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# Pellet technical quality of feeds for Atlantic salmon


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Bhaskar Pandey

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# Abstract

Different physical parameters of Atlantic salmon feed were examined with respect to change in oil composition, change in temperature at vacuum coating and storage. The extruded 4 mm pellets and 2.96 % fat were brought from Nofima, Bergen, Norway. The oils used were fish oil (FO), rapeseed oil (RO), coconut oil (CNO). The different samples were prepared by vacuum coating with oil blend A (0% CNO, 25% FO, 75% RO), B (2.5% CNO, 25% FO, 72.5% RO), C (5% CNO, 25% FO, 70% RO) at 30°C and 70°C and storage at 4°C and 23°C. The vacuum coating was done by lab scale batch type vacuum coater designed by NMBU Extrusion and Pelleting innovation Centre.

Results obtained in this study showed the Pellet durability index (PDI), texture (force, areas) and appearance (colorimetric values) showed no significant variation with increase in coconut oil concentration in oil blend. However, significant difference on fat leakage and floating of pellets were absorbed when vacuum coated with oil blend C then oil blend A and B. The dry matter content showed significant difference among feeds with different oil blend, with increasing dry matter content with increase in CNO in the oil blend.

Vacuum coating pellets at 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C and 70°C (storage at 23°C) resulted in increasing dry matter content and decrease in floating pellet both in seawater and fresh water.

It is concluded that texture analyser can be used as method for analysis of fat leakage with some modification. The value of PDI is higher when vacuum coated pellets is stored at 23°C then stored at 4°C. The vacuum coating temperature (35°C and 70°C) will have significant effect on PDI of diet that is vacuum coated with same oil blend (a or b or c) and stored at same temperature (4°C or 23°C). The inclusion of CNO (0%, 2.5% and 5%) will not show any meaningful change in colour. The vacuum coating temperature (35°C and 70°C) will not show any significant effect on fat leakage for diet vacuum coated with same oil blend (A or B or C) and stored at same temperature (4°C or 23°C). The fat leakage will be higher when vacuum coated with oil blend A and stored at 24°C then with oil blend B and C stored at same temperature. For same vacuum coating temperature 35°C and 70°C and oil blend (A, B, and C) the dry matter content will be higher for pellet stored at 4°C then 24°C. The increase in coconut oil content (0%, 2.5% and 5%) at vacuum coating will decreased in floating pellets at freshwater. The increase in Dry matter content in a diet vacuum coated with oil blend A and stored at 24°C will decrease in floating pellets both at freshwater and seawater.

Key words: Coconut oil, Extruded fish pelleted feed, Physical Properties, Texture analyser, Saturated fatty acid, Atlantic salmon feed, Vacuum coating.

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# 1. Introduction

The population of the world is believed to reach 9.7 billion in 2050. Changes in climate, uncertain conditions, and overuse of natural resources have resulted in an unsustainable situation to feed those over 9 billion world population. So, the international community, and especially The United Nations member states, accept the sustainable development 2030 agenda in terms of economy, social and environmental to face those challenges. The aquaculture industry is a growing, thriving business. The world fish production and consumption increased from 67% to 87% from 1960 to 2014. So, the UN also set some sustainable development agenda for aquaculture production (FAO, 2016).

The world production of aquaculture species was around 65 million metric tons in 2014. The seafood business is a fast-growing food business throughout the world, and the farmed aqua food production is considered to contribute to about 50% of total fish consumption (Miller, Nichols, & Carter, 2011). There is an increasing number of distinct species farmed in the world, and by 2030, the aquaculture production will increase by 60-70% to meet the increased demand for seafood (Rollin & Larondelle, 2008; Subasinghe, Soto, & Jia, 2009). The total production of Atlantic salmon was 3.6 million tonnes in 2016, where most of them were farmed salmon (2 million tonnes) and rest are from wild catching (Oecd-fao, 2016).

The increase in aquaculture production of different species is made possible by feed produced by the aquafeed industry and modern intensive farming systems. Various ingredients are mixed in fish pellets to fulfil the energy requirement of the fish. The fats/oil are nutritionally valuable, comparatively inexpensive and often more readily available then another energy source like protein and carbohydrates. So, oil became a choice of preference to fulfil the energy requirement in aquaculture feed (Vesa & Scott, 1998)

The feed covers the highest cost of production in fish farming. Previously, the aquafeed industry entirely depended on marine-based traditional feeding ingredients, i.e., fishmeal and fish oil, as the protein and lipid sources. Due to sustainability issues, raw materials for aquaculture from the wild fishing business have limited growth potential(Michael & Wijkström, 2002). The shortage in fish raw materials results in hiking of price. The unavailability of those ingredients put an intense pressure in aquafeed industry to find an alternative source for fish mean and fish oil. American consumer research shows that some consumer prefers a fish product with reduced fishy test (Stead & Laird, 2002). So the use of blend oil seems to have the potential to fulfill consumer demands for high sensory quality.

The feed for intensive pisciculture is usually extruded dry pellets that can be very hard. Uneaten or undigested pellets can accumulate under the net pens and ultimately cause water pollution and create problems forhealth in aquaculture. Alternatively, the feed produced by extruder technology can produce porous feed with a lot of voids in the pellets. It can remain at the surface of the water for a long time and will not break when water is absorbed and is consider as superior then pellet mill feed, and causes less pollution in water. The void is made as a result of a sudden evaporation of water from the extruded pellets just after vigorous Hightemperature short time (HTST) process at high pressure and mechanical kneading of the feed ingredients inside the extruder towards the mouth of die(No, 2002).

Furthermore, fish oil contains a high concentration of polyunsaturated fatty acids (PUFA), and the use of this ingredient in aquafeed make feed pellets more vulnerable to oxidative rancidity. The use of fish oil might cause a problem in the high-fat diet of some carnivorous species like salmonids. The feed contains fat up to 40% for salmon. The research on finding alternative feed source is mainly targeted on finding ingredients that are easy to process, give low losses during pre- and post-processing, provide essential nutrients required for fish, improve the health of fish, have low costs and are readily available throughout the year.

Due to the growth in consumer demands regarding carnivorous species like salmonids, the growth for feed with high level of protein and fat has also increases. Fish meal was already used in salmon feed from a long time, and is believed to be a palatable superior source of protein with high digestability, rish in essential amino acids and some growth promotional factors. Approximately 90% of the fish oil produced in the world is considered to be used in aquafeeds. Fish oil used in aquafeed has already exceeded the sustainable level.

For the aquafeed industry expansion, dietary fish oil and fish meal is the main hurdle in aqua farming expansion. In the beginning, fish meal was only considered as a limiting factor, but after the development of high lipid twin shaft extrusion techniques and vacuum coating techniques, the use of fish oil also increased, and both of these biological resources are considered to be the limiting factors for the aquaculture expansion.

The profit of aquaculture farm business with a lot of input and output is determined by the cost of the feed that is believed to be 40-50% of the total production cost in fishfarm business (Craig, 2009).

A lot of research work has been carried to find sustainable alternatives to fish meal and fish oil in aquafeeds. For dietary fish oil replacement, in particular rapeseed oil is being used. Effects of oil blends on fish performance, health and quality have received more attention than their effects on the pellet quality. Using rapeseed oil may be challenging because of the low melting point of the oil, hence risk for oil leakage during storage. Previously, feed manufacturers added small amounts of palm oil (0.5%) to the salmon feed as a binding agent (https://salmonfacts.com). The growing palm oil industry has however led to deforestation, including of tropical rainforests. Because of negative consumer attitudes to using palm oil in salmon feeds, other alternatives to binding agents are required. which has resulted in negative consumer responses attitude .

# 2. Aim of Study

The aim of this thesis was to test pellet quality as effected by

Oil blend: inclusion of coconut oil at two levels in a standard oil blend for adult salmon

Coating temperature: 35°C or 70 °C for the different oil blends.

Storage temperature: 4°C or 23 °C

Standard physical parameters will be analysed, but method for analysis of pellets quality are limited. In this thesis work the aim was also to develop a novel and rapid technique to analyse fat leakage from the pellet objectively, by using Texture analyser.

# 3. Background

Lipid as a whole is a heterogeneous group of a chemical compound which are insoluble in water but are soluble in a non-polar solvent like chloroform, alcohols, etc. (Gurr, Harwood, & Frayn, 2002)

The Major macronutrient includes the class of proteins, carbohydrates, and lipids. Macronutrients are considered as an essential source in providing essential nutrients and building block for cell and tissues. Similarly, they are also used by the body to provide energy, growth, development, and mentinence of cell and tissues, and they are also used by an organism with the backbone to maintain metabolic equilibrium. There is much research is going on in fish nutrition. Among lipids and nonlipids, much research is going on in lipid ingredients. The main reason is due to the complex nature of lipids and is also sophisticated in calculating the daily requirement level in the diet (Ng, Tocher, & Bell, 2007; Turchini, 2016).

The farm Salmon is fed with commercial feeds which are usually supplied in a big bag or a bulk. The recent trend of raw materials for feeding Atlantic salmon in Norway had changed a lot from marine origin to the vegatible origin.

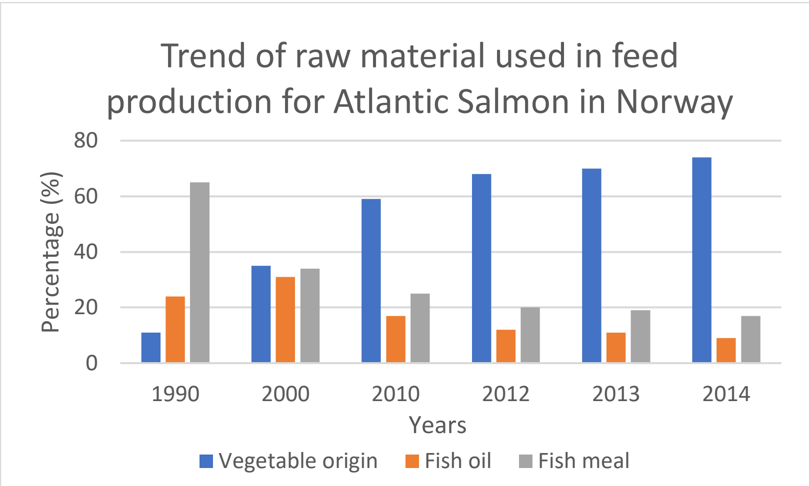



**Figure 1** Trend of feed raw materials for Atlantic Salmon in Norway (FAO, 2010; Marineharvest, 2017; Ytrestøyl, Aas, & Åsgård, 2015).

Morethen 70% of the world of Atlantic Salmon production is commercial farmed Salmon. They are rare in specially design net cage that floats in the sea water, fjords or bays (Marineharvest, 2017). The feed is then pneumatically conveyed to the cage. To prevent fragmentation and abrasion, oil leakage loss of the pellet during conveying and transportation the pellet should have superior physical quality. Different feed mix had a different response to extrusion process the properties of this mix determines the quality of pellets (Samuelsen, 2015). The extrusion cooking process is used in feed industry to produce such a high-quality pellet followed by vacuum coating to produce high-fat diet, especially for salmon feed or pets food.

The raw material cost, the feed conversion ratio and waste/leakage of the feed play the vital influence on the total production cost of fish.

The half of the global aquaculture production depends upon the aquafeed (Jobling, 2011). The production operation and ingredients used in the production of aquafeed depends upon feeding behavior of aquatic animals. Some species prefer floating feed, some of them prefer slow sinking feed, and some of them prefer to eat feed that is at the bottom of the sea or pound. Feed containing high fat (like Atlantic salmon feed) should retain fat inside the pellet and absorb fat to prevent leaching out in the water (M. Sørensen, 2012).

The extruder is used from 1930 in food processing, and different development and modification happen from the 1960s and 1970s in the extruder. After the 1980s the extruder was used in fish feed production due to its ability to produce high-quality pellet with high lipid inclusion possibility and with different density (Sk, Surshuwlhv, Vlfdo, & Txdolw, 2015).

Feed Production involves various unit operations viz. feed formulation, receiving, weighing, transferring and storing of feed ingredients, grinding, mixing, liquid addition, filtration, standardization, pasteurization, conditioning, expander, pelleting, extrusion, cooling and drying, vacuum Coating, Bagging off and Dispatch

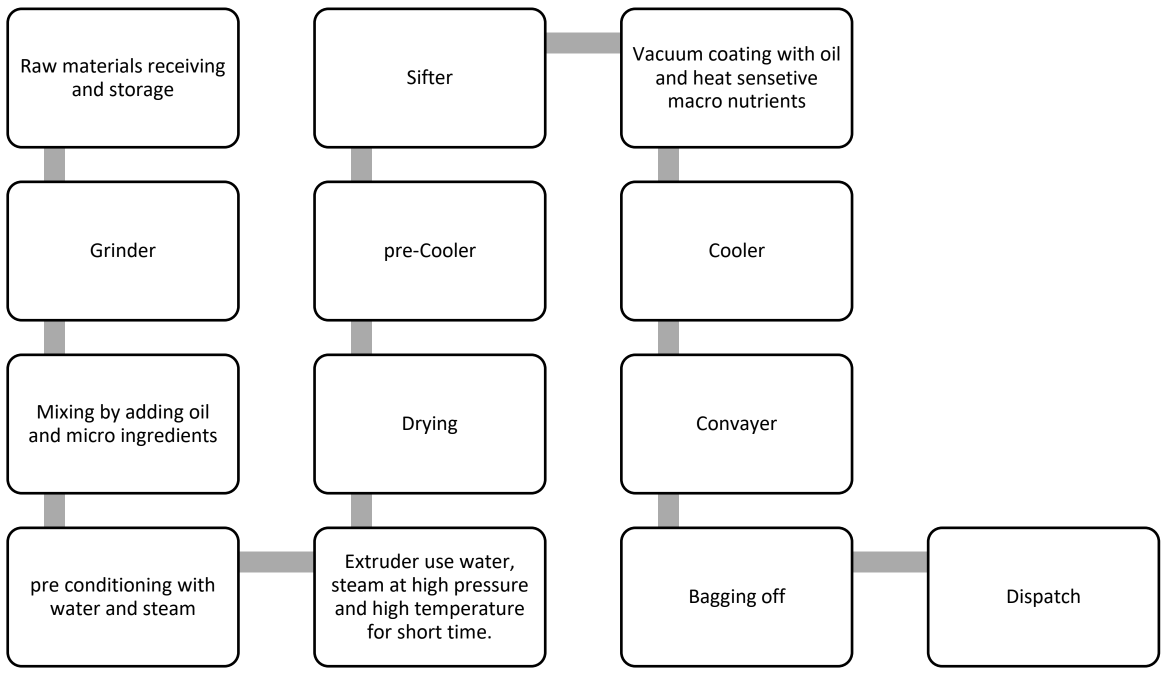



**Figure 2** Flow Chart of the vacuum coated pelleted fish feed by Extrusion process.

The primary Objective of extrusion processing is to avoid selecting feeding, improve hygienic quality and improve technical properties like physical durability, sinking velocity, water stability, etc. So that it will be easier for transportation, handling in the feeding system and storage with a reduction in feed loss. Another reason involves

Improve feed intake by reducing wastage.

- Balancing cost and benefits.
- Improve Self-life.
- Pathogenic organism elimination.
- Increase nutrients concentrate.
- Removal of anti-nutritive factor.
- Gelatinization of starch.
- Expansion (Increase the size of the pore in the pallet which helps in absorption of lipid in the pellet during vacuum coating to produced high lipid pellets like for Salmon and pet feed)

## 3.1. Feed formulation

Feed formulation is essential steps in the feed industry. It is Necessary steps to meet the feed standard requirements of dietary nutrient and energy, i.e., Dietary fat and digestible fat for digestible energy requirement and some essential fatty acids for nutrients requirements. Apart from nutrient and energy requirement Feed formulation is also very important from the economic point of view as we know feed ingredients cost constitute a very high portion of the variable cost in the feed industry. Generally, in feed industry, almost 80% cost involves in purchasing of feed ingredients, so it should be in systematic analysis on a specific key criterion.

Feed formulation in a modern feed industry is done by different available commercial linear programming tools and is known as least - cost formulation (Turchini, 2011). In this the feed ingredients of the known nutrient composition to meet the nutrient target and the least cost value of those ingredient used for feed production. Lipid ingredients that are mostly used in the least cost formulation are refined fats and oil from animal and vegetable origins and also from wild /catching fishing sources (Turchini, 2011).

## 3.2. Feed Production

There are different Processes/treatments involved during the preparation of feed. They may be Mechanical, Thermal, Thermal/mechanical or chemical treatment. The main reason behind these treatments is to modify the size of feed ingredients as we know feed ingredients are of distinct size and shape. Feed manufacturing starts with the selection of feed ingredients as it provides macro and micronutrients for the growth and development of animals.

The general process of ingredients to prepare feed is listed as below;

- Receiving, weighing, storing, and conveying of feed ingredients.
- Dosing and micro dosing
- Grinding
- Mixing
- Extruding.
- Drying.
- Vacuum coating
- Packing.

