Fishmeal physicochemical properties

Impact on the fish feed extrusion process, phase transitions and physical pellet quality

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Scientific environment

The research studies presented in this thesis has been carried out at the Norwegian Institute of Food, Fisheries and Aquaculture Research (Nofima). The principal supervisor has been Dr. Åge Oterhals, Nofima and co-supervisor, Dr. Svein Mjøs, University of Bergen, Department of Chemistry. The experimental work on the pilot scale extruder, physical feed- and phase transition analysis was performed at Nofima Feed Technology Centre in Bergen. Other analytical work was performed at Nofima BioLab (accredited according to ISO 17025).

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Abstract

Norway is the world largest producer of Atlantic salmon (*Salmo salar*). In 2013 the Norwegian aquaculture industry produced 1.2 million metric tons salmon with the use of 1.5 million metric tons of commercial feeds. The feeds are delivered to the farms in big bags or in bulk and are conveyed pneumatically to the sea cages. Such harsh treatments expose pellets to stress that may give product loss due to abrasion and fragmentation. Feed loss in pneumatic feeding systems is estimated to be in the range of 0.3% to 1.5%. This equals a yearly additional expense of around 40 to 200 million NOK for the Norwegian salmon industry.

To minimize product loss the feed has to be of a consistent and high physical quality. Extrusion processing is a technology that enables production of such feed quality. Physical pellet quality is normally improved by the addition of starch and other binders, but recent research has shown that the protein ingredients in the feed mix also impact the physical quality of extruded feed products. During the last decade several new plant derived protein ingredients has been introduced and partly replaced fishmeal. This has introduced new challenges in fish feed extrusion and stressed the importance to improve the knowledge related to the technical properties of the individual ingredients.

The main objectives of this work have been to quantify fishmeal physicochemical properties with significant effects on the extrusion cooking process and physical pellet quality, and to study the plasticization effect of water solubles in fishmeal. Various multivariate analytical techniques have been applied in the studies, such as principal component analysis, partial least squares -and multiple linear regression. In Paper I and II, the impact of variation in fishmeal physicochemical properties were assessed based on standardized extrusion, drying and coating conditions. In Paper III the effect of water-soluble protein level and moisture content on the extrusion process, extrudate phase transitions and physical quality of feed were studied. In Paper IV the influence of fishmeal water solubles and added moisture on glass transition and flow-starting temperature were quantified.

The studies in Paper I and II document the complexity of fishmeal as a protein ingredient with significant impact on the extrusion process, starch gelatinization and physical pellet quality. Large differences in technical quality within and between the studied fishmeal types (i.e. herring and sand eel) were observed. The research quantifies a positive effect of increased levels of water-soluble protein on pellet durability and hardness. This can be explained by two different mechanisms: a cross-linking effect of large polypeptides and a plasticizing effect of smaller peptides and amino acids. Differences in peptide size distribution between the two studied groups were identified with the highest level of large polypeptides for herring meal. At an equal level of water-soluble protein, extruded feed containing fishmeal from sand eel had significantly lower physical quality than feed containing herring meal. This can be attributed to differences in thermal and rheological properties between the two studied groups, and improper cooking in the extruder barrel for sand eel based feed mixes. Incomplete cooking or transformation may result in increased levels of particles in the extrudates and poor physical feed quality. The studies also document that fishmeal specifications normally used on the world commodity market inadequately describe the technical properties of fishmeal.

In Paper III the effects of water-soluble protein level in fishmeal on extrusion behaviour, phase transitions and physical feed quality were studied. The plasticizing effect of water-soluble protein was comparable to that of moisture. However, in contrast to moisture, addition of water-soluble protein had a positive effect on specific mechanical energy and physical pellet quality. No loss of water-soluble protein during the extrusion process could be observed, confirming that the amino acids and peptides do not form any new covalent bonds in the extrusion process. A non-volatile plasticizer like water-soluble protein will not be removed in the drying process. It will therefore influence the viscoelastic properties of the final product and have a positive effect on physical pellet quality by establishment of an intermolecular binding network through hydrogen-, ionic bond, and hydrophobic interactions. It can be concluded from the study that water-soluble protein can be used as a processing

aid for the fish feed industry, serving multiple purposes as nutrient, plasticizer and binder in extruded fish feed.

In Paper IV significant effects of fishmeal water solubles and moisture level on the glass transition and flow-starting temperatures have been documented. The effect of solubles level on the glass transition temperature could be modelled based on the Gordon-Taylor equation. The documented plasticizing effect of water solubles was lower than the effect of moister addition per unit mass, but higher on a molar basis. The plasticization effect can be attributed to the content of low molecular nitrogen-compounds. The studied fishmeal model system showed a large composition region of water solubles and moisture with a higher difference between the flow-starting and glass transition temperature than for other reported protein components (i.e. casein, gluten and soya protein isolate). This indicates a reduced temperature effect on viscosity reduction in the rubbery phase for fishmeal in this region. Combined with significantly lower glass transition temperatures, such differences in physicochemical properties may contribute to explain the unique functional properties of fishmeal compared to plant based proteins and casein. This will have positive impact on physical pellet quality and open up the possibility to obtain a satisfactory thermomechanical transformation in the extrusion process at reduced moisture level.

List of publications

Paper I Samuelsen, T.A., Mjøs, S.A. & Oterhals, Å. (2013) Impact of variability in fishmeal physicochemical properties on the extrusion process, starch gelatinization and pellet durability and hardness. *Animal Feed Science and Technology*, **179**, 77-84. DOI: 10.1016/j.anifeedsci.2012.10.009.

Paper II Samuelsen, T.A., Mjøs, S.A. & Oterhals, Å. (2014) Influence of type of raw material on fishmeal physicochemical properties, the extrusion process, starch gelatinization and physical quality of fish feed. *Aquaculture Nutrition*, **20**, 410-420. DOI: 10.1111/anu.12093.

Paper III Samuelsen, T.A. & Oterhals, Å. (2015) Water-soluble protein level in fishmeal affects extrusion behaviour, phase transitions and physical quality of feed. *Aquaculture Nutrition*, published online 27.01.2015. DOI: 10.1111/anu.12235.

Paper IV Oterhals, Å. & Samuelsen, T.A. (2015) Plasticization effect of solubles in fishmeal. *Food Research International*, **69**, 313-321. DOI: 10.1016/j.foodres.2014.12.028.

Contents

Abbreviations

ANOVA	Analysis of variance
C_1, C_2	Parameters in Williams-Landel-Ferry equation (Equation 5)
CCD	Central composite design
C_p	Specific heat capacity
DDC	Atmospheric double differential preconditioner
DJ	Dyno-Jet indirect air dryer
DM	Dry matter
E'	Storage or elastic modulus
E''	Loss or viscous modulus
FD	Flash dryer
FMH	Herring meal
FMSE	Fishmeal from sand eel
H	Hetland indirect air dryer
K	Function of coefficient of expansion (Equation 1); consistency index (Equation 4)
Lignin	Lignin sulfonate
MLR	Multiple linear regression
Mod corn	Modified corn starch
Mod potato	Modified potato starch
n	Flow behaviour index (Equation 4)
PC	Principal component
PCA	Principal component analysis
PCR	Principal component regression
PLSR	Partial least squares regression
PTA	Phase Transition Analyzer
RMSEC	Root mean square error of calibration
RMSEP	Root mean square error of prediction
RSF	Refrigeration by fresh water
RSW	Refrigeration by seawater

SD	Standard deviation
SD+	Indirect steam dryer used as pre-dryer
SEI	Sectional expansion index
SME	Specific mechanical energy
Synthetic	Polyvinylpyrrolidone
T_{die}	Temperature upstream the extruder die
T_f	Flow-starting temperature
T_g or T_{gMid}	Glass transition temperature
T_{gEnd}	Endpoint of the glass transition range
TMA	Trimethylamine
TMAO	Trimethylamine N-oxide
TVN	Total volatile nitrogen
V	Indirect vacuum dryer
WLF	Williams-Landel-Ferry equation
WSDM	Water soluble dry matter
WSP	Water-soluble protein
$\dot{\gamma}$, $\dot{\gamma}_{app}$	Shear rate, apparent shear rate
η , η_{app}	Viscosity, apparent viscosity
τ , τ_{app}	Shear stress, apparent shear stress

1. Introduction

The global production of fish to human consumption was about 128 million metric tons in 2010 with aquaculture production accounting for approximate 47% of the total supply (60 million metric tons; FAO 2012). The capture fisheries remain stable and to meet the demand of fish to an increasing global population future needs have to come from aquaculture (FAO 2012). Aquaculture has increased at an annual rate of 8.8% from 1980 to 2010 and is expected to increase at the same rate over the next decade (Tacon *et al.* 2011; FAO 2012). Approximately 46% of the global aquaculture production is based on intensive feeding by use of farmed made or commercially manufactured feeds. On a global basis the commercial fish feed production is estimated to increase from 35 to 71 million metric tons from 2010 to 2020 (Tacon *et al.* 2011), which will create a growing demand for supply of nutrients (i.e. protein, lipids, minerals and vitamins) and binders.

The total aquaculture production of Atlantic salmon has increased by 5.5% the last decade and is expected to reach 2.8 million metric tons in 2020 (Tacon *et al.* 2011). Salmon is farmed in floating net cages (on-growth phase) based on commercial feeds. The feeds are delivered from the producers to the farms in big bags or bulk and most commonly conveyed pneumatically to the sea cages (Aarseth 2004; Aarseth *et al.* 2006); systems demanding consistent and high physical pellet quality to minimize product loss due to abrasion and fragmentation. Extrusion processing is a technology that enables the manufacture of such quality and is therefore the dominating technology used in commercial salmon feed production. Compared to other thermal processes, extrusion is also energy efficient, has lower processing costs and can handle a variety of feed ingredients (Riaz & Rokey 2012). Products with different degree of expansion (sinking or floating feed), different shape and sizes and with nutritional values tailor made for the fish species and age can be produced.

Norway is the main global producer of Atlantic salmon (Tacon *et al.* 2011) with a production of 1.2 million metric tons in 2013 (Statistics Norway 2015). The feed consumption was 1.5 million metric tons with an average feed price of 9.19 NOK/kg

(Akvafakta 2015; Directorate of Fisheries 2014). The economic feed conversion ratio was 1.25. The feed cost accounts for more than half of the production costs per kilo of fish produced (Directorate of Fisheries 2014). Changes in feed cost, conversion ratio and waste will therefore have a major impact on the total production costs.

Each percentage of waste feed or non-utilized feed is equivalent to a loss of around 130 million NOK for the Norwegian salmon industry. Approximately 5% of the feed is lost through the environment during feeding and 12.5% is nondigested feed ejected in faeces (Findlay & Watling 1994; Brooks & Mahnken 2003; Institute of Marine Research 2012). Possible causes, as studied and discussed by Oehme (2013), include 1) suboptimal feeding practice, 2) nutritionally imbalanced diets and 3) suboptimal physical pellet qualities that reduce feed intake and feed utilization by fish. A few studies have examined the impact of physical pellet quality on the biological response of the fish (as reviewed by Sørensen 2012); however, data is inconsistent and there is a need for further investigations (Oehme 2013). Harsh treatments, such as transport and pneumatic conveying expose feeds to stress that may increase abrasion and fragmentation. Loss in pneumatic feeding systems is affected by physical feed quality, transportation distance, conveying velocity and bend radius and is estimated to be in the range of 0.3% to 1.5% (Brooks & Mahnken 2003; Aarset 2004; Aarseth *et al.* 2006; Aas *et al.* 2011). Feed ingredients respond differently to extrusion processing (Sørensen *et al.* 2009; Glencross *et al.* 2010; Draganovic *et al.* 2011; Kraugerud *et al.* 2011) and changes in feed mix properties may lead to feed pellets with a low physical quality not suitable for transport and pneumatic conveying. This enforces reprocessing of feeds that will increase energy consumption and production costs at a feed factory. Figures on amount of reprocessed feed are not publically available. Based on my knowledge, a rough estimate will be in the range 0.5% to 2.0% of total production. Non-utilized, wasted and reprocessed feeds have all negative economic and environmental impacts and should be minimized. This thesis focus on the underlying causes to the observed variability in physical fish feed quality and how to handle feed ingredients and the extrusion process to improve the product quality. This knowledge can be used to develop optimal pellets for biological

response, reduce reprocessing costs and minimize loss during transport and pneumatic feeding.

