by products also have been reported in animal protein sources (Lee et al., 2001).

Apart from animal protein sources, plants proteins sources are also the valuable source of protein nutritionally and economically due to be their nutrients consistent and cheap price (Tantikitti, 2014). Plant proteins such as soybean protein concentrate (SPC), wheat gluten,

and corn gluten have been used in feeds to replace FM. However there are many nutritional drawbacks in plant proteins when compared to FM, especially carnivores specie that are not adopted to plant feed such as salmon, shrimps and other (Olsen & Hasan, 2012). Plant protein sources have been reported to induce toxicity, interfere the vitamin functions as well as reduction of digestion and absorption of nutrients (Krogdahl et al., 2010; Gatlin et al., 2007; Francis et al., 2001). Although plant protein has the mentioned nutritional drawbacks, the improvements were achieved though different processes such as mechanical or chemical processes (Barrows et al., 2007; Gatlin et al., 2007). Biological methods (enzyme treatment) that can remove some ant nutrients such as phytic acid (Storebakken et al., 1998). Some effects of ant nutrient factors can be reduced though heat processes (New 1987: Sookying et al. 2013. Studies shows that it is possible to substitute FM with the proper chosen plant protein or other source of protein (Suarez et al., 2009; Amaya et al., 2007a; Davis &Arnold, 2000; Mendoza et al., 2001). Despite of the mentioned challenges and solutions for plant protein sources, their sustainability is doubtful due to competitive demand with human for resources such as arable land, food and water (Fry et al., 2016). Hence much focus are on the use of microbial / Single Cell Protein (SCP) sources in recent years (Balan, 2014; Tacon et al., 2006).

1.4 Use of Single Cell Protein (SCP)

The replacement of FM in aquaculture field has been further more researched on the use of single cell protein (SCP) either from microalgae, bacteria or yeast (Tacon et al., 2006, Anupama & Ravindra, 2000). Microbial biomass extract protein that is known as Single cell protein (SCP) (Anupama & Ravindra, 2000). Bacteria, algae and fungi can be utilized as SCP and they are the chief source of microbial protein Anupama & Ravindra, 2000). SCP price and nutritional qualities such as amino acid profiles are the determinant of the best source to replace FM and it was implied to other feed ingredients too (Olsen & Hasan, 2012). Protein source should also be environmentally friendly, sustainable, and non-competitive with human for resources such as food, water and land (Fry et al., 2016; Øverland & Skrede, 2016; Øverland et al., 2013).

The availability of nutrients in yeast, bacteria, and algae such as B- vitamins, proteins and other carbohydrates, make them potential source of protein and unconventional ingredient

for aquatic species (Hezarjaribi et al., 2016; Anupama & Ravindra, 2000 ; Sanderson & Jolly, 1994).

Bacterial species such as *Bacillus megaterium*, *Aeromonas hydrophilla* and *Bacillus subtilis* are used in feeds as the SCP origins (Dhanasekaran et al,. 2011). Substrates such as sugars, some liquid hydrocarbons like methane and starch is where bacteria cultivation is possible. About 80% of protein is available in bacteria in a dry matter basis, their 2.2%-3.0% of essential amino acids such as methionine and 15%-16% of nucleic acids (Al-Harbi & Uddin, 2005). High cost have been reported on the production of bacterial proteins so it is unlikely to be used as the protein source in the future unless if it can be obtained from industrial or agricultural waste stream (Tacon et al., 2006).

Several species of microalgae has been reported to be used in aquaculture such as *Skeletonema, Tetraselmis*, *Chaetoceros, Pavlova, Isochrysis* and *Nannochloropsis*, the use *spirulina* and *chlorella* (Spolaore et al., 2006). Due to good nutritional status and safety, microalgae have been approved as the protein source or substitute to other source of protein such as FM or soyabean meal (Becker, 2007). Also astaxanthin that is used in natural pigmentation in fish (Lorenz et al., 2000: Spolaore et al., 2006). Reasonable lipid and fatty acids content (Tokuşoglu & üUnal 2003), minerals and vitamins (Anupama & Ravindra, 2000), Caretoneids example β-carotene (Spolaore et al., 2006). High cost in production of microalgae is the challenge to use as the alternative protein source to replace FM unless the cost is reduced (Becker, 2007; Ratledge, 2011).

Great nutritional value has been reported in brewers yeast and reported to be a good protein source to replace FM partially (Øverland & Skrede, 2016; Oliva-Teles and Goncalves, 2001). Yeast depend less in water, climatic fluctuation and land, also yeast has ability to covert the forestry bio-mass from low value into high value feed ingredients, this makes it potential sustainable ingredient (Øverland et al., 2013). Among mentioned SCP, the most used one is the yeast (Tacon, 1994).

1.5 Yeast species and origin

There are several species of yeast such as *Pseudozyma, Trichosporon, Torulopsi, Cryptococcus, Kluyveromyces, Leu- cosporidium, Williopsis, Debaryomyces, Hansenula, Filobasidium, Pichia, Rhodotorula, Rhodosporidium, Yarrowia, Torulopsis, Candida* / TY and other. Origin of yeast determine their nutritional values for example, yeast produced in carbohydrates are not as better as those grown in alkaline (Olvera-Novoa et al., 2002). Different species of yeast such as *Kluyveromyces marxianus* and *C. utilis* that grow on lignocellulosic biomass have been reported to contain excellent nutrient properties (Øverland et al., 2013). For economical industrial scale, exploitation of the future yeast products based on lignocellulosic biomass are promising as the protein sources that are sustainable (Øverland and Skrede, 2016). Ability to grow in different biomass accumulation such as industrial waste streams, as well as high rate of growth has been reported by yeast (Klein & Favreau, 1995). No negative health effect has been reported when using yeast in aquatic species. (Li & Gatlin, 2003; Li & Gatlin, 2004). Among the mentioned species, Torula yeast is the species of choice in this paper for some additional reasons explained in subtitle 1.6 of this paper.

1.5.1 Chemical Composition of yeast

Growing conditions, growth media, strain and downstream processing after fermentation are factors that determine the chemical composition of whole cell of yeast (Halasz and Lasztity, 1991: Øverland & Skrede, 2016). Yeast has been reported to contain about 65. 3% of protein (Zhang et al., 2009). *Saccharomyces cerevisiae*, has been reported to contain about 62.5% of protein (Yamanda & Sgarbieri, 2005). About 50-55% of the protein content has been reported in *K. fragilis* yeast (Kim et al., 1998). Minerals such as sodium, copper, zinc, calcium, manganese, iron and selenium are available in yeast (Cheng et al., 2004; Chanda and Chakrabarti, 1996). Yeast such as *S. cerevisiae* has (unsaturated fatty acid), fatty acid desaturase also can produce linoleic and α-linolenic acids (Stukey et al., 1989: Yazawa et al., 2009). *kluyveromyces lactis* yeast has been reported to produce linoleic and α-linolenic acids (Dujon et al., 2004). Table 6 report *K. marxianus, C. utilis,* and *S. cerevisiae* proximate composition. For the three yeasts, the crude protein was similar but *K. marxianus* show limited comparable data. Low fiber content and variation in nucleic

acid content, in these three yeasts may be due to different analytical methods, Culture condition and stage of growth (Øverland & Skrede, 2016; Mydland et al., 2008; Yamanda & Sgarberi, 2005; Halasz & Lasztity, 1991).The content of nucleic acids is higher in yeasts compared to animal food and plants, and the cell density is what determines the content of nucleic acid in foods (Øverland & Skrede 2016). On a basis of crude protein, FM has similar contents of indispensable amino acid profiles as yeasts Table 7. Although yeast have low methionine level compared to FM, and higher content of non – protein nitrogen in nucleic acid form. The amino acids reported in Table 7 agree well with the yeast protein reported ranges (Parajo et al., 1995).

Table 6: Concentration (g/kg DM) of crude protein (N. 6.25), nucleic acids, lipids, fibre, Carbohydrates and ash of yeast (Øverland & Skrede 2016).

Organism	Crude protein	Nucleic acids	Lipids	Fibre	Carbohydrates	Ash	Reference
Saccharomyces cerevisiae	445	–	–	6.3[¶]	–	64	43
	485	75[*]	34[§]	122[**]	329[¶¶]	83	61
	539	–	06[‡]	23[††]	50[***]	68	116
	473	48[*]	–	–	77[***]	–	117
	475	60[†]	21[‡]	–	11.4[***]	66	8
	456	–	80[§]	–	232[¶¶]	103	42
	466	–	10[§]	–	–	63	64
	396	9.0[*]	5	314[**]	–	45	37
Candida utilis	501	–	–	–	–	–	55
	462	11.5[†]	24[§]	12[¶]	–	95	44
	482	–	16[§]	6.5[¶]	–	–	54
	598	99[†]	3.2[‡]	–	40[***]	58	8
Kluyveromyces marxianus	544	109[†]	9[‡]	–	9[***]	81	8

[*]RNA; [†]Total nucleic acids; [‡]HCl-EE; [§]Ether extract (EE); [¶]Crude fibre; [**]Total fibre; [††]Acid detergent fibre (ADF); [§§]Neutral detergent fibre (NDF); [¶¶]Total carbohydrates; [***]Starch.

1.5.2 Yeast effects on growth performance, utilization of nitrogen and composition of the carcass

Yeast strain, yeast substrate, post fermentation processing, diet formulation and specie of the animal can determine protein utilization and response on growth of the animal fed yeast diet (Øverland & Skrede, 2016). Studies reported the increase in nitrogen retention and rate of growth to different species of fish for example *Piaractus mesopotamicus*, sea bass and other when FM was substituted partially with brewers yeast (*S. cerevisiae*) (Oliva-Teles &

Goncalves, 2001). High level of (*S. cerevisiae*) of around (500 g kg^{-1} and 750 g kg^{-1}) has been reported to reduce growth rate in rainbow trout while moderate amount (250 g kg^{-1}) was reported to improve the performance of growth in the same specie (Rumsey et al., 1991:Øverland & Skrede, 2016).

Table 7: Average amino acid composition (g 16 g N^{-1} ± STD of non-hydrated amino acids) of yeast compared with fishmeal and soybean meal (Øverland & Skrede, 2016).

Amino acid	Fishmeal[46]	Soybean meal[46]	*Saccharomyces cereviciae*[8,37,43,61,64,116]	SD	*Candida utilis*[8,44,54,55,118]	SD	*Kluyveromyces marxianus*[8,119,120]	SD
Indispensable amino acids								
Arginine	5.74	7.38	4.68	0.60	5.20	0.71	4.34	0.20
Histidine	2.36	2.67	2.47	0.87	1.97	0.28	1.80	0.18
Isoleucine	4.53	4.94	4.43	0.76	4.29	0.34	4.20	0.14
Leucine	7.06	7.80	6.73	1.16	6.19	0.61	6.81	1.37
Lysine	8.18	5.53	6.95	0.70	7.71	1.16	7.39	0.65
Methionine	2.87	1.41	1.81	0.49	1.08	0.23	1.33	0.25
Phenylanine	3.84	5.26	4.18	0.72	3.64	0.51	3.96	0.25
Threonine	4.00	4.03	4.71	0.78	4.71	0.23	5.11	0.33
Tryptophan	1.05	1.41	1.08	0.15	1.17	0.12	0.98	
Valine	4.87	5.51	5.24	0.82	5.08	0.56	5.11	0.62
Dispensable amino acids								
Alanine	–	–	6.13	1.17	5.75	0.74	8.49	1.39
Aspartic acid	–	–	9.51	1.48	8.30	1.59	10.59	1.11
Cysteine	1.31	1.53	1.23	0.65	0.81	0.21	0.58	0.17
Glutamic acid	–	–	13.01	1.02	10.43	1.37	13.63	1.92
Glycine	–	–	4.32	0.77	4.15	0.27	4.47	0.45
Proline	–	–	3.69	0.71	3.85	0.83	3.99	0.66
Serine	–	–	4.48	0.94	4.07	0.74	5.34	0.34
Tyrosine	3.08	3.21	3.69	1.01	3.07	0.50	3.18	0.28

Langeland et al. (2013) reported no significance reduction in rate of growth when *C. utilis* is partially replaced FM in rainbow trout diet. Also 40% of FM was replaced by *C. utilis* without any negative impacts on feed conversion, feed intake and growth rate and nitrogen retention in Atlantic salmon (Øverland et al., 2013). Replacement of FM with 350gkg^{-1} *C. utilis* was reported to have positive impact on feed conversion and growth rate (Olvera-Novoa et al., 2002). In shrimp (*Litopenaeus vannamei*) varying proportions of *C utilis* and FM in the diet reported to have similar growth and survival rates (Gamboa-Delgado et al., 2016).