### 3.2.1. Receiving, weighing, Storing, and Conveying of feed ingredients.

Quality feed depends on quality ingredients, and it is all producer responsibility to confirm the material used in feeds is pure and safe. Important care should be taken before receiving feed ingredients: scheduling, weight verification, initial inspection, sampling and quality control of each ingredient. QC personal should periodically check it for their quality parameters were meet or nor during and after receiving. We can also categorize the received ingredients as below:

- Unprocessed Ingredients (grain, cereals)
- processed ingredients
- Macro Ingredients
- Micro ingredients (vitamins, minerals, enzyme)
- Liquids and semisolid (oil, flavor, color, Syrups, and fats)

The ingredient may be received in bulk trucks, train, road tankers, depending upon size. These ingredients must be checked for different parameters like moisture content in grains (in general moisture content should be less than 13% in Grain), color, order, pesticide residue because it may cause physical, chemical, and microbiological hazards in animals. The wholesaler, retailer, and transportation provider should be certified so that they will not cause any cross-contamination in raw materials and finished goods. Receiving of raw materials also varies depending upon size;

- Dry and liquid ingredients in large bulk bags, (500kg to 1500kg per bulk bag)
- Standard-sized bags, (25kg or 50kg)
- Barrels

Storing depending upon characteristics of materials. Usually, grains are stored in big silos, tank, warehouse, and other liquid, vitamins, fat are stored in the way to prevent oxidation/ discoloration, etc. store must be protected from rodents, animals, insects, microbes, etc

To convey feed ingredients from receiving pit to production area and from production area to different production unit we need different types of transporting system some of them are listed down:

- Screw conveyor
- Drag conveyor
- Belt conveyors
- Bucket elevator
- Pneumatic conveying

### 3.2.2. Grinding

It is a process of reduction in the size of cereals and grain partials. Grinding has been practiced from an ancient age when people use cereals and grain as food. During ancient age, they use the stone to reduce the size. Today we have several types of grinding machine available in the market. This can be operated according to types of grain and can produce desired particle size. In general, the grinding process cost around 0.5% to 2 % of the total cost.

The smaller size of the feed ingredients gives better and quick heat transfer and mixing effect. On the other hand, if the ingredients are finely ground then to prevent rancidity, the suitable antioxident should be added (Kossmann, Heinrich. Ludvigsen, 1997).

The cost of grinding varies according to characteristics of grain, as high fibrous cereals like oats and barley. The grinding price is high then soft cereals and legumes. During feed processing, we should not neglect the cost and benefits of processing. The Main principles behind grinding are

- Compression
- Impact
- Shear
- Cutting
- Abrasion

### 3.2.3. Dosing and Micro dosing

Two methods are widespread in Dosing process.

- Volumetric dosing
- Gravimetric dosing

Volumetric dosing based on the volume of ingredient it occupies and gravimetric dosing is based on weighing of ingredients.

### 3.2.4. Mixing

It is one of the critical processes in the feed industry. The recipe that is prepared by nutritionist should be uniformly mixed so that every pallet contained required minimum % of every ingredient in feed. To achieve proper mixing the particle size of ingredients should be same, and it is critical to have an optimum load. There are distinctive designs of mixture available some of them are listed below.

- Twin-shaft pedal mixer
- Horizontal ribbon mixer
- Vertical screw mixer

Among them, continuous twin shaft pedal mixer is widely in use by big feed industries. It has some advantage than other:

- Uniform mixing of ingredients
- Less time consumption
- Less energy consumption

### 3.2.5. Conditioner

There are three main principles of conditioning

- Water Addition
- Heating
- Mixing

They are all affected by retention time in the conditioner. There should be optimum retention time in conditioner. Double shaft conditioner almost dominates the pallet feed industry. Double shaft conditioner consists of two insulated chambers. In first chamber feed, steam (live and from extruder barrel); water, and other liquid are taken from the top as shown in the figure. This chamber is also called mixing chamber where feed ingredients are mixed with a high-speed agitator (approximately 1000 rpm). Then mash travel to retention section where the retention time can be maintained from 60 seconds to 600 seconds The RPM of motor can be regulated. Thus, we can have maximum retention time for almost one-third of gelatinization of starch. Temperature is maintained not more than 96ºC in conditioner. To determine temperature sensor like PT100 are used. Then the feed is transferred to extruder (Rokey, 2012).

### 3.2.6. Extruding

It is thermal, mechanical process of feed manufacturing often called high temperature and short time process (HTST). In this, the feed ingredient is forced to flow from a barrel fitted with a screw under a high temperature around 110ºC to 140ºC and high pressure of around 20 to 30 bars with a retention time of about 25 seconds. The high pressure in an extruder tube helps water to retain in its liquid state although the temperature is higher than the boiling point of water. The pressure inside the extruder barrel is too high and push the materials to the several openings toward the outside of the extruder. The pressure outside is 1bar (atmospheric pressure). The immediate decrease in pressure results in the transformation of process water into vapor/steam and this results in expansion of feed and produce a porous structure of the pellet. This porous structure of feed kernel when dried is responsible for absorption of fat/lipid. The bigger the pore volume the capacity of fat absorption will be higher which is used by modern feed industry in the production of a high-fat diet.

The extruder consists of a barrel of 5 sections which are of equal size, and total length is 1.25meters. From 1 to 5 as shown in figure 11 feed enters from section 1 from conditioner at this section water is also added while steam is added from the fourth section to achieve pasteurization effect of feed. Usually, twin screw with different screw configuration is fitted, but single screw system is also available. Single screw system is more products dependent can handle less fat (approximately less than 10%), the output rate is narrow, with improper mixing, product quality varies, and self-cleaning is not possible. Due to the distinct disadvantage of single screw extruder now twin screw with co-rotating are widely used in the feed industry. It is product independent, can handle inlet moisture up to 60%, can treat high fatty ingredients up to 20% fat, with good mixing effect, gas removing and self-cleaning by rinsing and produce uniform product quality. In a continuous system, raw material/ingredients can analyze by using GSM (Guided Microwave spectrometry), MIR (Medium Infra-Red) or NIR (Near Infra-Red) technology. The analysis can be used to standardize the fat and oil content in a continuous system (Kossmann, Heinrich. Ludvigsen, 1997).

The advantage of Extrusion feed production.

- Improve digestibility (e.g., starch gelatinized, protein denaturation)
- Reduce microbial population
- Destroy anti-nutritional factor, enzyme (e.g., Trypsin from soya been)
- Reduce toxin
- Improve texture
- Improve self-life
- Improve transportation

### 3.2.7. Cutting

Different types of cutting machine available. Sliding and direct mounting type are most commons, and is located at the tip of the die. During this time the temperature of feed will be around 60°C to 90°C and moisture content will be around 20-25%.

### 3.2.8. Drying

It is a process of removing free water by evaporation. Temperature, time and airflow are the critical driving factor in the drying process. The main advantage is to increase the shelf life of the product by reducing chemical, enzymatic, and microbial activity. There are different dryer available while selecting dryer it must have produce uniform drying, must reduce moisture around 6-10% (in feed) (Kossmann, Heinrich. Ludvigsen, 1997), should not burn the product. The temperature of the feed at the outlet of the dryer is usually 70°C to 100 °C.

There are different types of dryer categorized according to different parameter among them depending upon handling method they are of two kinds. Batch dryer and continuous dryer there are different types of continuous dryer, and they are cross flow, concurrent flow, counter Flow, mixed flow, fluidized bed dryer, rotary dryer, etc. Vacuum coating is done just after drying, but before the cooling process.

### 3.2.9. Vacuum coating

Vacuum coating is essential steps to make a high-fat diet and to include heat sensitive fat-soluble nutrients like vitamins and enzyme as the temperature during extrusion process is too high than during vacuum coating. It is also used in aquafeed and pet feed where finished product quality is in top priority (Lamichhane, Sahtout, Smillie, & Scott, 2015). The fat coating is also possible in the atmospheric condition, but special feed diet like high-fat feed for Salmon grower with around 35-40% of fat inclusion cannot be achieved without the use of vacuum coater. The vacuum in a coater speed up the process of coating. There is a different type of design available. The vacuum coater with twin shaft paddle mixture and ribbon screw type are most common.

The extruder cannot handle more fat as the increase in fat content in extruder work like a lubricant and which results in loss of mechanical shear. As a result, it causes a hydrophobic character in the pellet. The high moisture and temperature inside the extruder may cause oxidation of lipid. Oxidation of lipid results in degradation of pellet quality(Miller et al., 2011). So vacuum coating is done after the extrusion when the feed is dried and before the cooling to add extra lipid in the diet of Atlantic salmon.

The primary process in pellet coating/ vacuum coating is listed below.

- The feed usually contains 7 to 8% of moisture after drying (No, 2002). Pellet is then pre-cooled and sifted in specially design sealed coating equipment to prevent evaporation and accumulation of dust.
- The pressure in a coating vessel is reduced by a vacuum pump and is maintained up to 10 to 20 % below than 1 Bar. Then the desired volume of oil and oil mix is spread in a vessel. The evaporation of water also occurs due to negative pressure at a lower temperature.
- The oil and pellet are mixed for around 10 seconds.
- Then the negative pressure is released slowly.

- The pressure inside pellet is lower than the pressure outside the pellet this results in a flow of oil inside the tiny hole of the pellet until the pressure equilibrium to atmospheric pressure is reached.

The pellet quality remains unaffected in extrusion when oil is added below 7%, from 7-12% the study shows there is an increase in density of the pellet with an increase in oil inclusion. Similarly, if the oil level will increase from 12% – 17% the pellet will be durable, but expansion will be reduced. After 17 % and more inclusion level the cause the decrease in the durability of the pellet (Riaz & Aldrich, 2007). So if we need extra fat level in the diet, then we need to coat excess fat in an extruded pellet. Different methods can be used to increase the inclusion of fat into the pellet. Initially, the pellets are bath in hot oil to raise the level of oil, but now a day's modern techniques are used called Vacuum infusion coating process where the salmon diet fat level can be increased by 30-36% very effectively and efficiently(Stead & Laird, 2002).

## 3.3. Lipid / Oil as feed materials for fish

Fats and oil provide twice the energy than the protein and carbohydrates and in fish feeds it is used as the suitable substitute for protein. Besides, they are a rich source of essential fatty acids and help as a medium to transport fat-soluble vitamins on a body.

The various kinds of fats and oil had been tried by many aquafeed industries throughout the world. They are mostly based on blend oil (fish oil with plant oil) with the purpose of improving physical quality (increasing self-life, decreasing oil leakage, improve durability, etc.), lower the price of feed ingredients and enhance animal health and performance**.**

### 3.3.1. Fish oil

Fish is an essential basis for the food, nutrition, and source of economy. Lipid is considered as a primary source of energy in fish and aquaculture nutrition. It is used in a high level in high energy feed diet. The total world production of aquatic lies is more than 65 million metrics tons. The farmed aquaculture contributes about 50% of entire fish that we consumed. The world demands of aquaculture are increasing day by day with an increase in population. Feed cost cover more than 50 % to 70% of the production cost during harvesting fish and is the main factor that affects the profitability. Fish meal and fish oil are the primary sources of protein and fat in commercial feed production. Fish oil is considered as a primary source in providing health beneficial long-chain polysaccharide especially ω-3 fatty acid, EPA(eicosapentaenoic acid, 20:5n-3) and DHA (docosahexaenoic acid, 22:6n-3) for farmed aquaculture and human health. The use of this ingredient is very high to produce feed for the aquaculture. More than 90% of the fish oil marketed in the world is used in the production of feed for aquatic animals (Turchini, 2011). Supply and cost fluctuate seasonally. There is an increase in wild harvest of marine life in unsustainable Manner from the ocean to fulfill the growing demand of fish oil. Biodiversity in aquatic life disturbed. Similarly, the demand is very high that it is now challenging to satisfy the requirements and at the same time, this results in hiking in the price of this ingredient in a dramatic way.

Many research is ongoing in finding some suitable alternative to replace fish oil with some animal/vegetable origin fat and oil. The high fish oil-based diet required a high level of antioxidant and needed to store in cold storage during transportation and storage to prevent it from rancidity (Watanabe, 2002). At first fish meal was only considered as the primary source of feed ingredient in feed production. The development of highly sophisticated extrusion technology which can produce pellet with high expension and handle high lipid absorving capacity the use of fish oil and oil blend was also increased.

The 100 kg of fresh fish raw material input in reduction process which will result in an output of 20 kg fish meal and 5 kg of fish oil in general (Turchini, 2011).

There is remarkably fluctuation in the production of fish oil in this era, with the highest production was 1,6 million metric tons, was at 1987 and 1990 but now it was gradually decreased and reached to less than one million metric tons (Turchini, 2011).

**Table 1** Different group of fatty acid found in fish oils and Plant oils (Miller et al., 2011).

| Fatty acids (FA) | Fish oil | | Plant oil | |
|---|---|---|---|---|
| | Maximum | Minimum | Maximum | Minimum |
| Saturated FA | 34.3 | 16.8 | 47.2 | 7.5 |
| Monoenes | 60.8 | 23.9 | 62.2 | 19.1 |
| n-3 FA | 33.4 | 17.4 | 55.6 | 0.4 |
| n-6 FA | 5.1 | 2.1 | 52.2 | 10.2 |

The physical properties of fish oil very significantly according to the origin, session, and type of fish used in the reduction process. There are different physical analysis methods are available for determining the physical parameters some of them are listed below:

- Refractive index analysis
- Density
- Acid number
- Colour analysis
- Iodine value

The chemical composition of fish oil varies considerably according to Stage of development, dimensions of fish, reproductive status, geographic location, type of fish, time of catching, storage. Fish oil is considered as a suitable source of n-3 long chain PUFA, which is believed to have a good impact on human health. Similarly, it is the reach source of EPA, DHA, and ARA (arachidonic acid 20:4n-6). Distinct species like capelin, pollock, and sand eel, etc. they store lipid in their body muscles, and they are high in triacylglycerol (TGA). Generally fish oil content fatty acid with chain length from C12-C24 which are dominated by 14:0, 16:0, 16:1n-7, 18:1n-9, 20:5n-3, and 22:6n-3 but north Atlantic, i.e. higher latitude species, fatty acid composition is dominated by monounsaturated fatty acids(MUFA). The diet that contains sufficient amount of wax esters, such as zooplankton results in increased in concentration of 20:1n-9 and 22:1n-11, fatty acid composition of a fish oil (Turchini, 2011).

**Tables 2** Crude fish oil guidelines (Hertrampf & Piedad-Pascual, 2000).

| **Specification** | Units | Guideline |
|---|---|---|
| Free fatty acids | % | 2-5 |
| Moisture and impurities | % | 0.5-1.0 |
| Peroxide value | meq/kg | 3-20 |
| Anisidine | No. | 4-60 |
| Iodine value of oil From | | |
| capelin | | 95-160 |
| Herring | | 115-160 |
| Menhaden | | 150-200 |
| Sardine | | 160-200 |
| Anchovy | | 180-200 |
| Color | Gardner scale | 12-14 |
| Iron | | 0.5-7.0 |
| Copper | | Max.0.3 |
| Phosphorus | | 5-100 |
| Sulphur | | Max. 30 |

**Plant oil and alternative to fish oil (vegetable oils (VO) and lauric oil mostly used in aquaculture feed).**

There is some global issue of using vegetable oil and lauric oil as a feed ingredient in fish feed. There is also an argument for the use of dietary fish oil, because the world production of aquafeed and aquaculture is increasing, and the use of fish oil is also increasing. In european aquaculture the blue growth initiative is more target to adress the sustainability issue

(Fao, 2015). The feed produced by using this high unsaturated fatty acid (HUFA) like fish oil is also very susceptible to auto-oxidation of lipids, so we should take necessary action on decreased the level of unsaturated fatty acid and increase the use of a saturated fatty acid source to prevent rancidity of fat used in feed. If the rancid oil contains in the feed, then it will decrease the palatability of feed, and also it will affect the cellular biomembranes of fish (Watanabe, 2002). In this respect, we must sustainably use the fish oil and need some viable and environmentally friendly alternatives. The palm oil and some saturated fatty acid rich plant oil like coconut oil and palm Kernel oil is considered as a future alternative to the fish oil. The production of this oil is high enough and is regarded as viable and sustainable plant source for the replacement of fish oil (Turchini, 2011). However, while replacing the fish oil in aquafeed, the feed producer should consider the essential fatty acid requirement of the fish.

- **Vegetable oil: Palm and Rapeseed oil**
- **Lauric oils: Coconut oil**

**Table 3** Oil production and price in US$/ Metric ton in 2007 to 2008 (Miller et al., 2011).

| Oil | Production Million Metric tons | Price in Europe |
|---|---|---|
| Oil Palm (Palm oil + Palm keral oil) | 42.4+4.9 | 1058 |
| Rapseed | 19.4 | 1410 |
| Coconut | 3.1 | 1306 |
| Fish oil | 1.1 | 1500 |

### 3.3.2. Oil pam

The oil pam is extracted from the palm tree (Elaeis guineensis) and is a combination of palm kernel oil and crude palm oil. The yield/hector of oil palm is higher than soybean, coconut and rapeseed and is considered as the more efficient oil-producing plant in the world. The Crude palm oil (CPO) is the highest produced vegetable oil in the world (Turchini et al., 2011). The plant grows mostly in tropical regions. Malaysia and Indonesia are the largest producers of oil Palm. The feed containing high fat like in Salmon feeds, the increase in the concentration of CPO can decrease in oil Leakage from the pellet (Ng, Tocher, & Bell, 2007). This oil is one of the chipset sources of vegetable oil in the world. In recent year due to the level of trans fatty acid, the use of palm oil decreased due to health consciousness.