The use of fishmeal in feeds to the global aquaculture production is expected to decrease in the long-term. The reasons are 1) high prices due to increasing market demand, 2) expected static or decreased supply, 3) public demand for improved sustainability and 4) use of more cost efficient fishmeal replacers such as other marine ingredients, plant and microbiological ingredients, marine and terrestrial animal by-products and insect meals (Tacon & Metian 2008; Sørensen *et al.* 2011; Tacon *et al.* 2011). In Norway the inclusion levels of fishmeal in Atlantic salmon feed have been reduced from approximately 65% in 1990 to 18% in 2013 (Ytrestøyl *et al.* 2014). In Norway, fishmeal has mainly been replaced by less expensive plant derived alternatives (Sørensen *et al.* 2011; Ytrestøyl *et al.* 2014). The changes in diet feed composition have stressed the importance to improve the knowledge on technical properties of the individual feed ingredients and the possible interactions between them, to better understand and control the extrusion process and physical product quality. Effects on physical feed quality from the replacement of fishmeal with plant-derived alternatives have been reported in several studies (Sørensen *et al.* 2009; Glencross *et al.* 2010; Draganovic *et al.* 2011; Kraugerud *et al.* 2011). However, little is published about the variability in physicochemical properties within different types of ingredients and the influence of this variability on the extrusion process and physical feed quality. Nofima Feed Technology Centre in Bergen, former part of Norwegian Herring Oil and Meal Industry Research Institute (SSF; until 2003) and Norwegian Institute of Fisheries and Aquaculture Research (Fiskeriforskning; 2002-2008) has considerable experience in the research area of feed technology and feed raw materials and has worked with extruded fish feed products since 1990. Several studies have documented that fishmeal is one of the most variable ingredients used in aqua feed production (Nofima, unpublished results). However, the industry has acknowledged the unique technical properties of fishmeal compared to plant-derived proteins, and has suggested establishing a new knowledge platform based on

fishmeal extrusion properties with the aim to improve the processability of plant proteins (Draganovic *et al.* 2011).

1.1 Objectives of the thesis

The main objective for the research activity was to explore fishmeal physicochemical properties influencing the fish feed extrusion process, phase transitions and physical pellet quality.

Sub goals:

1) To characterize intra- and inter variability in fishmeal physicochemical properties.
2) To identify fishmeal physicochemical properties with significant effect on the extrusion cooking process and pellet binding properties.
3) To study the effect of water-soluble protein level and moisture content on the extrusion process, extrudate phase transitions and physical quality of feed.
4) To quantify the plasticization effect of water solubles in fishmeal.
5) To assess the impact of variability in fishmeal psychochemical properties on industrial feed processing.

2. Background

The extrusion process used for salmon feed manufacturing is a complex and multivariate process with several adjustment possibilities. In industrial manufacturing operations, the final feed product has to meet several target product characteristics that depend on the physicochemical and rheological properties of the feed ingredients, processing conditions and extruder type and configuration. A commercial salmon feed has to meet the following requirements (Oliveira 1990; Sørensen 2012; Draganovic 2013):

- Be balanced for optimal feed intake and feed utilization. This is mainly controlled by the diet feed mix composition but may also be affected by physical feed quality (Hilton *et al.* 1981).
- Be of high physical quality to minimize product loss during transport and pneumatic feeding, but not of a durability that prevents complete digestion by the fish.
- Have a water stability that minimizes degradation. Water stability of a feed is most important for aquatic slow eaters (e.g. Sea Urchin) but may also impact the degradation pattern in the gastrointestinal tract of the fish (Hilton *et al.* 1981; Baeverfjord *et al.* 2006).
- Have a size customized for the different life stages of the farmed fish. This is mainly defined by the extruder die size but is also controlled by the degree of pellet expansion.
- Have sufficient expansion to adsorb desired amount of oil but still be dense enough to sink at a speed that enables the fish to catch the feed. Feed that floats or sinks too fast may escape the net cages and increase feed loss. There is a negative effect of increased expansion on physical quality (Sørensen 2012; Paper III) indicating the challenge to meet both these requirements during production.
- Have optimal microstructure to minimize oil leakage during transport, storage and pneumatic feeding. Pellet pore structure is difficult to control and for high energy salmon feed (up to 40% fat content; Sørensen 2012) oil leakage may be a problem.

To meet the above requirements, the aqua feed manufactures must manage the variability in extrusion- and binding properties between and within feed ingredients and also learn how to handle and control this variability during feed processing. This is a demanding task and commercial fish feed production is known to be very dependent on skilled operators. The work in this thesis is a step towards a knowledge based control of the extrusion process and physical feed quality.

3. Fishmeal and feed technology

3.1 Fishmeal and oil process

The production of fishmeal and fish oil was developed in northern Europe and North America in the beginning of the 19th century and has grown to be a global industry supplying ingredients to aquaculture and terrestrial animal feeds (Schmidtsdorff 1995; Hall 2011). World fishmeal production was 4.7 million metric tons with Peru, China, Thailand, Chile, USA, Japan, Denmark, Ecuador, Mexico, Iceland, Vietnam and Norway as the main producing countries in descending 2013 order (IFFO, 2014). Current Norwegian production is around 100 000 metric tons (IFFO, 2014). Fishmeal (Fig. 1) is produced by use of heat coagulation combined with mechanical fat separation and thermal dewatering steps (Schmidtsdorff 1995). The process, Fig. 2, is fairly standardized worldwide, although some differences can be observed in the technology used (Oterhals & Vogt 2013).

Figure 1. Typical appearance of a fishmeal (Frank Gregersen, Nofima).

3.1.1 Fish raw material

Fish used for fishmeal and oil can be divided into three categories 1) fish caught for the purpose of fishmeal production (industrial fish), 2) by-catches and 3) fish offcuts

and offal from filleting, gutting and other fish processing operations (FAO 1986). The latter is estimated to be 22% to 24% of the total worldwide fishmeal production (Hall 2011). The major sources of industrial fish in Norway, Denmark and Iceland are blue whiting, sand eel, herring, capelin, Norway pout, sprat, horse mackerel and mackerel (in descending 2014 order, tons delivered in Norway; Norges Sildesalgslag 2015). The sources in Peru and Chile are anchovy, jack mackerel and pilchard (sardine), USA; Alaska pollock and menhaden, Japan; pilchard, South Africa; anchovy and pilchard and various species in Thailand and China (Hall 2011).

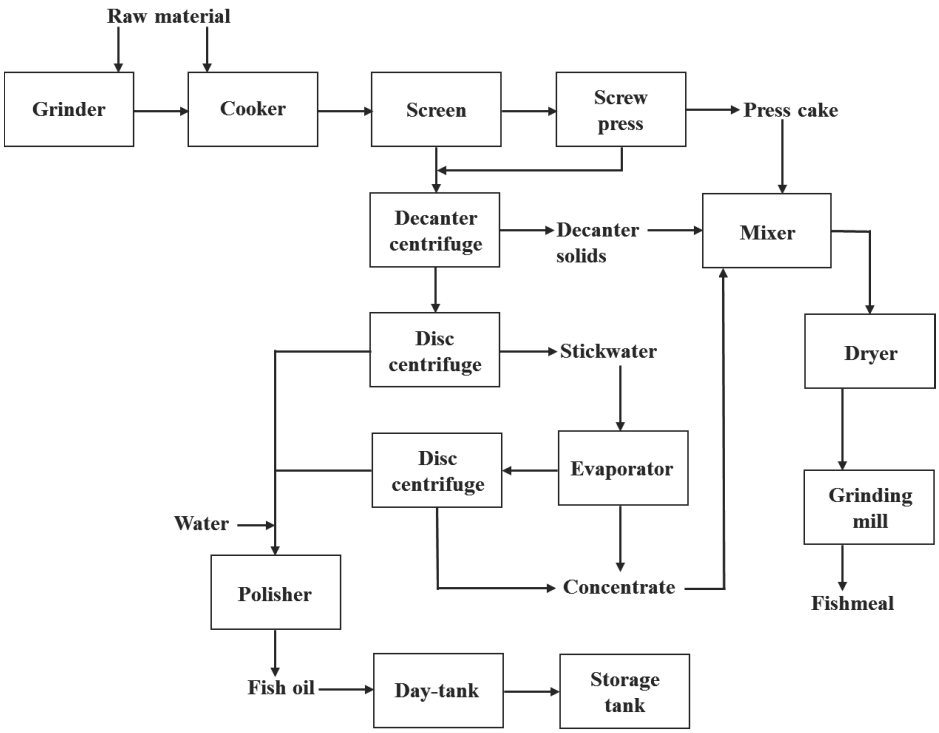


Figure 2. Simplified flow diagram of the fishmeal and fish oil process (after Oterhals & Vogt 2013).

During transport at sea and storage there will be a risk of partial spoilage of the fish raw material. Spoilage is dependent on both storage time and temperature and can be autolytic and microbiological. The autolytic process degrades the tissue to water soluble peptides and amino acids and is dependent on the level of endogenous

proteolytic enzyme activity in the fish. The activity will vary with the content of feed (zooplankton) in the fish stomach and gut (seasonal variations). Bacteria contribute to the proteolytic activity and also convert amino acids to biogenic amines (e.g. putrescine, cadaverine and histamine). Bacterial breakdown of trimethylamine N-oxide (TMAO) produces ammonia and trimethylamine (TMA) respectively, which increase the total volatile nitrogen (TVN) content (Aksnes 1988; Aksnes & Brekken 1988; Aksnes & Mundheim 1997; Opstvedt *et al.* 2000; Bragadottir *et al*. 2002). The TVN content can therefore be used as a guide to raw material freshness. To produce fishmeal with the highest yield and quality the fresh raw material should not exceed a TVN value of 50 mg N 100 g^{-1} during production (Schmidtsdorff 1995). The most common methods for preservation of the fish on-board the fishing vessel are refrigerated or chilled water systems and ice-slurry/fish mixing systems (FAO 1986; Schmidtsdorff 1995). In some cases, the cooling medium is added acetic acid. Other factors affecting quality and yield will be type of raw material (fish species) and seasonal variations in fat content and level of roe and milt (McBride *et al.* 1959; Suzuki 1981; Schmidtsdorff 1995; Bragadottir *et al*. 2002; 2004)

3.1.2 Unit operations in fishmeal processing

A general flow diagram of the fishmeal and oil process is given in Fig. 2. The main unit operations are explained based on FAO 1986; Schmidtsdorff 1995; Hall 2011 and Oterhals & Vogt 2013.

Heat treatment

Heat treatment is performed in a continuous screw cooker at 90 to 95 °C for approximate 20 minutes. The treatment coagulates proteins, disrupt fat deposits and release oil and water. This is a key process as it conditions the raw material for the downstream separation processes.

Mechanical pressing

After heat treatment the raw material passes a strainer to remove any free oil and water before it enters the screw press. The purpose of the screw press is to squeeze out oil and water from the coagulated material (presscake). Oil yield depends on fish

species and seasonal variations in fat content. Water solubles, containing most of the water soluble nitrogen compounds (protein, peptides, amino acids, putrefaction products etc.), vitamins and minerals, and suspended fine particles will also follow the liquid fraction. The amounts depend on the endogenous proteolytic enzyme activity and freshness of the fish. High enzyme activity and/or spoilage give increased level of solubles and a high content of suspended solids in the soluble phase (Høstmark 1987).

Oil separation

The liquid fraction containing water solubles and suspended particles are mixed with the oil/water fraction from the strainer, heated to 90 to 95 °C, and run over a decanter centrifuge to remove the suspended particles (decanter solids). The separation of oil and water solubles (stickwater) is thereafter performed in a disc centrifuge. The oil is polished with water over a second disc centrifuge, pumped to a day tank to settle residual impurities and finally pumped to a storage tank (Oterhals & Vogt 2013).

Solubles concentration

The stickwater, usually with water content of 90 to 94%, is concentrated in the evaporators to water content of approximately 70 to 80%. The concentration potential depends on the viscosity of the concentrate, which will vary with the amount of suspended solids in the concentrate, peptide size distribution, fish species and season (McBride *et al.* 1959; Høstmark 1987).

Mixing, drying and milling

To produce a “normal” or “whole” meal the presscake and decanter solids, which are mainly composed of myofibrillar protein with a variable degree of fragmentation (Suzuki 1981), are mixed with stickwater concentrate and dried to a final water content of 6 to 10%. The normal range of WSP in “whole” meal is 20 to 30% of the total protein content, although levels above 35% can be observed in some cases (Oterhals *et al.* 2001). The temperature in the drying material should be kept low and not exceed 70 °C if high quality fishmeal is the target. Higher temperature may damage the nutritional value of the meal (Aksnes & Mundheim 1997). Different types of dryers are used, both directly and indirectly heated. The types most

commonly used in Norway are indirect steam dryers and hot air rotary dryers. The indirect steam dryers are mostly used as pre-dryers because of the higher heat load on the product due to the high surface temperatures within the dryers (Flesland *et al.* 2000). Other dryers used are vacuum dryers and flash type dryers. Downstream the drier, the fishmeal is ground typically with the use of a hammer mill before storage.

