Acknowledgments

I am greatly thankful to my main professor Trond Storebakken for all his guidance, support and encouragement to me throughout the periods of this thesis and living in Norway. Without the hands from Trond, I could not have accomplished this task.

I send my appreciation to Ole Petter Humborstad, the CEO of Ecobait, for providing the great opportunity to me. Due to his support, I was able to complete my experiments when I stayed in Måløy.

I am thankful to Dejan Knezevic for all his help during the production and experiments. Because of him, I was able to research in this highly interesting topic.

I am grateful to Sandra Milosevic to help on the analysis of the videos of experiments. Also, the nice meals she cooked in the factory was warming up stomach and heart.

I am also happy to collaborate with Prajwal Pradhanang in the same project which focused on artificial bait based on rest raw materials and surplus fish. We also reviewed together in the literature search.

I am also thankful to my girlfriend, family, and friends, for everything.

Ås, August 13th, 2018

Lifeng Zhao

Abstract

Atlantic cod is one of the main fish specifies for commercial fishery in Norway which largely utilizes the longline technique for fishing. This method requires baits as an important element. Ideal longline baits should be strong, palatable and attractive to cod. This experiment aims to attain the ideal properties of bait by optimization of the extrusion and coating process under correct diet formulations. Five diets (diet - A, B, C, D, and E) were extruded, and each of them was coated with four variations of coating solutions (coating - A, B, C, D) to produce a 5x4 matrix of 20 different samples. Samples were either cooled (coated-cooled) or dried (coateddried) at 10°C for the strengthening of the baits. All samples were subjected to strength test which showed coated-dried baits stronger than coated-cooled baits. Thus, dried baits were further analyzed for moisture and water activity analysis along with their feeding trials to cod in tanks. The strength-test results among the coating-solutions within diets showed higher strength for coating B and D (9% gelatin) than A and C (5% gelatin) regardless of the difference in water types used (i.e., fresh or seawater). Among the diets, diet B and C showed the highest strength than diet A, D and E. Moisture analysis among coating-solutions showed freshwater samples (coating A and B) with significantly higher moisture content than seawater samples (coating C and D) and 5% gelatin samples (coating A and C) with higher moisture than 9% gelatin (coating B and D). This represents coating A (5% gelatin + freshwater) having the highest water content in all diets. Among diets, diet D and E contain lower moisture than rest diets. Water activity analysis showed a small difference among all samples ranging from 0.789 to 0.913. Even though statistical analysis showed a significant difference between the samples; they did not follow the explainable trend. Feeding trials with the coating-solutions within the diets showed the highest preference for coating D (9% gelatin + seawater). Among five diets, diet D showed the highest preference for cod than the rest of the diets. The ideal baits properties of having high strength for longline hooks and attractive to cod did not find in the same diet which calls for further studies in this field.

Keywords: Atlantic cod, baits, longline, extrusion, coating, gelatin, strength

Abbreviations

NOK	Norwegian kroner					
kg	Kilogram					
g	Gram					
min	Minutes					
mm	Millimeter					
m	Meter					
R20	20 mm in length with the turning direction of right					
R40	40 mm in length with the turning direction of right					
R60	60 mm in length with the turning direction of right					
R80	80 mm in length with the turning direction of right					
R100	100 mm in length with the turning direction of right					
P45-4/R120	120 mm in length with the turning direction of right 4 comportments					
had a 45° between each o	ther					
P45-4/R60	60 mm in length with the turning direction of right 4 comportments					
had a 45° between each o	ther					
60/ L20, 90°	20 mm in length with the turning direction of left, the diameter is 60					
mm, with a 90° with the between to Archimedean Spiral (screw alike)						
Ν	Newton					
SD	Standard Deviation					
ANOVA	Analysis of variance					

Table of Contents

1.	In	ntroduction	1
1.	1 Air	m of the study	2
	1.2 I	Hypothesis	2
	1.	2.1 Physical tensile strength	2
	1.	2.2 Taste	2
2.	Lite	erature review	3
	2.1	Cod feeding mechanism	3
	2.2	Application of natural baits in longline cod fishing	4
	2.3 I	Efforts to develop alternative longline fish baits for cod	5
	2.	3.1 Norbait	5
	2.	3.2 Artificial bait alternatives mainly based on fish waste	6
	2.4	Development of baits based on extrusion technology	7
	2.	4.1 Extrusion cooking	8
	2.	4.2 Vacuum coating	9
	2.5	Gelatin as a binder added on the coatings	9
3.	Met	hod and Materials	11
	3.1	Preparation for extrusion	11
	3.2	Mixing	12
	3.3 I	Extrusion	12
	3.4	Coating	16
	3.5	Formulas for coatings	18
	3.6	Drying	19
	3.7	Strength test of baits	19
	3.8 I	Filming of the cod feeding trails	19
	3.9 V	Water activity analysis	21
	3.10	Moisture content	21
	3.11	Statistical Analysis	
4.	R	esults	
	4.1 I	List of the extruded baits	23
	4.2 I	List of the extruded diets with four different coatings	24
	4.3 I	Results of the tensile strength of baits after one-night on cooling	
	4.4 I	Results of the tensile strength of baits after soaked in seawater for 2 hours	27
	4.5 N	Moisture content	29

4.6 Water activity	
4.7 Results of feeding trail observation for cod	
5. Discussion	35
5.1 Importance of the tensile strength of baits for longline cod fishing	
5.2 Water activity and moisture content	
5.3 Feedback of cod feeding behaviors on the experimental samples	
6. Conclusion	
References	

List of Figures:

Figure 1. Fish behaviors on baited hooks	4
Figure 2. Norbait fish bait 600 E	6
Figure 3. Different bait preparation for the same material	7
Figure 4. Schematic representation of an extruder	8
Figure 5. Gel strength as a function of concentration, at 10 °C	10
Figure 6. Twin-Screw Bühler BCTG 62 extruder	12
Figure 7. Screw configuration A for the production of diet A, B, C from the inlet (botto outlet (top).	m) to 14
Figure 8. Screw configuration B for diet D, E, from the inlet (bottom) to the outlet (top)	15
Figure 9. The die applied in extrusion	16
Figure 10. Experimental vacuum coating system.	17
Figure 11. Experimental Vacuum Coating System.	18
Figure 12. Measuring the tensile strength of the sample	19
Figure 13. Large fiberglass fish tank	20
Figure 14. Water activity is a measuring instrument (Rotronic Hydro C1)	21
Figure 15. Sartorius Moisture Analyser MA 50	22
Figure 16. Extruded diet A, B, C, D, and E.	23
Figure 17. Vacuum coated samples of diet A.	24
Figure 18. Vacuum coated samples of diet B	24
Figure 19. Vacuum coated samples of diet C	25
Figure 20. Vacuum coated samples of diet D.	25
Figure 21. Vacuum coated samples of diet E	25
Figure 22. The tensile strength of baits after one-night cooling	26
Figure 23. Results of all samples after one-night drying.	
Figure 24. Results of moisture content for diet A.	30
Figure 25. Results of moisture content for diet B.	30

Figure 26. Results of moisture content for diet C.	31
Figure 27. Results of moisture content for diet D.	31
Figure 28. Results of moisture content for diet E.	31
Figure 29. Results of water activity of diet A.	33
Figure 30. Results of water activity of diet B.	33
Figure 31. Results of water activity for diet C.	33
Figure 32. Results of water activity for diet D.	33
Figure 33. Results of water activity for diet E.	33
Figure 34. Results of feeding trail observations for cod.	35

List of Tables:

Table 1. Ingredient composition (%) of formulated cod baits for extrusion.	. 11
Table 2. Extrusion processing parameters	. 13
Table 3. Parameters of vacuum coating.	. 17
Table 4. Results of tensile strength (kg) of baits after one-night cooling	.26
Table 5. Results of tension strength (kg) for baits after soaked in seawater 2 hours	. 28
Table 6. Results of moisture content for all samples.	. 30
Table 7. Results of water activity (aw).	. 32

1. Introduction

Atlantic cod (*Gadus morhua*) is one of the important fish species for commercial fisheries in Norway. The catching volume of cod in Norway was 412,567 tonnes in 2016, with the value of NOK 18,7 billion in 2016 (StatisticsNorway, 2018). Currently, around 25% of the cod quotas are contributed on longline ("hooks & line")(ICES, 2017). This system work by luring the target fish close to baited hooks which motivates the fish to bite and get hooked(Kumar et al., 2016). Longline gears consist of the main line, branch line, hooks and baits. The materials and lengths of longline gear elements and their deployment strategies could be changed to manipulate the target fish catch (Watson & Kerstetter, 2006).