Chemical and physical properties of palm oil and palm kernel oil differ considerably from each other even though they are yield from the same palm tree.

Palm oil contains mostly 16:0 and 18:0 fatty acids and on the other hand palm kernel oil is mainly made up of 12:0 and 14:0 fatty acid. Monoacylglycerol content in CPO is very low below 0.5%, and the diacylglycerol content is from 5% to 8%. The major phosphatide component is phosphatidylcholine and galactosyldiglyceride respectively. Phosphorous content in CPO is mostly inorganic phosphorous. It also reaches the source of vitamin E and carotenoid the deep red color intensity determines the carotenoid content. In crude palm oil due to the low content of PUFA and around 48% Saturated fatty acids prevent oxidation in the feed (Watanabe, 2002).

RBD (refined, bleached, deodorized) Palm oil and Palm olein contains almost the same amount of saturated and unsaturated fatty acids (Turchini, 2011).

The high content of saturated fatty acid unusually short chain crude palm kernel oil has a high melting point and is highly stable to oxidative rancidity then crude palm oil. The commercial crude palm oil (CPO) had the acidity of 3.5% in an average. At room temperature the CPO is Semisolid, and melting point is higher than fish oil.

This oil is used in food, feed and fuel/ biodiesel production (Turchini, 2011). Crude palm oil (CPO) can be used in high-fat content diet like the diet for Atlantic salmon at low cost. Furthermore, it does not increase oxidation so, maintain its freshness and palatability. It was also absorbed that the Extruded diet containing CPO can result in low leakage. Palm kernel oil (PKO) is different forms are mainly used in the production of cocoa butter, ice cream, margarine, filled milk, milkfat replacement, biscuits, and also used in the production of medium chain triglycerides (MCT) (Pantzaris & Ahmad, 2001).

The yield of oil has increased due to some extent due to rising in the plantation of oilseed plants (Miller et al., 2011).

### 3.3.3. Rapeseed (canola) oil

This oil is being used in food for a long time, and it is often called as colza oil. It belongs to family Brassicaceae. The color of the flower is bright yellow. It is being used and harvested in Asia from an ancient age. The unselected breed of Rapeseed oil contains a high level of erucic acid (ERA, 22:1n-9) but provide a very low level of Gadoleic acid, 20:1n-9. On the other hand, it also contains sulfur and nitrogen compound called glucosinolates and it is better in the test and considered toxic if consumed in large quantity. So, this acts as antifeedants. So, this is mainly used as a feed for animal and industrial use as the production of biodiesel. Mostly now a day by plant breeding and genetic modification it is possible to produce oil with the low level of ERA and glucosinolates, so rapeseed is also highest in oilseed production. This oil is a reach source of monounsaturated fatty acids (MUFA) and is easily digestible.

The oil is considered as a suitable alternative to replace fish oil and is regarded as a most utilized fish oil Substitute in an aquafeed diet (Turchini, 2011). RBD rapeseed oil is used in salad oil; hydrogenated RO is used for cooking, baking, margarine preparation, anti-sticking cooking spray, topping of food, fat coating, and frying. Similarly, the use of RO to replace FO in aquafeed is also increasing day by day.

The use of RO in the production of biodiesel is increasing day by day. It is a reliable source of MUFA which is suitable for Biodiesel production, and in future, there may be hiking of price, and maybe this oil will not be available for other purposes (Turchini, 2011).

RO is winterized to eliminate high melting point fraction. Thus, canola oil is produced with low erucic acid (less than 2%) with a melting point -10°C. The smoking point of RO and CO is 226oC-234 °C and 220-230 °C respectively. The relative density of RO and CO is 0.910 to 0.912g/cm3 and 0.914-0.920 g/cm3 respectively. The refractive index of RO is around 1.465 to 1.469 and CO is approximately 1.465 to 1.467 (Gunstone, 2004). The saponification value of RO and CO usually is 168-181 and 182-193 respectively (Turchini, 2011).

#### 3.3.4. Coconut oil (CNO)

This oil is mainly grown on the land near the Pacific Ocean. It is produced from the kernel of coconut (*Cocos nucifera* L.). Mostly in tropical place this plant has great importance and is considered as a source of food for human, feed for an animal, it is also used as a shelter, and some oleochemical industry used it as a raw material. From CNO we can produce more MCT then Palm Kernal Oil (PKO) this contain Short chain, fully saturated fatty acid due to which it is more stable to oxidation and has a very low viscosity (Pantzaris & Ahmad, 2001). Coconut oil is used to improve the shelf life of the product as it is resistance to oxidative rancidity.

Non-food application of coconut oil includes used in the production of soap, used as animal feed, and used by oleochemical industry.

It is a reach source of medium chain saturated fatty acid mainly lauric acid (12:0), so it is also called as a Lauric oil. It also contains notable amount of Short- chain fatty acid.

As CNO can prevent the oxidative rancidity thus increase shelf-life of the product. In the food industry, this is widely used as a surface-active compound, non-dairy creams/milk fat replacer, coffee whiteners, etc., By enzymatic and chemical treatment this oil can be used in the production of margarine and shortenings. It has a melting point of around 32-34°C after hydrogenation process. The primary producer of coconut oil is the Philippines, Indonesia, and Malaysia they use this RBD coconut oil as frying oil.

**Table 4** Physical and chemical properties of some vegetable oils (Firestone, 1999)

| **Oil types** | **Specific Gravity (°C)** | **Refractive index (°C)** | **Iodine value** | **Saponification value** | **Melting point (°C)** |
|---|---|---|---|---|---|
| **Coconut** | 0.908-0.921 | 1.448-1.450 | 5-13 | 248-265 | 32-34 (Hydrogenated) |
| **Palm kernal** | 0.899-0.914 | 1.448-1.452 | 14-21 | 230-254 | 24-26 |
| **Palm** | 0.891-0.899 | 1.454-1.456 | 49-55 | 190-209 | 33-40 |
| **Rapseed (Low erucic)** | 0.914-0.920 | 1.465-1.467 | 110-126 | 182-193 | -10 |

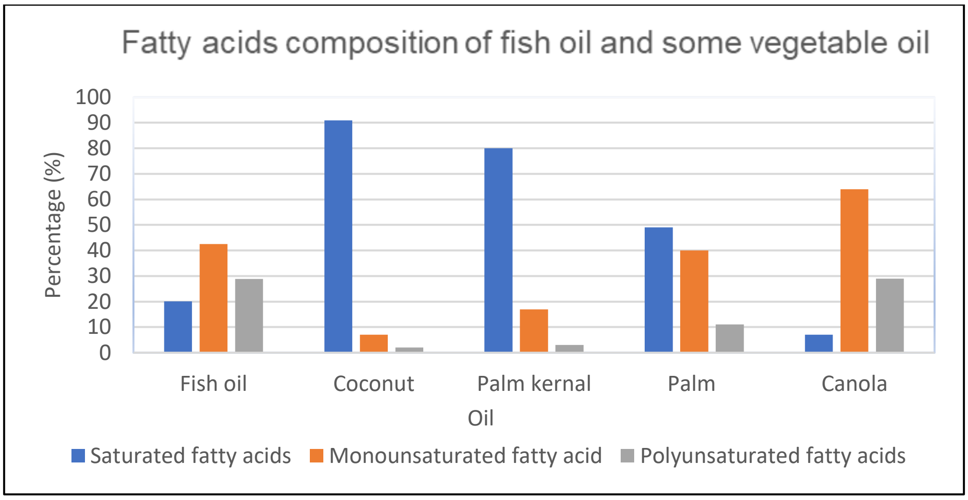



Figure 3 Fatty acid composition of some dietary oil (Carlos Zambiazi, Przybylski, Weber Zambiazi, & Barbosa Mendonça, 2007; Ng et al., 2007).

## 3.4. An analytical method to determine the physical quality of Vacuum Coated Feed for Atlantic salmon.

The physical quality of vacuum coated Pellet feed for Atlantic salmon can be determined by different techniques.

During feed production of Atlantic salmon product loss, float ability, water Solubility, expansion ratio, bulk density, Pellet durability, water absorption, sinking velocity, oil leakage, moisture content and microstructure of the feed should be considered.

In general, the definition of physical quality in high energy Pelleted feed used in intensive Atlantic Salmon production is the capacity of the pellet to withhold the lipid content (no or low oil leakage) and dust particles from the pellet during handling, transportation, and pneumatic feeding system (Aarseth, 2004). The main force act on pellet during this process are compression, impact, and shear. However, the pellet should also maintain its shape and size that help in high feed consumption (Hardy, 1989) and should also consider the efficiency of feed digestion by aquaculture (Baeverfjord, Refstie, Krogedal, & Åsgård, 2006). In the pelleting process, small particles are combined to form a macro-particle (pellet) by the help of attractive force that exists between a particle with the aim to facilitate transport, storage, homogeneity and concentrates feeding that suit the modern aquaculture demands. The hardness of pellet also affects the digestibility if fish (Pillay & Kutty, 2005). If the fish is feed with a high amount of hard pellet, then this may cause fermentation and gas production in the fish stomach this results in inflammation and rupture. However, the soft pellet may cause osmoregulatory stress and abdominal distension syndrome in rainbow trout by oil bleaching due to the separation of oil inside stomach (Baeverfjord et al., 2006). The wearing occurred in the extruded pelleted feed are of two types. Fragmentation and abrasion. Fragmentation is defined as breakage of the extruded pellet into big particle and usually occurred as a static tension, compression and collision during storage and transportation. Conversely, abrasion is defined as wearing a way of the small fine particle from the side of the pellet and is caused mainly by friction during transport and handling. Among these losses, the abrasion is considered as a most damaging type from the nutritional and environmental point of view. Durability and hardness are used to evaluate these losses in physical quality of extruded pellet. Bulk density and water stability helps to determine the sinking and floating characteristics of the pellet and can be controlled during processing. This should be corrected according to the eating behavior of farmed fish (M. Sørensen, 2012). There are different methods, and the device had been developed for analysis of high energy diet for salmon feed some of them are listed below:

### 3.4.1. Hardness

It is defined as a maximum force or fragmentation strength needed to rupture/ break the pellet. The well-known instrument used to determine the ability of the pellet to withstand compression are texture analyzer (Thomas & van der Poel, 1996), and Kahl pellet hardness tester. The pellet is placed between the two metals usually steel, then the pressure/force is applied. The force required to crack the pellet is measured and recorded in the form of Newton. The hardness of pellet depends upon degree of expansion, raw materials used and processing parameters (M. Sørensen, 2012). This instrument resembles like the external force on the pellet during transportation, storage, pneumatic feeder and the biting of the pellet by teeth of fish to break the pellet so can be used to measure the breaking resistance of the pellet. It is general practice to measure force to break more than ten pellets and take the average due to variation between the pellets.

### 3.4.2. Durability

Pellet should be appropriately tested by authentic method before sending it to the market. This test is based on the abrasion resistance of pellet when subject to mechanical or pneumatic agitation (Thomas & van der Poel, 1996). The measurement of durability mimics the acting force on pellet during storage, transportation, and pneumatic feeding system. The pellets should have sufficient physical combination that can survive during transportation, storage and handling from feed industry to the farm and through the different feeding system.

There is different instrument available to access the pellet quality of extruded diet, but only few can be used to examine the durability of high energy extruded pellet. Especially high oil vacuum coated pellet diet for a grower of Atlantic salmon. Pallet durability is usually analyzed by using Holmen pellet tester, but this instrument is used only for that pallet with low-fat content. ligno tester and Doris Tester is used if pellet contains high fat Some of the methods are listed below:

1. Holmen durability tester
2. LignoTester
3. Doris Tester
4. Tumbling box.

Among them, Holmen durability tester, LignoTester, and Doris Tester are most commonly used in European fish feed industry. Tumbling box is mostly used in feed industry as a standard method in the US and some other countries (Engineers, 2003).

**Holmen durability**

It is considered as a most appropriate durability measurement method. In this instrument the fixed weight of pellet usually 100g is transported around the closed circuit with 90-degree bends tube for a standardized time typically 30 to 120 seconds. The small broken partial is formed when it hit the right-angled corner of the tester. Then the feed material is sieved in a sieve size of 80% of the pellet diameter. The Holmen pellet durability index (PDI) is interpreted as a percentage and is calculated as the mass of remaining pellets divided by the mass of initial pellets multiplied by 100%.

$$PDI = \frac{Final\ weight\ of\ pellet\ remain}{Initial\ weight\ of\ pellet} \times 100\%$$

**LignoTester**

This is another durability testing device which uses pneumatic shear force to stress the pellet to simulate the degradation caused by pneumatic transportation/feeding. In this process, 100-gram sample is used. The sample is prepared by pre-sifted to remove dust from it and is placed in a perforated hopper. Air is blown around the perforated chamber for 120 seconds. The fines are removed continuously, and the amount of pellet left after the end of this procedure is calculated to find the pellet durability index (PDI) as in Holmen test (M. Sørensen, 2012; Wolska, Holst, Adlercreutz, & Jonkers, 2016).

**Doris Tester**

Norwegian aquafeed industry develops this method. The instrument is made from an Archimedes screw. The sample of approximately 350 g is placed in a DORIS tester. The Archimedes screw hit the pellet into a narrow vane. This effect resembles like the stress that pellets are exposed into the Pneumatic conveying feeder. Doris value is calculated as the percentage sum of Fracture and Fines after sieved. Three replication is usually done for same sample (Aas et al., 2011).

**Pfost Tumbling box method**

In these 500 grams of pellet sample is pre-screened. The sample is placed in a small square container attached to a rotating shaft. This square container rotates around at a speed of 100 rpm for 10 minutes. Then the pellet is sieved in a mechanical sieve shaker. The sieve size is 80% of pellet diameter. PDI of Tumbler method is calculated similarly like Holmen durability tester (M. Sørensen, 2012).

#### 3.4.3. Water stability

Some fish like Shrimp and Sea urchin they are a bottom feeder and eat feed at the slower rate. The feed for those species should be water stable and must reserve the oil and nutrients without leaching into water (M. Sørensen, 2012). Therefore, the eating behavior and water stability of feed should be standardized. Low water stability also causes oil belching in rainbow trout causing osmoregulatory stress due to oil-belching (Baeverfjord et al., 2006).

Water stability is measured by using following procedure. 10 gram of pellet sample is weighted. The weighted sample is then transferred into a circular wire net basket with 8 cm diameter and 3 mm net mesh size. Each test is done three times. The feed sample was placed in a beaker with 600ml and 300 ml of ordinary tap water. Then the beaker is set in a shaker attached water bath maintained at 23°C. The speed of shaker is maintained as 100 shakings per minutes, and the sample is placed in a shaker for 30, 60, 120, 240 minutes. Then the incubation is turned off, and the basket is gently dried with tissues paper, and weighing is done. Then the basket is placed in the heating chamber maintained at 105°C for 18 hours for drying. After drying each basket is measured to determine the final weight of the residual dry matter. The water stability is calculated as a difference in dry matter weight before and after incubation divided by dry matter weight of the feed before incubation (Baeverfjord et al., 2006).

### 3.4.4. Sinking and Floating Pellet

The test is done in a transparent tube, the diameter, and Hight of the tube is 3cm and 200 cm respectively. The tube is filled with fresh water or water with a particular concentration of salt. The temperature and salinity both affect the sinking speed of the pellet, so it should regularly be monitored. To achieve the constant temperature the salt water with defined salinity is left for a day. The fixed point is marked on 10 cm and 160 cm. Then the sinking velocity to travel 150 cm is measured by using stop-watch (Lekang, O.I., Andersen, J., Bøe, J.K. & Berre, 1991). The fourty pellet is haphazardly sampled from each diet, and those pellets which come in contact with the wall of measuring cylinder during dropping is not considered. The pellet that do not sink until 15seconds is considered as a floating pellet (Milanovic, 2015).

### 3.4.5. Bulk density

The bulk density is fundamental characteristics of the feed pellet. Expansion during extrusion is the determining factor in the production of floating or sinking pellet. Bulk density is measured by filling the pellet in measuring cylinder of known volume. Genital Scraping is done to remove the excess feed from the surface of the measuring cylinder. It is a standard practice to take a triplicate measurement, and bulk density per duplicate sample is calculated as mass per unit volume of the sample. It is Express as $gl^{-1}$ (M. Sørensen, 2012). For more accurate result the specific density of the pellet can also be measured by Volumetric displacement methods (Draganovic, Van Der Goot, Boom, & Jonkers, 2011). The bulk density should be greater than 525 $gl^{-1}$ to sink in the sea water with 35 $gl^{-1}$ of salinity (Glencross, Rutherford, & Hawkins, 2011).