The resulting physical properties of the fishmeal powder are dependent on species and type and combination of dryers and are important for the handling, storage and production of feed (Flesland *et al.* 2000; Paper I and II). In Paper I and II fishmeal from herring (*Clupea harengus*, FMH) and sand eel (*Ammodytes tobianus* and *Ammodytes marinus*, FMSE) were produced by applying different drying technology (Table 1) and flow-figure and bulk density were measured. These properties could be interpreted as an indirect measure of friction forces between the fishmeal particles. Flow-figure and loose bulk density show a significant negative correlation and for FMH reported in Paper I, the main impact on these properties was related to the type and combination of dryers (Fig. 3; Samuelsen, Nofima, unpublished results). Fishmeal with lowest friction forces (low value of flow-figure and high value of loose bulk density) were produced on a Hetland indirect hot air dryer and the fishmeal with highest friction forces was produced on a Jäckering Ultra-rotor mill dryer (flash dryer). In between were combinations of steam pre-dryer and final air/vacuum dryers with the increasing friction forces in the order Hetland indirect hot air dryer < Dyno-Jet indirect hot air dryer < indirect vacuum dryer. The findings are consistent with Flesland *et al.* (2000) and Høstmark *et al.* (2001). Differences in the relationship between flow-figure and bulk density were found for FMSE compared to FMH (Paper II). Comparing fishmeal from different species, these properties will therefore give an inaccurate measure of particle friction forces.

Table 1. The independent fishmeal batches used in Paper I and II

Batch#	Species	Preservation method[1]	Factory	Drying technology	Screen aperture (mm)[2]	Paper
1	Herring	Ice	C	SD+V	8	I
2	Herring	Ice	C	SD+V	2	I
3	Herring	Ice	B	SD+DJ	5	I
4	Herring	Ice	B	SD+DJ	2	I
5	Herring	Ice	B	FD	-[3]	I
6	Herring	Ice	B	SD+DJ	5	I
7	Herring	Ice	A	H	5	I, II
8	Herring	Ice	A	H	5	I, II
9	Herring	Ice	A	H	5	I, II
10	Herring	Ice	A	SD+H	5	I, II
11	Herring	Ice	A	SD+H	5	I, II
12	Herring	Ice	A	SD+H	5	I, II
13	Herring	Ice	B	DJ	5	I
14	Herring	Ice	B	DJ	5	I
15	Herring	Ice	B	DJ	5	I
16	Sand eel	Unknown	A	SD+H	6	II
17	Sand eel	Unknown	A	SD+H	6	II
18	Sand eel	RSW	A	SD+H	6	II
19	Sand eel	RSF	A	SD+H	6	II
20	Sand eel	Ice	A	SD+H	6	II

DJ, Dyno-Jet indirect air dryer (Stord International A/S, Bergen, Norway); FD, flash dryer (Ultra-rotor mill dryer, Altenburger Maschinen Jäckering GmbH, Hamm, Germany); H, Hetland indirect air dryer (Kværner Hetland A/S, Bryne, Norway); RSF, refrigeration by fresh water; RSW, refrigeration by seawater; SD+, indirect steam dryer used as pre-dryer; V, indirect vacuum dryer (Stord International A/S, Bergen, Norway).

[1] Acetic acid not used.

[2] Ground in hammer mill (Jesma-Matador AS, Vejle, Denmark).

[3] Ground directly during the drying operation.

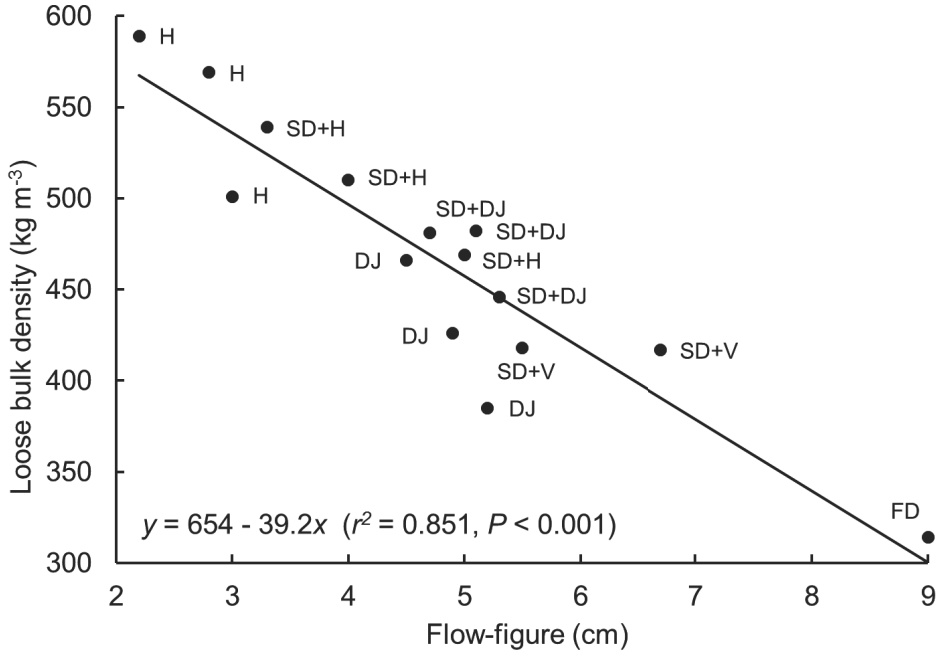


Figure 3. Relationship between flow-figure and loose bulk density for herring meal dried at different types or combination of dryers (Samuelsen, Nofima, unpublished results). Abbreviation, see Table 1.

3.1.3 Fishmeal quality

As outlined above fishmeal chemical composition, physical properties and nutritional value are all influenced by raw material type and freshness, seasonal variations and applied process conditions and dryer type. The two main fishmeal qualities used in Norwegian aquaculture feed production are NorSeaMink and the high quality Norse-LT 94 (Table 2), or other fishmeal on the world commodity market with similar specifications (Schmidtsdorff 1995). The specifications are based on a limited set of chemical and biological analysis (Table 2) and give to a less degree relevant information about the technical properties of the fishmeal (Paper I and II).

Table 2. Specification for NorSeaMink and Norse-LT 94 (Norsildmel 2015)

	NorSeaMink	Norse-LT 94
Crude protein (%)	Min. -, Typical 71	Min. 68, Typical 71
Water-soluble protein (% of crude protein)	-	Max. 32, min. 18
Moisture (%)	Max.10, min. 5	Max.10, min. 6
Fat (Soxhlet) (%)	Max. 13	Max. 13
Ash, without salt (%)	Max. 14	Max. 14
Salt (sodium chloride) (%)	Max. 4	Max. 4
Total volatile nitrogen (%)	Max. 0.20	Max. 0.18
Cadaverine (g kg^{-1})	Max. 1.8	Max. 1.0
Histamine (g kg^{-1})	Max. 0.7	Max. 0.5

3.2 Fish feed extrusion process

The use of extruders for food processing was developed between 1930 and 1940 with expanding number of applications in 1960s and 1970s. Extruders was introduced to fish feed processing in the early 1980s because of their high capacity, high mixing and kneading capabilities and their possibilities of manufacturing high quality feeds with target density specifications and high lipid levels (Hilton *et al.* 1981; Oliveira 1990; Huber 2000). The fish feed extrusion process (Fig. 4) is used globally and is fairly standardized. The process involves use of moisture and high temperature achieved by water/steam injection and mechanical energy dissipation to obtain acceptable physical product quality and density specifications.

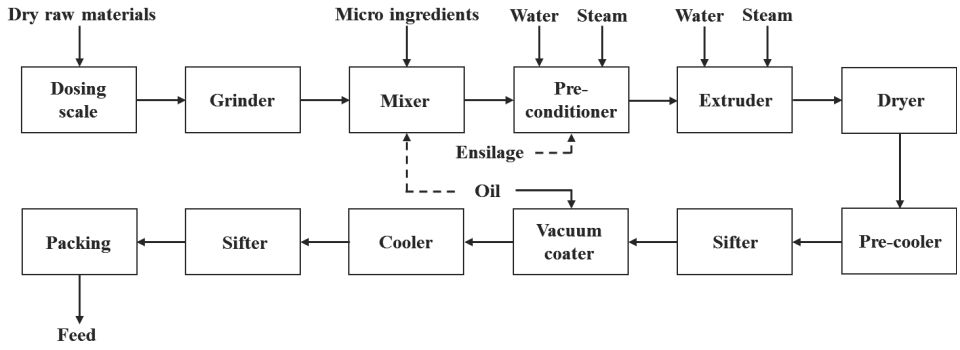


Figure 4. Simplified flow diagram of the fish feed extrusion process.

3.2.1 Feed ingredients

Extruded salmon feed consists of protein, starch and lipids as the major ingredients. In Norwegian aquaculture, proteins comes from fishmeal and various vegetable sources as soybean, sunflower, pea, beans, wheat and corn and in near future also lupins, canola and distiller dried grains with solubles (Glencross *et al.* 2007; Sørensen *et al*. 2011). The major source for starch is whole wheat, but other sources such as pea, potato, manioc (tapioca) and corn can be used (Rokey 1994; Sørensen *et al*. 2011). The lipids are mainly based on a blend of fish and rapeseed oil. Small amounts of soybean- and palm oil may also be added (Sørensen *et al*. 2011). The feed consists also of micro ingredients such as vitamins, minerals and amino acids. Ensilage or other fish protein concentrates may also be added. A typical ingredient composition of a Norwegian aquaculture feed is given in Table 3.

Table 3. Percent of ingredients used in a Norwegian aquaculture feed based on information from there feed companies in 2013 (Ytrestøyl *et al*. 2014)

Ingredient source	Percent used
Fishmeal	18.0
Krill meal	0.3
Plant protein	36.7
Starch	11.2
Marine oil	10.9
Plant oil	19.2
Micro ingredients	3.7

3.2.2 Unit operations in fish feed extrusion processing

A general flow diagram of the fish feed extrusion process is given in Fig. 4. The main unit operations are explained based on Harper (1989); Frame (1994); Guy (1994); Rokey (1994); Huber (2000); Strahm (2000) and Riaz & Rokey (2012).

Dosing and grinding

Major ingredients (protein and starch) are dosed to a grinder. In the grinder, usually a hammer mill, the particle size is reduced to <1 mm with use of a screen aperture at

1.5 to 1.0 mm. Grinding is advantageous. Uniform and small particles prevents segregation during mixing with micro ingredients, and small particles are easier to hydrate and will heat up more quickly than coarser particles in the preconditioner. It is also possible to add lipids (oil) in the mixing step but in a limited amount. Lipids will prevent water uptake to the powdery raw material and act as lubricants in the system. This will affect feed melt homogeneity and lower the viscous dissipation in the extruder, with poor physical feed quality as the result. As a rule of thumb it is possible to add up to 12% of total fat in the feed mix with limited effect on feed quality (Rokey 1994).

Preconditioning

Due to the low residence time in the extruder (<1 min) the feed mix is conditioned prior to extrusion in a preconditioner by use of steam and water, and with a typical residence time of 1.5 to 4 min. In the preconditioner the feed particles are hydrated and heated to a typical moisture content of 18 to 30% and a temperature of 77 to 95 °C (Rokey 1994; Strahm 2000; Riaz & Rokey 2012). Both hydration and temperature increase are time dependent and determined by particle size, water diffusivity and heat capacity. A preconditioner also have high mixing capabilities and fish ensilage or fish protein concentrates can be added in this stage. Ensilage and concentrates have high water content (typical range 60-70%) and addition restricts the use of moisture for controlling the extrusion process, pellet expansion and oil adsorption capacity (Samuelsen, Nofima unpublished results). There are different types of preconditioners on the marked. In Paper I, II and III an atmospheric double differential preconditioner (DDC; Fig 5; Wenger Manufacturing Inc., Sabetha, KS, USA) was used. The DDC is a double shafted counter rotating conditioner where the shafts have different dimensions and are run at different speeds to maintain dynamic mixing and long retention time.

Extrusion

Extrusion is the key process in feed manufacturing, and the physical product quality as well as pellet expansion and oil adsorption capacity are defined in this unit. In the extruder barrel the feed mix is cooked by mechanical energy dissipated into heat

(internal energy) and the addition of water and/or steam. During this treatment the mix is transformed into a plasticized and flowable material (melt) that can be shaped through a die and cut into pellets.