The bait plays an important role during the longline fishing. The cod is widely fished in longline using natural baits that are preferred in their wild habitats such as squid, mackerel, and herring. (He, 1996; Løkkeborg & Bjordal, 1992). As these baits are also used for human foods, the price of baits is increased and fluctuated annually. (Løkkeborg et al., 2014). For instance, the price of the baits has been reported to increase by 6 NOK per kg in recent year to more than 15 NOK per kg. Also, fishermen are also not satisfied with the high loss of natural bait in longlines. As in a few cases, the new baits were easily dislodged from the hooks being not able to withstand the repeated 'attack' or 'bite' (Johnstone & Hawkins, 1981).

The common demand of longline fishermen lies in having efficient alternative baits that contains the good holding ability on the hooks. It should also be available in large quantities, low price and sufficiently resilient to storage (Kumar et al., 2016; Løkkeborg et al., 2014). Atlantic cod is a very picky fish on their diet selections. The common way to produce the high-quality alternative baits for Atlantic cod is to minced fish species preferred by cod and held in some support structure that can be hooked in lines (Løkkeborg, 1991). Previous efforts to produce good longline fishing baits have not shown consistently satisfactory results.

This paper aims to overcome the existing problem in longline fishing of cod by producing strong, cheap and attractive bait by exploiting the widely adapted extrusion and coating technology prevailing in fish feed industries.

1.1 Aim of the study

It is important to develop good alternative baits for longline fishing of Atlantic cod, which had good physical qualities as strength, available for large quantities and cheap in prices. The extrusion and coating technology could be used to obtain this.

The objective of this thesis was to make a semi-dried alternative fish bait through the coating technology on the extruded baits for longline cod fishing. Through food and feed, manufacturing technic to test gelatin solutions with attractants coated on extruded fish baits provide physical strength and highly palatable taste to cod.

Objective 1:

To find the effects of increasing gelatin concentration on the tensile strength of extruded baits.

Objective 2:

To define fish bite preferences on extruded bait after coating with attractant and gelatin solution at similar inclusion levels as those of natural baits namely squid and herring.

1.2 Hypothesis

The general hypothesis of the thesis is the coating of the attractant-gelatin solution on the extruded fish baits strengthen and improve the taste of baits.

1.2.1 Physical tensile strength

H_{1-sub}: Increasing gelatin concentration strengthen the tensile strength of the baits.

 H_{1-sub} : Seawater will enhance the tensile strength of the baits with unchanged attractant characteristic as when fresh water is used to solubilize the gelatin.

1.2.2 Taste

 H_{1-sub} : fish bite preference on the bait with attractant and gelatin solution reaches the same levels as that of natural or traditional baits namely squid and herring.

2. Literature review

This project was based on the production of alternative baits for cod using extrusion-coating technology as a part of the project performed in this thesis and stuffing technique as the other part performed by Prajwal Pradhanang. Thus, it forms the shared review of the literature, focusing mainly on extrusion-based technology in this part of the thesis as discussed below.

2.1 Cod feeding mechanism

Cod is omnivorous fish in their diets. The cod is adapted to feed on the bottom of the sea. Cod captures mainly by suction, not in intense suction, but it also performs well for sizing and biting. As the cod had large esophagus and stomach, it can swallow large and heavily spine prey upon in a vigorous way.

Cod prefer to live in an environment of devoid of light but rich in dissolved compounds since that cod highly developed the chemosensory or chemical signaling systems rather than the visual food search system. The chemoreception is important for the detection and location to search for food for cod (Løkkeborg et al., 2014). The cod chemoreception consists of the olfactory and gustatory system (Hara, 1994). The olfactory system enables the cod to locate or narrow the distance to an odor source from a long distance (Løkkeborg, 1998). The cod normally encountered downstream twice than upstream to water currents known as rheotaxis (Carton & Montgomery, 2003; Løkkeborg, 1998). However, the olfactory system does not provide directional vector or signal where sound play the main role (Hawkins, 1986).

After cod get close to a food source, their eating motivation is directed by gustation system. This system identifies the taste of the food or prey of cod for whether to reject or swallow (Caprio et al., 1993; Kasumyan & DÖving, 2003). In the longline fishing system, if they are stimulated to swallow the food items, then they get easily hooked. Therefore, in longline fishing, baits play the important role in stimulating olfactory and gustation system for cod to approach and getting hooked.

As shown in Figure 1, Cod feeding behaviors also influence by the internal factors and external factors. The internal factors are the hunger state, reproductive status, daily rhythms and previous experiences. The external factors as light, temperature, current and prey density mainly had affected cod feeding behaviors (Gerstner, 1998; Løkkeborg et al., 1989; Løkkeborg et al., 2014; Stoner, 2004).

On the longline fishing, the cod olfactory searches the bait which highly attracts the cod, where the attractant type and concentration also play important roles. When cod visually observed fish bait, the bait size and shape are mainly responsible factors for cod to attack the baits. Under the gustation system motivation, the cod decide to swallow or swim away from the bait. However, the cod escape from the hooked baits also happens even though they have swallowed the baits.



Figure 1. Fish behaviors on baited hooks.

2.2 Application of natural baits in longline cod fishing

The squid, saury, mackerel, and herrings are well-known natural or traditional fish baits for the longline cod catch (Bjordal, 1981). The traditional baits usually come from the cod preferred to prey fish species in the wild.

Several studies on different types of natural baits are done for longline fishing of Atlantic cod. Mince raw materials of different fish are tested for cod using nylon bags as supporting structure for hooking in longlines. However, these bags act as a de-attractant therefore, naturally cut bait are generally preferred Løkkeborg (1991). Among the natural baits, the bait comparisons showed that cod preferred squid better than mackerel and herring (Løkkeborg, 1991). Squid is the ideal bait for cod that is preferred because the squid not only provide high strength in baiting and hooking machine but also highly stimulate the cod to prey and swallow ((Furevik & Løkkeborg, 1994; Johnstone & Mackie, 1990)). Lie et al. (1989) concluded that the feed

attraction properties of squid and prawn for cod were higher than the feed based on saithe fillet. However, the utilization of mackerel and herring are more widespread than the squid for the longline fishing (Bjordal, 1983) since the mackerel and herring are both abundance and easy to catch than the squid. In the 19th century, mackerel was dominating the longline cod fishing baits. As there is no waste for mackerel in making baits for cod, the head, gut, and tails are all valuable parts for cod baits (WALKER, 2009). Disadvantages of herring bait in longline not only the taste preference lower than the squid but also the physical strength lower than that of squid on the hooks (He, 1996). Since the squids are not abundance as mackerel and herring but also the price of those natural baits increased because the most natural baits using in longline cod fishing are valuable nutrient food for human consumption.

2.3 Efforts to develop alternative longline fish baits for cod

Several developments try to replace traditional baits for cod. Mainly, the two methods applied to use. One based on natural resource (e.g., surplus products from fish industries) and the other was using synthetic ingredients as attractants (Løkkeborg et al., 2014). In both methods, the baits consist of three main components: the attractants, reinforcement, and binders (gelling agent). The attractants stimulated the targeted fish species to express food searching behaviors. The fish and crustaceans had feeding stimuli on the low-molecular nitrogenous substances like amino acids, peptides, bile acid (Carr & Derby, 1986; Kasumyan & DÖving, 2003). On the research of the bottom food search behaviors in cod, the amino acids from shrimp extract presented high attractant for cod. Among them, glycine is a potent component in shrimp, followed by alanine (Ellingsen & Døving, 1986). The reinforcement mainly gives a physical structure and strength to the bait which cannot lose during the periods of fishing. The purpose of binders is not only forming a gel but also ensuring the fair release of attractants during the period of longline fishing.

2.3.1 Norbait

Norbait DA Company, in Norway, developed fish bait based on the fish waste and surplus product from fish industries. The alginate applied as a binding agent for Norbait (Figure 2). The cotton stocking is reinforcement. The production of Norbait more or less the same as the production of sausages (Norbait, 2018). For the raw materials, the most come from the fish processing industries. However, the Norbait also used the other raw materials that well-known for making a good fish bait for particular fish species. Norbait gets the success of catching haddock, but for cod, the catchability is low.



Figure 2. Norbait fish bait 600 E (Norbait, 2018).

2.3.2 Artificial bait alternatives mainly based on fish waste

The EU project "Artificial bait alternatives, mainly based on fish waste (Q5CR-2000-70427) " planned to develop an alternative fish bait mainly for cod, haddock, and hake, for longline deep-sea fishing fleet (Tryggvadóttir et al., 2002). The raw materials for making this kind of bait were from the fish and shellfish waste from the fish meal production. To successfully produce longline fishing baits, the fish freshness is superior to determine the quality of alternative baits. The freshness can have influenced by the process. As they found out, the thawing, mincing, mixing, stirring and re-freezing the bait materials reduced the attractant effectiveness. The handling of raw materials influences the bait also demonstrate by the other experiment simply shown in Figure 3. The bait material in the left looks quite fresh, and the catch was 27-80% compared to the traditional bait. The bait material in the middle through thawed then minced and packed in the packing machine then frozen already get grey meat color. The catch rate was 16-40% of traditional bait. However, the bait material at right is grated frozen and then packed still frozen. The material color is similar to the original meat color and the catch rate also from 66% to 112%.