## 3.4.6. Fat leakage

The fat leakage in high energy diet is a severe problem in aquafeed. The oil leakage results in oil loss from the pellet this results in loss of nutrients. Furthermore, if the oil is accumulated in pipeline the pneumatic conveying system, then the small pellet partial is piled up and block the pipe of the pneumatic feeder (M. Sørensen, 2012). Both the coated feed and mainly designed plastic bucket equipped with a plastic-coated diaper is placed at the bottom of the bucket. Then the bucket with the testing pellet is closed by lead and is stored at a room temperature maintained at 20-22°C for seven days. Then after seven days, the feed is removed from the bucket, and the bucket is weighted for oil leakage (Øverland, Romarheim, Ahlstrøm, Storebakken, & Skrede, 2007).

There is also another rapid method available to calculate the oil leakage. In this method, the plastic box containing absorptive lining is weighed. Then 100 grams of pellets is placed in that container. The box is incubated for a day at 40°C. Then the pellet and dust particle is removed from the box, and final weight of the box and an absorptive Strip is measured to determine the fat leakage (Mette Sørensen, Nguyen, Storebakken, & Øverland, 2010).

#### 3.4.7. Dry matter content

The dry matter can be determined by drying the fixed amount of sample usually 3 or 5 gram at 105°C for 4 hours (Henken, Lucas, Tijssen, & Machiels, 1986).

# 4. Material and Methods

## 4.1. Feed

Feed pellets were for Atlantic Salmon (Salmo Salar, L.) were obtained from Nofima Bergen, Norway. The 4-millimeter pellet were prepared by using following ingredients as shown in table 6. The feed were prepared by extrusion methord and packed in a 25 kg bag on 14/02/217 and was brought to Nofima Ås, Norway on 16/02/2017 and immediately store at cold storage maintained at 4°C for a week and vacuum coating was done at two different temperature ie 35°C and 70°C with three different oil blend (A, B, and C). The vacuum coated pellets were labeled and stored at two different temperature ie; 23°C and 4°C for a week and were tested for their physical properties.

Table 5 Composition of Atlantic salmon pelleted feed

| Composition of Diet | % Inclusion |
|---|---|
| Fish meal | 7.5 |
| Wheat | 10.05 |
| Wheat gluten | 22.45 |
| Soya protein concentrate (SPC) | 26 |
| Oil blend | 2.96 |
| Mineral mix | 0.59 |
| Vitamin mix | 2 |
| MSP (26% P) | 2.5 |

| | |
|---|---|
| Yttrium Oxide | 0.01 |
| Betafine | 0.5 |
| DL-Methionine | 0.8 |
| L-Lysine | 1.7 |
| Threonine | 0.2 |

## 4.2. Oil blend Preperation

The Oil blende was prepared at Nofima, Ås, Norway. The three Oil blend A (Control), B, and C were made with industrial grade Fish oil (FO) (Felleskjpet, 35 kg capacity Jar), Refined Rapeseed oil (RO) (Idum, 10 Liters Capacity jar) and Harden coconut (CNO) (Delfia Kokos Matfett, 250 Gram, 95% saturated fat) with following composition as shown in table 6. The Harden coconut (CNO) was prepared by melting at oven (Termaks) maintained at 70°C for 15 minutes in a 250-ml beaker (Borosilicate Glass). Then all three-oil blend was prepared by weighing a different portion of oil in a balance (Mettler Toledo XS603S) and homogeneously mixed with stirrer rod in a conical flask (Borosilicate Glass).

Then the oil mix is transferred to the bottle with led at room temperature (23°C) to maintain the final weight of 1.5 Kilogram for each blend.

Table 6 Percentage composition of oil blend.

| Oil | A | B | C |
|---|---|---|---|
| Coconut oil (CNO) | 0 | 2.5 | 5 |
| Rapeseed oil (RO) | 75 | 72.5 | 70 |
| Fish oil (FO) | 25 | 25 | 25 |
| % | 100 | 100 | 100 |

- For Oil blend, A was prepared by mixing 1.125 kg RO, without CNO.
- For Oil blend, B was prepared by mixing 0.0375 kg CNO, and 1.0875 kg RO.
- For Oil blend, C was prepared by mixing 0.075 kg CNO and 1.05 kg RO.

All of them were finally mixed with the constant pre-weighted amount of fish oil 0.375 kg to get final amount of 1.5 kg of oil blend A, B, and C.  All three-oil mix was stored in a cold store maintained at 4°C at Nofima, Ås, Fish Laboratory for 24 hours.

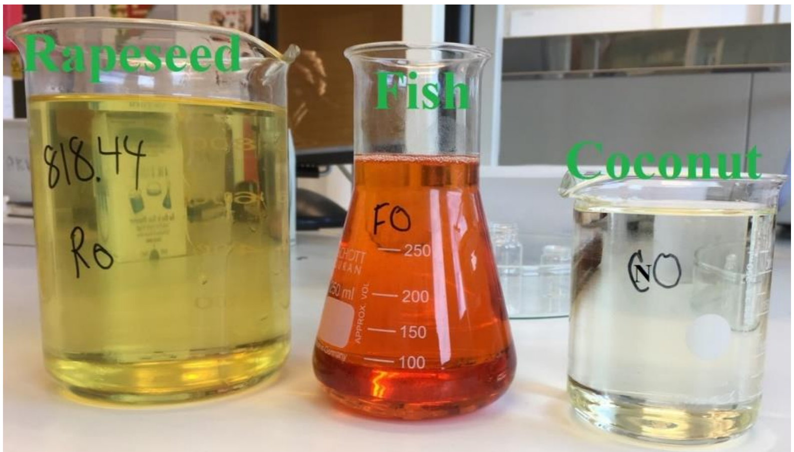



Figure 4 Rapeseed oil (RO), Fish oil (FO) Coconut oil (CNO) prepared for making oil blend.


Figure 5 Oil blend A, B, and C ready for Vacuum coating.

## 4.3. Sample preparation, labeling and vacuum coating of pellet

The Vacuum coating was done in a specially designed vacuum coater, designed by NMBU Extrusion and Pelleting innovation Centre. The coating was done at 35°C and 70°C temperature. For this, the oil blend and pellet were put in an incubator Matforsk SENS2007111 at Nofima, Ås, laboratory to maintained 35 °C and 70°C temperature of oil and pellet.

After the desired temperature is obtained the pellet and then oil mix was transferred into specially designed coating jar. Initial fat content in raw pellet was 2.96% and final estimated in hold of 30.96% fat was achieved by adding 140 grams (28% W/W) of oil blend in 500 grams of the pellet.

The oil blend and pellets were mixed manually for 10 seconds by rotating the coating jar containing pellet and oil blend. Then the pressure in the coating vessel was reduced to 80 to -90 Bars by using vacuum pump. The oil blend and pellets are left for 15 seconds then pressure was released slowly.

## 4.4. First Vacuum coating

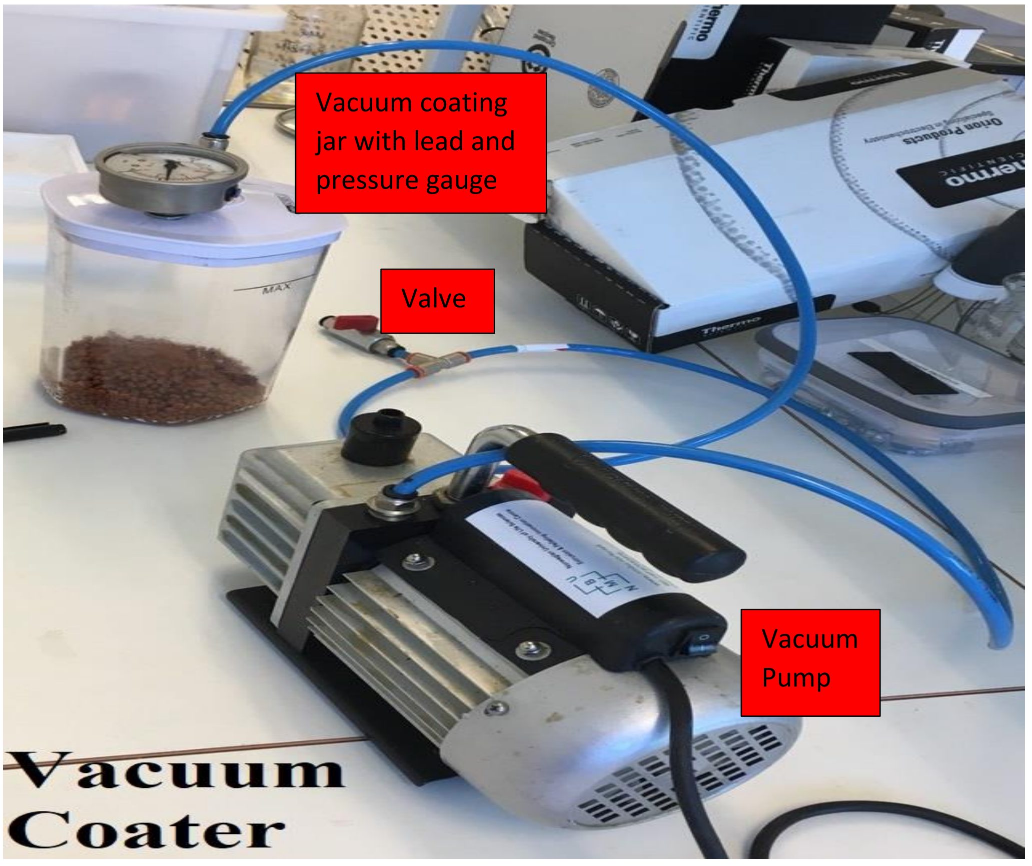



Figure 6 Vacuum coater assembly design by NMBU Extrusion and Pelleting innovation Centre.

Sample were prepared by vacuum coating with oil blend A, B and C at 35°C and 70°C temperatures and Stored at 4°C and 23°C. For each sample, 1.5 Kg of Pellet and 0.420kg of oil blend was used. The size of vacuum coater was small, so each pellet sample of 1.5 Kg was divided into three Portion, and Vacuum coating was performed with 0.5 Kg of pellet and 140grams of the oil blend. After vacuum coating, all the three portions of a same sample were placed in a plastic container and homogeneously mixed with a glass rod. Then 0.25 Kg grams of the coated pellet was randomly sampled and transferred into four plastic containers with blotting paper at the bottom. Then the Plastic container was labeled as according to a temperature of coating, oil blend used and temperature of Storage after coating as shown in the Figure 7 and Table 7.

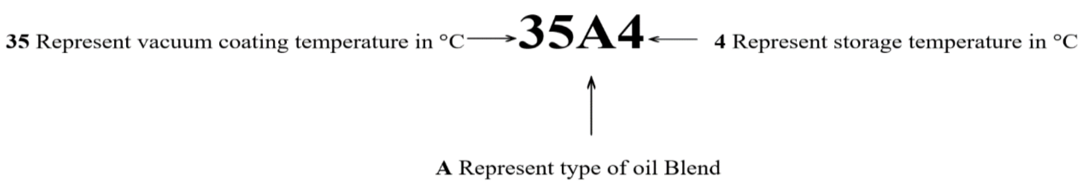



**Figure 7** Nomenclature of diet

**Table 7** Plastic container labelling code representing vacuum coating temperature, type of Oil blend and storage temperature.

| | | | | |
|---|---|---|---|---|
| **A** | **35A4** | **70A4** | **35A20** | **70A20** |
| **B** | **35B4** | **70B4** | **35B20** | **70B20** |
| **C** | **35C4** | **70C4** | **35C20** | **70C20** |

The sample prepared was then stored at room temperature and cold storage respectively for one week then the different test was performed to determine the Physiochemical properties of the pellet after storage.

## 4.5. Second Vacuum coating (part 2)

The next vacuum coating was done as before with 50 gram of pellet and 14 gram of oil blend A. The vacuum coating was done at 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C and 70°C temperature with the remaining raw pellet that was stored at the Cold store (4°C) at fish laboratory Nofima, Ås. The same parameter as above was used except the incubator Termaks (Tek 980144) was used to heat up the blend oil and pellet to get the coating temperature of oil and pellets. The vacuum coated feed was stored at 23°C for a week. The variation in floating and sinking velocity was measured by preparing plastic tube and salt water as below.

## 4.6. Plastic tube preperation

For this test, the transparent plastic cylindrical hollow tube of 4 cm diameter and height of 98 cm was marked 13 cm from the top and 25 cm from the bottom so that the length between the two marks will be 60 cm. The hole at the bottom of tube was closed by sealing tape and plastic cap as shown in figure 8.

## 4.7. Fresh water and Sea water preparation

Then 3.5% w/v of sea water was prepared with five litters of fresh water. The five litters of water were mixed with 175g of salt. The salt water was then stored for 24 hours in a cold store. Furthermore, the tube was filled with fresh water (6°C) and then with sea water (5°C) until 8 cm above the top mark to measure the sinking time and floating pellet at fresh and sea water.

## 4.8. Durability analysis

Pellet durability was tested for all twelve sample by using Ligno tester serial N LT110; Borregaard Lignotech, Sarpsborg, Norway at NMBU, IHA laboratory. The mechanical strength was tested by using 30 grams of dust and broken particle free pellet sample. Pellet was placed in a tester with the specially designed perforated hopper. The diameter of the perforated hole was 2.5 mm. The hoper was cover with filter paper and lead as provided by the manufacturer. The test was run for predefined time, i.e. 120 seconds, and the air pressure was also recorded on every test. The sample was sieved automatically through the perforated hole. The lead was then open, and the entire pellet was transferred to sample jar and weighed to find the final weight of the whole particle. Each sample was tested three times to get concurrent results, and ligno tester machine was cleaned after each test to reduce error. The pellet durability index (PDI) was calculated by using the formula below and expressed as a percentage (%).

$$PDI = \frac{Final\ weight\ of\ pellet\ remain\ at\ hopper}{Initial\ weight\ of\ pellet} \times 100\%$$

## 4.9. Colour Measurement

Colour measurement was done by CHROMA METER CR-400 at NOFIMA laboratory. The coated pellet was ground in a coffee grinder (Delaonghi), and then approximately 50 gram of grind sample were put in a white plastic plate. Then the colorimetric analysis was done by placing the lens sensor at the top of grinded feed that was placed in a white plastic plate to get the value of L* (light) a* (red) b* (yellow).

## 4.10. Floating and Sinking velocity of Pellets

The floating pellet was analysed at Nofima, Ås laboratory according to (Sk et al., 2015). The pellets were dropped from approximately 20 cm height in a 250-ml glass beaker containing 200 ml tap water. For each analysis, 60 pellets were dropped, and the number of floating pellet was coated. The water was changed with every five droppings.

## 4.11. Analysis of Floating and Sinking pellet

For each test sample, 60 pellet was randomly selected. The pellets were then dropped from the top of the pipe (5cm above the water level) by using a tweezer and then the time required to travel from the first mark to the last mark (60 cm) was recorded by using a stopwatch. Those pellets which do not sink until 15 seconds were marked as F. similar procedure was repeated for salt water. The result is listed as floating pellet was given number 1, and for sinking pellet, the number 0 was given. Similarly, the time taken was measured in second to travel 60 cm distance was measured by using a stopwatch and recorded

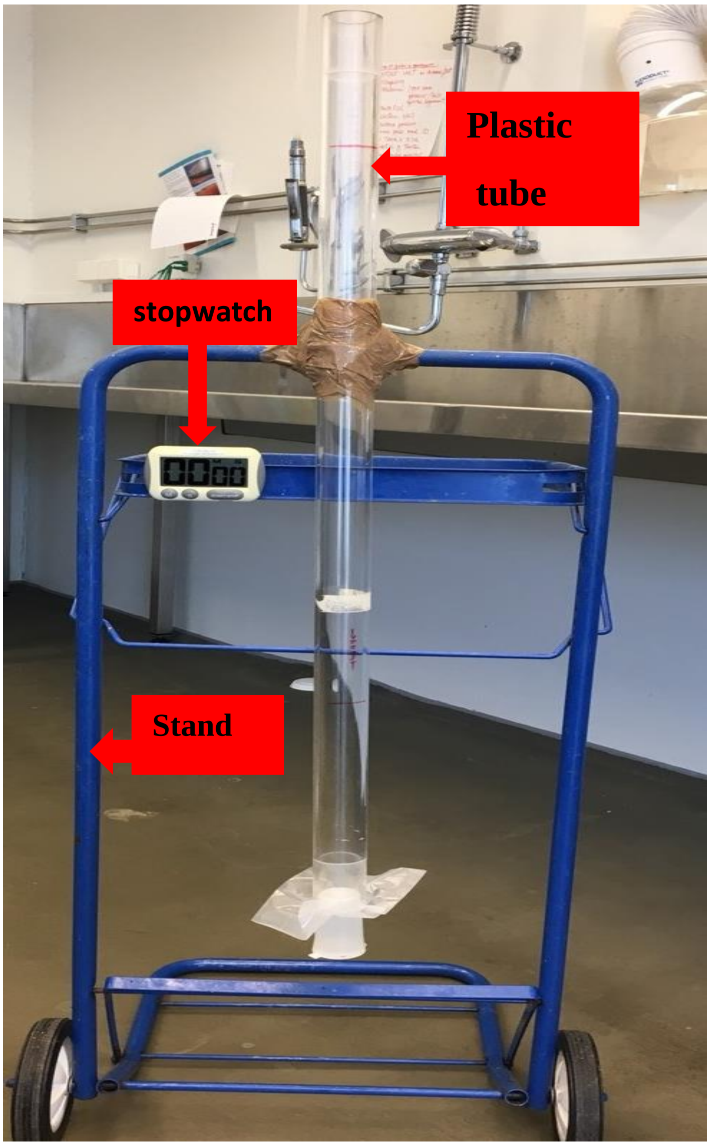



Figure 8 Preparation of tube for sinking and floting pellet analysis.