Fig. 5 illustrates an extruder set up for fish feed processing. The dry feed mix is transported with use of a screw feeder from a bin to the preconditioner. The conditioned feed mix is then fed to the extruder. The most commonly used extruders for fish feed processing are single screw or co-rotating, fully intermeshing twin-screw extruders. A single screw extruder is easier to operate, cost about half that of a twin-screw extruder and has lower maintenance costs, whereas a twin-screw extruder is more flexible in use (handles viscous, sticky, oily and wet materials), has higher mixing capacity and better heat transfer and is self-cleaning. In Paper I, II and III a TX-52 co-rotating, fully intermeshing twin-screw extruder (Wenger Manufacturing Inc., Sabetha, KS, USA) was used. A typical twin-screw design for fish feed processing consists of conveying sections, kneading sections (kneading elements and/or reverse screws; Della Valle *et al.* 1993) and a final cooking section (cone final screws). The screws in the conveying sections are partly filled with resulting insignificant dissipation of mechanical work on the feed mix. The screw filling takes place in the kneading sections because of reduced conveying capacity and backflow. As the resistance increases the feed is compacted and transformed at a rate depending on the mechanical energy input. Interchangeable dies restrict the extruder discharge, and shape and texturize the final product. A cutting device cut the product to the desired length by controlling the knife speed. The temperature upstream the extruder die during fish feed production is typically in the range of 120 to 145 °C (Sørensen *et al.* 2009; Sørensen *et al.* 2010; Paper III). The extrusion process is mainly operated by adjusting the water and steam level, feed rate and screw speed. The adjustments influence residence time, filling rate, specific mechanical energy (SME), temperature and pressure upstream the extruder die and control the physical product quality, pellet expansion and oil adsorption capacity (Meuser *et al.* 1984; Della Valle *et al*. 1989; Paper III). Other on-line systems have been introduced as pellet density control systems based on vented head (Munz 2004), back-pressure (Hauck & Wenger 2004)

and pressure chamber regulation (Oddsen *et al*. 2000; Hauck & Wenger 2004) at extruder outlet, and SME regulation based on mid-barrel restriction (Rokey & Plattner 2009). These have improved the adjustment possibilities.

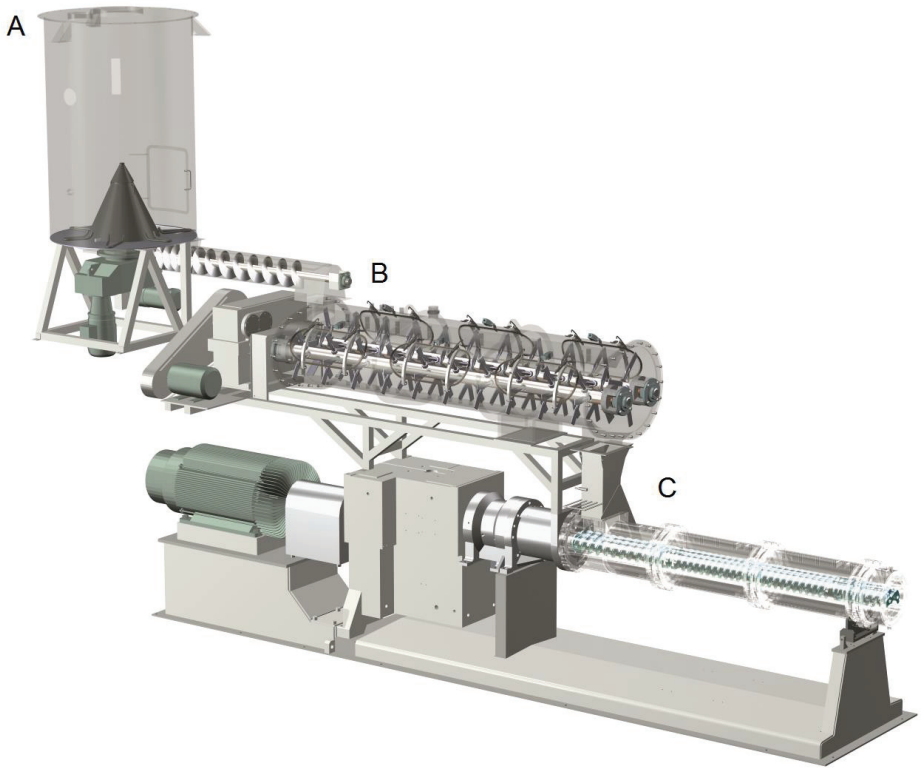


Figure 5. A cross-sectional view of an extrusion cooking system. (A) Raw material bin, (B) Atmospheric double differential preconditioner and (C) Extruder barrel (with permission from Wenger).

Drying

The wet extrudate has to be dried to prevent mould and bacteria growth and to fix the final porous structure and physical quality. The extrudate has water content of approximately 18 to 30% (Rokey 1994; Sørensen 2012) and is dried to around 8% (Sørensen 2012). The most commonly used dryers in fish feed processing is conveyor dryers (single pass, multi pass, two stage or multi stage) where the air flows transversely through the product bed in separate zones with the lowest air temperature

in the outlet zone. Other used dryers are carousel dryers where hot air enters through the bottom against the product flow. In Paper I and II a dual layer carousel dryer (Model 200.2; Paul Klöckner GmbH, Nistertal, Germany) was used. Product depth, air flow, temperature, humidity and residence time can be adjusted to suit the product characteristics. The drying step represents around 65% of the total energy consumption in the feed extrusion process. Due to higher moisture level during processing of plant based diets these requires more drying (about 30%) compared to fish meal based diets (Draganovic 2013; Draganovic *et al*. 2013).

Vacuum coating

Prior to vacuum coating, the feed is pre-cooled and sifted to prevent evaporation and dust accumulation in the coater. Most of the oil (lipids) is added in the vacuum coater. In this unit the air is withdrawn from the dry pellets before adding oil into the coater. After a predetermined mixing time the air is slowly released back in order to let the oil be drawn into the porous pellet structure (Strauch 2005).

Cooling and packaging

After vacuum coating the finished feed (Fig. 6) is cooled and sifted to prevent evaporation and dust during packaging.

Figure 6. Extruded fish feed pellet after vacuum coating of oil (Frank Gregersen, Nofima).

3.2.3 Analytical methods used to assess physical feed quality

Requirements for physical salmon feed quality are given in chapter 2 and can be assessed by different measuring techniques:

Product loss:

Product loss is due to attrition of the feed pellets, which generates particles and dust (Aarseth *et al.* 2006). Attrition of feed pellets comprises two phenomena, fragmentation and abrasion. Fragmentation involves the fracture of pellets into smaller particles and fines at the fracture area and abrasion involves the fracture on the edges of particles (Aarseth *et al.* 2006). Hardness is determined by using equipment that measures the force needed to fragment the pellet (fragmentation). Durability is measured by instruments that measures mechanical resistance (abrasion) or pneumatic resistance (fragmentation and abrasion) (Thomas & van der Poel 1996). In a hardness tester the peak breaking force is measured. Different equipment is used, such as texture analysers with different probes (knife or flat ended) for individual pellets (standing or laying) and the Kramer shear press for multiple pellets (Thomas & van der Poel 1996; Sørensen *et al.* 2012). Pneumatic resistance is measured in a pneumatic durability tester were pellets is conveyed by high velocity air for a predetermined time in a closed circuit or around a perforated chamber. After the test cycle, the weight-percentage of pellets collected on a screen (about 0.8 times the pellet diameter; Sørensen 2012) is measured, and durability expressed as the percentage retained. In a newly developed device, the DORIS tester (AKVAsmart, Bryne, Norway), pellets are transported in a screw conveyor to a rotating fan. Impact with the fan and the walls downstream the fan generates cracks and fines which are measured using different screen sizes (Aas *et al.* 2011). Hardness and pneumatic durability tests are well suited to evaluate differences in physical quality of extruded fish feed (Sørensen *et al.* 2010).

Water stability

Water stability can be measured as described in the study of Baeverfjord *et al*. (2006). Feed samples are placed in steel-mesh buckets inside glass beakers filled with water.

The beakers are shaken in a thermostatted water bath for a predetermined time and the remaining amount of dry matter (DM) is measured.

Expansion parameters

Expansion parameters are important to control sinking properties and oil adsorption capacity. Bulk density is usually measured by loose pouring of pellets from a funnel into a measuring cylinder. Pellet size (length and diameter) can be measured with use of an electronic calliper.

Oil adsorption capacity and leakage

Oil adsorption capacity can be investigated in a lab-scale vacuum coater using the same principle as for a full scale coater. If maximum oil adsorption capacity is the target, oil has to be in excess amount in the coater. Oil leakage can be measured in a plastic box with blotting paper incubated at predetermined temperature and time.

Sinking velocity

Sinking velocity can be measured in a transparent pipe filled with saline water with a given temperature. A stopwatch can be used to measure the time it takes for a pellet to sink a predetermined distance.

The different methods used for measuring physical feed quality, expansion, oil adsorption capacity and oil leakage are reviewed and discussed in detail in Sørensen (2012). It can be concluded that reported measurements of feed quality parameters in published studies are inconsistent in type of equipment used, methodology and the implementation of the methods, and also with conflicting results (Sørensen 2012; Oehme 2013). The feed producers also use different methods and different target values in their product quality control, indicating a need to standardize the different methods used to evaluate fish feed

4. Physicochemical properties of ingredients

4.1 Starches

Starch acts as a binder and gives network structure, strength, elasticity and expansion to the finished feed product (Colonna *et al*. 1989). Starch is composed of linked glucose molecules in the form of amylose and amylopectin. Amylose is a linear and amylopectin a highly branched molecule (Southgate 1991; Appelqvist & Debet 1997; Liu 2005). Different starch sources have different ratios of the two types of molecules, which affect the rheological behaviour of the starch and the properties of the end product (Colonna *et al*. 1989; Liu 2005; Xie *et al.* 2009).

Gelatinization

Native starch granules exist in an amorphous and partially crystalline state. Gelatinization of starch is a phenomenon associated with the disruption of the granular starch structure, hydration and swelling, solubilisation of starch molecules and formation of new molecular aggregate structures by hydrogen bonding during cooling (Appelqvist & Debet 1997; Liu 2005). The gelatinization process results in a rapid increase in viscosity (Appelqvist & Debet 1997; Liu 2005; Tan *et al.* 2008). When starch is heated in excess amounts of water (above 60%, wet basis) gelatinization takes place at a temperature range of about 60 to 75 °C depending on type of starch. At water content lower than 60% (wet basis) gelatinization or melting takes place at increasing temperatures with decreasing moisture, reaching a temperature at about 100 to 175 °C at the moisture content used in extrusion processing (Wang *et al*. 1992).

Dextrinization

During extrusion, dextrinization of starch molecules can occur, which may have a negative impact on physical feed quality. Dextrinization is a process that reduces starch molecules to smaller fragments (dextrins). Enzymatic dextrinization depends on the α-amylase concentration in the wheat. Native α-amylase is inactivated at temperatures >60 °C (Southgate 1991) and would not survive in the extrusion process

as shown in the study of Chouvel *et al.* (1983) and Vasanthan *et al.* (2001). Thermomechanical treatment may also lead to macromolecular degradation of starch (Vergnes *et al.* 1987; van den Einde *et al.* 2004). The degradation pattern depends on both the maximal shear stress in the system and the achieved feed melt temperature (van den Einde *et al.* 2004).

Retrogradation

Gelatinized starch can undergo structure transformation as reassociation and recrystallization upon storage, which change the texture and physical properties of the product (retrogradation). The transformation pattern is not clearly understood and is dependent on several factors. These can be starch source, amylose/amylopectin ratio, molecular chain length and distribution, pH, presences of other biopolymers, processing temperature, cooling regime and storage conditions (Appelqvist & Debet 1997; Liu 2005). It is therefore of great importance in technical extrusion studies to standardize drying conditions, storage conditions and storage time before performing physical measurements on the feed pellets.

Complex formation

Amylose-lipid complexes can also be formed during extrusion, which also affects expansion and bulk density (Bhatnagar & Hanna 1994). Amount of starch that can complex with lipids depends on the processing conditions and type of starch and lipid with monoglycerides and free fatty acids being more active than triglycerides (Bhatnagar & Hanna 1994).

4.2 Proteins

Proteins will also contribute to network structure and pellet strength. The protein biopolymers are formed essentially from 20 primary amino acids resulting in many possibilities of sequential arrangements with a wide range of interactions and possible chemical reactions (Hernandez-Izquierdo & Krochta 2008). Consequently, extrusion of proteins is recognized as more complex compared to extrusion of starches. In the extrusion process the protein biopolymers undergo both physical and chemical

changes as denaturation, association, associate disruption, bond formation (both covalent and non-covalent) and final transition to a rigid and expanded porous structure upon drying and cooling. These changes are all dependent on processing conditions, moisture content, pH, ionic strength, heating -and shear rate (Rhee *et al.* 1981; Simonsky & Stanley 1982; Stanley 1989; Dahl & Vilotta 1991; Mitchell & Arêas 1992; Roos 1992; Sheinerman *et al.* 2000; Schreiber 2002).