Figure 3. Different bait preparation for the same material (Tryggvadóttir et al., 2002).

2.4 Development of baits based on extrusion technology

There are only a few studies been done in making baits based on thermal-treatment technology like the well-known processing as pelleting and extrusion processing which is usually confidential with very less information. The extrusion technology widely been studied in making fish feed in an aquaculture system. The knowledge can be foraged from these studies to develop cod baits based on extrusion technology. In most fish feed, extruded products are coated with nutrient liquids as attractants and fats to meet their palatability and nutritional requirements. The coating is improvised further by using a vacuum technique. Binders are also seldom used to hold the coated materials in the pellets.

2.4.1 Extrusion cooking

Extrusion processing in food and feed production had become very popular. Extrusion had opted in so many diverse operations; they are regarded as a versatile process (Riaz, 2000). The extrusion system consists of a barrel housing with one or two rotating screws (single or twin screw extruder) (Sørensen, 2012). For the extruding processes, the mixture of ingredients is steam conditioned, compressed and through the die of the extruder (Rokey & Plattner, 1995). Extrusion technology had potential advantages as known as low price, the variability of products shape, higher productivity, inactivation of anti-nutrient factors. Twin-screw extruders are composed of two axes that rotate inside a single barrel. For the extruder, the variations of screw speed, barrel temperature, screw, and barrel configuration, die type and opening and feeding rate are some important parameters that affect the extrusion (Navale et al., 2015). The feed composition, moisture content and particle size of raw materials had determined the performance of extrusion cooking. The starch, lipids/ fats, and protein content contributed to product qualities. Feed moisture is the critical variable factor in extrusion processing because it contributed to thermos-mechanical liquefaction and gelatinization of starch (Gomez & Aguilera, 1984). "Increase in particle size for a given biochemical composition" gave extruded products that were harder, with an unexpected expansion and low bulk density (Desrumaux et al., 1998). The schematic representation of an extruder (Figure 4) illustrates the main drive motor given force to screw in the extruder barrel to drive. The extruder cooking zones mainly consist of the feeding zone, the kneading zone, and high-pressure zone. At the outlet of the extruder, the die meets high pressure and forms the product shapes. The barrel jacket limited the high temperature and pressure escape and isolated heat transparent into the air from the extruder.



Figure 4. Schematic representation of an extruder(Navale et al., 2015).

2.4.2 Vacuum coating

Extrusion and steam pelleting are widely applied heat treatment processes by the feed suppliers. However, it comes with the demerits of the destruction of heat-sensitive nutrients such as vitamins and enzymes(Lucas & Southgate, 2012). This brings an additional step to be included during feed manufacturing. Among post pelleting treatment, the vacuum coating exhibits positive results for extruded products, especially.

Vacuum coating system invented in Norway started in feed industries since late of the 1980s. Originally, Dinnissen vacuum core coating line applied by Dutch feed manufactures for pelleted broiler chicken feed. The aim of vacuum coating mainly designs to reinforce better pellet quality, higher liquid content in the pellets and better protection of heat sensitive micro-ingredients. The vacuum coating cannot be applied in the pelleted feed as same for extruded feed. The high density of pelleted press feed removed much interior air but for extruded pores feed, the pellets density is lower than the pelleted press and containing high porosity(GILL, 2000). Extruded feed absorbs liquids easier than the pressed pellets. As an example, the vacuum infusion is widely applied in fish feed. The coating procedures seem to begin by withdrawing air from the pellets, and then releasing the liquid or fats reach to pellets when the desired vacuum pressure achieved. The capillary forces promote the penetration of oils or liquids into the feed pellets. The oils or liquids content of pellets are up to the maximum with 40% of whole pellets weight while ensuring a dry surface (A.Brisset, 2006).

2.5 Gelatin as a binder added on the coatings

Gelatin, one of the well-known biopolymers, provide well physical properties since that the applications of gelatin is widely separated in food, drugs, photographic films and other products including paints, matches, and fertilizers as gelling agent, foam stabilizer, and structure enhancers (Gudmundsson, 2002; Hou & Regenstein, 2004; Yang et al., 2007). Gelatin comes from the collagen of animal connective tissues, skin and bones, mainly from pigs, fish, and cattle (Boran & Regenstein, 2010). Gelatin form a gel with high physical strength. Gelatin gel had thermo-reversible property. Gelatin gel well forms from a gelatin solution with 5% or higher than 5% concentration under the temperature approximate 35-40 °C on cooling (GMIA, 2012). To perform a good gelatin gel, the partial random return of gelatin converts to collagen-like helices to form locally ordered regions. Besides, a three-dimensional fibrillar network of micelles forms the non-specific bonds throughout the segment of the chains. The most time-consuming part is forming of crossbonds so that when increasing the crossbands forming, the

strength of gelatin gel increases with time. However, also, A strength of gelatin gel decided by the gelatin concentration, intrinsic strength of gelatin, PH, temperature and the presences of any additive.

The standard method to measure the strength of gelatin gel is developed by the Gelatin Manufacturers Institute of America (GMIA, 2012). The gel forms on the concentration of 6.67% of gelatin content and then the gel strength must be measured by 10°C (Boran & Regenstein, 2010). The strength of gelatin gel also had the increasing trend when the increase in the gelatin concentration in the aqueous samples (Figure 5).



Figure 5. Gel strength as a function of concentration, at 10 °C(GMIA, 2012)

"Bloom" strength for gels, named by the Oscar T.Bloom in 1925. The definition for "bloom" strength was first, the force required for a 12.7mm diameter flat probe to penetrate 4 mm into the gel with the speed of 1mm/s. This force expresses by the gram reported as the gel strength.

3. Method and Materials

3.1 Preparation for extrusion

Five different cod attractants-based diets were extruded at Centre for Feed Technology (Fôrtek) at Norwegian University of Life Science (NMBU), Ås, Norway. Formulations and composition of diets presented in Table 1. Since the product and recipes are confidential, there are no any possible names of attractant used in this thesis. Briefly, the diets mainly distinguished in attractant content and attractant items shown in Table 1. Four different attractants formulated in five diets. Namely, these were Diet A, Diet B, Diet C and Diet D which were added to 25% of attractants, respectively. Moreover, the Diet E had lower attractant content diets, which was 19%. To achieve good expansion and high porosity, the wheat flour was utilized for binding and expanding during extrusion. Chitosan was used as binder helper to strengthen the physical properties of bait. Monosodium glutamate was included as a flavor enhancer.

Table 1. Ingredient composition (%) of formulated cod baits for extrusion.

Diet ADiet BDiet CDiet DDiet E(%)(%)(%)(%)(%)(%)Attractant A25Attractant B-25Attractant C25Attractant D2519Wheat Flour64.264.264.264.270.2Chitosan8.88.88.88.88.8Monosodium22222glutamate						
Attractant A(%)(%)(%)(%)(%)25Attractant B-25Attractant C25-Attractant D2519Wheat Flour64.264.264.264.2Chitosan8.88.88.88.8Monosodium2222glutamate		Diet A	Diet B	Diet C	Diet D	Diet E
Attractant A 25 - - - - Attractant B - 25 - - - Attractant C - - 25 - - Attractant D - - 25 - - Attractant D - - 25 19 Wheat Flour 64.2 64.2 64.2 70.2 Chitosan 8.8 8.8 8.8 8.8 Monosodium 2 2 2 2 2 glutamate - - - - -		(%)	(%)	(%)	(%)	(%)
Attractant B - 25 - - Attractant C - - 25 - - Attractant D - - 25 19 Wheat Flour 64.2 64.2 64.2 70.2 Chitosan 8.8 8.8 8.8 8.8 Monosodium 2 2 2 2 2 glutamate - - - - -	Attractant A	25	-	-	-	-
Attractant C25Attractant D2519Wheat Flour64.264.264.264.270.2Chitosan8.88.88.88.88.8Monosodium22222glutamate2519	Attractant B	-	25	-	-	-
Attractant D - - 25 19 Wheat Flour 64.2 64.2 64.2 70.2 Chitosan 8.8 8.8 8.8 8.8 Monosodium 2 2 2 2 2 glutamate - - - 25 19	Attractant C	-	-	25	-	-
Wheat Flour64.264.264.264.270.2Chitosan8.88.88.88.88.8Monosodium22222glutamate	Attractant D	-	-	-	25	19
Chitosan 8.8 8.8 8.8 8.8 8.8 Monosodium 2 2 2 2 2 glutamate - - - - -	Wheat Flour	64.2	64.2	64.2	64.2	70.2
Monosodium 2 2 2 2 2 2 2 glutamate	Chitosan	8.8	8.8	8.8	8.8	8.8
	Monosodium glutamate	2	2	2	2	2

Ingredient

3.2 Mixing

The ingredients mixed in the twin screw shafts with 30 kg capacity batch mixer with manual steering with steering panel. The batch mixer is a horizontal mixer, which provides complete mixing of the ingredients. Mixing the ingredients of various densities was followed by adding the liquid in the mixer. The mixer for each batch runs around 2 min with full power. After mixing, the feed mash depends on formulation put into different buckets and marked with tips prepared for extrusion processing.