### 4.12. Dry Matter (DM) content

Twenty grams of each sample of feed pellet was grinded by using delaonghi coffee grander at Nofima. Around 5 grams of grinded pellet of different diet and aluminium plate was weighed by using Mettler Toledo, XS603S. The pre- weighted grinded pellets were put in to the pre- weighed aluminium plates. Then the plate with pellet was dried for 4 hours at 105°C temperature (Henken et al., 1986) at Tormaks incubator (Tek 980144) at Nofima, Ås, laboratory. Then the DM content of different diets were calculated by using following formula.

$$\%DM = 100 - \frac{(Initial\ weight - weight\ after\ drying)}{Initial\ weight} \times 100$$

## 4.13. Fat leakage using Texture analyser

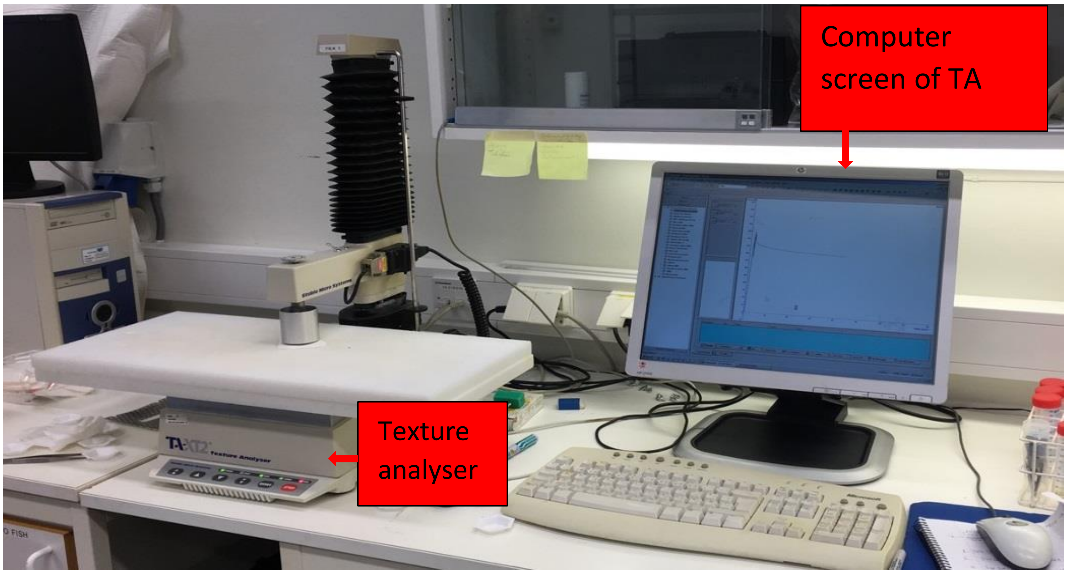



Figure 9 Texture analyser assembly

**Sample preparation**

All the pellets were brought to same room temperature 19.5°C and left it for 3 hours. Then the weight of 7 pellet was taken to increase the accuracy, and the pellets were placed in a white plastic plate with the label.

**Filter paper preparation**

The 589[3] Blue ribbon ashless S & S Filter Paper Circles with 125mm Diameter was used. It was folded and cut at the corner to increase the stability during measurement. Then the paper is weighed.

**Parameter of test**

Fat leakage analysis was performed by using new methodology with the help of Texture analyser TA-XT2, Stable Micro System, Surrey, UK. It was connected with special designed cylindrical prove with proving diameter of 5cm. The trigger force of 0.04903 Newton was applied. Test speed 1 mm/s with 80% strain for 90 seconds. The parameter recorded was area under from the force time graph, force required to break the pellets and oil leakage.

Table 8 Test mode of Texture analyzer for fat leakage analysis

| CAPTION | VALUE | UNITS |
|---|---|---|
| Test Mode | Compression | |
| Pre-test speed | 10 | mm/sec |
| Test speed | 1 | mm/sec |
| Post-test speed | 10 | mm/sec |
| Target mode | Strain | |
| Strain | 80 | % |
| Holding Time | 90 | Sec |
| Trigger type | Auto (force) | |
| Trigger force | 0.04903 | N |
| Advance options | Off | |

**Calibration of TA**

The calibration of the weight of TA was done by using 5 kg weight in Calibration platform, and height was calibrated by putting the folded filter paper in between basement and probe.

**Test for oil leakage and pellet hardness**

The seven pellets were placed in a horizontal position at the top of filter paper. Then the tests were done according to parameter above in table 10. Then the broken pellet particles were removed from the paper gently and papers were folded and put it in a dryer for 2 hours at 105°C to remove the moisture. Then the filter papers were weighted to determine the fat leakage by using the formula below.

$$\% \text{ Fat loss} = \frac{W2-W1}{W} * 100$$

Where,

W2 = Final weight of Paper

W1= Initial weight of Paper

W = Weight of Pellet used for analysis

Each sample was tested three times. The max force (N), Area ($m^2$) and length (mm) were also noted at the same time as shown in table 11.

## 4.14. Statistical analysis

Different data from the various sample were statistically analyzed by using SAS (version 9.4 TS Level 1 M2, SAS Windows Version; SAS Institute, Cary, NC, USA) and some data was analysed as LSMEAN ± SE. All the results are presented as a mean value and standard error (SE) of the means. If the $P \leq 0.05$ the sample was considered as significantly different for all statistical analysis presented in this thesis. The number of repetation for the physical analyses were: PDI (n = 3), fat leakage (n = 3), texture analyser (n = 3), colorimeteric analysis (n = 3), floating pellets (n = 60), drymatter content (n = 2).

# 5. Results

## 5.1. Part 1

The relation between variables and physical analysis are shown in table 9. The statistical models explained as low as 21.1% of the data for the force required to break the pellets (Force, N) to as high as 96.6% of the data for the dry matter. PDI was found to be significantly affected ($p \leq 0.05$) by vacuum coating temperature, storage temperature, and the interaction between oil blend and vacuum coating temperature. Similarly, fat leakage was found to be significantly affected by oil blend and storage temperature only. L* value was found to be significantly affected by interaction between oil blend and vacuum coating temperature and interaction between oil blend and temperature of storage. No significant variation was found in b* value and Area (N*s) with treatment variables. The number of floating pellets were found to be significantly affected by oil blend and vacuum coating temperature. Similarly, the dry matter content was also found to be significantly affected by oil blend, storage temperature, and interaction between oil blend and temperature of storage.

Table 9 P-values for different treatments from statistical analysis

| | Variables / Physical analysis | Oil blend | Vacuum Coating temperature | Storage temper ature | Oil blend * Vacuum coating temperature | Oil blend * Tempera ture of storage | P Model | $R^2$ (%) |
|---|---|---|---|---|---|---|---|---|
| **1** | **PDI** | 0.3297 | <0.0001 | 0.0045 | 0.0119 | 0.5436 | <0.0001 | 75.8 |
| **2** | **Fat leakage** | 0.0128 | 0.2054 | 0.0004 | 0.8694 | 0.2303 | 0.0032 | 54 |
| **3** | **Force, N** | 0.8503 | 0.0895 | 0.3927 | 0.9874 | 0.2415 | 0.5298 | 21.1 |
| **4** | **L*** | 0.6078 | 0.0658 | 0.039 | 0.0072 | 0.0033 | 0.0016 | 56.8 |
| **5** | **a*** | 0.2502 | 0.3323 | 0.2126 | 0.394 | 0.1535 | 0.2278 | 29.8 |
| **6** | **b*** | 0.1853 | 0.9822 | 0.2413 | 0.1291 | 0.0569 | 0.0885 | 37 |
| **7** | **Area (TA)** | 0.1586 | 0.3867 | 0.2022 | 0.6816 | 0.9631 | 0.46 | 27.7 |
| **8** | **Floating pellets** | 0.0001 | 0.0001 | 0.0985 | 0.5597 | 0.7755 | <0.001 | 86.4 |
| **9** | **Dry matter** | 0.0001 | 0.0828 | 0.0001 | 0.6467 | 0.0001 | 0.0001 | 96.6 |

Table 10 LS MEAN ± SE for different physical analysis of pellets with different coconut oil content in the oil blend: A 0%, B 2.5%, C5%; The results are presented irrespective of vacuum coating temperature and storage temperature.

| S.no | Source/ Test | Oil blend (LS MEAN ± SE) | | |
|---|---|---|---|---|
| | | A | B | C |
| 1 | PDI | 91.18 ± 0.54 [a] | 91.14 ± 0.54 [a] | 92.07 ± 0.54 [a] |
| 2 | Fat leakage | 3.58 ± 0.25 [a] | 2.77 ± 0.25 [a] | 2.45 ± 0.25 [b] |
| 3 | Force (N) | 313.10 ± 14.74 [a] | 302.09 ± 14.74 [a] | 311.99 ± 14.74 [a] |
| 4 | L* | 37.98 ± 0.31 [a] | 38.03 ± 0.31 [a] | 37.70 ± 0.31 [a] |
| 5 | a* | 22.10 ± 0.19 [a] | 21.73 ± 0.19 [a] | 22.12 ± 0.19 [a] |
| 6 | b* | 34.6 ± 0.4 [a] | 33.7 ± 0.4 [a] | 33.8 ± 0.4 [a] |
| 7 | Area (f*s) | 12785.2 ± 1476.7 [a] | 8101.6 ± 1241.8 [ab] | 9656.3 ± 1241.9 [a] |
| 8 | Floating pellets | 3.1 ± 0.2 [a] | 1.25 ± 0.2 [a] | 0.63 ± 0.2 [b] |
| 9 | Dry matters | 92.76 ± 0.2 [c] | 94.12 ± 0.2 [b] | 94.93 ± 0.2 [a] |

- **PDI vs. oil blend**

Numerically, the diet coated with oil blend C had highest and diet coated with oil blend B had lowest PDI value. The result revealed that oil blend C with high CNO (5%) shows the highest PDI value in diet but statistical analysis showed no significant difference in PDI value of diet prepared by coating with different oil blends.

- **Fat Leakage vs. oil blend**

Numerically, the diet coated with oil blend A had highest and diet coated with oil blend C had lowest value of % fat leakage. These results revealed that oil blend C with high CNO (5%) showed the lowest % fat leakage in the diet. Statistical analysis revealed that most significant variation was absorbed between diet prepared by coating with oil blend A and B Vs. Oil blend C.

- **Force (N) vs. oil blend**

Numerically, the diet coated with oil blend A required highest and diet coated with oil blend B required lowest force for breaking the pellets. This result revealed highest breaking force for oil blend A with 0% CNO, but statistical analysis showed no significant difference between the treatments.

- **L* vs. oil blend**

Numerically, the diet coated with oil blend B had highest and diet coated with oil blend C had lowest L* value. These results revealed that oil blend B with 2.5 CNO showed the highest L* value but statistical analysis showed no significant difference in L* value of diets prepared by coating with different oil blends.

- **a* vs. oil blend**

Numerically, the diet coated with oil blend C had highest and diet coated with oil blend A had lowest a* value. This result revealed that oil blend C with 5 % CNO showed the highest a* value in diet, but statistical analysis showed no significant difference in a* value of diets prepared by coating with different oil blend.

- **b* vs. oil blend**

Numerically, the diet coated with oil blend A had highest and diet coated with oil blend B had lowest b* value. These results revealed that oil blend A with 0 % CNO showes the highest b* value in diet, but statistical analysis showed no significant difference in b* value of diets prepared by coating with different oil blend.

- **Total force/Area vs. oil blend**

Numerically, the diet coated with oil blend A had highest and diet coated with oil blend B had lowest Area. These results revealed that oil blend A with 0 % CNO showed the highest Area value in diet, but statistical analysis showed no significant difference in the area of diet prepared by coating with different oil blend.

- **Floating pellets vs. oil blend**

Numerically, the diet coated with oil blend A had highest and diet coated with oil blend C had lowest number of floating pellets. These results revealed that oil blend A with 0 % CNO showed the highest number of floating pellets in diet. Statistical analysis revealed that most significant variation was obsorbed between diet prepared by coating with oil blend C than another oil blend.

### 5.1.1. Ligno tester

The Pellet durability index (PDI) % is presented in figure 10. Diet 35A4 showed highest 96.03 and Diet 70A4 shows the lowest 84.76 PDI values among all diets. The durability increased by increasing the concentration of coconut oil during vacuum coating for all diet stored at 4°C and 23°C but in case of diet vacuum coated at 35°C and stored at 4°C (35A4, 35B4, and 35C4), an opposite trend was observed. The diet (70A4, 70B4, 70C4 vs. 70A23, 70B23, 70C23) vacuum coated at 70°C and stored at 4°C and 23°C showed significant difference between this group, and PDI values were higher in 23°C storage pellet than in 4°C stored pellet.

Further, the Diet (35A23, 35B23, and 35C23) and (70A23, 70B23 and 70C23) despite numerical differences in LSmean, they were not significantly different ($P \leq 0.05$) from each other with change in oil blend during vacuum coating. The vacuum coating temperature will have significient effect when coated with same oil blend and stored at same temperature.

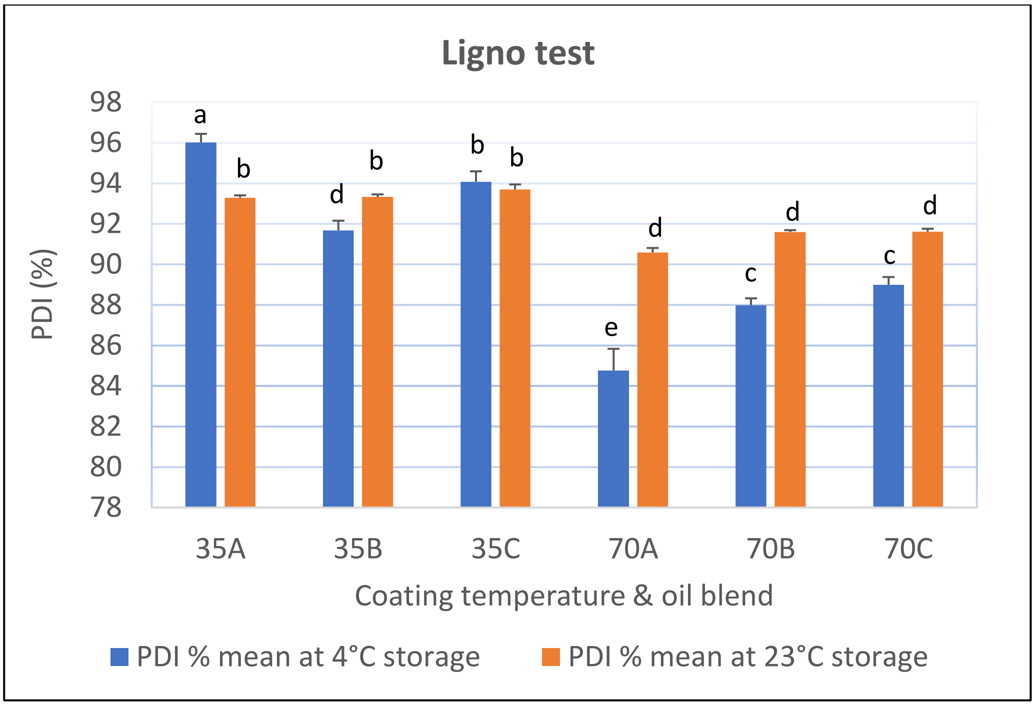



Figure 10 Pellet Durability Index (PDI) results of 12 different vacuum coated diets analyzed by Ligno tester by following standard methods. Result are presented as LSmean ± SE (n=3). Treatments not sharing the same superscripts above the error bars are significantly different. The alpha level was set to 5% ($P \leq 0.05$ was considered as significant difference).

### 5.1.2. Colorimetric analysis

The colorimetric analysis was done on the 8th day after vacuum coating of the pellet and stored at 4°C and 23°C.

Figure 11A

Numerically, the lightness (L*) value analyzed showed the mean value ranged from 35.98 (35B23) to 39.92 (70B4). The diet coated with oil blend B had a higher value of L* then oil blend A and B at 4°C stored pellets for both coating temperatures.