Denaturation

Denaturation of protein is a transition where the arrangement of the polypeptide chains within the molecule is changed from a native, folded structure to a more disordered and unfolded arrangement. The protein fraction in food and feed materials is composed of a large number of proteins and will exhibit several denaturation temperatures, typically in the range of 60 to 130 °C. As for starches, these temperatures are reduced with addition of water (Roos 1992).

Association and disruption of associates

After denaturation new protein-protein complexes are developed by electrostatic and hydrophobic forces (associates; Sheinerman *et al.* 2000; Schreiber 2002). The associates disrupt by heat and shear in the extruder and form a biopolymer melt (Mitchell & Arêas 1992). Fishmeal, because of the heat treatment during processing, is composed of denatured and associated proteins.

Interaction, bond formation and texturization

Both covalent bonds and non-covalent intermolecular interactions can be formed during extrusion. The different states of the proteins, including the prevalence of disulphide and covalent bonds, can be determined semiquantitatively by use of a combination of a buffer, urea and disulphide cleaving agents (Hager 1984). Based on a study on soya protein concentrate, Hager (1984) concluded that extrusion at temperatures <150 °C formed structured protein consisting primarily of covalent intermolecular disulphide bridging accompanied by intermolecular interactions (hydrogen-, ionic bond, and hydrophobic interactions). This is also confirmed in the study of Stanley (1989) and Mitchell & Arêas (1992) and assumed valid for fish feed extrusion (120 to 145 °C). The achieved cross-linked binding network is then locked

upon drying and cooling of the extrudate (Rhee *et al.* 1981; Stanley 1989; Mitchell & Arêas 1992).

Maillard reactions

Maillard reactions can occur between the carbonyl groups of the reducing sugars and amine groups of the amino acids during extrusion. Maillard compounds are intermolecular cross-linked products that may be texture promoting (Stanley 1989) but with a negative nutritional effect due to loss of e.g. lysine (Björck *et al*. 1984; Cheftel 1986; Opstvedt *et al*. 2003). In Paper I, II and III, baking quality whole wheat flour was used (falling number >200; Hagberg 1961) to secure low amount of reducing sugars and to minimize possible Maillard reactions.

4.3 Binders

To meet target physical feed quality, manufactures may incorporate binders in their diets. There are several different binders on the marked (Thomas *et al.* 1998; Sørensen *et al.* 2011) e.g. starches from different plant sources, modified starches, lignin sulfonate, synthetic binders and gluten based binders. Except for gluten and to some extent starch, they all have low to zero nutritional value.

In the work on developing a protein based marine binder with high nutritional value (Samuelsen & Oterhals 2000) a competitor analysis was performed (Samuelsen, Nofima, unpublished results) with different binders incorporated in a control feed. The feed was composed of 450 g kg^{-1} DM protein, 120 g kg^{-1} DM carbohydrate, 320 g kg^{-1} DM lipids and 111 g kg^{-1} DM ash. The ingredients used were blue whiting fish meal (Norse-LT 94; Norsildmel AS, Bergen, Norway), baking quality whole wheat flour (Norgesmøllene AS, Vaksdal, Norway) and fish oil (NorSalmOil; Norsildmel AS). The binders (Table 4) where incorporated in the feed at the levels given in Table 4.

Table 4: Type of binders and levels used in the competitor analysis

Type of binder	Level 1 [1] g kg^{-1}	Level 2[1] g kg^{-1}
Tapioca	20[2]	50[2]
Modified potato starch	20[2]	50[2]
Modified corn starch	20[2]	50[2]
Spray dried water-soluble protein	50[3]	
Corn gluten	50[3]	
Wheat gluten	50[3]	
Lignin sulfonate	5[2,4]	10[2,4]
Synthetic (polyvinylpyrrolidone)	10[2,4]	20[2,4]

[1] g kg^{-1} of binder based on finished feed (300 g kg^{-1} lipid and 70 g kg^{-1} moisture on wet basis).

[2] Whole wheat flour reduced and replaced with chosen level of the starch based, lignin sulfonate or synthetic binder.

[3] Fishmeal reduced and replaced with equivalent amount of protein based binder. The binders are digestible and only one level was used.

[4] Lignin sulfonate and synthetic binder are non-digestible and the recommended levels given from the suppliers are used.

The marine binder was based on a spray-dried WSP fraction from herring. Each of the 14 feed mixes where processed with standardized extrusion, drying and coating conditions by use of the same processing equipment and extruder screw profile as used in Paper I. The calibrated feed rate was 150 kg h^{-1} with a standardized moisture level at 192 g kg^{-1} (wet basis) in the DDC and 260 g kg^{-1} (wet basis) in the extruder. The wet extrudates were dried at 80 °C to approximate 70 g kg^{-1} (wet basis) and coated with NorSalmOil to approximate 300 g kg^{-1} (wet basis) prior to measurements of durability and hardness as defined in chapter 7.3.2.

Fig. 7 shows the results from the competitor analysis. In the hardness vs. durability plot (Fig. 7) the potential of a WSP based binder is shown with an effect near that of modified corn starch and the synthetic binder (polyvinylpyrrolidone), but slightly lower than modified potato starch, tapioca (50 g kg^{-1}) and lignin sulfonate. Of the three protein based binders (WSP, corn and wheat gluten) WSP gave the best result relative to the control diet. There is a linear relationship between the two physical

quality tests (r^2 = 0.690, P < 0.001) with only 20 g kg^{-1} tapioca and 10 g kg^{-1} lignin sulfonate deviating from the straight line.

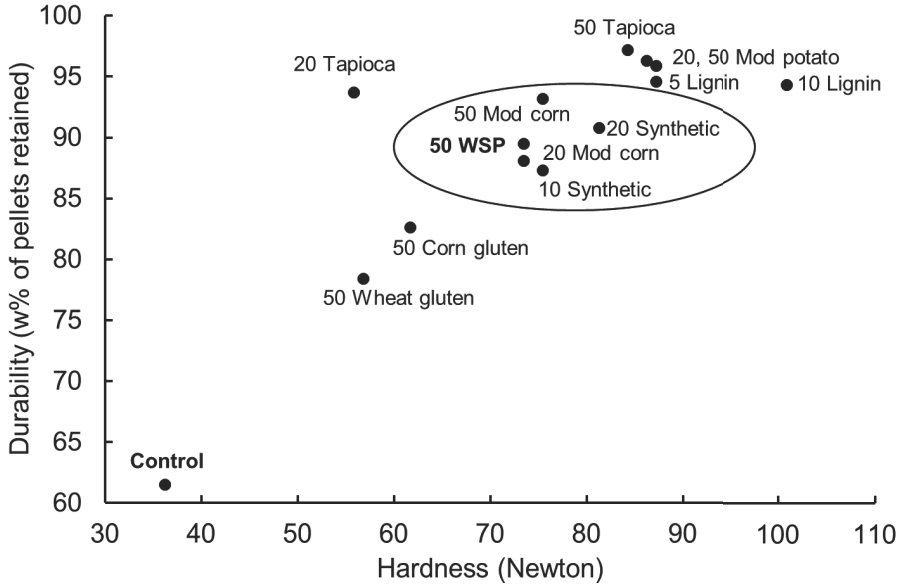


Figure 7: Hardness vs. durability for the control feed (Control) and 13 feeds with different types and levels of binders. Markers represent level (g kg^{-1}) and type of binder. The circle marker encloses binders comparable with spray-dried water-soluble protein (WSP). Lignin, lignin sulfonate; Mod corn, modified corn starch; Mod potato, modified potato starch; Synthetic, polyvinylpyrrolidone.

An important outcome for the scientific work presented in this thesis is to establish plausible explanations for the underlying mechanisms behind the binding effect of WSP.

5. Phase transitions and plasticizers

5.1 Phase transitions

The glass transition of an amorphous solid is a temperature range where the solid transits from a brittle glassy to a soft rubbery state. The transition is a function of temperature, time, molecular weight, composition, water activity and moisture content. During the transition a smooth change in heat capacity and coefficient of expansion is observed (Abiad *et al.* 2009). The glass transition temperature (T_g) is most often defined as the inflection or midpoint of the transition range, but sometimes the onset temperature where the change start is used (Abiad *et al.* 2009). The rubbery polymer reaches a state where it can be considered as a highly viscous melt when heated above T_g. The melt or flow-starting temperature (T_f) can be defined as the temperature where a melt starts to flow through a capillary die at a constant pressure (Fujio *et al.* 1991).

5.2 Plasticizers

The extrusion process involves plasticization of the biopolymers into a flowable melt and establishment of new intermolecular bindings in the biopolymer matrix. To reduce the plasticization temperature, which will increase flowability and cooking efficiency a plasticizer is added. A plasticizer is a low molecular weight compound incorporated into an amorphous solid with the aim to depress both T_g and T_f by 1) increasing the free volume and freedom for motion of polymer molecules, 2) disruption of polymer-polymer interactions forces and 3) lubricating to facilitate movements of the macromolecules (Abiad *et al.* 2009; Cuq *et al.* 1998; Fujio *et al.* 1991; Igura *et al*. 1997; di Gioia & Guilbert 1999). In the extrusion process the added plasticizers interpose themselves between the biopolymers at elevated temperatures transforming the mix from a rubbery state ($>T_g$) to a free flowing melt ($\geq T_f$) upstream the extruder die. The wet soft and rubbery extrudate leaving the extruder is then dried and cooled down below T_g to a hard crushable product. The most important

plasticizer in food and feed systems is water (Roos 1995), which is widely used and studied in the extrusion process (Meuser *et al.* 1984; Bhattacharya & Hanna 1987; Alvarez-Martinez *et al.* 1988; Della Valle *et al.* 1989; Wang *et al.* 1992; Akdogan 1996; Blanche & Sun 2004). Other common plasticizers that can be of hydrophilic, hydrophobic or amphiphilic nature include polyols (Pouplin *et al*. 1999), sugars (Carvalho & Mitchell 2001), organic acids (Pommet *et al*. 2005), fatty acids (Pommet *et al*. 2003; di Gioia & Guilbert 1999), amines (Irissin-Mangata *et al*. 2001) and mono-diglyceride esters (di Gioia & Guilbert 1999). They have been studied in relation to bio-plastic formulations, but none of these are to our knowledge used in the feed industry, due to lack of nutritional value or formulation constraints. Amino acids in combination with glycerol are found to plasticize starch-based biodegradable plastics (Stein & Greene 1997; Stein *et al.* 1999). The plasticizing effect of fishmeal water solubles was documented in Paper III and IV, with a significant reduction of both T_g and T_f in feed extrudates and fishmeal.

5.3 Models for prediction of the glass transition temperature

A feed mix consists of a mixture of polymers with different sequential arrangements, a wide range of interactions and molecular sizes and may exhibit one or several glass transition temperatures or a broad glass transition range. Several models and equations have been proposed to predict T_g in such systems (Abiad *et al.* 2009).

The glass transition of a binary amorphous polymer mixture can be described by the Gordon-Taylor equation (Gordon & Taylor 1952):

$$T_g = \frac{x_1 \times T_{g1} + K \times x_2 \times T_{g2}}{x_1 + K \times x_2} \quad (1)$$

where T_g is the glass transition temperature of the mixture, x_i is the weight fraction and T_{gi} is the glass transition temperature of the component i (1 = diluent or plasticizer and 2 = amorphous polymer), and K is a function of the coefficient of expansion of the components as they change from the glassy to the rubbery state

(Bengoechea *et al*. 2007; Abiad *et al*. 2009). The equation is based on the assumption of ideal volume additivity and that the change in volume is linear. The plasticization effect of solubles addition to fishmeal can be predicted by use of this equation (Paper IV).

Couchman & Karasz (1978) proposed an equation to predict the transition temperature of amorphous polymer mixtures by the assumption that the transition is a thermodynamic effect:

$$T_g = \frac{x_1 \times \Delta C_{p1} \times T_{g1} + x_2 \times \Delta C_{p2} \times T_{g2}}{x_1 \times \Delta C_{p1} + x_2 \times \Delta C_{p2}} \quad (2)$$

where T_g is the glass transition temperature of the mixture, x_i is the molar fraction (or weight fraction), and T_{gi} is the glass transition temperature of the component *i* and ΔC_{pi} is the change in heat capacity (molar or weight) for component *i* at T_g (Rouilli *et al.* 2001; Abiad *et al.* 2009). The *K* value in Eq. 1 is equal to $\frac{\Delta C_{p2}}{\Delta C_{p1}}$, which gives Eq. 2 = Eq. 1 (Couchman & Karasz 1978). Exact ΔC_p values are difficult to obtain experimentally (Roos 1995).