3.3 Extrusion

The mixed ingredients were added to a small size feeder (Katron feeder), and the ingredients were sent from the feeder to the extruder barrel. The meal mixtures processed by a fivehead Twin-Screw Bühler BCTG 62 extruder from Switzerland. Production capacity is 800 kg per hour for fish feed production. Figure 6 is showing the representation of the main parts and zones of an extruder. A die plates consisting of one nozzle that shape the product appearances for diet A, B, C, with 20.0 mm diameter. The other nozzle for diet D and E, with oval shape 20.0 mm in length. The expectations of extruded products are the diameter or width $\pm 30 - 40$ mm. The extruder parameters used in the present experiment is presented in Table 2.



Figure 6. Twin-Screw Bühler BCTG 62 extruder(Miladinovic, 2014)

Table 2. Extrusion processing parameters

	Diet A	Diet B	Diet C	Diet D	Diet E
Adjustable extruder parameters					
Screw speed(Rpm)	575	625	623	700	699
Dependent extruder parameters					
Die temperature (°C)	116	112	106	106	114
Motor load (torque N m)	355	191	153	329	319
Die pressure (bar)	16	4,3	3	8,7	10,3
SME (kWht ^{-1b})	790	486	335	960	1032

^a Rotation per minute.

^b Specific mechanical energy(SME) was calculated using the expression (Hu et al., 1993)

 $SME(kW h kg^{-1}) = \frac{Screw speed \times Power (kW) \times Torque(\%)}{Max. screw speed \times Throughput (kg h^{-1}) \times 100}$

To obtain fine expansion and physical appearances of extrusion products, two different screw configurations and extrusion dies were utilized for extrusion. The Diet A, B and C product on screw configuration A (Figure 7) and extrusion die A (Figure 9) and the diet C and D run on screw configuration B (Figure 8), and extrusion die B (Figure 9). In the figure of screw configuration, R means right turn of the screw element and L mean left (backward) turn of the screw element. Numbers are representing the length of the Archimedean Spiral (screw alike) for each screw element. The unit of length is millimeter (mm). When running on the extruder, the motor force the feed mash by the screw of turning the direction of right. For screw configuration A, ten of R 80 screw elements from the inlets transport the feed mash to the polygon elements (P 45-4/R120) mainly for kneading, and then the feed mash met the backword element (60/ L20, 90°) offset the force from the polygon. After a short offset, the flow of feed mash was transport by right direction elements of R100, R80 and three R 60. Before the outlet, the feed mash decreased the flow speed by the screw elements of R 20 and R 40.

For screw configuration B, three R80 and a R60 screw elements from the inlets transport the feed mash to the polygon elements (P 45-4/R60) mainly for kneading, and then the feed mash met the backword element (60/L20, 90°) offset the force from the polygon. After a short offset, the flow of feed mash was transport by screw elements of two R80 and two R 60. The feed mash after those screw elements transport to the polygon elements (P 45-4/R120) mainly for kneading also, and then the feed mash met the backward element (60/L20, 90°) offset the force

from the polygon. After the backward elements of offsetting, the flow of feed mash was transport by right direction elements. Before the outlet, the feed mash decreased the flow speed by the screw elements of R 60 and R 40.



Figure 7. Screw configuration A for the production of diet A, B, C from the inlet (bottom) to outlet (top).



Figure 8. Screw configuration B for diet D, E, from the inlet (bottom) to the outlet (top).



The extrusion die A and Extrusion die B Figure 9. The die applied in extrusion.

3.4 Coating

All products after extrusion stored at freezer under -20° C in Ås then freezing transfer to Måløy, Norway. The vacuum coating was conducted at the artificial bait products company, Ecobait, in Måløy, Norway. All diets were coated with four gelatin coating solutions which were prepared at dissolving the gelatin either sea-water or fresh water at 45°C and then mixed with Ecobait attractant either sea or freshwater at10°C. The gelatin solutions cooked in a water bath with 50 °C till to obtain pure liquid gelatin solutions. A lab-scale vacuum coater was applied during the experiments shown in Figure 10.

The procedures of vacuum coating experiments were finished experimental vacuum coating system as shown in Figure 11. The extruded samples of five diets de-freeze at room temperature (20°C). Therefore, the sample ready to vacuum coat. First, extruded baits cut into small pieces with 10 cm in length with the weight of 40 g for diet A, B, and C but for that of diet D and E was 20 g. Second, the prepared coating solutions pour into the coater filling up to half of it. Third, the pellets were out in the vacuum coating, and the coating solutions have soaked the samples. There is a weight of approximately 3 kg weight to push the samples into coating solutions. Each coating operation put ten samples inside the coater and the coating pressure to -0.8 bar. Fourth, the pressure was slowly released under the manual control until the atmospheric pressure. The pressure is releasing time controlled around 2-3 min for each batch of coating. The coated samples were semi-dried at *ten* °C on cooling or drying. The process parameters are shown in Table 3.

Diets Parameters	Diet A	Diet B	Diet C	Diet D	Diet E
Total vacuum	10	10	10	10	10
time (min)					
Vacuum	-0.8	-0.8	-0.8	-0.8	-0.8
pressure (bar)					
Coating	45	45	45	45	45
temperature					
(°C)					

Table 3. Parameters of vacuum coating.



Figure 10. Experimental vacuum coating system.



1. Vacuum pump. 2. Pressure gauge. 3. Vacuum pot. 4. Sieve. 5, 6. Valve. 7. Weight.

Figure 11. Experimental Vacuum Coating System.

3.5 Formulas for coatings

For the coating, four different mixed coating solutions based on different attractants coated on five different diets (diet- A, B, C, D, and E). The four coating solution formulas (formula 1-4) are shown in below:

Formula 1:

```
Coating A(100\%) = ecobait attractant(47.5\%) + fresh water(47.5\%) + gelatine powder(5\%)
```

Formula 2:

```
Coating B(100\%) = ecobait attractant(42.5\%) + fresh water(42.5\%) + gelatine powder(9\%)
```

Formula 3:

Coating C(100%) = ecobait attractant(47.5%) + sea water(47.5%) + gelatine powder(5%)

Formula 4:

 $Coating \ D(100\%) = ecobait \ attractant(42.5\%) + sea \ water(42.5\%) + gelatine \ powder(9\%)$

3.6 Drying

The drying process was manipulated at one night after coating. During the experiment, pellets were placed into a dryer which consists of a fan and a small room framed by wood and covered with foil. The dryer had an inlet and outlet. The fan blows the cool air from the inlet into the room and air escape by the outlet.

3.7 Strength test of baits

The sample was fixed on board, hooked on the probe at strength test scaler. By pulling on the scaler gave force load to the sample, the bait deformation is given strength to probe then the strength recorded on the scaler screen. The measurements were three times repeated for each bait, and the results were written down the figure for analysis. The procedure is shown in Figure 12.



Figure 12. Measuring the tensile strength of the sample.

3.8 Filming of the cod feeding trails

Juvenile cod were caught by trawl and transported to the aquarium at Ecobait in Måløy, Norway.18 Atlantic cod with the body weight of 4 kg were used by the experiments to test. The cod were randomly divided into six groups and kept in large fiberglass tanks (2.5m Ø, 0.6m water depth) in a recirculated seawater system shown in Figure 13. All tanks were supplied with running seawater pumped from a depth of 30 m with an average water temperature of 4 ± 0.5 °C. The fish were kept under the Ultraviolet light regime on a 17: 7 light-dark cycle. All of them starved two weeks before the experiments. During the periods of experiments, the cod fed by frozen chopped mackerel pieces, in 3 cm in length and around 30 g in weight. The vision is the primary sense for cod in midwater feeding to attack and prey (Brawn, 1969). Each feeding trails had a 24 h of the interval. The experiments commenced from March to June.