Body growth and condition may determine the characteristics of fillet fed yeast (Olvera-

Novoa et al., 2002). Researchers reported positive impacts on muscle proximate composition or the whole body of fish fed diet with FM replaced by different yeast species (Pongpet et al., 2015; Hauptman et al., 2014). Rainbow trout has been reported to have significant positive impact on carcass composition once FM replaced by *C. utilis* in the diet (Martin et al., 1993 : Øverland and Skrede, 2016). No clear effects has been reported on carcarss composition of tilapia fed diet replaced with high level of *C. utilis* (Olvera-Novoa et al., 2002).

1.5.3 Constraints of yeast

The major constraint of yeast processing industries is the tough cell wall (Kim et al., 1998). Structural components and intra structural components have been reported in yeast (Feofilova et al., 2010). Structural component comprises of mannoproteins, b-glucans and a small amount of chitin that is crossed linked in some ways (Orlean, 2012). Yamanda & Sgarbieri (2005) also reported the presence of mannan, glycan and monoligosaccharides (MOS) in the intrastructural layer that makes it harder to release protein and other nutrients. Bioactive and immunostimulating compounds like mannan oligosaccharides and β-glucan are riched in the cell wall of yeasts (Øverland and Skrede 2016). Depression of growth-rate reported to the fish fed intact yeast cell may be due to interference with the release of the intracellular ingredients (R umsey et al., 1990: IN Oliva–Teles & Goncalves 2001).

To increase the digestibility of the nutrient contents of yeast, different methods can be used to destruct the cell wall of yeast (Nasseri et al., 2011, Murray & Marchant, 1986). Cell wall of yeast can be ruptured by the processes such as enzymatic, mechanical, chemical and physical methods (Nasseri et al., 2011). Enzymatic hydrolysis or mechanical rupturing of the cell wall of yeast that increases nutrient digestibility (Tukmechi & Bandboni, 2014). Pre-treatment of Torula yeast by enzymes followed by the mechanical homogenization by high pressure have been reported as efficient (Baldwin &Robison 1994). The process of extrusion increase the digestibility of the amino acids and proteins as it may cause the partial disruption of the cell wall of yeast (Vidakovic et al., 2015).

Deficiency of Sulphur amino acid and presence of high carbohydrate level has been reported in yeast (Rumsey et al. 1992). Low level of methionine has been reported in *C. utilis, K. marxianus* and *S. cerevisiae* (Øverland et al., 2013). To improve growth performance of aquatic animals, methionine supplementation is very important when using yeast due to its deficient in sulfur (Mohanty et al., 1996; kuhad et al., 1997; Murray and Marchant 1986).

1.6 Torula yeast (*Candida utilis)*

For more than 70 years Torula yeast has been commercially nutritionally supplied in animal feed (Bekatorou et al., 2006). *C.utilis* is commonly used as the additive in food due to its high palatability, safety and umami flavor (Øverland & Skrede 2016). Nutrient contribution and protein sources of both FM and TY are suitable or complement well nutritionally due to their relatively similar nitrogen contribution (Gamboa- Delgado et al., 2016).

1.7 Objectives

The objective of this study was to analyze the influence of Torula yeast as a fishmeal replacer on rheological and quality characteristics of pelleted shrimp feed. The rheological and physical quality qualities of shrimp feed were determined through several parameters such as moisture contents of the mash before pelleting, moisture content of pellets after pelleting, tensile strength of the pellets, water activity (a_W) ,water surface contact angle, oil surface contact angle, underwater pellet swelling (UPS) as well as estimation of energy consumption (P-Max).

Chapter II: Rheological and physical qualities characterization of the pellet

2. Rheological mechanisms and its applications in the feed

Agglomeration of the wet powders during pelleting process and determination of physical qualities of the pellet involves rheology (MacRitchie et al., 2002).

2.1 Water movement during pelleting

MacRitchie et al. (2002) reported the two stages of water movement during pellet formation is stress dependent and it involves two stages are such as,

- Pushing of the mash into the die
- The pushing of the wet mash along the die

The induction of stress in the die causes the water migration to the pellet surface and hence pellets agglomeration (MacRitchie et al., 2002). The variations of water content after the two stages might determine the easiness of the movement of water into the membrane (Boutell et al., 2002; Fielden et al., 1992). As well as variation of moisture content after pelleting (Baert et al., 1992; Tomer and Newton, 1999a, b). The mechanism of binding of particles is described in subtitle 2.1.1

2.1.1 Mechanism of binding of feed particles

According to Rumpf, (1958): Thomas and Van der Poel, (1996), the mechanism of binding of food particles is divided into (figure 4) stages such as;

- Solid –solid; this is the interactions between solid to solid particles
- Liquid necking; this is the capillary forces between air, water, and solid phase (three phase system)
- Cohesive and adhesive forces between binders and particulates and interaction between particles due to plying and folding.

The interaction between solid to solid consist of sintering, ingredients crystal growth or recrystallization, chemical reactions, thermoplastic materials are melted and solidification of the melted materials into the state that is crystalline (Rumpf, 1958: Thomas & Van der

Poel, 1996)). During cooling/ drying process bonds are established between the particles in the solid-solid interaction.

Liquid necking holds particles in porous agglomerate that can be distinguished into three phases that is air, water and the solid materials from the particles. Radius (r) of the neighboring particle (m), binding liquid y (N/ m), and Surface tension (Δ p) might determine the force of the bond (Rumpf, 1958 :Thomas & Van der Poel, 1996).

Laplace equation (Δ p = 2 times (Y/ r)) gives the binding pressure of the bond (N), in this case there is equal radius of the neighboring particles. Binding strength increase as the radius of the particles decreases, this is derived from the equation.Water as the binding agent can be distributed around the particles without any negative impacts / loss of the bonds that were established. Moisture bridges will shrink in the case that the pellets moisture level is decreasing and due to less number of bridges the decrease of the total binding forces will occur. Firstly, evaporation of moisture in the larger capillaries leads to the increase in force per bond. Then the bonds between the smaller particles are established by the remaining water. As it shown in the equation that the decrease in the radius lead to the strong binding force between the neighboring particles. Distance between particles decrease while the surfaces interaction between particles increases. When pressure is applied between particles then the decrease in the distance between particles occur and this is how solid-solid interaction came into effect. Change of the structural integrity may occur with storage time when there is large amount of bonds in the feed. Particles smaller than 0.2905 the binding forces are carried out by Van der Waals' forces (Rumpf, 1958: Thomas & Van der Poel, 1996). Van der Waals' forces decreases when the particles increase and then the binding agents and capillary forces can take over.

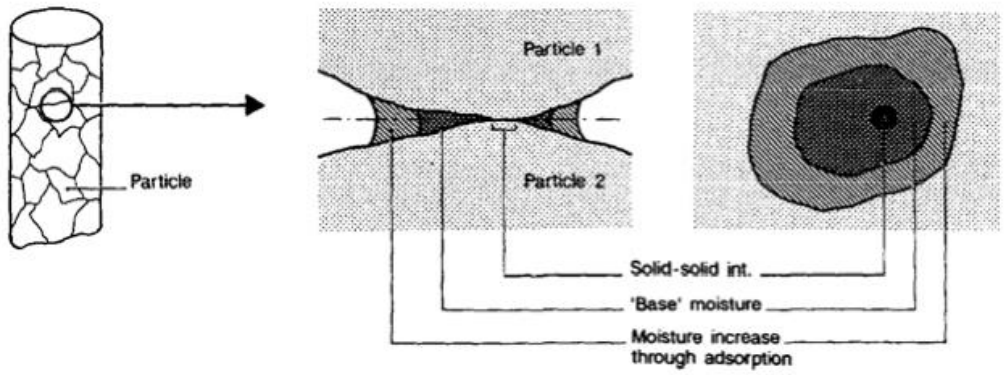


Figure 4: General binding forces model figure between two particles (Modified after (Rumpf, 1958: Thomas & Van der Poel, 1996).

2.2 Tensile strength/Hardness

Flow behavior of materials can be affected by some physical attributes such as size and texture, and hardness of the pellets (Pahk & Klinzing, 2008). Movement of hard and soft pellets is different. Soft pellets move in the random way compared to soft pellets that move along the direction of flow of air and hence less interaction (particle – particle interaction) or the pipe (Pahk & Klinzing, 2008).

According to Thomas and van der Poel, (1996), the necessary force to crush the pellet is known as hardness. Hardness can be measured by several devices such as Texture analyzer or Kahl device (Aas et al., 2011). Undersized particle and dust have no value in feed as they represent increase in the cost of production and loss of feed. Also emission of nutrient to water can be caused by too small particles. Also sticking of small particles into surfaces example pipe surface can lead to microbial growth. Although no established hardness or durability standards. Physical qualities established standards is a challenge (Sørensen et al., 2010).

For nutritional reasons, hardness had been considered as the must due to its effects on the intestinal absorption of nitrogenous components (Thomas & Van der Poel, 1996). Disintegration of pellets may occur during handling, collisions between the pipe, wall or pellets pneumatic conveying (Aarseth et al., 2006a). Small particle and fines are resulted

from abrasion and attrition of the pellet surface. The degradation of pellets may occur due to the interaction between pellet-pipe, pellet-pellet and pipe wall. Chipping is considered as the fragmentation of large particles from the pellets. Corners and edges are more susceptible for chipping. Hard impacts, usually at high bends of the pipe or high air velocities lead to fracture of pellets (Aas et al., 2011).

2.2.1 Rheological of compact

Diametral compression test, tensile test and simple compression test are common tests used in describing compaction rheology (Salas-Bringas, 2011). Among the three the diametral compression test is the most used one in animal feed pellets (Salas-Bringas et al., 2007), wood pellets, pharmaceutical tablets as well as in food powders (Salas-Bringas et al., 2010). Pellet tensile strength, is measured by the force that determines the strength divided by the failure plane cross-section area (Pietsch, 2002). Due to undefined ends of the pellet, the area of stress is difficult to be determined because of the pellets are breaking into failure plane that are random. Density of the compact determines the failure line location (Salas - Bringas, 2011).

2.2.2 Mechanism of stress transmission into the pellet

During breaking of pellet, transmission of stress is as follows fig 1.The overall strength agglomerate is determined by the lowest strength (Pietsch, 2002).

(a) Represent the strength of the pore volume –binder substance tensile strength.

(b) Represent strength of the particle boundary-caused by the adhesion between solids and binder.