5.4 Measurement techniques

Several techniques have been developed to measure T_g in amorphous polymers (Abiad *et al.* 2009) with differential scanning calorimetry as the most widely applied method. The principle for this method is that when a sample undergoes a phase change the sample requires more heat to increase its temperature at the same rate as a reference sample. This is due to the absorption of heat as it undergoes the endothermic phase transition from glass to rubber (Abiad *et al.* 2009; Kaletunç 2009). The heat capacity change (ΔC_p; Eq. 2) can be measured and T_g is defined as the midpoint/inflection point of this change (Rouilli *et al.* 2001; Bengoechea *et al*. 2007; Kaletunç 2009).

Most foods, feeds, dough and melted materials are both solids and liquids. They are called viscoelastic because they simultaneously exhibit some of the elastic properties

of an ideal solid and some of the flow properties of and ideal liquid (Schramm 2000; Bourne 2002). Dynamic mechanical analysis is a thermal analysis technique that measures the viscoelastic behaviour of materials as they are deformed under periodic stress during heating (Woo *et al.* 1994). The principle is that an oscillation sinusoidal strain deformation is applied and the resulting sinusoidal stress response is measured. The phase difference is combined with the amplitude of the stress and strain waves to determine material parameters as storage (*E'*, elastic) and loss (*E''*, viscous) modulus (Schramm 2000). Due to the increase in molecular mobility during the glass to rubber transition a sharp fall in *E'* and a maximum in *E''* is observed. T_g can then be defined as the temperature where the maximum in $\frac{E''}{E'}$ or *E"* is observed (Bengoechea *et al.* 2007).

An alternative method is closed-chamber capillary rheometry, which enables the measure of both T_g and T_f at elevated moisture levels and high pressure and temperatures (Fujio *et al*. 1991; Igura *et al*. 1997). The method reflects the softening of the material and resistance to flow through a die encountered in the extrusion process. The Phase Transition Analyzer (Wenger Manufacturing Inc., Sabetha, KS, USA; Strahm *et al*. 2000) is a closed-chamber capillary rheometer developed for this purpose (see chapter 7.3.3). As pressure has minor effect on T_g (Bianchi 1965; 1971) the technique gives T_g values consistent with the information obtained from differential scanning calorimetry and dynamic mechanical thermal analysis (Bengoechea *et al*. 2007). The Phase Transition Analyzer (PTA) was used to measure T_g and T_f in extrudates in Paper III and fishmeal in Paper IV.

6. Feed melt rheology

6.1 Melt viscosity

Viscosity (η) is the main measure of resistance to flow and can be expressed as:

$$\eta = \frac{\tau}{\dot{\gamma}} \quad (3)$$

Where τ is the shear stress and $\dot{\gamma}$ is the shear rate. If the viscosity is constant and independent of the shear rate it is defined as a Newtonian liquid. Melted biopolymers are non-Newtonian and show a pseudoplastic behaviour. It means that the viscosity decreases with an increasing shear rate (shear tinning). When a pseudoplastic melt is at rest it maintains a minimum energy state and will have a high resistance against flow (high viscosity). With increasing shear the molecules will orientate in parallel to the driving force allowing the molecules to slip past each other more easily i.e. viscosity is reduced (Schramm 2000). A pseudoplastic melt can be fitted to the power law model as shown in the following equation (Vergnes *et al.* 1987; Bourne 2002):

$$\eta = \frac{\tau}{\dot{\gamma}} = K\dot{\gamma}^{n-1} \quad (4)$$

Where *K* is the consistency index and n is the flow behaviour index (dimensionless). For shear thinning fluids, $0<n<1$, the closer to zero the more shear thinning is the fluid. If $n = 1$, constant viscosity is obtained (Newtonian liquid). Viscosity can be measured in molten materials by use of capillary rheometry (Vergnes *et al*. 1987; Schramm 2000; Paper IV). Calculations can be performed with or without corrections (true or apparent viscosity; apparent = calculations on a non-Newtonian fluid as it was a Newtonian liquid).

The viscosity of a polymer in the glassy state is above 10^{12} Pa s and is dramatically reduced during the transition from glass to rubber and further into a high viscous melt (Roos 1995). As an example, the apparent viscosity at T_f for fishmeal was estimated in the range of 3-8 x 10^5 Pa s (Paper IV). The temperature dependence of viscosity at

T_g to T_g+100 °C can be described by the Williams-Landel-Ferry (WLF) equation (Williams *et al.* 1955):

$$\log\left[\frac{\eta(T)}{\eta(T_g)}\right] = \frac{-C_1(T - T_g)}{C_2 + (T - T_g)} \quad (5)$$

where η(T) and $\eta(T_g)$ are viscosity at temperature T, and the selected reference temperature T_g, respectively. The parameters C_1 and C_2 are not universal for food systems and they are highly dependent on type of biopolymer and conditions such as moisture content and water activity (Roos 1995; Matveev *et al.* 1999; Yildiz & Kokini 2001; Abiad *et al.* 2009). For fishmeal the parameters, C_1 and C_2, were dependent on both moisture content and water solubles level (Paper IV).

6.2 Melt homogeneity

A low level of plasticizers and/or moisture and/or low SME in the extruder barrel may give improper cooking or transformation of the feed mix with particles still intact in the feed melt upstream the extruder die (Fig. 8). If the amount of the solid particles suspended in the fluid-like melt is high this will give improper flow and no final structure is possible (Arêas 1992). Lack of homogeneity is the major reason for the difficulty in understanding the rheology of biopolymer melts (Mitchell & Arêas 1992). Studies on heterogeneity in extrudates have been reported for starch rich systems. Farhat *et al.* (2003) extruded blends of amylopectin and sucrose at relatively low SME and temperature. They documented that these conditions resulted in non-homogenous melt within the extruder barrel that resulted in heterogeneous pellets. This is consistent with Chuang & Yeh (2004) who found that low degree of starch gelatinization resulted in the presence of particulates in the extrudate. In Paper I and II a negative effect on physical feed quality was observed with increased level of the non-soluble protein in fishmeal. This could be explained by a reduced level of efficient plasticizers (i.e., lack of fishmeal water solubles). One of the main reasons for the large variation in physical feed quality documented in Paper I and II can be the degree of unmelted solid particles in the extrudates, which depends both on the

water soluble/non-soluble protein ratio, the thermal properties of the non-soluble protein fraction and the amount of effective plasticizing compounds in the water soluble phase.

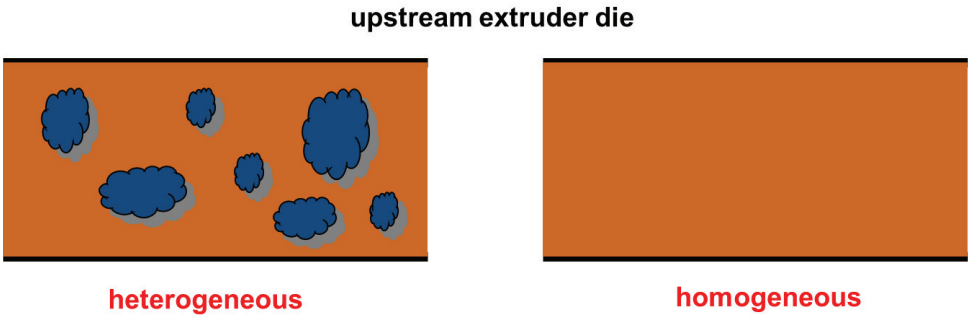


Figure 8: Improper cooked feed melt with particles to the left (heterogeneous). Homogeneous feed melt where all particles are transformed to the right (after Mitchell & Arêas 1992).

6.3 Specific mechanical energy

The SME (Wh kg^{-1} or kJ kg^{-1}) is a measure of the work input from the extruder motor to the material per unit mass and could be defined as (Akdogan 1996):

$$SME = \frac{net\ motor\ torque \times screw\ speed}{mass\ flow\ rate} \quad (6)$$

The energy is mainly converted into heat in the material through viscous dissipation and is the most important contribution to the energy input in the extruder barrel (Godavarti & Karwe 1997; Della Valle *et al*. 1989).

SME is a measure of the sum of the total mechanical energy dissipated over the total length of the screw (Akdogan 1996) and increases with increasing torque and/or screw speed and decreasing flow rate (Eq. 6). Motor torque can be increased by changing transport elements to elements that increases mixing and backflow (i.e.

reverse screw, kneading elements and cut flight screws) or by use of a mid-barrel restriction or back-pressure regulation (Della Valle *et al*. 1993; Hauck & Wenger 2004; Rokey & Plattner 2009). If screw design, screw speed and mass flow rate are standardized, an increase in motor torque and consequently an increase SME, reflects an increase in the viscosity of the feed mass (Bhattacharya & Hanna 1987; Akdogan 1996). Moisture and temperature are the two most important factors affecting viscosity, with decreasing values at increased moisture and temperature (Meuser *et al*. 1984; Bhattacharya & Hanna 1987; Della Valle *et al*. 1989; Akdogan 1996; Paper III). In Paper III it was shown that while increased moisture had a negative effect on SME, an increase in WSP gave the opposite effect, caused by the much higher viscosity of WSP compared to moisture and differences in biopolymer interactions. Biopolymers show pseudoplastic behaviour, and a lower *n* (Eq. 4) means higher viscosity reduction with increased shear. As the viscosity drops, the motor torque needed to rotate a segment of the screw will decrease, and the heat generated from this part of the screw will therefore be reduced. Opposite, biopolymers with high *n* are less shear thinning and will likely generate more heat through viscous dissipation. The studied fishmeal model system in Paper IV showed a large composition region of WSP and moisture with a higher difference between T_f and T_{gMid} than for other reported protein components (i.e. casein, gluten and soya protein isolate; Bengoechea *et al.* 2007). This indicates a reduced temperature effect on viscosity reduction in the rubbery phase for fishmeal in this region and will consequently also generate more heat in the extruder barrel. For starches or mixes containing starch based ingredients the melt viscosity increases with higher degree of starch gelatinization (pasting) with resulting positive contribution to SME (Appelqvist & Debet 1997; Liu 2005; Tan *et al*. 2008). The study reported in Paper I indicate that increased level of small and fibrous particles in the feed mix increased SME, probably due to increased particle to particle contact and friction.

6.4 Extrudate expansion

Expansion is an important parameter in fish feed extrusion. For high energy fish feed most of the oil has to be added to the texturized product after drying. This means that the product has to be expanded and porous enough to adsorb the correct amount of oil, but at the same time dense enough to sink (Fig. 9). The extrudate will expand both in a radial and longitudinal direction (Alvarez-Martinez *et al.* 1988). Since pellet length is controlled and defined by the knife cutting speed, radial expansion is the most important parameter in fish feed extrusion. Bulk density is negatively correlated to both radial and longitudinal expansion and to oil adsorption capacity (Alvarez-Martinez *et al.* 1988; Draganovic *et al.* 2011; Paper III).

Figure 9. Expanded porous fish feed pellet after drying (lower right) and further coating (upper left) (Jon-Are Berg-Jacobsen, Nofima).

The mechanism of expansion can be explained by the following: Upstream the extruder die, because of elevated temperature and pressure, moisture is in its liquid state in the feed melt. When leaving the extruder die the melt enters atmospheric conditions and steam flashes off. This process creates bubbles by nucleation of steam in the feed melt. Because of high steam pressure, the bubbles will rupture through the

cell walls and an open porous structure in the extrudate is formed. During the flash of steam there will be a sudden decrease in extrudate moisture and temperature, which will dramatically change the viscoelastic properties (Fan *et al.* 1994). As steam pressure is the main driving force for feed melt expansion, expansion can be increased with higher steam flashing rate, i.e. increase in temperature upstream the extruder die (T_{die}, Paper III). Expansion will also be controlled by viscoelastic properties, by the viscosity of the extrudate at die exit and by die configuration (Fan *et al.* 1994; Faller *et al.* 1995; Arhaliass *et al.* 2003).