For the feeding trails, five different extruded diets coated with four attractant coatings, so that, in total, 20 independents artificial baits fed for cod. Each bait had three times repetition in different fish tanks. The three cod in each tank were fed three pieces of bait with 3 cm in length and around 30 g in weight for one tank of cod.

A camera acquisition system with two orthogonal cameras and a digital image analysis program was used to observe patterns of foraging. The waterproof cameras fixed before the test under careful movement to avoid stress for cod in the tank. The sample was dropped carefully in the middle of the fish tank just over the surface of water follow the water current to avoid the splash. After the test, the releases of cameras also follow the role 'no stress' for cod.

After filming the cod feeding, video analysis will give the results of how cod perform on the experimental baits. The results are the main data from a large number of smell and taste preferences, grading different diets according to their ability to elicit a bite response of the cod.



Figure 13. Large fiberglass fish tank

3.9 Water activity analysis

The water activity analysis was carried out in the experimental lab at the Animal Science Department, NMBU. Five different diets (diet- A, B, C, D, E) coated with four different solutions for every single one was analyzed by Rotronic Hygrolab C1 (Figure 14) from Switzerland. The samples were filled up to the level of the ring of the cut but not to the brim. The sensor of probes was placed in the right position to record the amount of moisture content in the sample at a given temperature. The probes were reset and run for 10 minutes. The values of each probe were recorded after the estimated time (10 Mins).



Figure 14. Water activity is a measuring instrument (Rotronic Hydro C1).

3.10 Moisture content

The Sartorius Moisture Analyser MA 50 used for all moisture content of samples shown in Figure 15. Preparation of measurements was firstly turning on the machine that needs 30 minutes to be ready for the test. At the same time, the samples need to crush by a pestle or a shredder. After preparation, there is an aluminum pan (Sartorius sample pans) to put measuring mash. There is only one chamber in the machine, so each time can measure one pan of the sample. The weight of the sample is approximately 2g. The results will be displayed on the screen. Each sample measured three repeats and wrote down the average results as the moisture content of that sample.



Figure 15. Sartorius Moisture Analyser MA 50.

3.11 Statistical Analysis

The data collected during the production and the physical analysis as moisture content and water activity were treated by R studio (ANOVA, p<0.05) for statistical significance. Then, in the case of significant differences, the bar plot method has been realized at a 5% level to identify the significant difference of each treatment. The results of tensile strength and feeding behaviors analyzed by Excel 2016, presented in the tables and bar charts, which provide the averages and standard divisions.

4. Results

4.1 List of the extruded baits

There are five diets (diet- A, B, C, D, and E) from the extrusion shown in Figure 16. The surfaces of the pellets do not unveil many variations in the surface structure of different diets. The cross-section profiles unveil differences in the internal microstructure of pellets. The pore sizes on the extruded baits are not homogeneously distributed. The diet A, B and C produced from same screw configuration A and extrusion die A. The diet D and E produced for same screw configuration B and the extrusion die B.



Figure 16. Extruded diet A, B, C, D, and E.

4.2 List of the extruded diets with four different coatings

Each diet had coated with four different liquid solutions namely coating A, B, C and D. In total, five different extruded diets (diet- A, B, C, D, and E) coated on four different liquid coatings (solution- A, B, C, and D). The results were 20 samples as the final baits shown in below (Figure 17-21). For diet A, B and C from screw configuration A and Extrusion die A, the sample weights were 40 g before coating, but after coating, the sample weights were already approximately 120 g for per weight of the sample. For the diet D and E from screw configuration B and Extrusion die B, the sample weights were 20 before coating, but after coating, the sample weight of the sample weight.



Figure 17. Vacuum coated samples of diet A.



Figure 18. Vacuum coated samples of diet B.



Figure 19. Vacuum coated samples of diet C.



Figure 20. Vacuum coated samples of diet D.



Figure 21. Vacuum coated samples of diet E.

4.3 Results of the tensile strength of baits after one-night on cooling

The results of tensile strength for 20 samples from five different diets (diet- A, B, C, D and E) coated with four different solutions (coating- A, B, C and D), after one night on cooling at room temperature (10°C), shown in Table 4 and Figure 22. All samples had tensile strengths below 1 kg (9.8 N). The difference in results was observed particularly in the different gelatine concentrations, i.e. 5% and 9%. 9% gelatine-coating formulations gave higher strength than 5% within all five diets. However, the fresh or sea-water on the tensile strength were not influenced.

The results (Table 4) of all samples after a night cooling did not produce the expected results on the tensile strength. So, drying was performed on the coated diets.

Tensile	diet A	diet B	diet C	diet D	diet E
strength (kg)					
coating A	0.42±0.04	0.52±0.04	0.53±0.02	0.33±0.02	0.41±0.05
coating B	0.79±0.05	0.84 ± 0.07	0.87±0.04	0.54±0.02	0.56±0.04
coating C	0.37±0.05	0.53±0.06	0.47±0.05	0.39±0.03	0.41±0.02
coating D	0.78 ± 0.04	0.86 ± 0.06	0.91±0.03	0.50±0.02	0.58 ± 0.06

Table 4. Results of tensile strength (kg) of baits after one-night cooling.



Figure 22. The tensile strength of baits after one-night cooling.

4.4 Results of the tensile strength of baits after soaked in seawater for 2 hours

The coated- dried samples were soaked in water for two hours before to strength measurement. The results for 20 samples from 5 different diets (diet- A, B, C, D, and E) coated with four different coating solutions (coating- A, B, C, and D) shown in Table 5 and Figure 23.

For all diets, the results of tensile strength from the coating of freshwater groups (coating A and B) were similar to the seawater groups (coating C and D). The results of the coating of the high content of gelatine (coating B and D) were higher than the coating of the low content of gelatine (coating A and C). The results of diet B and C had higher strength than the rest of diets.

For the diet A, the results of coating A and C were the mean tensile strength of 1.21 ± 0.10 and 1.28 ± 0.12 kg but for those of coating B and D were 1.59 ± 0.03 and 1.69 ± 0.04 kg. The results of 9% gelatine groups had a higher strength of approximately 0.4 kg than the 5% gelatine groups.

For the diet B, the results of coating A and C were the mean tensile strength of 2.14 ± 0.15 and 2.11 ± 0.06 kg, but those of coating B and D were 3.09 ± 0.07 and 3.29 ± 0.14 kg. The strength from 9% gelatine groups had a strength of approximately 1 kg than those of 5% gelatine groups.

For the diet C, the results of coating A and C were the mean tensile strength of 2.14 ± 0.02 and 2.11 ± 0.04 kg. The results of coating B and D were the mean tensile strength of 3.09 ± 0.06 and 3.29 ± 0.05 kg. Compared the groups on their gelatine content, the results of coating B and D had 1 kg of the higher strength than that of coating A and C.

For the diet D, the results of the mean tensile strength for coating A and C were 1.72 ± 0.05 and 1.74 ± 0.03 kg, but for the coating B and D, the results of the mean tensile strength were 2.42 ± 0.08 and 2.78 ± 0.03 kg, respectively. The 5% gelatine-coated samples had 0.8 kg of strength lower than that of 9% gelatine-coated samples.

For the diet E, the results of tensile strength for coating A and C were the mean tensile strength 1.24 ± 0.01 and 1.38 ± 0.06 kg. The tension strength for coating B and D were the mean of 2.13 ± 0.11 and 2.31 ± 0.15 kg.

Coated-dried samples were better than the coated-cooled samples. For all coating solutions, the increase of gelatine concentration in coating solutions enhanced the tensile strength of the baits, but for the variation in fresh and seawater did not find the difference. Among the five different diets, the diet B and C showed higher tensile strength which is close to ideal bait properties

compared to other diets. Within diets, each diet at coating B and D had a strong tensile strength than that of coating A and C.

Tensile strength (kg)	diet A	diet B	diet C	diet D	diet E
Coating A	1.21±0.10	2.14±0.15	2.14±0.02	1.72±0.05	1.24±0.01
Coating B	1.59±0.03	3.09±0.07	3.09±0.06	2.42±0.08	2.13±0.11
Coating C	1.28±0.12	2.11±0.06	2.11±0.04	1.74±0.03	1.38±0.06
Coating D	1.69±0.04	3.29±0.14	3.29±0.05	2.78±0.03	2.31±0.15

Table 5. Results of tension strength (kg) for baits after soaked in seawater 2 hours.



Figure 23. Results of all samples after one-night drying.

4.5 Moisture content

The results of moisture content for five diets (diet- A, B, C, D, and E) coated with four different coatings (coating- A, B, C, and D) are presented in Table 6 and Figure 24-28. In total, 20 different samples were analyzed for moisture contents. The values in the table represented mean values \pm SD of the samples. Superscript in the bar plots shows significant differences among the samples (p < 0.05). It was observed significant differences among the coating solutions within the diets.