(c) Represent solids strength- that form the agglomerate.

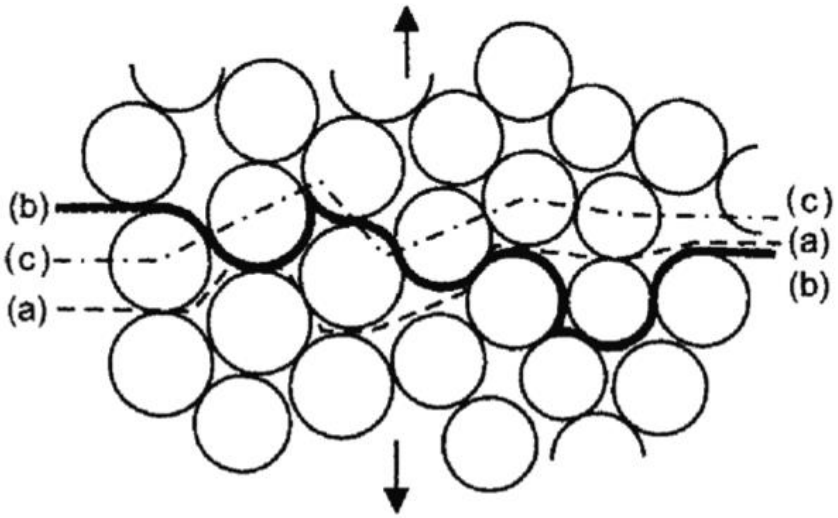


Figure 1: Two-dimensional failure lines schematic representation, describing agglomerates strength with a binder (Pietsch, 2002: Salas-Bringas, 2011)

2.3 Contact angle (CA)

Contact angle is defined as the interface of the liquid- solid intersection and the interface of the liquid – vapour application of the tangent line, from the points of contact in the droplet profile along the interface of the liquid – vapour (Yuan & Lee, 2013). The primary data in contact angle measurement is the study of wettability. When liquid and solid interact, the wetting degree is indicated (Yuan & Lee 2013).

Three-phase contact line is the co- existence of solid, liquid and vapour interface (Yuan & Lee, 2013). Solid surfaces that are suitably prepared (example powder to pellet) can ease measure the contact angle (Kwok and Neumann 1999). Wettability of the solid surface is important for several inter face phenomenon such as wettability, friction, adhesion and adsorption (Yuan & Lee, 2013). Young in 1805 that recognized the possibilities using contact angle to estimate the solid surface tensions (Kwok & Neumann 1999). Three interfacial tensions can define the drop mathematical equilibrium that can define the liquid contact angle on the solid surface (Kwok & Neumann 1999). Young's equation (Figure 5.2c) where by θ_Y is the contact angle that can be inserted (Young contact angle).
Solid-vapor Y_{sv}, solid-liquid, Y_{sl} and liquid-vapor Y_{lv}. This equilibrium relation is known

as Young's equation:

$y_{lv} \cos\theta_Y \; y_{sv} \; y_{sl}$

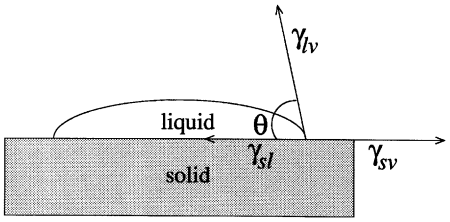


Figure 5.2c: Sessile-drop contact angle (Kwok & Neumann 1999).

According to Yuan and Lee, (2013), when the liquid beads on the surface the large contact angle is observed while observation of the low contact angle is when the liquid spread on the surface. When the contact angle is 0°, it means there is complete wetting while the contact angle greater than 150° indicates that the lotus effect, that means there is no contact at all between surface and the drop of liquid (Yuan & Lee, 2013).

Hysteresis (H) is the difference between the receding and advanced contact angle. Advanced contact angles θ_a are those formed by the and expansion of the liquid while Receding contact angle are those formed by the liquid contraction:

$H = \theta_a - \theta_r$ (1.2)

Heterogeneity and surface roughness is what cause hysteresis of contact angle (Phillips and Riddiford, 1972).

2.3.1 Mechanism of oil adsorption

The adsorption of oil increase with time and remain constant once reaches the maximum equilibrium (Carmody et al., 2007). The absorption depends ion airflows. Absorption of oil is dependent on the olephilic property of the material and time of exposure of adsorption.

According to Kumagai et al., (2007), several mechanism steps are involved in oil

adsorption that is:

- Interaction of the surface functional group with the oleophilic compounds
- Physical trapping which is fineness, density and irregularity related
- Capillary action though diffusion of oil into the pores
- High-speed adsorption (Dedov, 2006) where by the oil fills the capillaries and is dependent on materials bulk density.
- Low speed adsorption when the surface is wet, in this stage the rate of absorption is determined by the adsorbent oleophilicity and morphology of the surface.

Capillaries diameter decrease when the bulk density of the adsorbent increase and hence high penetration height that is dependent on the oil thickness. In the layer of adsorbent, the small the penetration height caused by the higher density of oil.

2.4 Water activity (a_w)

Water activity is the mole fractions of effective content of water that reaches the equilibrium in a sealed container once the reflected its relative humidity where solution or hygroscopic product is placed (Grant, 2004). Jarvis et al., (2016) reported the ranges of water activity to be from 0 to 1 that is, from absolutely dry to pure water. There is scarce information about a_w in feed, more studies are from food. Water availability in a particular environment can be defined in the use of water activity (a_w) term (Grant, 2004). Water activity (a_w) helps to determine quality of products and shelf life of products as well as susceptibility of materials to the growth of molds (Pasanen et al., 2000). Low a_W from the diets hinder the growth of most microorganism and hence less storage problems (Figura & Teixeira 2007). According to Houtsma et al., 1996, the below 0.90 suppress the growth of most of pathogens. Exception to *Staphylococcus aureus* that can grow 0.82 a_W.

Relative humidity of about 90 -95% is required by hydrophilic microorganisms such as yeast, bacteria, fungi and molds (Grant et al., 1989; Viitanen, 1994:Pasanen et al., 2000). Although microbial population can be affected by other factors such as anti-microbial inhibitors, oxygen, temperature and pH (Houtsma et al., 1996). Xerophilic or Xerotolerant

are organisms that are capable to grow into low water activity. The suffix “philic” means requirement of an organism to grow into low a_W while the suffix “tolerant” means capacity of organism to grow into low a_W conditions although it is not necessary to have conditions of low a_W (Grant, 2004). Proper food production environment can be improving the safety level of Low a_W foods. Heat treatment has been reported to be the initial stage in the reduction of microbial contamination in low a_W food products example to reduce Salmonera (Podolack et al., 2010).

2.4.1 Determination of water activity

(Rahman & Labuza 1999) reported three general methods for measuring the water activity although there are several other methods. The three general methods are;

- (Desiccators method or isopiestic technique)- in this, the sample is brought into equilibrium with the constant relative humidity that is known from a closed atmosphere.
- (Water activity meter)- in this, equilibrium head space atmosphere surrounding sample is measured for its relative humidity with the sample
- Dynamic method-in this, simultaneously weighing and exposing of sample to the atmospheric pressure of relative humidity.

Loss or gain of moisture will change the sample moisture automatically. Periodically, the sample then is measured and recorded until it reaches the equilibrium of the surroundings relative humidity. The approach is sometimes known as isopiestic technique since with time, the sample will neither gain nor loose the weight, and the relative humidity used throughout the experiment will have the same water activity as sample. Constant known moisture of the food sample is placed into the sealed enclosure that has small headspace. Partial pressure or relative humidity changes are measured and monitored by the probed atmosphere headspace until it reaches the equilibrium. Water activity of the sample is how the measured relative humidity is taken. Software for the extrapolation to the water activity is equipped due to the time consuming example several hours taken to reach equilibrium. At this the sample will neither loss nor gain moisture. In the dynamic method the automatic

weighing of the sample exposed to various relative humidity of a flowing air is performed. The useful technique for this purpose is isothermal thermo gravimetric method. It is important to note that, in all three approaches during conducting an experiment, temperature must be controlled and kept constant because the sorption is the temperature dependent.

2.5. Under water pellet swell (UPS)

The amount of time required by the shrimps / fish to consume the pellet is what we refers about pellet water stability (Lim & Cuzon 1994). Studies show that, decrease in retention of crude protein and crude lipid at the increasing immersion time presents loss of free amino acids and fatty acids as the leaching and disintegration results (Ighwela et al., 2017). Proteins can be broken down and amino acids can be lost in form of nitrogen (N) due to moisture (Richard et al., 2011; Falayi et al., 2005).

2.5.1 Mechanism of water adsorption

According to Langorsse et al. (2005), three steps are involved in water adsorption in micropores materials such as;

- Hydrogen –bonding is the means of water adsorption in hydrophilic groups. In this, the nature of materials will determine the directly attachment of number of water molecules
- Around the hydrophilic groups, the clusters of water grow until number of water molecules reaches the critical size
- The water molecules can be released from the hydrophilic group (as if they are a single molecule) due to its enough energy of dispersion.

Hydrogen bonds are formed when water contact the surface. At first the adsorption of water occurs into the hydrophobic group (carbon layers) with non-carbon element (oxygen) (Montes-Morán et al.,2004). Carbonyl, carboxyl are some of the example of the oxygen groups that might be found in the carbon later (Boehm, 1994). Adsorption of first molecule other molecules are also adsorbed due to influence from interactions of adsorbate – adsorbate (Langorsse et al., 2005). Water molecule might interact the oxygen fictional

groups and form maximum number of hydrogen bonds through unshared pairs of electrons (Langorsse et al., 2005). Production process of carbon material might determine the nature of surface functional group as well as concentration. Although they can be modified by post-treatment or thermal processes.

2.5.2 Determination of underwater pellet swell

Methods such as mechanical agitation of the pellet in a static water and stimulation of the water movement and pellet in a particular cultural system have been used to measure the pellet stability (Obaldo et al., 2002). No single method to date that can serve as a standard for routine laboratory analysis (Obaldo et al., 2002). Considerations on the method flexibility to mimic conditions in actual shrimp culture condition are important (Obaldo et al., 2002).

Leaching rate of the pellet immersed in water as the parameter of stability has been used in the last two decades (table 2). No much changes occurred so the method is still used with some errors such as:

- Stacking of some wet material can be stacked into the filter paper so it does not give out the total wet material.
- The vibration of the tank in the laboratory might be too strong compared to the movements of shrimps in water since they are crustaceans with the slow move.
- Leaching time test cannot give direct information on the physical point of view, how stable in water it is. It only gives information about environments in the ponds and perhaps some leak in nutrients.

The objective of the measurement will determine the choice of the method to be used such as nutritional purposes, handling, or fines production study due to abrasive stresses or fragmentation. Different information can be obtained from different evaluation method. Hence the use of each type of device for every pellet type is not possible.

According to (Thomas &Van der Poel, 1996), more information's can be obtained in tensile and bending tests compared to compression tests. Though it has low value due to the

attrition types that the pellet undergoing in the animal feeding trough and manufacturing in the feed mill. Abrasion and fragmentations are both involved in handling and transportation hence Holmen pellet tester would be the best choice than tumbling can device due to in between stimulator act of the Holmen pellet tester.