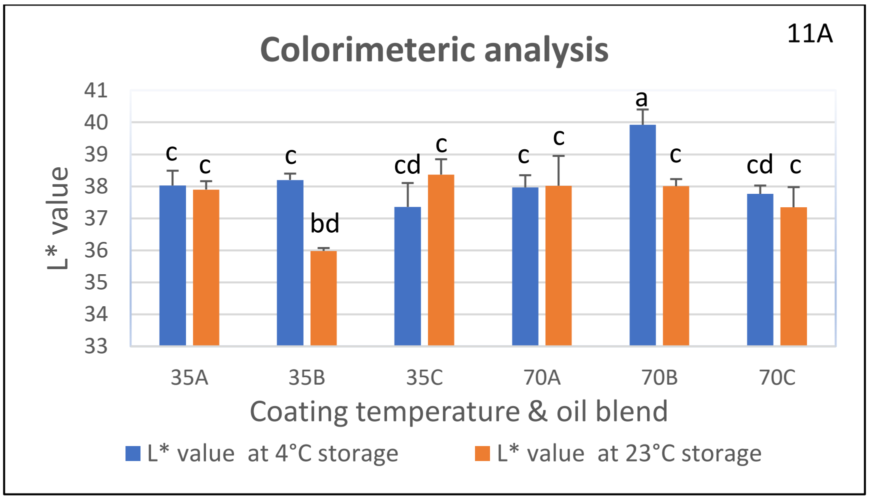
There were not any significant variation between the diets prepared. The exception being 70B4 diet which significantly different from all other treatment.

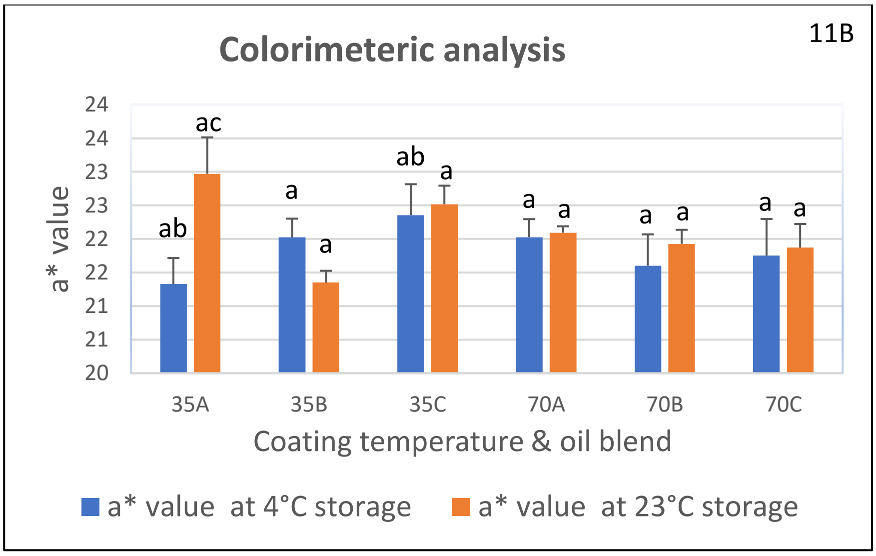
Figure 11B

The redness (a*) value analyzed showed that mean values ranged from lowest value 21.33 to highest value 22.97 for treatment 35A23 and 35A4 respectively. Pellets stored at 4°C showed increase in a* value but showed decrease in a* value when pellets were stored at 23°C with increase in coconut oil concentration during vacuum coating. Despite some variation in average number, no significant difference was observed between diets.

Figure 11C

The yellowness (b*) value analyzed showed a mean value range from lowest value 32.2 to highest value 36.16 in diet 35A23 and 35B23 respectively. The pellets when vacuum coated at 70°C showed decreased in b* value with increase in concentration of coconut oil at both storage temperature. The statistical analysis showed that the pellet stored at 4°C showed no significant variation. The diet 35A23, 35C23 and 70B23 were not significantly different, but all of them were significantly different from other treatment.



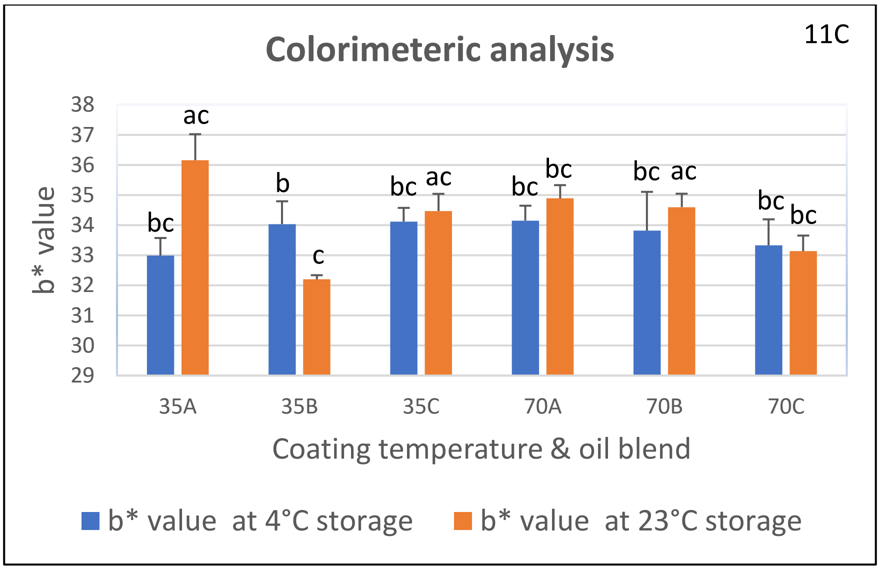



Figure 11 11A, 11B, and 11C represent L*, a* and b* value respectively for colorimetric analysis of different diets. Results are presented as LSmean ± SE (n=3). Treatments not sharing the same superscripts above the error bars are significantly different. The alpha level was set to 5% ($P \leq 0.05$ was considered as significantly difference).

### 5.1.3. Texture analyzer (TA)

**Fat Leakage**

The fat leakage was analyzed by using Texture analyzer of 12 different diets. The average value of fat leakage % ranged from 1.7 to 4.7.

Numerically, the highest oil leakage were found in diets prepared by vacuum coating at 70°C with control oil blend A and stored at 23°C temperature, i.e. 70A23. On the other hand, the lowest oil leakage was found in diet prepared by vacuum coating at 70°C by using oil blend C and stored at 4°C i.e. (70C4).

The increase in CNO shows lower fat leakage for pellets stored at both 4°C and 23°C. On the other hand, the pellet vacuum coated at higher temperature showed more oil leakage then vacuum coated at a lower temperature. Also, the higher the storage temperature, the more the oil leakage was absorbed by the increase in the concentration of CNO.

The statistical analysis shows that diets stored at same temperature and prepared by vacuum coating with same oil blend are not significantly different with change in vacuum coating temperature. Similarly, the diet (35A23 and 70A23) that was stored at 23°C and prepared by vacuum coating at 35°C and 70°C degree with oil blend "A" showed significant difference with most of the diet exception being 70B23 and 70C23.

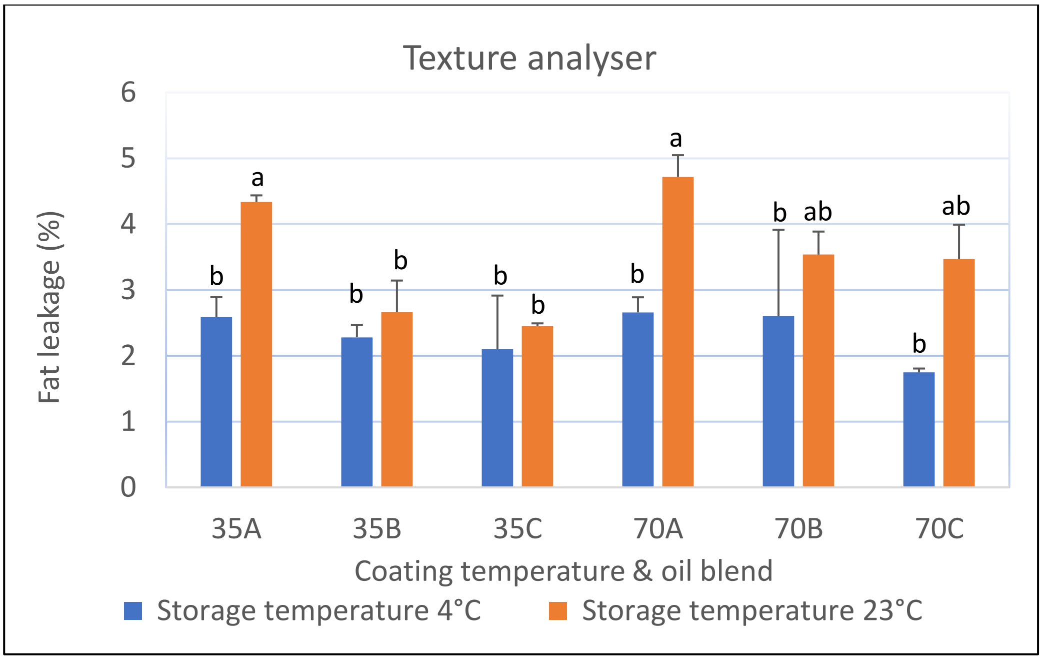



Fig 12 Fat leakage analysis results of 12 different vacuum coated pellet diet analyzed by Texture Analyzer. Results are presented as LSMEAN ± SE (n=3). Diet not sharing the same superscripts above the error bars are significantly different. The alpha level of data analysis was set to 5% ($P \leq 0.05$ was considered as a significant difference).

**Maximum compression force (Force, N)**

The maximum compression force (Force, N) was analyzed by using texture analyzer. The mean value of maximum compression force ranged from 250.7 of diet 35A23 to 357.2 of diet 70B23.

Numerically, the increase in the concentration of CNO during vacuum coating showed decreased in force to compress the pellet stored at 23°C but the pellets were harder when stored at 4°C and prepared by vacuum coating at both temperatures (i.e., 35°C and 70°C).

The diet with 2.5 % of CNO showed softer pellet then others. The exception being diet 70B4 coated with oil blend B which shows higher numerical force values then A and C oil blend.

Statistical analysis showed no consistent difference between treatment.

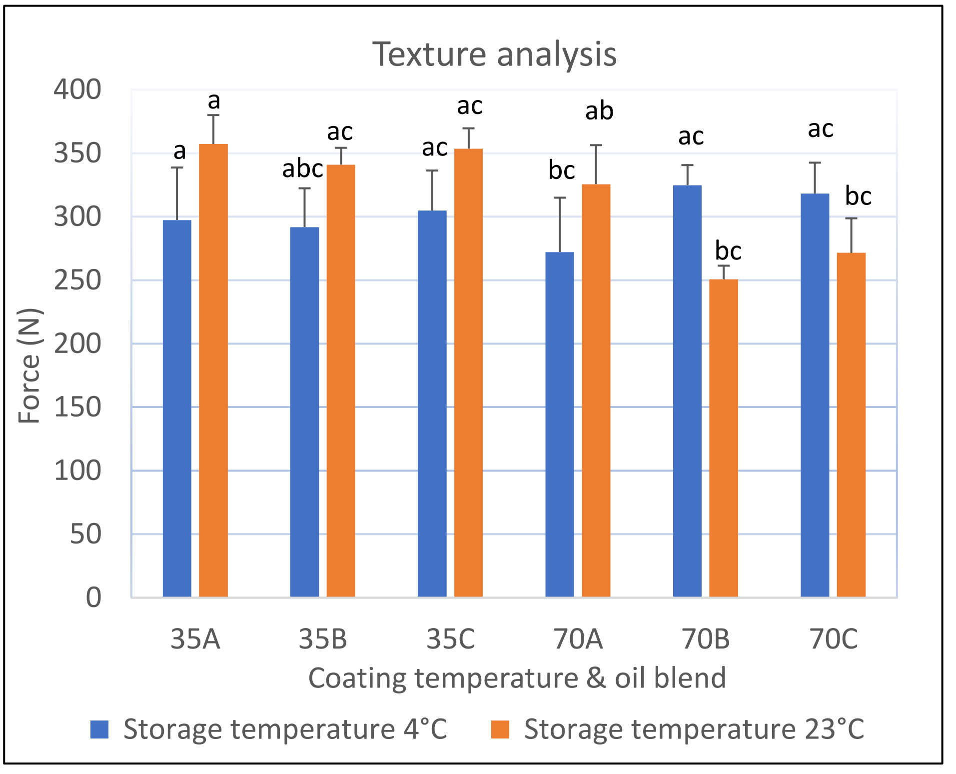



Figure 13 Maximum compression force (Newton) required to compress 12 different vacuum coated pellet diet analyzed by Texture analyzer. Results are presented as LSMEAN ± SE (n=3). Treatments not sharing the same superscripts above the error bars are significantly different. The alpha level was set to 5% ($P \leq 0.05$ was considered as significant difference).

**Area (N*s)**

The total work (Area, N*s) was analyzed by using Texture analyzer of 12 different diets. The mean value of area ranged from 5836.979 of diet 70B4 to 13641.81 of diet 70A23.

Numerically, the oil blend B showed the lowest area among the same storage temperature and coating temperature group i.e. (35A4, 35B4, 35C4), (35A23, 35B23, 35C23), (70A4, 70B4, 70C4,) and (70A23, 70B23, 70C23) among them 35B4, 35B23, 70B4, 70B23 had the lowest Area.

Statistical analysis revealed that there were no significant variations between the 12 treatments.

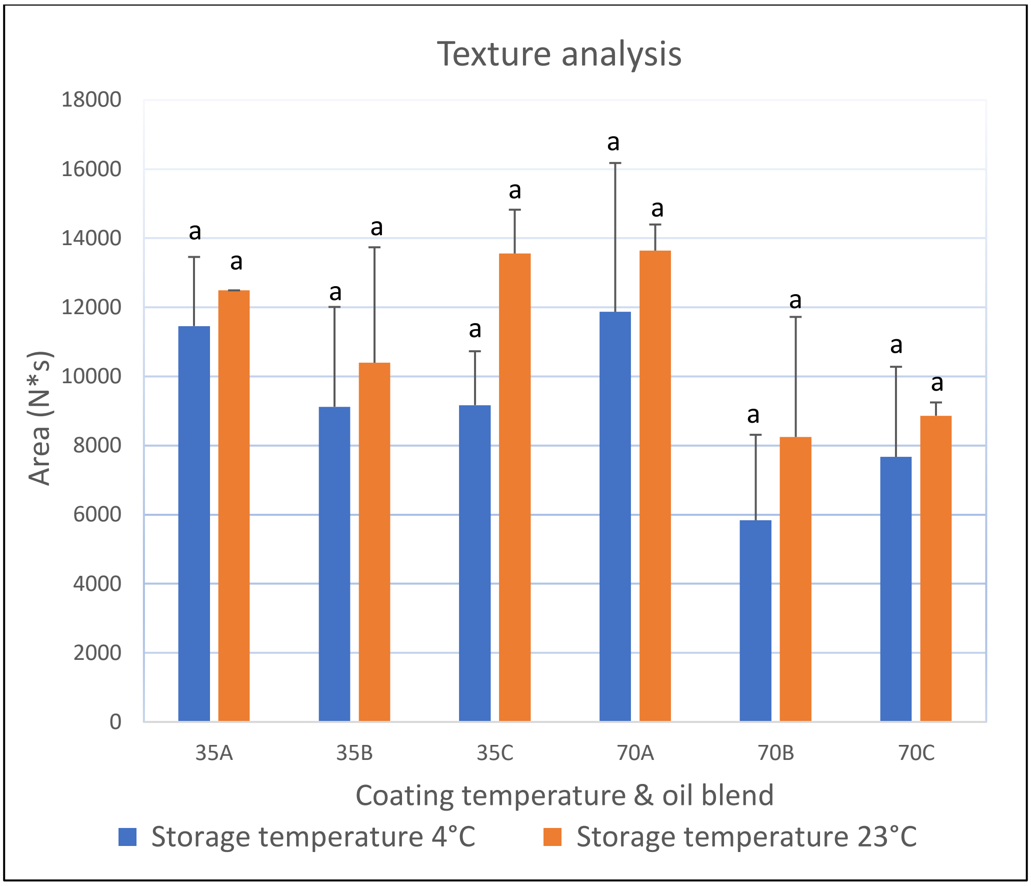



Figure 14 The total work (area, N*s) done on vacuum coated pellets, analyzed by Texture analyzer. Results are presented as LSMEAN ± SE (n=3). Treatments not sharing the same superscripts above the error bars are significantly different. The alpha level was set to 5% ($P \leq 0.05$ was considered as significant difference).

#### 5.1.4. Dry Matter (DM) content

The mean value of dry matter ranged from 90.8 of diet 35A23 to 95.6 of diet 70C4. The increase in CNO concentration in oil blend showed increased in dry matter content of diet when coated and stored at the same temperature.

Numerically, the DM content was higher when vacuum coated pellets were stored at a lower temperature 4°C then higher temperature 23°C for same oil blend and coating temperature. The statistical analysis revealed significant variation in dry matter content.