In all five diets, the statistical analysis among coating groups showed the trend of having coating A and B (i.e., fresh water) with higher moisture content than those of coating C and D (i.e., seawater). For both coating groups of fresh or sea-water, the 9% gelatine concentration samples had lower water content than the 5% gelatine concentration samples. The results of coating A with fresh water and 5% gelatine, had the highest moisture content among four different coating treatments. The results of coating D with sea-water and 9% gelatine, had the lowest moisture content among the four different coating treatments. Compared to the diets, diet A, B and C had significantly higher moisture content than that of diet D and E.

In the bar plot of diet A, the coating A had the highest moisture content. The coating B and C are not significant differences. The coating D was the lowest moisture content. However, the overall of the moisture content was between 46% and 35%.

In the bar plot of diet B, the coating A was the highest moisture content. The coating B and coating C placed the second and third of moisture content among the four coatings. The coating D was the lowest moisture content. However, the statistic results of four coatings were significant differences among the each other. However, the variations of moisture content for diet B were between 47% and 40%.

In the bar plot of diet C, the coating A and B had the higher moisture content than the coating C, and D. the variations of moisture content for diet C were approximately 10% among the four different coatings, which were from 39.48 % to 49.24 %.

In the bar plot of diet D, the results of coating A was significantly higher than the rest of the coatings. The results of the moisture content of coating A was approximately 5% higher than the rest of coating namely, coating B, C and D. For diet D, the results of moisture content were from 33.84% to 39.22%.

In the bar plot of diet E, the results of all coatings had small variations among each coating, which were around 4%. The results slightly decrease at the interval of approximately 1% among the coating A, B, C, and D.

Moisture content (%)	Diet A	Diet B	Diet C	Diet D	Diet E
Coating A	46.31±0.57	47.14 <u>±</u> 0.32	48.96 <u>±</u> 0.39	39.22 <u>+</u> 0.31	34.43 <u>+</u> 0.41
Coating B	43.63±0.06	45,32±0.31	49.24 <u>±</u> 0.13	33.84 <u>±</u> 0.34	33.78±0.96
Coating C	44.03±0.27	44.29±0.30	44.23±0.39	35.53±0.51	32.29±0.85
Coating D	36.37±0.45	40.05±0.33	39.48±0.31	34.11±0.21	30.66±0.34

Table 6. Results of moisture content for all samples.



Figure 24. Results of moisture content for diet A.

Figure 25. Results of moisture content for diet B.



Figure 26. Results of moisture content for diet C.

Figure 27. Results of moisture content for diet D.



Figure 28. Results of moisture content for diet E.

4.6 Water activity

The results of water activity for five diets (diet- A, B, C, D, and E) coated with four different coatings (coating- A, B, C, and D) are presented in Table 7 and Figure 29-33. In total, 20 different samples were analyzed for water activity. The values in the table represented mean values \pm SD of the samples. Superscript in the bar plots shows significant differences among the samples (p < 0.05). It was observed significant differences among the coating solutions within the diets. In all five diets, the statistical analysis among coating groups did not show the explainable trend for all samples.

For the results of diet A, the results of coating B was the highest water activity. The results of the rest of coatings namely coating A, C and D were lower than that of coating B. But, there are small variations among four different coatings, even though the statistical analysis showed

that there had. The overall results of water activity for diet A coatings were between 0.867 ± 0.004 and 0.913 ± 0.008 .

For the results of diet B, the results of coating A and B were not significant statistical difference. Meanwhile, the results of coating C and D also were not significantly different results between each other. The variations of results of water activity for diet B were from 0.868 ± 0.007 to 0.901 ± 0.013 .

For the results of diet C, the results of water activity for diet C had the small variations among the four different coatings. The variations in the results were from 0.851 ± 0.009

to 0.896<u>+</u>0.009.

For the results of diet D, the coating D was the highest the water activity among different coatings. The lowest result of water activity was for coating C. The variations of results of water activity for diet D were from 0.789 ± 0.002 to 0.863 ± 0.010 .

For diet E, the results of water activity for coating A and D were not statistically different. The coating B for diet E was the highest of water activity.

Water	Diet A	Diet B	Diet C	Diet D	Diet E
activity(a _w)					
Coating A	0.879 <u>±</u> 0.002	0.901 <u>±</u> 0.013	0.881 ± 0.001	0.825 <u>+</u> 0.011	0.827 <u>±</u> 0.017
Coating B	0.913 <u>+</u> 0.008	0.890±0.004	0.896 <u>±</u> 0.009	0.836 <u>+</u> 0.009	0.895 <u>+</u> 0.005
Coating C	0.867 <u>±</u> 0.004	0.868 <u>±</u> 0.007	0.851 <u>±</u> 0.009	0.789 <u>±</u> 0.002	0.872 <u>±</u> 0.008
Coating D	0.894 <u>+</u> 0.003	0.872 <u>+</u> 0.013	0.863±0.012	0.863 <u>+</u> 0.010	0.843±0.004

Table 7. Results of water activity (aw).





Figure 30. Results of water activity of diet B.



Figure 31. Results of water activity for diet C.





Figure 32. Results of water activity for diet D.



Figure 33. Results of water activity for diet E.

4.7 Results of feeding trail observation for cod

The video analysis results for the cod behaviors on the experimental baits were presented by a grading system which gives a score on the tested particles namely the baits depending on how the cod perform on the tested particles (Figure 34). The grading scores for feeding behaviors were named swallow, chewing, nibbling and touch. Each grade matches a score to reflect how the cod perform on the bait. The grading system simply as swallow scored 4, chewing scored 3, nibbling scored 2 and touch are scored 1.

For the definition of cod feeding behaviors, the touch scored one which means the cod arrived the tested samples and touched that over three times but not eat the tested particles. The nibbling scored two which means that the cod arrived tested samples and, nibbles and lubricated some small pieces and put into the mouth but not eat the sample. The chewing scored three which means that the cod attacked the tested samples and ingested but chewed in the mouth several times. The swallow scored four which means the cod instant swallowed the samples and ingested it.

For the diet A, the results of coating A were scored three namely that the cod feeding behaviors were chewing. The results of coating B were between the 2 and 3, which mean the baits for cod ingested by nibbling and chewing. The results of coating C were between 3 and 4, which mean the cod chewed and then swallowed the bait. The results of coating D were scored 4, which mean the cod instantly swallowed the bait.

For the diet B, the results of coating A and B were scored between 3 and 4, which mean the cod perform chewing and swallow. The results of coating C also between the score 3 and 4, but the score of coating C was higher than the coating A and B, which mean the cod perform more swallow and less chewing on the baits. The results of coating D got the score of 4 which mean the cod directly swallowed the baits.

For the diet C, the results of coating A scored between 2 and 3, which mean the cod perform nibbling and chewing on the baits. The results of coating B was the score of 3, which mean the cod perform chewing on that bait. The results of coating C were between 3 and 4, which mean the cod perform chewing and swallow on that bait. The results of coating D was scored 4, which mean the cod instant swallowing the baits.

For the diet D, the results of both coating A and B were scored between 3 and 4, which mean the cod like to chew and swallow the baits. The results of both coating C and D were 4, which mean the cod highly perform to swallow the baits.

For the diet E, the results of coating A were between 2 and 3, which illustrate the cod are nibbling and chewing on that bait. The results of coating B was 3, which mean the cod chewing the baits. The results of coating C were between 3 and 4, which express that the cod are chewing and swallowing the baits. The results of coating D was score 4, which means the cod instant swallowed the baits.

The results of coating D were the highest among the five different diets since the cod preferred the coating of sea-water with high gelatin content. But for the comparisons on the diets, the diet D illustrated the most cod favorited baits, and the second preferred diets for cod was by the diet B. the rest of diets there are not big differences.



Figure 34. Results of feeding trail observations for cod.

5. Discussion

In my study, the alternative baits for longline fishing cod can be produced by the extrusion and coating technology under the optimum operations and diet formulations with the same characteristic of tensile strength and taste as natural baits. The raw materials under the extrusion and coating will provide the properties of binding, reinforcement, and attraction to the baits. This development of alternative baits for cod longline fishing match with the recommendation from the study of Løkkeborg et al. (2014).

Due to the deep coating, all the weight of samples after coating increased. For the diet A, B and C, each sample weight after four different coatings were increased from 40 g to approximately 120 g. For diet D and E, each sample weight after four different coatings were also increased from 20 g to approximately 60 g.