Table 2:Water stability described analysis methods

Method	**Description of the method**	**Results**
1. Brass wire mesh baskets immersed in water **(**Rout and Bandyopadhyay, 1999)	Immersion of triplicate baskets with spread in one layer of 20g pellets into saline water flowing through a tank under air stone mild agitation for 30 and 240 min.	Ratio of the average of the dry pellet diameter to the die hole was measured as the expansion ratio. For five measurements the results were reported as mean ±S.D.
2. Stimulation of submerged pellets under the tanks / Feed pellets water stability under the tank (Fagbenro & Jauncey, 1995; Farmanfarmaian et al., 1982)	Series1; Recording of submerged pellets integrity and physical appearance after 1, 2, 4,6,8 and 22 hours of immersion Series 2: Weighing and sieving of sieves of 1.0 mm mesh for each type of the ten pellets. The sieves were left submerged for 6, 12, 18 or 24 hours periods	Series 1: Partial intact/ entire intact or stability appearance of the pellets were recorded Series 2: Reweighing of oven dried (at 75°C) sieved pellets. Leaching level of the particular feed/ pellets were obtained by the difference between the initial and final weight
3. Horizontal shaking method (Obaldo et al., 2002)	Heating circulating water bath with a shaker and a Lindberg/BlueM refrigerated. For each test run the shaker tray held up to eight flask of 250 mL	Buchner filtration apparatus with Whatman filter paper (5μ) used to remove all solids. After 24hourd drying (105°C) and

		Cooled of the sample. The ratio of the recovered dry matter after leaching to the percentage of the dry matter of the original sample is how Water stability is calculated.
4. Static water method (Obaldo et al., 2002**)**	The same procedures as in horizontal shaking method	
5. Vertical shaking method (Obaldo et al., 2002	Three lifted and lowered baskets in 1000mililitre beaker were placed into the basket rack. A Van Kel disintegration testing system is used. During leaching and during oven drying, long mesh basket cylindrical stainless steel with mesh cover that is removable is used to hold basket 2 gram of feed	The same procedures as the other two methods above.
6. Submerging of pellets under water in cone shaped pouches made of nylon cloth. (Ahamed Ali, 1988)	Initial weight of 50 pellets of 5millimeter kept in each pouch was recorded. The pouch with pellets were lowered into water carefully and placed into petri dishes at the bottom of the plastic lined pool with of 20% salinity and the depth of 40 and 90 centimeter respectively.	Recording of the physical shape of the pouch after taking out from water was done after one hour. The pouch is then dipped for 5 minutes into the fresh water container and oven dried (70°C) for constant weight. The procedures were repeated after every 2,3,4,5,6,12 and 24 hours. The difference between the weight before and after immersion is used to determine the weight loss.
7. Wet durability test	Pellets of each diet into the	Percentage dry matter loss

(Ighwela et al., 2014)	triplicate 5 grams samples were dropped into 800miles glass beaker of tap water .The glass beaker immersed four times in 1, 2, 4 and six hours respectively. The undissolved solids were filtered and oven dried 105^{o}c for 30 minutes and dried again for 65 ^{o}c to a constant weight and cooled in a desiccators.	was calculated through the differences in weights of beaker containing feed before immersion and after drying at respective intervals of time.

Chapter III- Analysis of the Influence of torula yeast on rheological and physical qualities of pelleted shrimp feed

3.0 Materials and methods.

3.1 Preparation, processing and mixing of ingredients

Ingredients for the shrimp feed formulation were obtained at the Centre for Feed Technology (FôrTek), located at Norwegian University of Life Sciences, Ås, Norway. Table 8 in the annex present the ingredients used in all six treatments. Grounded starch (cereals) ingredients in the hammer mill were sieved at 2mm. During mixing of the formulated diet, 10% of moisture was added and mixed thoroughly for about 800 seconds using high shear mixer that have tulip-form chopper and three impellers (Diosna P1/6, Germany). Mixed samples were vacuum packed and stored at 4^0C in the fridge for three days before further processing (conditioning and pelleting).

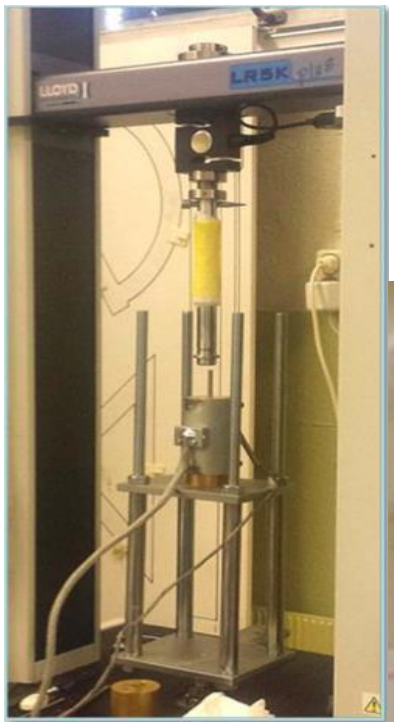
3.2 Steam conditioning of the mash

To make one pellet, mash was weighed at the average of 0.13g and placed in the epindolvs. Weighed ingredients in the epindolvs were dipped into the boiled water (100^oC) and cored for 3 minutes to allow steam conditioning to take place. Steam conditions mash were allowed to cool for five minutes before thoroughly mixing again to ensure equally steam distribution in the mash before pelleting.

3. 3 Pelleting process

For all six treatments, sixteen pellets were made and single die pellet press was used to make these pellets as shown in the Figure 5a. At the workshop in the IMT at the Norwegian university of life science is where the device was designed and fabricated. The die pelleting ring has a barrel that is made up of brass, and the compression channel at the center. Diameter of the compression channel was 5.5mm and the rod was 5.5 mm diameter. The rod was used to press the mash within the die. The limit compacting stress used was 285Mpa. After the compacting stress reached the limit, Unloading of the pellet from the die was performed through unscrewing of the die from the barrel. All pellets used in this research were produced in the laboratory die pelleting rig presented by Salas-Bringas et al. (2010). The rig was assembled in a Lloyd LR 5K .

During pelleting the channel was heated up to degrees 81^{o}C. Conditioned mash were poured into the channel and allowed to be heated for 90 seconds before the rod start to compress the mash and form a pellet. The compression stress was 10mm/min and the unloading stress was 2mm/min. After the maximum compacting capacity was reached, the pellet was unloaded and the length was measured using a digital Vernier caliper. The same procedures were done for all six treatments. The averages of six minutes were used to make one pellet (retention time). Figure 5b are some of the pellets produced.



5a 5b

Figure 5a: Laboratory single die pellet press, Figure: 5b pelleted pellets

3.4 Analyses

3.4.1 Moisture content (%)

The moisture content of the mash after steam conditioning and of pellets after pelleting was determined. The average mash weight of about 0.13g was determined its moisture content in a moisture analyzer scale with Mettler Toledo LJ16 Infrared (Sigma Aldrich,

U.S).

2.4.2 Tensile strength/ Hardness (MPa)

Diametric compression test as shown in figure 6 was used for testing the tensile strength of the pellets. The probe of about 60mm connected to a Lloyd LR 5K-texture analyzer (Lloyd Instruments, U.K.), as Salas−Bringas et al. (2011) reported. The speed of compression was 1mm/min.

Figure 6: Diametric compression test connected to a Lloyd LR 5K texture analyzer

3.4.3 Water activity (a_w)

Rotronic HygroLab C1 (Switzerland) instrument was used to measure the water activity (a_w) of the pellets. The instrument temperature sensor was measuring the temperature during analyses and 21.2 ± 0.2 °C was the average temperature.

3.4.4 Underwater Pellet swell (UPS)

3.4.4.1 Description of the analysis

The analysis for the swelling rate of the feed pellet under stagnant water was performed through software for image analysis and the Video lenses assembled in the figure 7, which

is from Krüss Tensiometer and its source of light, a Micro Viper Portable Computer with its software and Allen (Compact Video Microscope lenses). Figure 8 present the working body of Krüss Tensiometer that supports the video microscope. The equipment and the glass container (printed in 3D) were designed at the mathematical and technical department in Norwegian university of life sciences (Figure 9).

Capturing a picture at the intended time, was facilitated by the Micro Viper Portable computer contains the video microscope and the Micro Viper Portable software. Figure 10 present the Compacting video microscope (Allen), that have different capabilities in zooming depends on the size and distance of the solid to be examined. The assembled settings are then connected Micro Viper portable computer.

3.4.4.2 Underwater pellet swelling (UPS) rate measurement.

Recommendation for routine laboratory analysis of UPS measurements of the aquatic feeds using digitalized image analysis:

a. Purpose

-To characterize the physical quality of the aquatic feeds like fish and crustaceans, UPS rate is an important part. Historically, UPS described as "pellet stability" that is referred to the pellet's ability to maintain its form when submerged into water, without nutrient leaking before consumed by aquatic animals.

b. Scope and the equipment

-UPS rate can be analyzed by a video microscope lenses connected to a Micro Viper portable computer attached to a Krüss tensiometer body. As shown in figure 11, full assembled equipment consist of:

- Micro Viper portable computer
-Video microscope
- Krüss tensiometer
-100 mL graduated cylinder for measuring the 100 mL distilled water
- Assembly made in plastic by 3D printer

- Stopwatch

c. Analytical procedure

- Random selection of one pellet (at least 3 pellets as replicates are randomly selected for best results) from the diets is performed in order to observe the UPS rate
- As suggested in the figure 10, the video microscope lenses are assembled and as shown in (Figure 8) mounted on the tensiometer as shown in (Figure 12) the 3D printed holder parts.
- The optical tensiometer is mounted in the, mount the glass container (Figure 12, part5) as suggested in figure 8, and figure 11 part 4 pellet support bridge are introduced in the container.
- 100mL of distilled water was added in the glass container, and wait for 5 minutes for water to settle, or destroy the air bubbles with the laboratory pinzette. Air bubbles are prohibited for good results.
- The pinzette must be dry enough before touching the pellet for analysis.
- Pellet can be submerged into distilled water very fast with the long pinzette into the bridge for supporting the sample (Figure 11, part 4).
- Only the circle of the compressed cylinder (pellet) must be seen on the screen of micro Viper portable computer. If not, submerged pellet should be adjusted by touching it as long as you see the circle. This must be done under 60 seconds. Another pellet can be used incase
- Longer time is needed in this adjustment.
- As soon as the pellet is place on the support bridge, stopwatch is started without delaying and the image are taken once in a minute for 40 minutes.

Figure 7 present a complete analytical setup for measuring the rate of the ratio of UPS.

Figure 7: Equipment used for evaluation of under water pellet stability (UPS). Items indicated by latters (A) Krüss Tensiometer, (B) Allen Compact Video Microscope Lenses, (C) Micro Viper Portable Computer) (Catargiu, 2015)

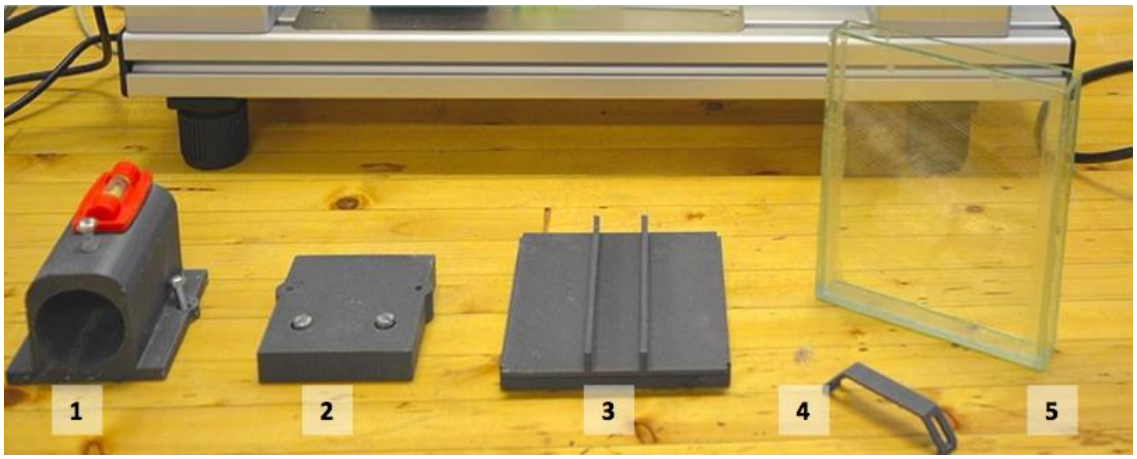


Figure 8: Materials made in the University's 3D printer for complementing the Tensiometer (Krüss G10). Items are indicated by numbers: (1) & (2) Support for stabilizing and mounting the video microscope on the Tensiometer, (3) Support for fitting the glass container to DataPhysics Optical Tensiometer, (4) Bridge for keeping and stabilizing the pellet wile in the glass container, (5) Glass container (Catargiu, 2015).