The statistical analysis revealed that the significant variation was also obsorbed with increase in coconut oil concentration when pellets were stored 4°C and 23°C for same coating temperature and same oil blend.

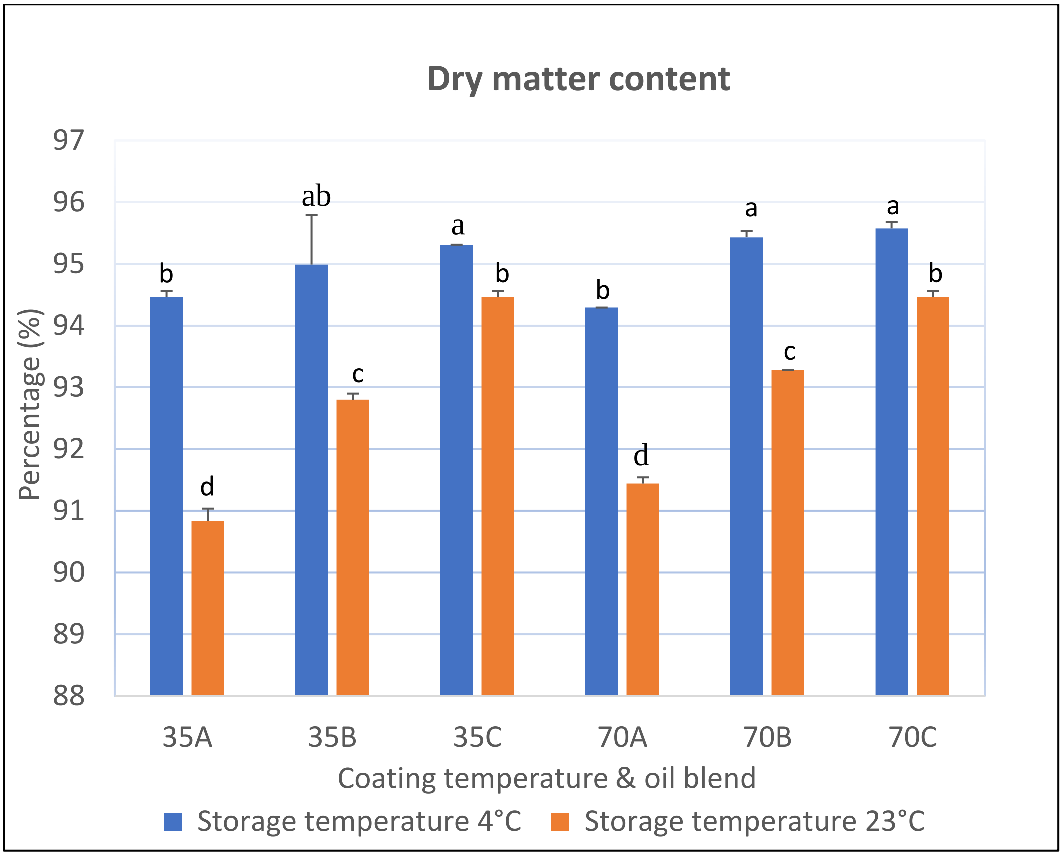



Figure 15 Dry matter content in % vacuum coated pellet. Results are presented as LSMEAN ± SE (n=3). Diet not sharing the same superscripts above the error bars are significantly different. The alpha level was set to 5% ($P \leq 0.05$ was considered as significant difference).

#### 5.1.5. Floating pellets

The mean value of floating pellets ranges from 0 (70C4 and 70C23) to 4 (35A4 and 35A23).

Numerically, the increase in CNO concentration in oil blend showed decrease in relative numbers of floating pellets of diet when coated and stored at the same temperature. The pellet stored at 23°C showed more floating pellet than at 4°C with some exception for Diet (35A4 vs. 35A23) and (70C4 vs. 70C23) where they showed equal numbers of floating pellets. Statistically there was no significant difference.

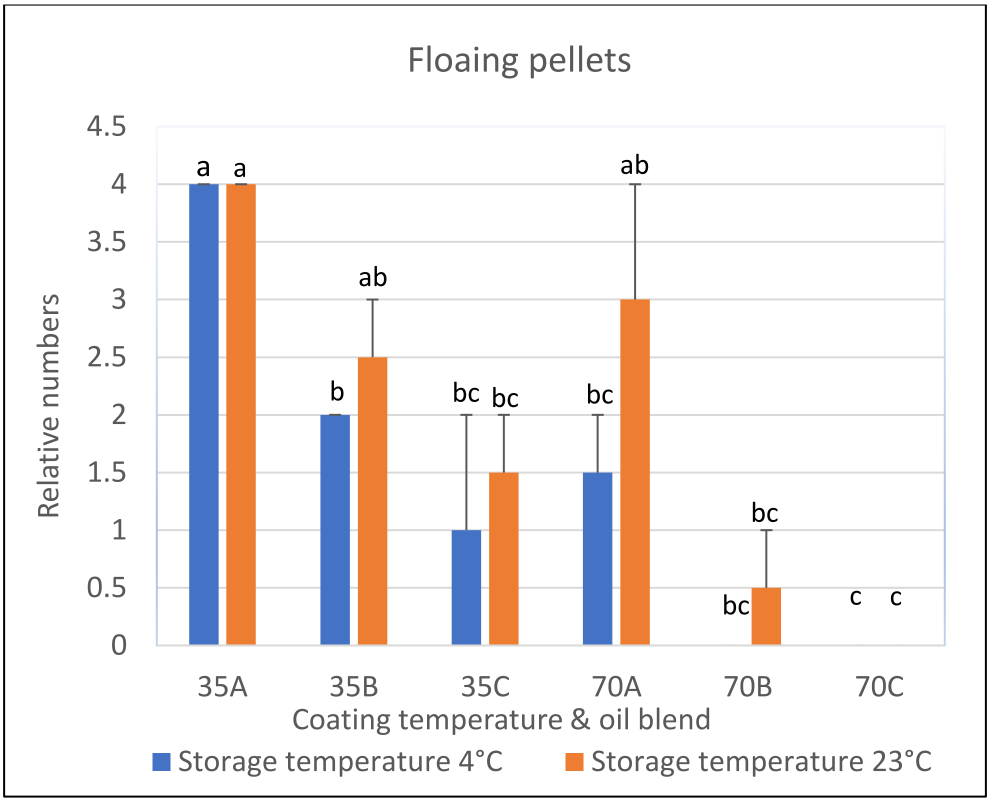



Figure 16 Floating pellets for vacuum coated pellets. Results are presented as mean ± SE (n=3). Treatments not sharing the same superscripts above the error bars are significantly different. The alpha level was set to 5% ($P \leq 0.05$ was considered as significantly difference).

## 5.2. Part 2

Vacuum coating of pellets at temperature (°C) 35, 40, 45, 50, 55, 60, 65, 70 by using control oil A was done and pellets were stored at room temperature.

### 5.2.1. Dry matter (DM) content

The percentage dry matter ranged from low 92.72% for raw pellets to 95.34 % for pellet coated at 65°C.

The dry matter content increases with increase in coating temperature until 45°C and then decreased at 50°C coating then DM content was again raised.

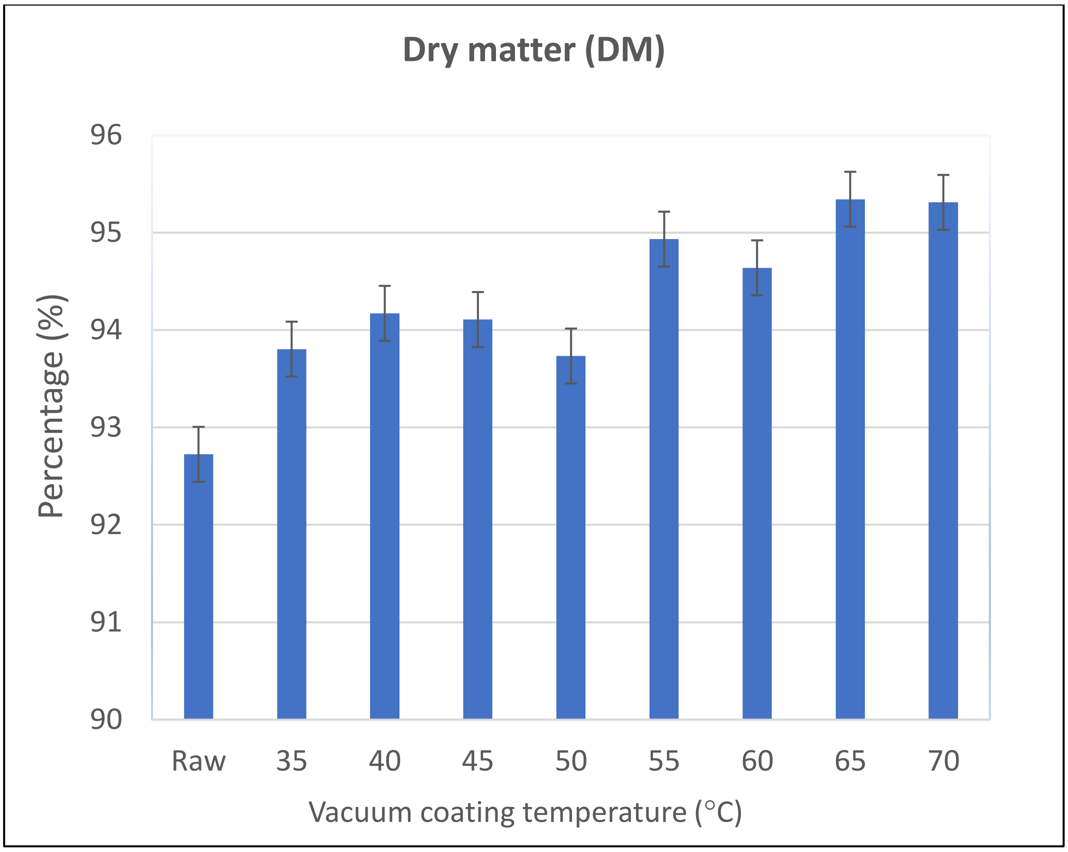



Figure 17 The dry matter content (%) of one raw and six vacuum coated batches of pellets. The vacuum coating was done by using control oil mix (A). The dry matter content results are presented as average values.

### 5.2.2. Floating and Sinking pellets

Figure 18A describes average relative numbers of floating pellets in freshwater.

In this graph, the floating pellets decrease with increase in coating temperature until 40°C and then again, the floating pellets increased until 50°C vacuum coating. Then again, the floating pellet decreased and remained below 0.6 relative numbers. From the statistical analysis, the raw pellets showed more significant variation with vacuum coated pellets. Similarly, the considerable difference was not absorbed between (35°C, 50°C and 70°C) and (40°C,45°C, 55°C, 60°C and 65°C) vacuum coated pellet.

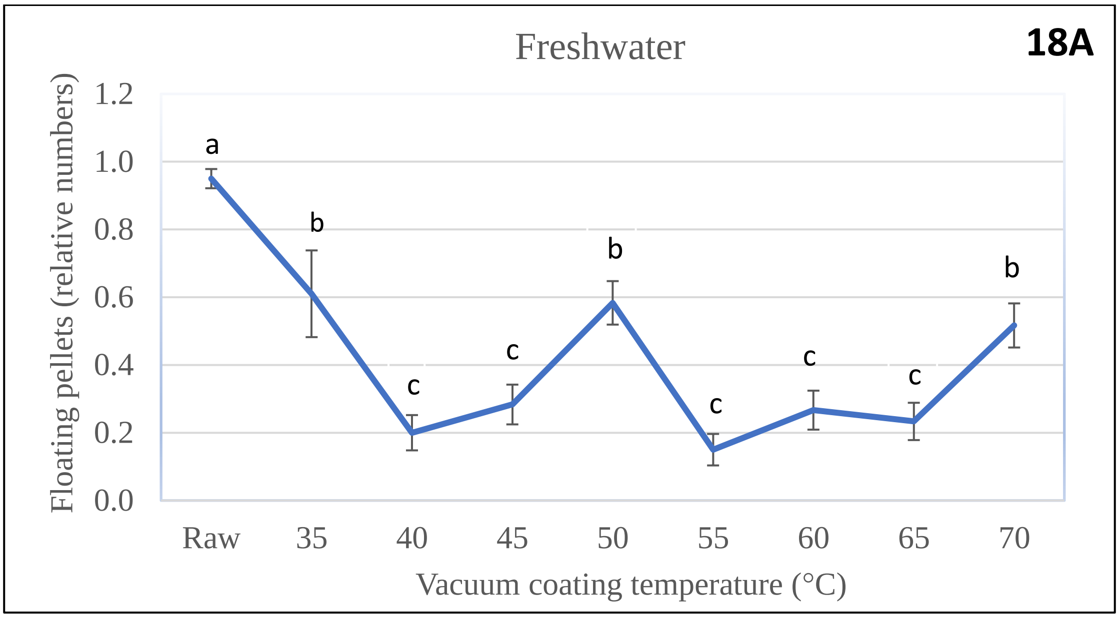
Figure 18B Describes average relative seconds of sinking pellets in freshwater.

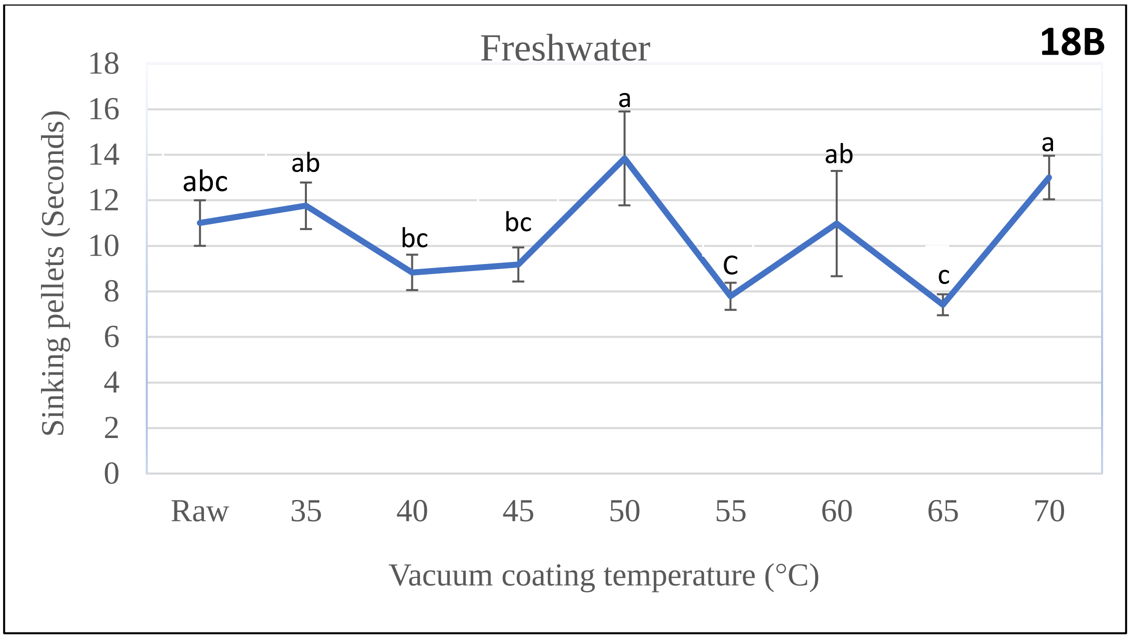
In this graph, the sinking time was highest (13.83) at 50°C and the lowest (7.41) at 65°C vacuum coated pellets. A lot of fluctuation between different coating temperature was absorbed. From the statistical analysis, the pellets coated at 55°C and 65°C were similar and significantly different from pellet coated at 35°C, 50°C, 60°C, and 70°C.

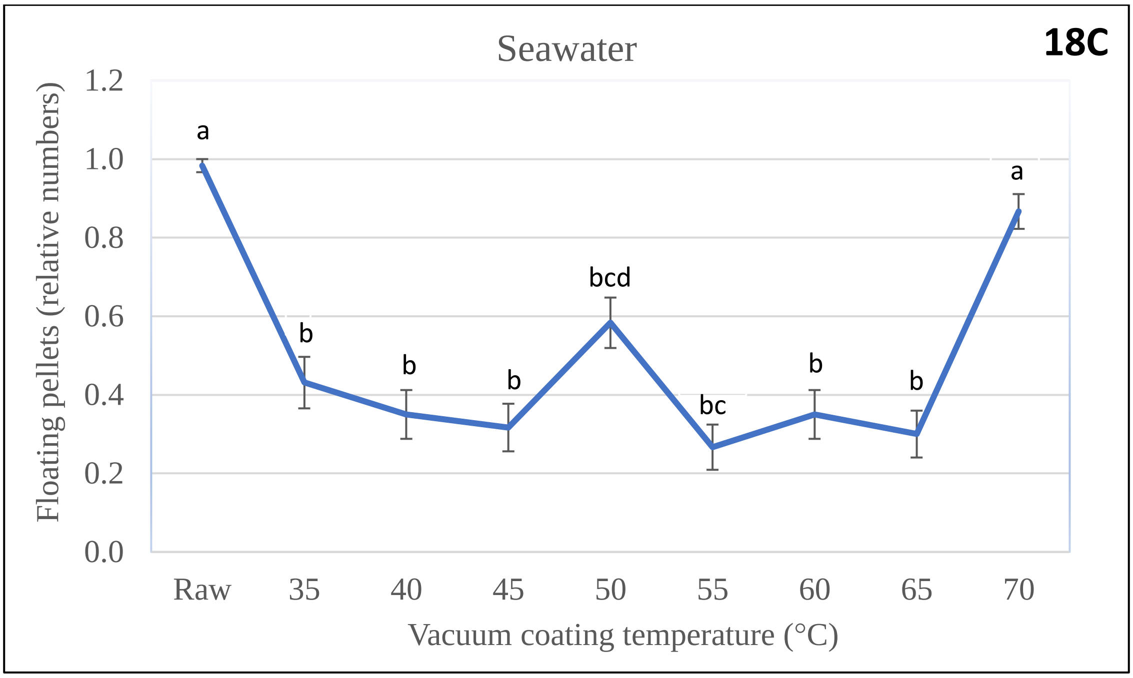
Figure 18C describes the average relative numbers of floating pellets at seawater.