5.1 Importance of the tensile strength of baits for longline cod fishing

For the longline cod fishing, the bait loss is the primary factor, which affects the efficiency of fishing. To get an efficiency fishing, the baits for longline fishing must have a specific texture and mechanical properties to hold on the hooks during whole fishing periods (He, 1996; Kumar et al., 2016). Good longline fishing baits must hold a good tensile strength to resist the force from the attacker like cod. He (1996) presented that in the bottom set longlines for cod, the loss of herring and capelin baits is twice than that of squid baits. The squid was found to be superior in the hook holding properties. The firm nature of squid flesh is determined the less bait loss rate than that of mackerel bait (Broadhurst & Hazin, 2001). The quality of bait estimates through how well the baits remain on the hook during the periods of fishing or fish hooked. The physical strength of the bait throughout the soaking time influences the effectiveness of bait (Kumar et al., 2015). After a soaking time of 56 min, the tensile strength of squid baits was approximately 30 N, which is higher than the herring (24 N) (He, 1996). That shows that a good longline fishing bait should hold significant tensile strength. Based on the results of experiments, the baits manufactured by extursion and coating, can provide the tensile strength as natural baits. The diets after coatings treated cooling or drying at the temperature of 10°C. The results of the sample after cooling were lower than 1 kg (i.e., 10 N), but for the same samples after coatings, the results of samples under drying treatment had a good tensile strength. Among the samples, the diet B and C had the tensile strength higher than 2 kg (i.e., 20 N). For all diets, the highest results of strength got on the coating B and D, which mean increasing the gelatine concentration will provide stronger strength. However, for the diets from the screw configuration, A and extrusion die A, the diet A had significant low tensile strength than the diet B and C. Among three diets, the only differences on the diet formulations so that the attraction variations for the diets will significant influences on the final product physical strength. For the diet D and E, the tensile strength slightly decreased when there was a decrease in the attractant content, increase in the wheat flour content. Compared results of sample strength from different screw configurations, the results of screw configuration A shown much better than that of screw configuration B under the same extrusion system.

For the strength of alternative baits for longline fishing of Atlantic cod, the diet processing procedures be optimized properly depending on the different diet formulations.

5.2 Water activity and moisture content

The results of moisture content and water activity for all samples were very high. The results due to that the samples were soaked in coater during the coating, not spray the coating liquid. The results of water activity (i.e., a_W) and moisture content for all samples already had the conditions to rear mould, bacteria growth and toxin at room temperature (Leistner et al., 1978). To avoid using up all the samples, before and after the test, samples had been stored in a freezer under -20°C.

"There is a direct linear relationship between the water activity and moisture content for the products of food and feed" (Lowe & Kershaw, 1995). The water activity increased with the increase of moisture content for the products. However, the linear relationship between the moisture content and water activity was not observed. In the five diets, the moisture content increase when the gelatine concentration decrease, and then the water activity also should be increased. However, on the results of five diets with four coatings, there is not any single diet express the linear relationship of moisture content and water activity.

From the results of moisture content obtain that the five diets with four coatings mainly vary on their screw configurations, and extrusion dies. The diets (diet A, B and C) from screw configuration A and extrusion die A had higher moisture content than the diets (diet D and E) from screw configuration B and extrusion die B. The differences of moisture among five diets were due to the screw configuration and extrusion die shape a high moisture content than those low moisture content diet from the other screw configuration and extrusion die, which provided differeces on the parameters of extursion.

For the moisture content of all samples for five diets, each diet with four different coatings expresses significant different results. The moisture content of Diet A and D had the trend of when the increase the gelatine concentration, the moisture content will be decreased. For the water activity, the results of the high gelatine concentration of coatings did not have the lower water activity than the low gelatine concentration of coatings. The ranging variations for water activity was not significant.

For observations from water activity and moisture content, the reason why there are not the directly linear relationship between the moisture content and water activity. First, the results of water activity for each diet were not significant differences among the four coatings, which

mean the variations of water activity were very low. Second, during the extrusion production, the parameters of processing are not constant. The feeder rate, screw speed, and barrel temperature had effects on the final pellets, mainly in the porosity of pellets and texture. Finally, the coating process also influences the properties of samples. The coater is not precise on the seal or isolating of air. The releasing time from vacuum pressure (-0.8 bar) to atmospheric pressure (0 bar) was difficult to control the timing during coating.

The results get from water activity, and moisture content was that the extruded baits based on coating technology had a high moisture content and water activity. Even though statistical results were not shown the linear relationship, the high moisture content gave high water activity observed from the comparison results of moisture content and water activity. The fresh or seawater for coating solution will not influence the results. However, the gelatin concentration of 5% and 9% in coating solution did not obtain significant influence on water activity and moisture content.

5.3 Feedback of cod feeding behaviors on the experimental samples

For the results of cod feeding behaviors, the feeding trails done in the glass fiber tanks. The external factors, like water temperature, current and light almost kept constant during the periods of feeding trails. So that on the field of longline fishing, the external factors were not considered in this thesis. Cod had a high hooking rate during the late of afternoon so that the feeding trail did that time (Løkkeborg & Fernö, 1999). For the longline fishing for Atlantic cod, Johnstone and Mackie (1990); Løkkeborg (1991); (Løkkeborg et al., 2014) also studied on the cod feeding behaviors in the wild or laboratory living environment.

Cod had specific feeding behaviors on the feed stimulant and attractions. The chemoreception namely, olfactory and gustation system, lead the cod to search and locate to the food source. Cod have species and size selectivity to prey, which means that the fish bait for longline cod fishing is superior to consider. For the bait types, the squid, mackerel, and herring as natural or traditional baits dominated in longline fishing for Atlantic cod (Løkkeborg & Bjordal, 1992). Cod also had size selectivity on the bait size, so in my study, the bait size limited in two sizes as 30 mm and 50 mm.

A successful longline cod fishing baits had the feeding behavior of swallow for cod, and then the cod could catch by the hooks. For my experiments, the definition of good baits for cod taste seems as cod perform the swallow on the tested baits. From the results of cod feeding behaviors on experimental baits, the coating and drying process helps a lot to develop a good alternative fish bait. The coating of attractant with binder well held the attractant tastes for cod.

Since cod inhibited in seawater, the results of feeding trails express the samples by coating solution through seawater was better than the freshwater. For all diets, their diet formulations content different cod attractants up to 25% for diet A, B, C and D, but for Diet E 19%. But, a little decreasing of attractant content was recommended when formulated the diets.

It is aware that the cod had variations at their diet selections during the year, so that the high palatable results from this study could not support the samples accepted by cod during whole year. The repetition of feeding trails on this alternative bait suggested to continue till fulfilled the records of whole year of cod feeding behavioral habits.

Developing alternative baits for longline fishing of Atlantic cod performed by the optimization of extruded baits with coating solutions under the accurate diet formulations and processing parameters. Both on the physical strength and taste already get to the levels of natural baits. However, there are still effort to test on the preliminary processing. First, all sample analyzed in the test were all drying at one-night, so the relationship of long or short drying time and physical strength should find out. Second, all samples had high moisture content and water activity. The limited or optimum points of water activity and moisture content should find out.

6. Conclusion

The general expectation of the alternative baits for longline fishing of Atlantic cod was achieved by optimization of the diet processing and formulations conducted in this thesis. The extrusion technology could have potential to fulfill the demand of large quantities for longline baits, good physical qualities, and attractive to cod.

Alternative longline fish baits developed sufficiently meet the basic demands of longline fishing of Atlantic cod, providing physical strength on the hooks in excess to natural bait with high palatability. Unfortunately, the high moisture and water activity of the experimental baits as shown by the present study indicated storage of the bait below freezing temperature (-20°C) to prevent the potential microbial contaminations. As cod had a species selectivity on prey, the baits based on feed manufacturing technical manipulations provided palatable baits to Atlantic cod. Based on the feeding trails completed in fish tanks, the feeding behaviors of cod perform the same as the natural baits on some of experimental samples. Due to the alternative baits produce by feed manufacturing line, it is able to accomplish the production in large quantity under the sufficient raw materials and proper equipment in once. The development of alternative fishing baits for Atlantic cod, in this study, was entirely based on experimental tanks, which does not completely represent the natural fishing conditions. Hence, full-scale longline fishing trail should be done in the real field to understand different factors affecting the catching efficiency of baits.