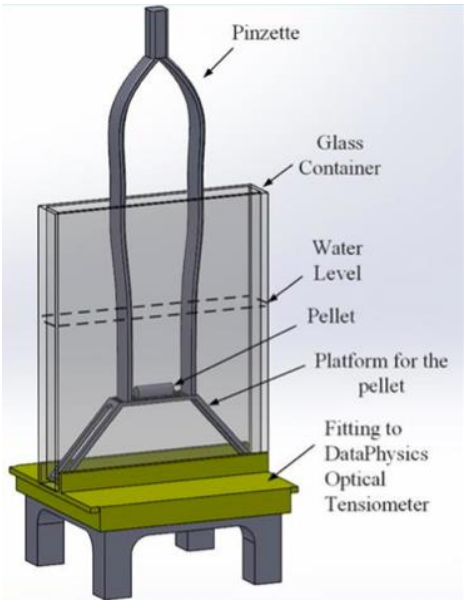


Figure 9: Image analysis equipment for the swelling rate of the pellet (Salas-Bringas et al., 2015)

Figure 10: Assembling the video microscope. Items are indicated by numbers: (1) CVM Video probe head, (2) Zoom lens, (3) 20x – 120x basic lens, (4) Contact head adaptor, (5) 60x-420x contact head) (Catargiu, 2015).

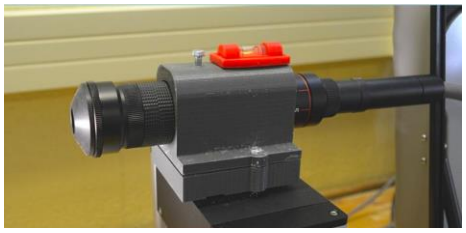


Figure 11: Final view of the assembled and fixed video microscope on the Tensiometer

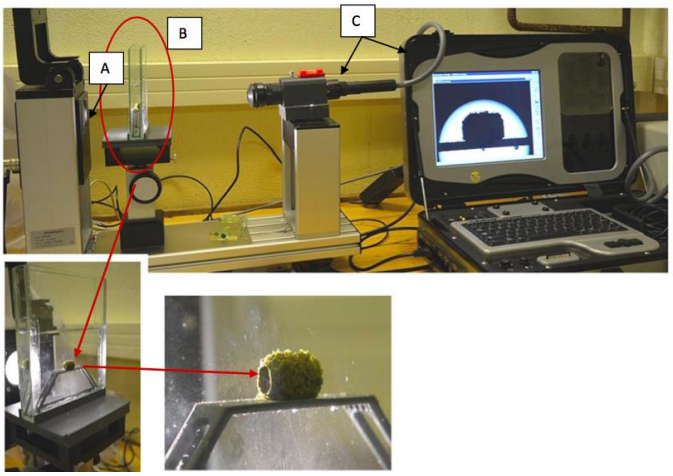


Figure 12: Experimental setup for the water stability measurement using Image Analysis. Items are indicated by letters: (A) light source; (B) Pellet in Glass Container; (C) Video Microscope (Catargiu, 2015).

3.4.5 Surface hydration / Contact Angle

The surface hydration / surface contact angle (SCA) of the pellet was conducted at the room temperature by the θ (angle) measuring device OCA 15EC (DataPhysics Instruments GmbH, Germany). The device took the video during the drop absorption on the pellet. Two types of liquid were used, that is distilled water and oil (glycerol). For both types of liquids (water and oil), the same device was used but the dose used was different. For the surface water contact angle (θ/ms) the dosing volume used was 2 µl at the rate of 0.2 µl/ms. However 5 µl of glycerol dosing volume at the rate of 2µl/s was use at the surface oil contact angle (θ/s). The distilled water/ glycerol was discharged to the upper surface of the pellet from the upper pace surface of the syringe. Changes of θ with time were calculated by SCA20 software.

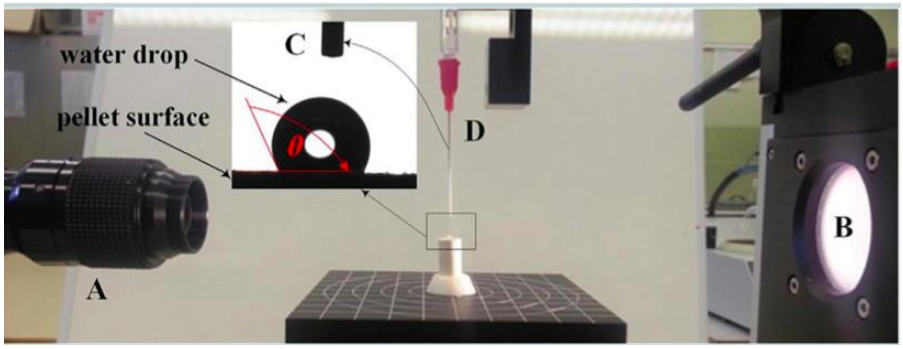


Figure17: Experimental setup for θ measurements. Items are indicated by letters: (A) camera; (B) light source; (C) image of a drop on top of a pellet surface for θ tests; (D) dosing syringe with a needle (Mišljenović et al; 2015).

The apparent rate of water and oil absorption was calculated after recording the changes of contact angle with time. Linear relationship slope between changes of angle with time was recorded before this calculation.

Figure 18: Example of the contact angle measurement by the sessile drop of compact (based on hard wheat flour) method. The initial contact angle (A) and after 3 min (B) (Roman-Gutierrez et al., 2003).

3.5 Statistical analyses

Statistical analysis was conducted using Minitab software (Minitab Inc., USA). Data were analyzed by ANOVA (One-way analyses of variance) for determining significant ($p < 0.05$) differences among tests and the means were separated using Tukey method.

UPS analysis and calculations

The analysis of images taken by the Micro Viper portable computer and lenses assembly was performed by the FIJI software. Calculation of increase in area was performed by the formula ($A = \pi r^2$), followed by Mintab and Turkey posthoc analyses. Pearson correlation was extended for the correlations among the parameters analyzed.

4. Results

4.1 Moisture content

Moisture content was statistically different among the treatments, both before and after pelleting (Figure 5.1). Before pelleting, the treatment with 0% yeast was significantly different (*p<0.05)* from treatment with 100% yeast. After pelleting, moisture content of the pellets was significantly different (*p<0.05)*, treatment with 5% and 10% yeast have significant differences to treatment with 100% yeast.

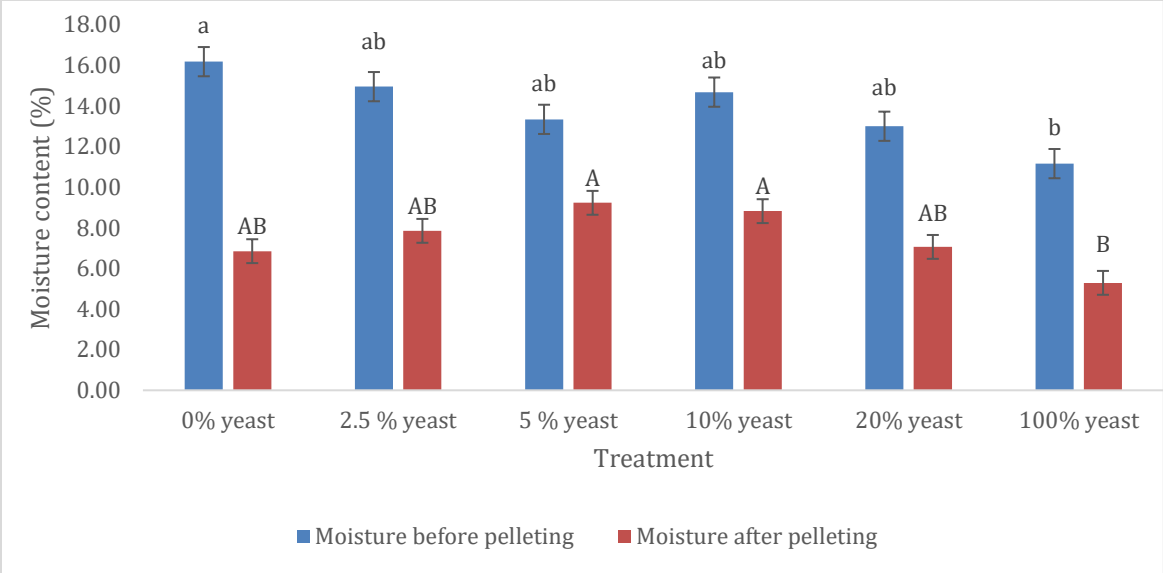


Figure 5.1: Mean moisture content (%) at 95% confidence interval for the six treatments before and after pelleting (±STD).

4.2 Tensile strength

Results for tensile strength between the six treatments were statistically different *(p<0.05*) (table 5.1). Tensile strength for treatment with 0%, 2.5%, 5% and 10% yeast levels were not different (*p>0.05*), however statistical difference (*p<0.05*) was observed in treatments with 20% and 100% yeast levels when compared to treatments with 0%, 2.5%, 5% and 10% TY.

4.3 Water activity (a_w)

In assessing the (a_W) of six treatments, the study showed that the treatments did not influence a_W values *($p>0.05$)* (Table 5.1).

Table 5.1: Tensile strength and water activity (a_w) for six treatments

Parameters	**Means and Turkey grouping for different amount of TY into treatments** (± STD)					
	0%	**2.5%**	**5%**	**10%**	**20%**	**100%**
Tensile strength (MPa)	18.08±0.77^{c}	34.05±7.38^{c}	30.68±1.55^{c}	44.19±3.31^{bc}	70.28±2.50^{b}	173.8±14.9^{a}
Water activity (a_w)	0 .35±002 ^{a}	0.46±0.004^{a}	0.35±0.004^{a}	0.40±0.004 ^{a}	0.40±0.001^{a}	0.35±0.003^{a}

**Different superscripts in the same row imply that such treatments were significantly different (0,05) using Tukey test.*

4.4 Surface water / oil Contact angle (CA)

The study observed the significance deference within time *($p<0.05$)* on both surface water CA (Table 5.2a) and surface oil CA (Table 5.2b) between the treatments. In surface water CA treatment with 0% TY was statistically different *($p<0.05$)* from treatment with 10%, 20%, and 100% TY at 0ms, 47ms and 94 ms. Also treatment with 100% TY surface water CA was significantly different *($p<0.05$)* from treatment with 2.5%, 5% TY at 0ms, 45ms and 90ms.