Fan *et al*. (1994) proposed a model for extrudate expansion based on a combination of the power law model (Eq. 4) and the WLF equation (Eq. 5) and showed that cell wall movements and bubble growth can start to occur at 30 °C above T_g with typical shear rates of 10^{-2} to 5.0 s^{-1} and critical viscosity levels of 10^7 to 10^8 Pa s. Opposite, for fixation of structure the extrudate during cooling must reach a viscosity higher than the proposed critical viscosity range. Strahm *et al.* (2000) have used PTA data to explain extrudate expansion, fixation or collapse of structure in starch based extrudates. They found a linear relationship between expansion and the temperature difference between T_{die} and T_f, with the exception of some samples where the structure collapsed. The linear relationship could be explained by a larger driving force for expansion with higher temperature difference and cooling to a temperature below T_f at the extruder die exit for fixation of structure. Structure collapse was explained by a moisture level and temperature in the expanded extrudate near or above T_f. Blanche & Sun (2004) observed a negatively linear relationship between bulk density and the temperature difference between T_{die} and moisturized native starch melting temperature (measured by use of differential scanning calorimetry). However, the results were also affected by mechanical shearing. The apparent viscosity at T_f for fishmeal (3-8 x 10^5 Pa s at shear rates of 0.1 to 0.6 s^{-1}; Paper IV) is below the critical viscosity level for bubble growth and a negative relationship between bulk density and the temperature difference between T_{die} and T_f was also found for fish feed extrudates (Paper III). However, more information is needed to verify if such simplified expansion models can be used or not.

7. Experimental and analytical approaches

7.1 Production of experimental fishmeal batches

The fish meal batches used in Paper I were produced from fresh (TVN = 16 to 23 mg N 100 g^{-1}) Norwegian spring spawning herring (*Clupea harengus*) at three different fishmeal factories in Norway (A, B and C) in accordance with the specifications for high quality fish meal (Schmidtsdorff 1995). The factories were selected to cover the types of drying technology applied at Norwegian fishmeal factories including a large scale test facility based on flash-drying technology (Table 1, Høstmark *et al.* 2001). The content of WSP was adjusted by varying the ratio of press cake to stickwater concentrate prior to drying. In addition, at factory A, and used in Paper II, five independent fish meal batches were produced from fresh (TVN = 17 to 39 mg N 100 g^{-1}) sand eel (*Ammodytes tobianus* and *Ammodytes marinus*). The variation in WSP content for the five sand eel meals was caused by the natural variation of endogenous protease activity in the raw material due to different content of feed (zooplankton) in the stomach and gut. The batches were ground (Table 1) prior to analyses and feed mix preparation.

The fish meal batches used in Paper III and IV was based on blue whiting (*Micromesistius poutassou*) raw material caught by one fishing vessel and preserved on board by a combination of acetic acid addition (2 g kg^{-1}) and chilling by circulation of fresh/seawater mixture. Hot air dried press cake, hot air dried normal fishmeal and stickwater concentrate were obtained from a Norwegian fishmeal factory (D). The fish was of high and consistent quality (TVN = 33 to 34 mg N 100 g^{-1}) during the production period. For the experimental work in Paper III a third fishmeal with high WSP content was produced by mixing of stickwater concentrate in the normal fishmeal and thereafter dried in a Forberg FT-200 pilot scale air dryer (Forberg AS, Larvik, Norway). The three fishmeal batches were ground in a hammer mill (Jesma-Matador AS, Vejle, Denmark) to a particle size <1.00 mm prior to the preparation of the feed mixes. For the study in Paper IV, five experimental fishmeal samples were prepared in laboratory scale by addition of the stickwater concentrate to

the press cake meal in a kitchen blender, followed by drying in a hot air Retsch TG1 fluid bed dryer (Retsch GmbH, Haan, Germany) at 70 ± 3 °C. The fishmeal samples were thereafter ground on a Retsch ZM-1 Centrifugal Mill (Retsch GmbH, Haan, Germany) with ring sieve aperture 0.5 mm. Prior to measurements, the water content in the five experimental fishmeal samples was adjusted to fit a 2-factor central composite design as reported in Paper IV.

7.2 Production of experimental feeds

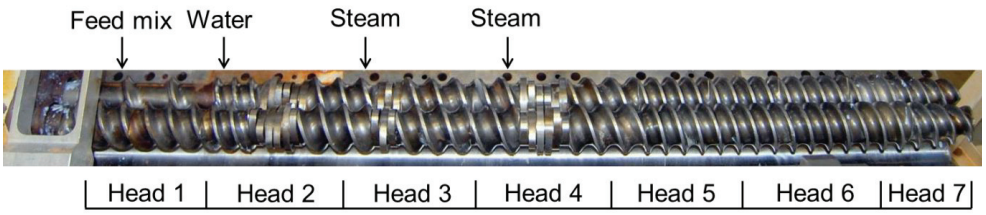
All production steps were performed at Nofima Feed Technology Centre in Bergen. The feed mixes were prepared and homogenized (30 min) using a horizontal mixer (Wolfking, William Jensen Maskinfabrik, Slagelse, Denmark). All powdery ingredients, oil (Paper I and II) and water (Paper III) were added in the mixing step. To secure even partitioning and adsorption into the feed matrix oil or water was sprayed homogeneously into the feed mixes at least 24 hours before processing. The feed mixes used in Paper I and II where processed according to pre-defined and standardized extrusion, drying and coating conditions developed with the purpose of ingredient screening. In Paper III a setup adapted for the production of high energy salmon feed was used. All feed mixes used in Paper I, II and III were calibrated to 150 kg h^{-1} prior to preconditioning in a DDC (Wenger Manufacturing Inc., Sabetha, KS, USA) followed by extrusion on a TX-52 co-rotating, fully intermeshing, pilot scale twin-screw extruder (Wenger; Fig. 10) as described in the respective papers. Two different extruder screw profiles were used and defined as the low shear profile (Fig. 11a, Table 5; Paper I and II) and the high shear profile (Fig. 11b, Table 5; Paper II and III). The differences of the profiles can be seen in head 6 and 7 where transport elements are replaced by reverse kneading elements and cut flight on the final cone screw (Fig 11, Table 5).

Figure 10. Rolf Egil Myrmel operating the Wenger extrusion system at Nofima Feed Technology Centre in Bergen.

Extruder torque was recorded during processing and SME (Paper I to III), specific thermal energy, throughput and moisture content behind the extruder die (Paper III) were calculated according to Riaz (2000) and by use of Wenger Extruder Analysis Software (Wenger).

In Paper I and II the wet extrudates leaving the extruder were dried in a pilot scale hot air dual layer carousel dryer (Model 200.2; Paul Klöckner GmbH, Nistertal, Germany) and coated with fish oil in a rotating coating reel (Model SU 145L; Susemihl GmbH, Neu-Ansprach, Germany). In Paper III the wet extrudates were dried in a laboratory scale fluid bed dryer (Model Retsch TG1; Retsch GmbH, Haan, Germany) prior to analysis.

a)



b)

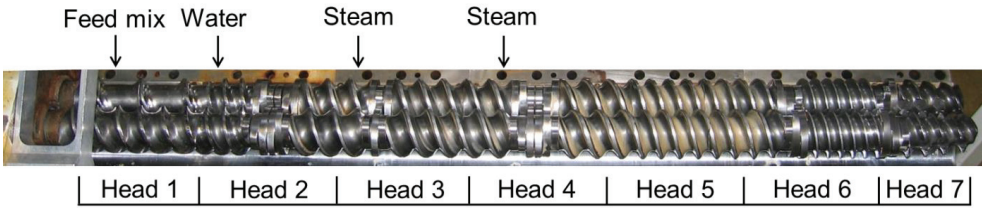


Figure 11: Used screw configurations a) low shear profile and b) high shear profile.

Table 5: Description of elements

Low shear profile		High shear profile	
Length[1] (mm)	Description of elements[2] (inlet to outlet)	Length[1] (mm)	Description of elements[2] (inlet to outlet)
156	Full pitch, single flight /full pitch, double flight	156	Full pitch, single flight /full pitch, double flight
52	¾ pitch, double flight	52	¾ pitch, double flight
52	Kneading, forward conveying	52	Kneading, forward conveying
78	Full pitch, double flight	78	Full pitch, double flight
26	Kneading, forward conveying	26	Kneading, forward conveying
130	Full pitch, double flight	130	Full pitch, double flight
52	Kneading, forward conveying	52	Kneading, forward conveying
78	Full pitch, double flight	78	Full pitch, double flight
286	¾ pitch, double flight	156	¾ pitch, double flight
73	¾ pitch, double flight, cone screw	26	Kneading, backward conveying
	-	78	½ pitch, doubled flight
	-	26	Kneading, backward conveying
	-	73	¾ pitch, double flight, cut flight, cone screw

[1] Total screw length is 983 mm.

[2] Screw diameter is 52 mm. Full pitch equals to 1 x diameter.

7.3 Applied analytical methods

7.3.1 Physical and chemical analyses

The following analytical methods have been applied to assess the physical properties and chemical composition of the materials studied in the respective papers.

Analytical method	**Reference**	**Paper**
Flow-figure	Høstmark 1985, Paper I	Paper I, II
Bulk density	ISO 5311, Paper I	Paper I, II
Oil adsorption capacity	Li & Lee 1996	Paper I, II
Water-holding capacity	Artz *et al.* 1990	Paper I, II
Particles size distribution	AOAC method 965.22, Paper I	Paper I, II
pH	Paper I	Paper I, II
Dry matter	ISO 6496	Paper I, II, III, IV
Water soluble dry matter	Paper IV	Paper IV
Crude protein (Kjeldahl/Dumas)	ISO 5983-2 / AOAC method 990.03	Paper I, II, III, IV
Water-soluble protein	Paper I and IV, ISO 5983-2	Paper I, II, III, IV
Peptide size distribution	Wang-Andersen & Haugsgjerd 2011, Paper I and IV	Paper I, II, IV
Degree of protein hydrolysis	Adler-Nissen 1979	Paper I, II
Total amino acid composition	Cohen & Michaud 1993	Paper IV
Cysteine and cystine	Cohen & Michaud 1993, Paper IV	Paper IV
Tryptophan	Miller 1967	Paper IV
Free amino acids	Bidlingmeyer *et al.* 1967	Paper IV
Lipid	AOCS method Ba 3-38	Paper I, II, III, IV
Lipid in stickwater concentrate	NS 9402	Paper IV
Total ash	ISO 5984	Paper I, II, III, IV
Salt (NaCl)	AOAC method 937.09	Paper I, II, III, IV
Total volatile nitrogen	AOAC method 920.03	Paper I, II, III, IV
Ammonia	Conway & Byrne 1933	Paper IV
Trimethylamine	Conway & Byrne 1933	Paper IV
Trimethylamine *N*-oxide	Conway & Byrne 1933	Paper IV

Biogenic amines (cadaverine, histamine, putrescine)	Mietz & Karmas 1978	Paper IV
Total starch and starch gelatinization	Chiang & Johnson (1977), Paper III	Paper I, II, III

7.3.2 Analyses of pellet properties

The following analytical methods have been applied to assess the extruded pellet properties.

Durability, hardness and cutting strength

Durability was measured on coated pellets in a Holmen pellet tester (Fig. 12; Holmen Feed Technology, Berkshire, UK) as described in Paper I. The test simulates pneumatic transport by conveying a pellet sample around in a closed circuit by a high velocity air stream for a predefined time. After the test cycle, the amount of pellets remaining is measured, and durability expressed as the weight-percentage of pellets retained. The method was used on pellets reported in Paper I and II.

Figure 12: Holmen pellet tester.

A hardness tester measures the mechanical resistance of the pellet. In Paper I and II, hardness was measured on coated pellets by use of a Pharma Test PTB 311 (Fig 13; Apparatebau AG, Hainburg, Germany). The measurement was performed on single laying pellets with the force applied on the diametrically side. In Paper III, hardness was measured on single standing pellets by use of a texture analyser (TA-HDi; Stable Micro Systems Ltd., Surrey, UK) equipped with a cylindrical flat-ended probe (Fig. 14) as described in Paper III. The measurement was performed on uncoated pellets. The texture analyser is a newer and more advanced instrument than the Pharma Test PTB 311 with several applications and possibility to use different probes, and it was therefore the preferred instrument for the study reported in Paper III. For both methods, pellets with curved ends were abraded carefully with sandpaper (P120) to a flat-ended cylindrical shape before measurements and the peak force before breakage was used as the value for hardness (expressed in Newton).

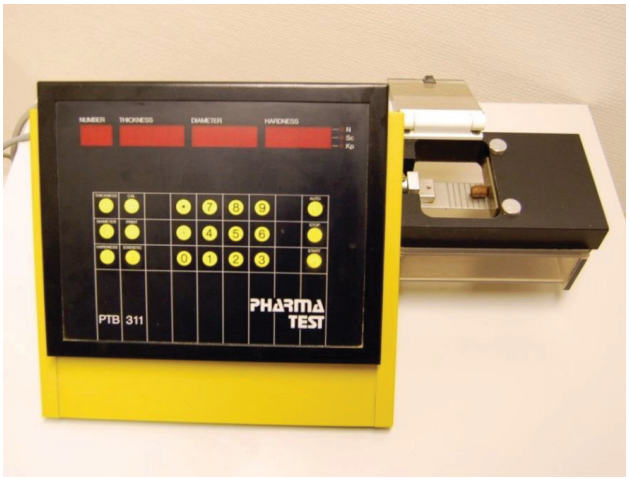


Figure 13: Pharma Test PTB 311. Measuring chamber with pellet to the right.