References

- A.Brisset, J. M. B. a. (2006). Aquafeed Twin Screw Extrusion Processing. Available at: <u>http://www.fao.org/fileadmin/user_upload/affris/img/Niletilapia_table/Bouvie_and_Br</u> <u>isset_2006_.pdf</u>.
- Bjordal, Å. (1981). Engineering and fish reaction aspects of longlining-a review: ICES.
- **Bjordal,** Å. (1983). *Effect of different long-line baits (mackerel, squid) on catch rates and selectivity for tusk and ling:* ICES.
- Boran, G. & Regenstein, J. M. (2010). Fish gelatin. In vol. 60 Advances in food and *nutrition research*, pp. 119-143: Elsevier.
- Brawn, V. (1969). Feeding behaviour of cod (Gadus morhua). *Journal of the Fisheries Board* of Canada, 26 (3): 583-596.
- Broadhurst, M. K. & Hazin, F. H. (2001). Influences of type and orientation of bait on catches of swordfish (Xiphias gladius) and other species in an artisanal sub-surface longline fishery off northeastern Brazil. *Fisheries Research*, 53 (2): 169-179.
- Caprio, J., Brand, J. G., Teeter, J. H., Valentincic, T., Kalinoski, D. L., Kohbara, J.,
 Kumazawa, T. & Wegert, S. (1993). The taste system of the channel catfish: from biophysics to behavior. *Trends in neurosciences*, 16 (5): 192-197.
- Carr, W. E. & Derby, C. D. (1986). Chemically stimulated feeding behavior in marine animals. *Journal of Chemical Ecology*, 12 (5): 989-1011.
- **Carton, A. & Montgomery, J.** (2003). Evidence of a rheotactic component in the odour search behaviour of freshwater eels. *Journal of fish biology*, 62 (3): 501-516.
- Desrumaux, A., Bouvier, J. & Burri, J. (1998). Corn grits particle size and distribution effects on the characteristics of expanded extrudates. *Journal of food science*, 63 (5): 857-863.
- Ellingsen, O. & Døving, K. (1986). Chemical fractionation of shrimp extracts inducing bottom food search behavior in cod (Gadus morhua L.). *Journal of chemical ecology*, 12 (1): 155-168.
- Furevik, D. M. & Løkkeborg, S. (1994). Fishing trials in Norway for torsk (Brosme brosme) and cod (Gadus morhua) using baited commercial pots. *Fisheries research*, 19 (3-4): 219-229.

- Gerstner, C. L. (1998). Use of substratum ripples for flow refuging by Atlantic cod, Gadus morhua. *Environmental Biology of Fishes*, 51 (4): 455-460.
- GILL, C. (2000). Vacuum liquid coating for pressed pellets. *FEED INTERNATIONAL*: 26-27.
- GMIA, G. H. (2012). Gelatin Manufacturers Institute of America. New York.
- Gomez, M. & Aguilera, J. (1984). A physicochemical model for extrusion of corn starch. *Journal of Food Science*, 49 (1): 40-43.
- Gudmundsson, M. (2002). Rheological properties of fish gelatins. *Journal of Food Science*, 67 (6): 2172-2176.
- Hara, T. J. (1994). Olfaction and gustation in fish: an overview. *Acta Physiologica*, 152 (2): 207-217.
- Hawkins, A. (1986). Underwater sound and fish behaviour. In *The behaviour of teleost fishes*, pp. 114-151: Springer.
- He, P. (1996). Bait loss from bottom-set longlines as determined by underwater observations and comparative fishing trials. *Fisheries research*, 27 (1-3): 29-36.
- Hou, P. & Regenstein, J. (2004). Optimization of extraction conditions for pollock skin gelatin. *Journal of food science*, 69 (5): C393-C398.
- Hu, L., Hsieh, F. & Huff, H. (1993). Corn meal extrusion with emulsifier and soybean fiber. LWT-Food Science and Technology, 26 (6): 544-551.
- ICES. (2017). Cod (Gadus morhua) in subareas 1 and 2 (Norwegian coastal waters cod). Available at: <u>http://www.ices.dk/sites/pub/Publication</u> <u>Reports/Advice/2017/2017/cod.27.1-2coast.pdf</u>.
- Johnstone, A. & Hawkins, A. (1981). A method for testing the effectiveness of different fishing baits in the sea: Department of Agriculture and Fisheries for Scotland.
- Johnstone, A. & Mackie, A. (1990). Laboratory investigations of bait acceptance by the cod, Gadus morhua L.: identification of feeding stimulants. *Fisheries research*, 9 (3): 219-230.
- Kasumyan, A. O. & DÖving, K. B. (2003). Taste preferences in fishes. *Fish and fisheries*, 4 (4): 289-347.

- Kumar, A. K., Pravin, P., Khanolkar, P. S., Remesan, M. & Meenakumari, B. (2015). Efficacy of bait species and baiting pattern on hooking rates and bait loss during longline fishing in Lakshadweep Sea, India.
- Kumar, K. A., Pravin, P. & Meenakumari, B. (2016). Bait, Bait Loss, and Depredation in Pelagic Longline Fisheries–A Review. *Reviews in Fisheries Science & Aquaculture*, 24 (4): 295-304.
- Leistner, L., Rodel, W. & Krispien, K. (1978). Microbiology of meat and meat products in high-and intermediate-moisture ranges. *Water activity: Influences on food quality: a treatise on the influence of bound and free water on the quality and stability of foods and other natural products.*
- Lie, Ø., Lied, E. & Lambertsen, G. (1989). Feed attractants for cod (Gadus morhua).
- Løkkeborg, S., Bjordal, Å. & Fernö, A. (1989). Responses of cod (Gadus morhua) and haddock (Melanogrammus aeglefinus) to baited hooks in the natural environment. *Canadian Journal of Fisheries and Aquatic Sciences*, 46 (9): 1478-1483.
- Løkkeborg, S. (1991). Fishing experiments with an alternative longline bait using surplus fish products. *Fisheries research*, 12 (1): 43-56.
- Løkkeborg, S. & Bjordal, Å. (1992). Species and size selectivity in longline fishing: a review. *Fisheries Research*, 13 (3): 311-322.
- Løkkeborg, S. (1998). Feeding behaviour of cod, Gadus morhua: activity rhythm and chemically mediated food search. *Animal behaviour*, 56 (2): 371-378.
- Løkkeborg, S., Siikavuopio, S. I., Humborstad, O.-B., Utne-Palm, A. C. & Ferter, K. (2014). Towards more efficient longline fisheries: fish feeding behaviour, bait characteristics and development of alternative baits. *Reviews in fish biology and fisheries*, 24 (4): 985-1003.
- Lowe, J. & Kershaw, S. (1995). Water activity-moisture content relationship as a predictive indicator for control of spoilage in commercial pet diet components. *Animal Feed Science and Technology*, 56 (3-4): 187-194.
- Lucas, J. S. & Southgate, P. C. (2012). *Aquaculture: Farming aquatic animals and plants:* John Wiley & Sons.

Miladinovic, D. D. (2014). *Extrusion line*. Available at: https://www.nmbu.no/en/services/centers/fortek/equipment/extrusion-line.

- Navale, S., Swami, S. B. & Thakor, N. (2015). Extrusion cooking technology for foods: a review. *Journal of Ready to Eat Food*, 2 (3): 66-80.
- Norbait. (2018). Products. Available at: http://www.norbait.com/index.htm.
- Riaz, M. N. (2000). Extruders in food applications: CRC press.
- Rokey, G. & Plattner, B. (1995). Process description. *Pet food production. Wenger Mfg, Inc., Sabetha, KS USA*, 66534: 1-18.
- Sørensen, M. (2012). A review of the effects of ingredient composition and processing conditions on the physical qualities of extruded high-energy fish feed as measured by prevailing methods. *Aquaculture Nutrition*, 18 (3): 233-248.
- StatisticsNorway. (2018). Quantity and value of catch from Norwegian vessels. Selected fish species. Preliminary figures. Available at: <u>https://www.ssb.no/en/fiskeri</u> (accessed: Fisheries).
- Stoner, A. (2004). Effects of environmental variables on fish feeding ecology: implications for the performance of baited fishing gear and stock assessment. *Journal of Fish Biology*, 65 (6): 1445-1471.
- Tryggvadóttir, S. V., Jónsson, G. P., Jónsdóttir, R. & Ólafsdóttir, G. (2002). Artificial bait alternatives, mainly based on fish waste: CRAFT Q5CR-200-70427. Project report to EU. Icelandic Fisheries Laboratories, 09-02.
- WALKER, S. (2009). *MACKEREL AND OTHER FISH BAITS*. Available at: <u>https://www.planetseafishing.com/mackerel-and-other-fish-baits/</u>.
- Watson, J. & Kerstetter, D. (2006). Pelagic longline fishing gear: a brief history and review of research efforts to improve selectivity. *Marine Technology Society Journal*, 40 (3): 6-11.
- Yang, H., Wang, Y., Jiang, M., Oh, J. H., Herring, J. & Zhou, P. (2007). 2-Step optimization of the extraction and subsequent physical properties of channel catfish (Ictalurus punctatus) skin gelatin. *Journal of Food Science*, 72 (4): C188-C195.