Table 5.2a: Surface water CA for the six treatments

Time	**Means and Tukey grouping for different TY amount into six treatments for surface water CA(±STD)**						
(ms)	**0%**	**2.5%**	**5%**	**10%**	**20%**	**100%**	**P-values**
0	63,12±1,25^{a}	59,68±4,35^{ab}	59,12±5,12^{ab}	50,98±3,76 ^{b}	49,85±5,68^{bc}	38,25±4,33^{c}	**0,001**
47	61,33±1,49^{a}	53,20±5,14^{ab}	49,25±6,53^{ab}	43,95±4,46^{bc}	44,88±4,7 ^{bc}	33,18±3,48^{c}	**0,001**
94	59,50±2,16^{a}	47,93±5,71^{b}	43,65±5,48 ^{b}	40,08±3,40^{bc}	42,53±4,63^{bc}	30,98±2,70^{c}	**0,001**

**Different superscripts in the same row imply that such treatments were significantly different (0, 05) using Tukey test.*

In surface oil CA treatment with 0% TY was statistically different within time from treatment with 100% TY *($p<0.05$)* at 0s, 47s and 94s (table 5.2b). Treatment with 20% TY

and treatment with 100% TY on surface oil CA were not statistically different *(p>0.05)* at 0s, 47s and 94s.

Table 5.2b: Surface oil CA for the six treatments

Time	Means and Tukey grouping for different TY amount into six treatments for surface oil CA (± STD)						
(S)	0%	2.5%	5%	10%	20%	100%	P-value
0	64,02±1,99[a]	63,33±1,53[ab]	57,47±10,06[ab]	61,2±2,98 [ab]	50,65±1,23[ab]	49,37±6,95[b]	**0,017**
47	62,92±2,05[a]	60,73±1.31[ab]	54,17±10,68[ab]	59,25±3,03[a]	49,63±2,06[bc]	48,12±6,74[c]	**0,028**
94	61,57±2,15[a]	58,83±0,97[ab]	57,37±4,92 [ab]	57,6±3,49 [bc]	48,7±2,71 [bc]	46,97±6,39[c]	**0,003**

**Different superscripts in the same row imply that such treatments were significantly different (0,05) using Tukey test.*

4. 5 Underwater Pellet swell (UPS)

The UPS results showed the significance difference *(p<0.05)* in stability among the six treatments within time, except in time zero (0 m) (Table 5.3a) and (Table 5.3b at the annex). Findings showed that, treatment with 20% yeast was more stable once stagnant water for other treatments (table 5.3a) and (5.3b). Also treatments with (2.5%, 5% and 10%) TY show small increase in stability although not as stable as treatment with 20% TY. Treatment with 0% yeast was also stable but not as treatment with 20%yeast. The increase in stability for all treatments within 40 minutes was as follows (table 5.3a) and (table 5.3b). At 1m,treatment with 2.5%% TY was statistically different *(p<0.05)* to treatment with 100%TY. At 2m, 3m, 4m, treatment with 100% TY was statistically different *(p<0.05)* to treatment with (0%, 2.5%, 5%, and 10%) TY. Although the significant difference *(p<0.05)* to 5%, and 10% shown from 5m to 8m. Statistical differences *(p<0.05)* shown at 10m to 12 m to the treatment with 5% TY to 100% TY. From 13 m to 19m, treatments with (5% and 20%)TY shows significant different *(p<0.05)* from treatment with 100% TY. From 20 m to 40 m only treatment with 20% TY had significant different *(p<0.05)* to treatment with 100%TY. The 100% TY shows small increase in are within time (hydrophobic) when compared to all other treatments, except the sudden increase and decrease in area shown at 9m and 10 m.

Table 5.3a: UPS (mm^2/m) from 0 to 20 minutes for the six treatments

Time (m)	UPS means and Tukey grouping for different TY amount into treatments (± STD)						
	0%	2.5%	5%	10%	20%	100%	P-value
0	All treatments has the same response at time zero second, no differences observed						
1	30,29±2,88 ^{ab}	36,12±1,75 ^{a}	28,36±6,92 ^{ab}	33,04±2,09 ^{ab}	28,81±0,77 ^{ab}	25,10±0,52 ^{b}	**0,02**
2	37,03±5,0^{ab}	37,9±1,46 ^{ab}	41,79±2,15 ^{a}	34,93±3,41 ^{ab}	30,764±0,84 ^{bc}	25,95±0,43 ^{c}	**0,001**
3	38,81±6,7^{a}	38,22±1,76^{a}	43,18±1,95 ^{a}	36,88±4,62 ^{a}	33,50±1,07 ^{ab}	26,56±0,47 ^{b}	**0,002**
4	39,47±7,24 ^{a}	38,96±2,47 ^{a}	44,40±1,61^{a}	38,58±5,44 ^{a}	35,11±1,091 ^{ab}	27,19±0,357 ^{b}	**0,004**
5	39,74±7,28 ^{ab}	39,86±3,06^{ab}	46,11±3,17 ^{a}	40,04±6,75 ^{a}	36,25±1,52 ^{ab}	27,69±0,389 ^{b}	**0,007**
6	39,86±7,28 ^{ab}	40,31±3,37^{ab}	46,74±3,84 ^{a}	42,25±7,36 ^{a}	37,75±2,00 ^{ab}	28,20±0,396 ^{b}	**0,01**
7	39,95±7,34 ^{ab}	40,56±3,37^{ab}	47,43±4,65 ^{a}	42,83±7,37 ^{a}	39,122±1,391^{ab}	28,843±0,788^{b}	**0,013**
8	39,99±7,34 ^{ab}	40,63±3,35^{ab}	47,68±4,74 ^{a}	43,30±8,04 ^{a}	40,028±1,173^{ab}	29,168±0,585^{a}	**0,017**
9	40,01±7,35 ^{ab}	40,66±3,38^{ab}	48,31±5,34 ^{a}	43,83±8,14 ^{a}	41,28±0,577 ^{ab}	40,01±0,464 ^{b}	**0,018**
10	40,04±7,36 ^{ab}	40,67±3,38^{ab}	48,63±5,40 ^{a}	44,09±8,31 ^{ab}	42,512±0,55 ^{ab}	29,89±0,46 ^{b}	**0,018**
11	40,05±7,37 ^{ab}	40,68±3,39^{ab}	48,89±5,60 ^{a}	44,39±8,66 ^{ab}	43,62±0,546 ^{ab}	30,20±0,454 ^{b}	**0,021**
12	40,08±7,38 ^{ab}	40,68±3,39^{ab}	48,99±5,65 ^{a}	44,6±8,77 ^{ab}	44,741±1,272^{ab}	30,563±0,444^{b}	**0,022**
13	40,11±7,39 ^{ab}	40,69±3,39^{ab}	49,01±5,66 ^{a}	45,04±9,08 ^{ab}	46,20±1,82 ^{a}	30,946±0,50 ^{b}	**0,023**
14	40,13±7,41 ^{ab}	40,73±3,44^{ab}	49,01±5,66 ^{a}	45,46±9,28 ^{ab}	46,84±1,79 ^{a}	31,19±0,497 ^{b}	**0,024**
15	40,15±7,41 ^{ab}	40,74±3,43^{ab}	49,02±5,66 ^{a}	45,59±9,23 ^{ab}	47,43±1,84 ^{a}	31,54±0,642 ^{b}	**0,024**
16	40,16±7,41 ^{ab}	40,75±3,45^{ab}	49,03±5,67 ^{a}	45,91±9,38 ^{ab}	48,43±1,88 ^{a}	31,75±0,574 ^{b}	**0,022**
17	40,17±7,43 ^{ab}	40,75±3,45^{ab}	49,04±5,68 ^{a}	46,04±9,45 ^{ab}	49,40±1,79 ^{a}	32,023±0,733^{b}	**0,021**
18	40,19±7,44 ^{ab}	40,76±3,45^{ab}	49,09±5,69 ^{a}	46,34±9,93 ^{ab}	50,01±1,83 ^{a}	32,32±0,725 ^{b}	**0,023**
19	40,20±7,45 ^{ab}	40,77±3,45^{ab}	49,10±5,70 ^{a}	46,82±10,53^{ab}	50,58±2,18 ^{a}	32,587±0,796^{b}	**0,026**
20	40,22±7,46 ^{ab}	40,78±3,47^{ab}	49,11±5,70 ^{ab}	47,16±10,84^{ab}	51,00±2,08 ^{a}	32,77±0,746 ^{b}	**0,027**

**Different superscripts in the same row imply that such treatments were significantly different (0,05) using Tukey test.*

4.6 Maximum Pressure for pellet discharge (P_{max})

Results from the study showed that P_{max} between the six treatments were statistically different ($p<0.05$) (Table 11). Treatment with 100% TY was statistically different ($p<0.05$) to all other treatments (0%, 2.5%, 5%, 10% and 20%) TY. No significant difference observed *($p>0.05$)* in treatments with (2.5%, 5%, 10%, and 20%) TY.

Table 11: Mean and standard errors for *p max* (MPa /mm^2) for the six diets

Treatments	**P_{max} (MPa/mm) ± STD**
0%yeast	0.0005 ± 0.00005[a]
2.5%yeast	16.65 ± 0.32[a]
5% yeast	17.38 ± 1.14[a]
10% yeast	19.72 ± 1.28[a]
20% yeast	23.86 ± 1.8[a]
100% yeast	128.73 ± 13.64[b]

**Different superscripts in the same column imply that such treatments were significantly different at 5% level using Tukey test.*

4.7. Correlation among the parameters

The results showed significant correlation *(p<0.05), (p< 0.001)* among the parameters (Table 5.1.1). TS had moderate correlation (p< 0.05) to MCBP and MCAP.

Table 5.1.1 Correlation between physical quality parameters of the pelleted feed

PQPP	**Physical quality parameters of the pellets**						
	TS ((MPa)	**MCBP (%)**	**MCAP (%)**	**CA Water** (θ/ms)	**CA Oil** (θ/s)	**UPS (mm^2/m)**	**a_w**
TS (MPa)		-0.61*	-0.49*	-0.78**	-0.58*	NS	NS
MCBP (%)	-0.61*		NS	0.48*	0.70*	NS	NS
MCAP (%)	-0.48*	NS		NS	NS	0.47*	NS
CA water (θ/ms)	-0.77**	0.48*	NS		O.52*	NS	NS
CA oil (θ/s)	-0.57*	0.69*	NS	O.52*		NS	NS
UPS (mm^2/m)	NS	NS	NS	NS	NS		NS
a_w	NS	NS	NS	NS	NS	NS	

[a] Numbers in the table indicates the coefficient of correlation: **NS,** non-significant; **p< 0.001 *p< 0.05; **TS**=Tensile strength, **MCBP**=Moisture content before pelleting, **MCAP**=Moisture content after pelleting, **CA water** = Surface contact angle water, **CA oil** = Surface contact angle oil, **UPS** = Under water pellet swell, **a_w** = Water activity, **PQPP** = Physical Quality Parameters of the Pellet.