In this graph, the number of floating pellets ranges from 0.983 at raw pellets to lowest value of 0.267 at 55°C coating pellets. The floating pellets decreased with increase in coating temperature until 45°C then again, the number of floating pellets increased and decreased until 65 then the floating pellet was increased and reached the highest values for vacuum coated pellet at 70°C. From the statistical analysis, the pellets coated at 70°C were significantly similar with raw pellets but most significantly different from other coating temperature.

Figure 18D describes the average relative sinking time of pellet in seawater. The value of sinking time was highest (14.12 seconds) of raw pellets and lowest (8.52 seconds) at 55°C. From the statistical analysis, there was no significant difference in sinking rate between raw and vacuum coated pellet.




Freshwater
18B
Sinking pellets (Seconds)
18
16
14
12
10
8
6
4
2
0
abc
ab
bc
bc
a
C
ab
c
a
Raw
35
40
45
50
55
60
65
70
Vacuum coating temperature (°C)


Seawater
18C
Floating pellets (relative numbers)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
b
b
b
bcd
bc
b
b
a
Raw
35
40
45
50
55
60
65
70
Vacuum coating temperature (°C)

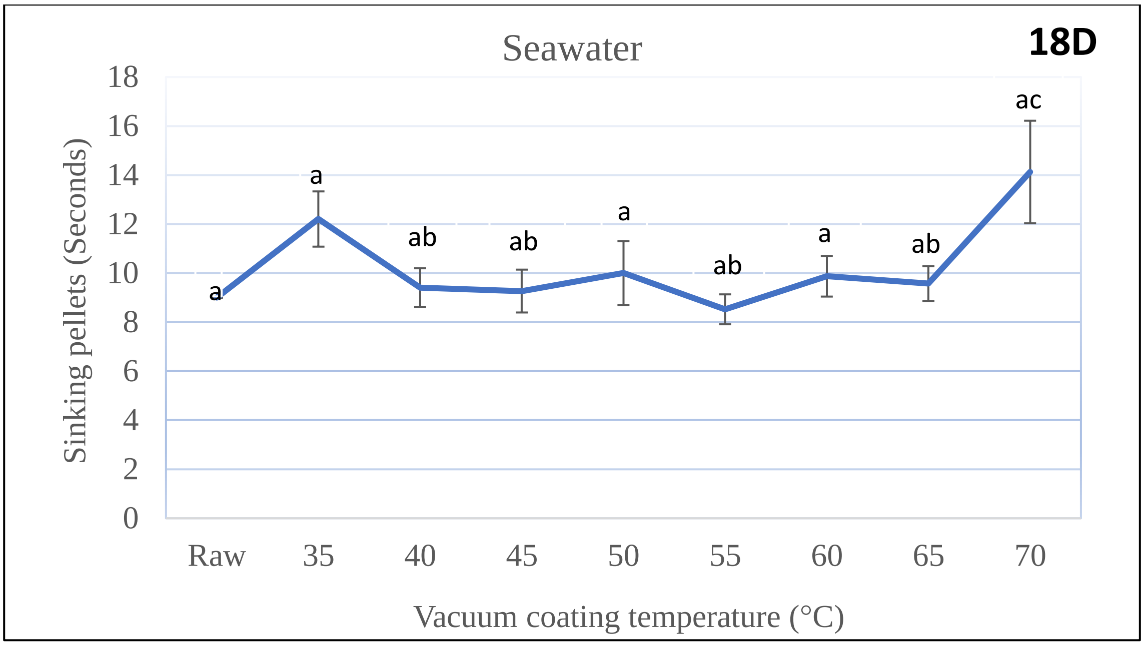



Figure 18 The Floating and sinking pellets of one raw and six vacuum coated pellets at a temperature °C (35, 40, 45, 50, 55, 60, 65, 70). Fig (18A) Describes the relative numbers of floating pellets at the freshwater of seven sample. Fig (18B) illustrates the time required to sink pellets in fresh water of seven sample. Fig (18C) explain the relative numbers of floating pellets in seawater of seven sample. Fig (18D) specify the time required to sink pellets in seawater of seven sample. Results are presented as LSMEAN ± SE (n = 60). Treatments not sharing the same superscripts above the error bars are significantly different. The alpha level was set to 5% ($P \leq 0.05$ was considered as significant difference).

### 5.2.3. Dry matter Vs. Floating pellets

Equation $y = 0.17x^2 - 31.97x + 1515.8$ and $y = 0.14x^2 - 27.04x + 1284.1$ describes the polynomial trend line of floating pellets vs dry matter for fresh and seawater respectively. The equation of fresh water describes 76 % of results whereas equation of seawater describes 62% of our results ($R^2$). The increase in dry matter content of diet resulted in a decrease in a number of floating pellets for both fresh and seawater.

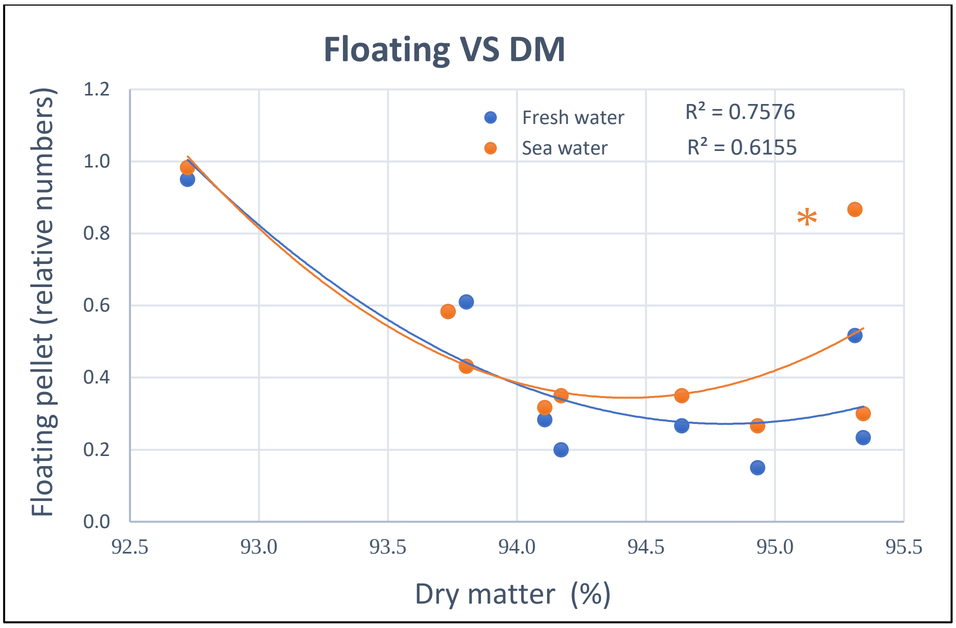



Figure 19 The dry matter content (%) vs. Relative number of floating pellets in fresh and seawater of one Raw and six Vacuum coated pellet batches. The dry matter content and floating results are presented as an average value. * represent value 0.87 of relative floating pellets of vacuum coated pellets at 70°C this was excluded from calculation due to high variation.

# 6. Discussion

The physical quality of feed pellets for Atlantic salmon was analysed using different techniques. The main challenges when studying the physical properties was from texture analyser because the particles got fragmented and moved out from the filter paper that was used for absorbing oil when force was applied by the probe. In future, this test could be optimized by using for example another probe type.

## 6.1. Part 1

### 6.1.1. Ligno test

The durability test is a useful indication of percentage fines produced during transportation, storage handling and through the pneumatic feeder. Increase in saturated fatty acids in the oil blend in feed pellets can decrease oil leakage during storage (Ng et al., 2007). High oil containing pellets showed improved elastic properties of pellets thus increase in durability of pellets (Borquez, 2007). In the present study, only vacuum coated pellets at 70°C and stored at 4°C showed equivalent results. Otherwise the results were contradictory. Similarly, Borquez’s also suggested that pellets coated at a lower temperature will have higher PDI value. Result in figure 10 also support the statement when pellets were stored at the same temperature.

### 6.1.2. Colorimetric analysis

From figure 11B and 11C, the color yellowness and redness of the pellets coated with oil blend A at 23°C and storage at 4°C showed a significant difference in respective color. The redness and yellowness increased when stored at a higher temperature. The unexpected increment in light color was absorbed in 4°C stored pellet when vacuum coated at 70°C with oil blend B. There is no research available for colorimetric analysis of feed pellets, but it is possible that rancidity of vacuum coated oil can affect the color. The salmon is good to identify colors, so this might be a useful topic for the further research of interest.

### 6.1.3. Texture analyzer

**Fat leakage**

Fats and oil are the primary sources of energy in salmon diets. High oil content in pellets might cause a problem due to leakage so proper oil blend and high oil holding capacity pellets might always be helpful to achieve the target oil inclusion in a feed.

High saturated fatty acid showed a decrease in fat leakage in extruded high-fat diet (Ng et al., 2007). High fat leakage was observed only for the pellet vacuum coated with oil blend A (0% CNO) and stored at 23°C, that might be due to high flowable nature of rapeseed oil. The high melting point of CNO shows semisolid nature at room temperature. This helps to prevent oil leakage and nutrient loss. Further, the oil leakage cause problem in feeding equipment and packaging materials. Thus the use of CNO might play significant roles in preventing oil leakage but should be included in higher concentration to get the effective results as the concentration of 5% in our analysis might not be enough to prevent fat leakage sufficiently.

**Maximum compression force (Force, N)**

Significant difference was not obsorbed between sample stored at 4°C and 23°C. This will signify that change in storage temperature did not affect the pellet hardness. Similarly, the shift in oil blend did not show any significant changes in compression force. The diet 35A23, 35C23 and 70A23 did not compress at 375 N. The maximum compressing force value was fixed to 375 N and the pellet that did not break at 375 N, were was all considered as 385 N during statistical analysis.

**Area (N*s)**

No significant difference was absorbed between work (Area, N*s) and other variables when stored at a different temperature. This confirms the findings of Yao's when examining the mechanical properties of the fish vertebra (Yao, 2017).

#### 6.1.4. Dry Matter (DM) content

The average dry matter content of feed pellets is assumed to be 94% (Ytrestøyl et al., 2015). The similar values were found in results above. The pellet stored at 23 °C showed increased in dry matter content with increase in coconut oil concentration. This might be possible due to flowable nature of rapeseed oil. The high content of rapeseed oil in oil blend A may leak out during storage in a blotting paper. On the other hand, the vacuum coated pellet with CNO containing oil blend might provide matrix (stable structure) in a pore which helps to block the rapeseed oil inside the poor, thus cause an increase in dry matter content at the increase in the concentration of coconut oil. No's describes that increase in pellet temperature will increase oil absorption in porous pellets (No, 2002). Results in figure 15 show that there was no significant increase in oil absorption when vacuum coated at a higher temperature as results did not show any significant increase in dry matter content when coated at lower and higher temperature.

#### 6.1.5. Floating pellet measurement of vacuum coated pellets.

High saturated fatty acid showed a decrease in fat leakage in the extruded high-fat diet (Ng et al., 2007). So increase in CNO should increase the dry matter content hence increase sinking pellets and decrease floating pellets. In the present study no such relations were observed. The floating pellets strongly depend on the interaction between macronutrient content in diet and water content and processing parameters of diet (Ayadi, Rosentrater, Muthukumarappan, & Brown, 2012). Due to some interaction between different vacuum coated pellets might also show variations in results. The salinity and water temperature might also cause fluctuations in results, but in the present study they were kept stable.

### 6.2. Part 2

During vacuum coating at 50°C the vacuum pressure was not built up sufficiently, and this might decrease or ununiform absorption of the oil, hence explain the unexpected results for this treatment.

### 6.2.1. Floating and Sinking pellets

- **Floating pellet fresh water**

In freshwater, significant differences were absorbed between raw pellets and vacuum coated pellets. The numbers of floating pellets decreased that might be due to increase in density of pellets during vacuum coating. The rise in coating temperature shows reduced in floating temperature, but the pattern was not uniform, so it's hard to say that the temperature of vacuum coating is the only parameters that affect the floating.

- **Sinking pellet fresh water**

The temperature of the coating does not show any significant correlation with sinking pellets in freshwater. No such research was found, so it's difficult to say if there is any relation of coating temperature with the sinking of pellets.

- **Floating seawater**

The unexpected results were absorbed with raw pellets, and vacuum coated pellets at 70°C. They are significantly different from other pellets. It's hard to say if the floating of pellets is related to the coating temperature.

- **Sinking seawater**

For sinking pellets at sea water, there was no significant difference for different coating temperature. The unexpected results might be possible due to improper or un homogenous vacuum coating or change in salinity and or temperature of the water during analysis.

### 6.2.2. Dry matter Vs. Floating pellets

The polynomial equation describes the floating pellets for both seawater and fresh water. This shows that both the sea and freshwater follow the same pattern. The sinking rate directly depends upon feed bulk densities (Adu, Cudjoe, & Vilhelm, 2015). The same results were observed; i.e. the floating pellets decreased with increase in dry matter content in diet.

# 7. Conclusion

The thesis was done with the aim of studying the potential of using Coconut oil to improve the physical quality of feed pellets for Atlantic salmon. Furthermore, to develop a new method for oil leakage analysis by using Texture Analyzer. The physical properties analyzed can be summarized in two Parts.

**Part 1** (Vacuum coating at 35°C and 70°C and storage at 4°C and 23°C)

- **Ligno test**

PDI value is higher for pellet stored at 23°C then pellet stored at 4°C.

The PDI value is higher in a vacuum coated pellet at 35°C then 70°C.

- **Colorimetric analysis**

Due to the low level of change in oil blend the color does not change in diet with some minor exception.

- **Texture analyzer**

The change in CNO from 0 to 5% in oil blend will not have significant change in pellet hardness but the oil leakage is very high when pellets are vacuum coated with oil blend A (0% CNO) and stored at 24°C.

- **Dry matter content**

For vacuum coated pellet with an increase in coconut oil content at oil blend during vacuum coating the dry matter content is higher for the pellet stored at 4°C then pellet stored at 23°C.

- **Floating pellets**

The increase in CNO from 0% to 5% in oil blend during coating will decreased in floating pellets. Comperatevely, the floating pellet will be higher when stored at 23°C.

**Part 2** (Vacuum coating at 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C)

With the increase in dry matter content in a vacuum coated pellet, the number of floating pellets will be decreased both in seawater and freshwater

# 8. Recommendation

- It will be better if the pellet is vacuum coated at automatic vacuum coater. For manual vacuum coater, the oil is not uniformly distributed or not homogeneously vacuum coated, so a lot of variation might occur between pellets for mostly high salmon diet.

- In this experiment, we just checked the response variable at two temperature of vacuum coating. Further research can be done by vacuum coating at 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 70°C to get the overall picture of change in pellet physical quality at different vacuum coating temperature.
- We assumed that the temperature of pellet and oil would remain at the same temperature during vacuum coating, but the vacuum coater was not insulated so the temperature may be decreased while coating the feed. Insulated vacuum coater should be used
- The use of alternative source of fish oil cause change in carcass composition and sensory characteristic of fish produced (Stead & Laird, 2002), so before adding "new oils" to salmon diets, effects on fillet quality should be analysed.

# 9. Implementation in commercial feed production

The oil leakage cause problem in feeding equipment and packaging materials. Thus the use of CNO might play significant roles in preventing oil leakage during storage, transportation and during pneumatic feeding. Similarly, floating pellets is one of the major problem in salmon farming so the proper use of coconut oil in diet may prevent floating pellets. The coconut oil contains more than 90% of saturated fatty acids and is more resistance to lipid peroxidation. On the other hand, it is believed that trans-fat and saturated fats increase the LDL blood cholesterol level and increase the risk of cardiovascular diseases. Saturated fat is considered as more harmful than trans-fat because it tends to decrease HDL cholesterol also. I have only done physical analysis in my thesis but would recommend to study adverse effect on palatability, growth performance, and effect of inclusion on human health before implementing CNO in feed formulation.

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