There was also strong correlation (*p< 0.001)* between TS and CA $_{water}$ and moderate correlation *(p<0.05)* was shown between TS and CA $_{oil.}$ MCBP was moderately correlated *(p< 0.05)* to the TS, CA $_{water}$ and CA $_{oil}$, while it was non significant to UPS and $a_{w.}$ MCAP was moderately correlated (p< 0.05) to the TS and UPS, while it was non—significant to other parameters (MCBP, CA $_{water}$, CA $_{oil}$, UPS and a_w). CA $_{water}$ was strongly correlated *(p< 0.001)* to TS and moderately correlated to (p< 0.05) CA $_{oil}$ and MCBP, while non-significant to other parameters (MCAP, UPS and a_w). CA $_{oil}$ was moderately correlated to *(p< 0.05)* to TS, MCBP, CA $_{water}$ while non significant to other parameters (MCAP, UPS, and a_w). UPS and a_w was non -correlated to all parameters

5. 0 Discussion

5.1 Moisture content

Significant differences (*p<0.05)* existed in moisture content of the mash before pelleting (figure 5.1). Treatment with 0% yeast and the treatment with 100% yeast were statistically different (*p<0.05).* This might happen due to the proportions of ingredients these treatments. Since 0% yeast had all other ingredients except yeast and treatment 100% has yeast alone (table 8). May be different ingredients has different absorption behavior. Mortitz et al. (2002) & Gilpin et al. (2002) reported that, different ingredients have different capabilities to absorption moisture due to some differences in physical and chemical surface conditions of the ingredients. Also maybe the steam conditioning of the mash for 3 minutes at 100°C enable the increase of moisture absorption capacity to the ingredients. Rehydration time (time of exposure to water) that is limited to few minutes and temperature have strong influence on water absorption ability for the mixed ingredients (Hemmingsen et al., 2008). Studies reported time as the determinant of the adsorption of water caused by structural changes of protein and starch (Thomas et., 1997). Also perhaps the addition of 10% moisture (water) content during mixing (for 800 seconds) had influence on the moisture content availability to the mash before pelleting. Studies show that, exposing ingredients to the water in the form of liquid state can influence more availability of water into the ingredients than when exposed into moist air (Hemmingsen et al., 2008; Svihus et al., 2004; Fairchild & Greer, 1999). Also ingredients have been reported to adsorb much water that is more available in liquid state than in moist air, although moist air has been reported to be the best way for water adsorption in protein ingredients while starch ingredients have effective adsorption of water in liquid water (Hemmingsen et al., 2008).

Grinding of ingredients especially cereal grains maybe improve the adsorption of moisture as well as the conditioning temperature of 100°C. The fraction size of each ingredient and temperature has been reported to have influence on the adsorption of water (Hemmingsen et al., 2008). Reduction of particle sizes has been reported to improve the water adsorption capacity as well as improving the pellet quality (Hemmingsen et al., 2008). However particle sizes has been reported to have less influence on water absorption when compared to temperature (Hemmingsen et al., 2008).

The decrease of moisture contents after pelleting might be due to the effect of temperature during pelleting process. Friedrich and Robohm, (1968) reported the evaporation of water through condensation and evaporation due to temperature elevation. The decrease in moisture content during pelleting lead to the shrinkage of bridges of moisture and establishment of bonds between that facilitate binding of particles (Thomas & Van der Poel, 1996).

5.2 Tensile strength

The significance difference *($p<0.05$)* in tensile strength may be was due to more additional of TY. The study shows that tensile strength of the pellets in the treatments (table 5.1), was increasing from 18N to 173N as TY was increased. Increase in tensile strength maybe was influenced by more amount of TY added (20% and 100%) TY (table 5.1). Studies show that higher strength of pellets can be influenced by the amount of protein (Haetami et al., 2017). This was shown in treatment with 20% and 100% TY that had yeast alone that became harder. Absence of significant difference *($p>0.05$)* in hardness between treatments with 0%, 2.5%, 5% and 10% TY) may was also due to less amount of yeast added (Figure 5.1).

Also conditioning process may have improved pellet tensile strength due the moisture and temperature addition. Salas-Bringas et al. (2012) reported the influence of higher moisture and temperature in the strength of the pellets. May be natural binders present in the feed ingredients facilitating the tensile strength of pellets. Since protein and starch has been reported to facilitate binding of ingredients due to moisture and heat treatments during conditioning (Hemmingsen et al., 2008). Also the cooling process after pelleting may be influenced the tensile strength of pellets. Literature reported that, tensile strength and quality of the pellet is improved by the addition of raw protein (Wood, 1987; Hemmingsen et al., 2008), although denaturation of protein is influenced by heat, the new bonds of protein might re-associates upon cooling (Thomas et al., 1997). Perhaps the compaction pressure also influence the tensile strength. Studies reported compaction pressure to influence the tensile strength of pellets, although after a certain threshold of high pressure strength may be reduced due to lamination and cracking in most materials, hence too much

pressure is not always desirable (Sinka et al., 2007).

5.3 Water activity (w_a)

The water activity obtained in this study for all treatments was low (< 0.6), (table 5.1). According to this study, the feed will not support the growth of microorganisms such as salmonella because the a_w obtained for all treatments was below 0.65. Studies shows that no growth of microorganisms below a_w 0.65 that is 65% relative humidity of the material (Pasanen et al,. 2000). Best a_w growth of salmonella has been reported to be 0.99 although they can be supported into food with a_w below 0.85 (Jarvis et al., 2016; Padolak et al., 2010).

5.4 Surface water / Oil CA

All treatments in surface water and oil CA were lipophilic because of their CA less than 90° (<90°), although there were some significance differences *($p<0.05$)* existed among them (table 5.2a). Lee & Yuan, (2013) reported that, high wettability is corresponded to contact angle less than 90° (<90°) while low wettability is corresponded by contact angle larger than 90° (> 90°). Treatment with 0% TY surface water CA Table 5.2a and surface oil CA Table 5.2b, was statistically different *($p<0.05$)* from treatment with 100% TY at 0ms, 47ms and 94ms and 0s, 47s and 94s for surface water and oil CA respectively. Since TY used was in the powder form (fine) while other ingredients were course (sieved at 2mm), may be the more increase in TY in the treatments lead to the more gluing of particles and smooth pellet surface. Perhaps this lead to better surface for liquid penetration and hence decrease in the CA. Studies reported that particle size fraction should be narrowly sieved for smooth and uniform surface (Bachmann et al., 2000). Also treatment with 100% TY was statistically different *($p<0.05$)* from treatments with 2,5% and 5% TY at 0s, 47s and 94s may be due to less amount of TY present on those treatments. Wondra et al., (1995) reported the small particles are softened easily and hydrated than big particle, and better gluing effect though liquid necking. Other studies reported that plain and homogenous surface is needed for the precise contact angle measurement (Drelich, 1997; Hazlett, 1992: Bachmann et al., 2000). Although no literature is stating how smooth the materials should be. Although texture formation might be affected by rheological properties or processes

such as attachment or gelation as well as adhesion and cohesion (Voragen et al., 1995).

Treatment with 0% TY for both surface water and oil CA (table 5.2a and 5.2b) had larger contact angle than treatments with 5%, 10%, 20% and 100% TY. This may be due to the roughness of pellets with 0% TY that was attributed by ingredients present (table 8). Contact angles are much larger on rough surfaces than smooth surface (Kwok & Neumann, 1999). This reveals that, more addition of TY in the treatments lead to the spread of the liquid water and / or oil on the surface of the pellet. Perhaps this led into smoothening of the surface of the pellets. Also, maybe the additional of TY in the treatments had no negative effect on the wettability of the pellets within time. Penetration of water and oil for all treatments were not less than 1 s and not more than 60 s. Studies show that Penetration times less than 60 s reveal slightly repellant or wettable material while the penetration time less than 1 s reveals the instantaneous infiltration (Dekker, 1998; Bachmann et al., 2000). Perhaps some fat due to accidental touching of the pellets experimental time. Researchers reported the interference of fat in the binding ability of water into feed components since fat is the compound that is hydrophobic (Haetami et al., 2017).

Treatments with 20% TY and 100% TY on surface CA oil were not statistically different at 0 s, 47 s and 94 s. The differences existed among the treatments maybe was due to roughness and homogeneity as discussed above. Determination of CA is very important in the determination of the hydrophobicity and hydrophilicity of the materials once they come into contact with water/ oil during wet storage conditions, condensation or rain (Catargiu, 2015). The replacing of TY into shrimp feed will not interfere further coating processes. Lubrication, printing, liquid coating, oil recovery and spray quenching are some of the roles of wettability in many industrial processes (Perelae et al., 2009).

5.5 Underwater Pellet swell (UPS)

Since all treatments had the same preparations and processing conditions (see materials and methods and table 8) that might influence pellet stability. Treatment with 20% TY shows high stability level within time when compared with treatments with (0%, 2.5%, 5% and 10%)TY. The significance difference in stability $(p < 0.05)$ shown by treatment with

20% TY might be due to the content and binding ability of TY. Binders have been reported to affect the materials into three ways such as; the reduction of void spaces that lead to more durable pellets due to more compaction, also they stick particles together by adhesion (Ruscoe et al., 2005). DeSilva and Anderson, (1995) reported that binders change the chemical action of the feed that results into pellets that are durable and stable. Treatment with 100% TY was hydrophobic when compared to all other treatments, this may be due to the presence of TY alone and absence of other ingredients (table 8). Hydrophobic character shown by TY may be shows its ability to reducing void spaces, sticking particles together once mixed with other ingredients. The sudden increase and decrease in area shown by 100% at 9m and 10 m may be was some mathematical, statistical or experimental errors that might happen during processing. Replacement of FM with 20%TY maybe can influence the stability of pellets into water within 40m until consumed by shrimps. After feed application into ponds, shrimps consume about 50% of the feed within 30 to 40 minutes (Hertrampf & Lumpur, 2001).

General other factors influencing pellet stability have been reported by several studies Steam conditioning and gelatinisation of starch (Ighwela, 2013), Moisture content (Jayaram & Shety 1981), and cooling (Thomas et al., 1997). Also settings in the pellet press manipulation, rate of feed production reduction as well as manipulation of cooling and drying system has been reported to have influence on pellet quality (Abdollahi et al., 2013). Other more factors you can find in Abdollahi et al. (2013) and other literatures.

5.8 Maximum Pressure for pellet discharge (P max)

Maybe higher pressure needed to produce the pellets in treatment with 100% yeast when compared to the pressure required to produce pellets in other treatments. This is different to treatment with 0% that has lower P_{max} value, may be lower pressure used to produce the pellets. Higher P_{max} has been reported to pellets produced in higher pressure (Shang et al., 2014; Ståhl & Berghel, 2011). The higher P_{max} value in 100% yeast maybe was due to low friction of the contact area of the pellet and the die and hence the P_{max} increased. This is different to treatment with 0% that has lower P_{max} value may be due to higher friction between the pellet contact area and the die. Studies show that the throughput is increased due to low friction between the pellet contact area and the die (Mišjenović et al., 2015).

No statistical differences ($p>0.05$) among the treatments with (2.5%, 5%, 10% and 20%) TY perhaps the same value of pressure was used initiated to unload the pellet due to the similarities existed. Also may be due to the same friction level and the contact area of the die and the pellet among the treatments with (2.5%, 5%, 10% and 20%) TY hence lead to the same pressure required to discharge the pellet. Studies show that generated friction level in the contact area of die and pellet was influenced by the pressure needed to initiate discharge of pellet (P_{max}) (Mišjenović et al., 2015). Reports show that single die pellet has been reported to estimate the consumption of electricity because it cannot be used to measure it (Mišjenović et al., 2014; Nielsen et al., 2009).

5.6 Correlation among the parameters analysed

The variables were significantly correlated at *($p< 0.05$* and *($p< 0.001$)*. The moderate to strong correlation observed shows the interdependent of analyzed parameters on the rheological and physical quality of the pelleted shrimp feed. TS was moderately correlated *($p< 0.05$)* to MCBP and MCAP may be due to the influence of moisture both before and after pelleting. Moisture addition influence agglomeration and strength of pellets (Thomas and Van der Poel, 1996). Strength of the pellet also reported to be influenced by heat and moisture (conditioning process) addition during pelleting process (Salas-Bringas et al., 2012). Moderate correlation ($p< 0.001$) of TS to the CA_{oil} and strong correlation $p< 0.001$ between TS and CA_{water} may be TS lead to better for the surface water and oil CA to take place. Other studies reported that plain and homogenous surface is needed for the precise contact angle measurement (Drelich, 1997; Hazlett, 1992: Bachmann et al., 2000). Although no literature is stating how smooth the materials should be.

MCBP was moderately correlated ($p< 0.05$) to the TS, CA_{water} and CA_{oil}, may be was due to the much availability of moisture to the ingredients and hence better binding property that influence better ST and surface for CA to take place. Much moisture is adsorbed by ingredients in liquid state (water) than in moist state (Hemmingsen et al., 2008). Better physical qualities are also influenced by pre conditioning moisture (water) additional (Abdollahi et al., 2013; Thomas and Van der Poel 1996). Salas Bringas et al., (2012) also

reported the influence of higher moisture and temperature in the strength of the pellets. While it was non significant of MCBP to UPS and a_w may be was due to few variables, perhaps more variables would have been influence the correlation.

MCAP was moderately correlated ($p< 0.05$) to the TS and UPS, perhaps due to high binding of the particles due to more crosslinking of the bonds and hence more TS and UPS. Decrease in moisture content during pelleting lead to the shrinkage of bridges of moisture and hence influence TS and UPS (Thomas and Van der Poel, 1996).

The parameters that are non-significance may be was due to the few variability, perhaps more variables would have been led to significant variations.

6. Conclusion

From the rheological and physical qualities point of view, replacing FM with torula yeast shows positive influence in this study. The adsorption of moisture was good as more torula yeast was replaced. Also the moisture content of the pellets after pelleting was not influencing microbial growth. The w_a was not affected by TY additional. Tensile strength of pellets was improves as more yeast was replaced. The wettability property was not affected negatively as more torula yeast was added which CA measured. Also the maximum addition of torula yeast in this study shows more stability of pellets in water as when compared to other treatments. The under water pellet swell (UPS) was stable within time. No significance increase in energy use when replacing FM with torula yeast. May be yeast can be used for the future replacement of FM since no any negative influence on rheological and physical qualities of pellets has been shown in this study.

Perhaps, replacement of FM with more than 20% torula yeast may be will have positive feedback on rheological and physical qualities of the pelleted shrimp feed.

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Annex

Table 3: Water quality parameters classified by monitoring frequency (Carbajal-Hernández et al, 2013).

Water quality parameters classified by monitoring frequency	
Monitoring frequency	**Water quality parameters**
Daily monitored	Salinity (Sal), pH, Temperature (Temp), dissolved oxygen (DO),
Weekly monitored	Nitrite (NO2), nitrate (NO_3), Total ammonia (NH), turbidity (Tb), non-ionized ammonia (NH_3).
Monitored by request	Suspended solids (Ss), Fecal coliforms (Fc), chlorophyll-A (ChA), Carbon dioxide (CO_2), potential redox (Px), total inorganic nitrogen (N), suspended solids (Ss), silicate (Si), total marine bacteria (Tmb), Vibrio (Vb).

Table 4: Estimated farmed species production, use Of FM and commercial feed in feed, and FCR[a] in 1995- 2020 (Tacon et al 2011: Olsen and Hasan 2012).

Species group	Total production[b]	% on commercial feed	Average FCR[a]	% fishmeal in feed	Total feed used[b]	Total fishmeal used[b]
Marine shrimps						
1995	925	75	2.0	28	1387	388
2005	2664	89	1.8	24	4268	1024
2010	4113	95	1.6	16	6251	1000
2015	6043	97	1.5	12	8793	1055
2020	8087	100	1.4	8	11,322	906
Marine fish						
1995	533	50	2.0	50	533	267
2005	1402	70	1.9	38	2050	779
2010	2137	73	1.9	26	2964	771
2015	3140	75	1.8	18	4239	763
2020	4613	80	1.8	12	6643	797
Salmon						
1995	537	100	1.5	45	806	363
2005	1382	100	1.3	35	1796	629
2010	1734	100	1.3	22	2255	496
2015	2213	100	1.3	16	2877	460
2020	2825	100	1.3	12	3672	441
Fed carps[c]						
1995	5154	20	2.0	10	2062	206
2005	9100	45	1.8	8	7371	590
2010	11,670	50	1.8	2	10,503	210
2015	14,198	55	1.7	1	13,275	133
2020	16,459	60	1.6	1	15,801	158
Tilapias						
1995	704	70	2.0	10	985	99
2005	1980	80	1.8	8	2852	228
2010	3386	85	1.7	3	4893	147
2015	5453	90	1.6	2	7852	157
2020	8012	95	1.6	1	12,178	122
Sum of all 5 groups						
1995	7853				5773	1323
2005	16,528				18,337	3250
2010	23,040				26,866	2624
2015	31,047				37,036	2568
2020	39,996				49,613	2424

[a] FCR: estimated feed conversion ratio of specie-group (total intake of feed/ increase in biomass

[b] In 1000 tonnes

[c] Silver carp excluded, Indian major carps and bighead carp

Table 8: Ingredients used in formulation of six treatments.

SBM; Soya bean meal, SPC; Soya protein Concentrate, MCP; Mono calcium phosphate, MnO; Manganese oxide; Vit; Vitamins.

Ingredients	**Weight (g) of specific ingredients in TY treatments**					
	0%	**2.5%**	**5%**	**10%**	**20%**	**100%**
Wheat flour	90.00	90.00	90.00	90.00	90.00	0.00
FM	67.50	60.00	52.50	37.50	7.50	0.00
SBM	30.00	30.00	30.00	30.00	30.00	0.00
Poultry meal	25.50	25.50	25.50	25.50	25.50	0.00
Rice flour	18.00	18.00	18.00	18.00	18.00	0.00
SPC	18.00	18.00	18.00	18.00	18.00	0.00
Squad meal	15.00	15.00	15.00	15.00	15.00	0.00
Yeast	0.00	7.50	15.00	30.00	60.00	273.81
MCP	4.80	4.80	4.80	4.80	4.80	0.00
MgO	0.90	0.90	0.90	0.90	0.90	0.00
Cholesterol	0.90	0.90	0.90	0.90	0.90	0.00
MnO	0.03	0.03	0.03	0.03	0.03	0.00
Vit/ minerals	1.50	1.50	1.50	1.50	1.50	0.00
MSP	1.68	1.68	1.68	1.60	1.68	0.00
TOTAL	**273.81**	**273.81**	**273.81**	**273.81**	**273.81**	**273.81**

Table 5.3b: UPS from 21min to 40 min for the six treatments

Time (Min)	UPS means and Tukey grouping for different TY amount into treatments (± STD)						
	0%	**2.5%**	**5%**	**10%**	**20%**	**100%**	**P-values**
21	40,23±7,46 ^{ab}	40,79±3,47 ^{ab}	49,12±5,70 ^{ab}	47,40±11,21 ^{ab}	51,25±2,19^{a}	32,97±0,759 ^{b}	**0,036**
22	40,23±7,46 ^{ab}	40,82±3,48 ^{ab}	49,13±5,71 ^{ab}	47,73±11,73 ^{ab}	51,64±2,51^{a}	33,33±0,831 ^{b}	**0,036**
23	40,25±7,46 ^{ab}	40,82±3,48 ^{ab}	49,14±5,71 ^{ab}	47,75±11,72 ^{ab}	52,31±3,15^{a}	33,49±0,803 ^{b}	**0,034**
24	40,26±7,47 ^{ab}	40,83±3,49 ^{ab}	49,15±5,71 ^{ab}	47,76±11,72 ^{ab}	52,41±3,18^{a}	33,664±0,847^{b}	**0,035**
25	40,26±7,47 ^{ab}	40,85±3,50 ^{ab}	49,15±5,71 ^{ab}	47,79±11,76 ^{ab}	52,55±3,15^{a}	33,927±0,811^{b}	**0,036**
26	40,26±7,48 ^{ab}	40,85±3,50 ^{ab}	49,17±5,71 ^{ab}	47,81±11,76 ^{ab}	52,67±3,08^{a}	34,05±0,867 ^{b}	**0,036**
27	40,26±7,47 ^{ab}	40,89±3,53 ^{ab}	49,18±5,72 ^{ab}	47,84±11,75 ^{ab}	52,80±3,04^{a}	34,294±0,858^{b}	**0,038**
28	40,27±7,48 ^{ab}	40,90±3,53 ^{ab}	49,35±5,79 ^{ab}	47,88±11,76 ^{ab}	53,01±2,99^{a}	34,49±0,853 ^{b}	**0,037**
29	40,27±7,48 ^{ab}	40,92±3,55 ^{ab}	49,45±5,83 ^{ab}	48,03±11,95 ^{ab}	53,21±3,06^{a}	34,73±0,887 ^{ab}	**0,039**
30	40,28±7,49 ^{ab}	40,94±3,55 ^{ab}	49,53±5,87 ^{ab}	48,11±11,97 ^{ab}	53,43±3,07^{a}	34,879±0,865^{b}	**0,039**
31	40,29±7,49 ^{ab}	40,95±3,56 ^{ab}	49,55±5,88 ^{ab}	48,15±11,95 ^{ab}	53,53±3,03^{a}	35,036±0,843^{b}	**0,039**
32	40,30±7,48 ^{ab}	40,98±3,56 ^{ab}	49,67±5,86 ^{ab}	48,21±11,95 ^{ab}	53,68±3,08^{a}	35,25±0,953 ^{b}	**0,039**
33	40,31±7,48 ^{ab}	40,99±3,57 ^{ab}	49,75±5,85 ^{ab}	48,25±11,98 ^{ab}	53,87±3,16^{a}	35,40±0,931 ^{b}	**0,039**
34	40,31±7,48 ^{ab}	41,01±3,59 ^{ab}	49,77±5,84 ^{ab}	48,30±11,96 ^{ab}	53,95±3,18^{a}	35,587±0,898^{b}	**0,04**
35	40,32±7,48 ^{ab}	41,07±^{3},62 ^{ab}	50,13±5,53 ^{ab}	48,40±11,95 ^{ab}	53,99±3,22^{a}	35,78±0,918 ^{b}	**0,038**
36	40,34±7,47 ^{ab}	41,07±3,62 ^{ab}	50,13±5,53 ^{ab}	48,40±11,95 ^{ab}	53,99±3,22^{a}	35,78±0,918 ^{b}	**0,038**
37	40,35±7,46 ^{ab}	41,11±3,65 ^{ab}	50,27±5,47 ^{ab}	48,82±11,70 ^{ab}	54,1±3,16 ^{a}	36,052±0,917^{b}	**0,034**
38	40,35±7,47 ^{ab}	41,10±3,64 ^{ab}	50,38±5,52 ^{ab}	49,02±11,79 ^{ab}	54,23±3,10^{a}	36,25±0,967 ^{b}	**0,034**
39	40,38±7,45 ^{ab}	41,11±3,64 ^{ab}	50,58±5,58 ^{ab}	49,22±11,70 ^{ab}	54,43±3,04^{a}	36,31±0,884 ^{b}	**0,031**
40	40,39±7,44 ^{ab}	41,13±3,66 ^{ab}	50,95±5,83 ^{ab}	49,52±11,83 ^{ab}	54,59±3,22^{a}	36,394±0,939^{b}	**0,031**

**Different superscripts in the same row imply that such treatments were significantly different (0,05) using Tukey test.*

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