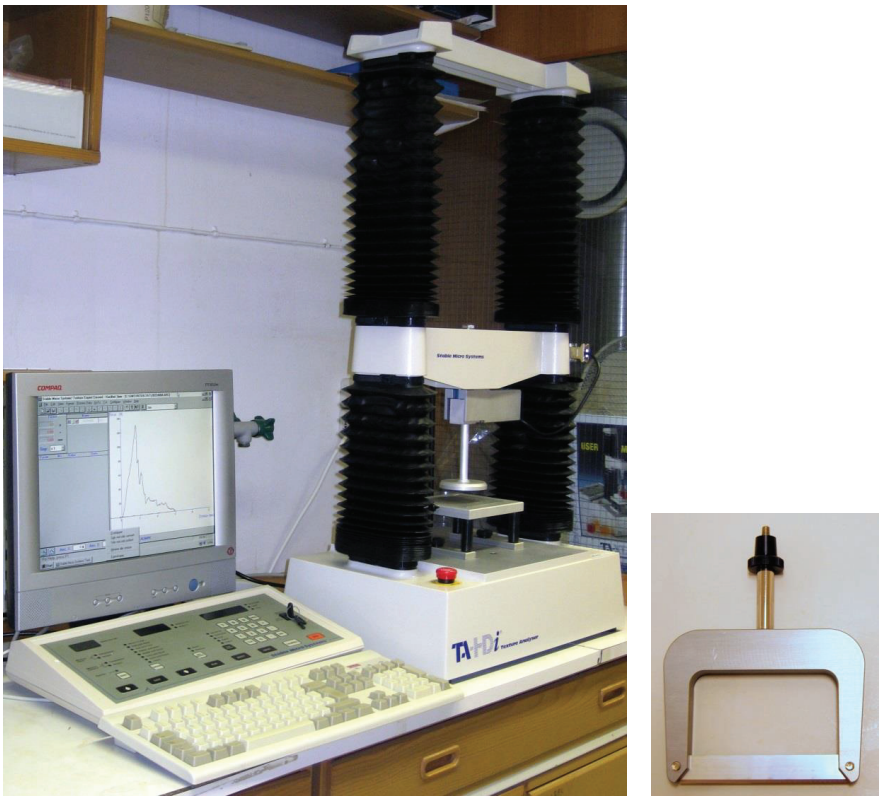


Figure 14: Texture analyser (TA-HDi) equipped with a cylindrical flat-ended probe. Extended craft knife to the right.

In Paper III, a method called cutting strength was introduced. The measurements were performed on single uncoated lying pellets by use of an extended craft knife (Fig. 14) mounted on the texture analyser and as described in Paper III. The knife penetrated the pellet cross-sectionally and the maximum cutting strength in Newton was recorded.

In Paper I and II the correlation between durability and hardness is discussed. Based on the conclusion from Paper I and II durability was not performed in the study reported in Paper III. In this paper cutting strength was introduced and discussed and compared to hardness.

Diameter, length, bulk density and oil adsorption capacity

Diameter, length, bulk density and oil adsorption capacity were performed on uncoated pellet reported in Paper III.

Diameter and length were measured with an electronic calliper. Diameter was expressed as sectional expansion index (SEI) defined in Eq. 1 in Paper III.

Bulk density was measured by loose pouring the uncoated pellets from a funnel into a 1000 ml measuring cylinder and the weight was recorded.

Oil adsorption capacity was measured using a lab-scale vacuum coater custom made by Nofima (Fig. 15) and the method described in Paper III.

The relationship between the different measurements is discussed in Paper III.

Figure 15: Lab-scale vacuum coater.

7.3.3 Determination of transition temperatures and viscosity in rubbery phase

The T_g and T_f (Paper III and IV) were measured by use of a Phase Transition Analyzer (PTA; Wenger Manufacturing Inc., Sabetha, KS, USA; Fig. 16). In the PTA compaction and flow relative to an initial sample height at constant pressure and at increasing temperature was measured by use of a displacement transducer.

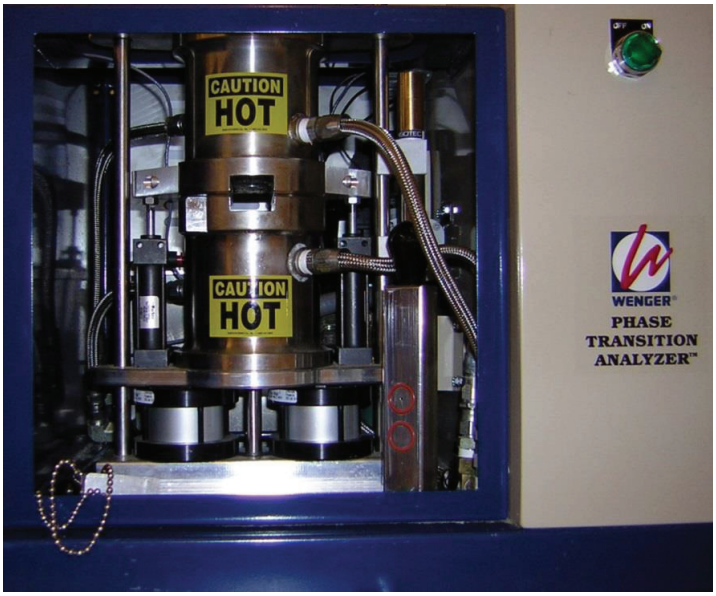


Figure 16: The phase transition analyser with sample bar to the lower right.

The sample bar (Fig. 16) was slid into the PTA and a 1.7 g sample was transferred to a cylindrical chamber which was sealed in the bottom by the sample bar. A piston connected to a pressure transducer was mounted at the top of the sample. The sample was compressed at 120 bars for fifteen seconds. The pressure was thereafter reduced to 100 bars and held constant through the test. The sample was heated at 8 °C min^{-1} (range, 3.6 to 180 ºC) and softening and compaction were measured by a displacement transducer. After complete compaction of the sample, the pressure was released and the blank die replaced with a die opening of 1.75 mm. The sample was compressed to 100 bars and heating continued at 8 °C min^{-1} until a mass flow was registered through the die opening by the displacement transducer (Fig. 17 and 18).

With use of the Gordon-Taylor equation (Eq. 1) the dependency of moisture content and water soluble level on T_{gMid} can be modelled with values comparable to the measured T_{gMid} values based on PTA (Paper IV).

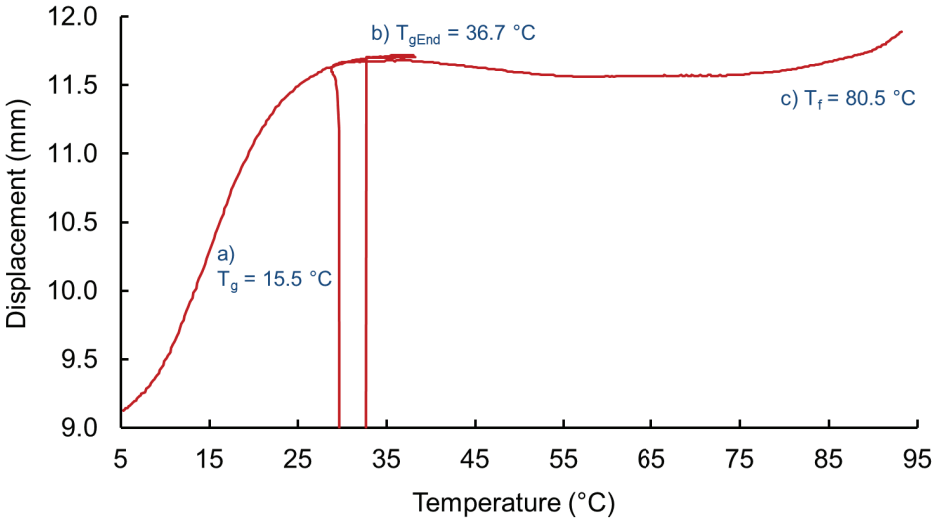


Figure 17: Typical displacement curve as a function of temperature at constant pressure. a) Glass transition temperature (T_g or T_{gMid}) defined as the inflection point of the steepest displacement slope of the glass transition temperature range. b) Complete compaction or endpoint of the glass transition range (T_{gEnd}). c) Flow starting temperature (T_f) defined as the temperature level at which the mass starts to flow through the die opening. Discontinuity in the graph at T_{gEnd} is caused by the replacement of the blank die with a die opening of 1.75 mm.

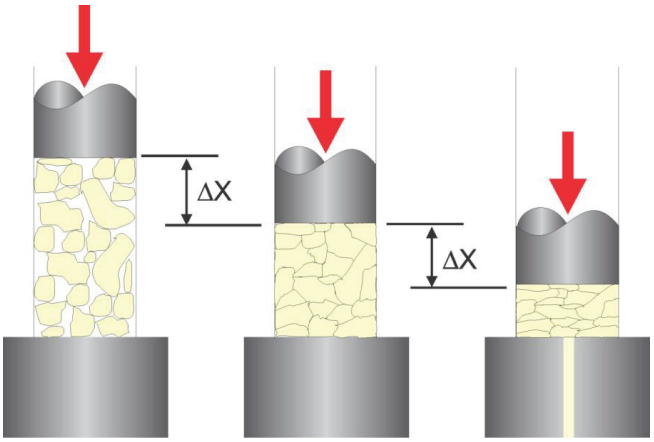


Figure 18: Sample compaction and flow during measurement. Blank die (left) replaced with a die opening of 1.75 mm (right) (with permission from Wenger).

The observed initial displacement speed at T_f for the samples reported in Paper IV was shown to be linear in the first 10 to 15 seconds and the PTA instrument could therefore be used as a constant pressure capillary viscometer. This enable us to calculate the apparent wall shear stress (τ_{app}), wall shear rate ($\dot{\gamma}_{app}$) and viscosity (η_{app}) at T_f based on equation 1, 2 and 3 reported in Paper IV.

With the use of the WLF equation (Eq. 5) and the measured values for T_{gMid}, T_f and viscosity at T_f, the dependency of moisture content and solubles level on the parameters C_1 and C_2 in equation 5 and the effect of temperature increase on viscosity reduction can be studied (Paper IV).

Apparent viscosity can also be calculated in extruder dies based on die pressure and mass flow rate and by use of equation 1, 2 and 3 reported in Paper IV (Della Valle *et al.* 1994). In Paper III the pressure in the last barrel head of the extruder was measured during extrusion but we were uncertain of the correctness of the measurements. A feed melt plugging of the space between the extruder barrel surface and the pressure sensor was observed and we therefore chose not to include pressure readings and such calculations in Paper III.

7.4 Multivariate methods

The extrusion process is a multivariate system where the physicochemical properties of the ingredients, process variables and screw configurations all have an effect on physical product quality. For fish feed the product quality also has to be characterised by several methods to confirm that the feed meets target requirements (see chapter 2 and 3.2.3). In this thesis several variables and responses has been studied and various multivariate methods been applied, such as principal component analysis (PCA) and partial least squares regression (PLSR) in Paper I to III and multiple linear regression (MLR) in Paper III and IV. In Paper I and II the investigations was designed to cover the variability in fishmeal physicochemical properties possible to obtain in commercial fishmeal processing. In Paper III and IV the experimental variables were investigated by use of factorial and central composite design.

7.4.1 Principal component analysis

Principal component analysis (PCA) is a statistical technique where the original values in a data matrix are projected to latent variables called principal components (PCs). The PCA technique decompose the data matrix (**X**) to a sets of column ($\mathbf{t_i}$) and row ($\mathbf{p_i^T}$) vectors, which gives a presentation of the dominant structure of the original objects and variables in **X**. The first PC (PC1) seeks the direction of the highest variance in **X** or the direction that minimizes the residuals ($\mathbf{E_1}$) and can be written.

$$\mathbf{X} = \mathbf{t_1 p_1^T} + \mathbf{E_1} \tag{7}$$

Where **t** is the column or score vector and $\mathbf{p^T}$ the row or loading vector. The loading vector defines the direction of PC1 relative to the original coordinate system and the score vector is the projection of the objects down to PC1. The second PC (PC2) is calculated from $\mathbf{E_1}$ by minimizing $\mathbf{E_2}$:

$$\mathbf{E_1} = \mathbf{t_2 p_2^T} + \mathbf{E_2} \tag{8}$$

The same calculations can be repeated until the number of PCs equals the numbers of objects or variables (what comes first). The PCs are set orthogonal to each other (i.e. do not co-vary) and in a decreasing order of explained variance.

Most of the variance in the data matrix is explained by the first two PCs. It is therefore most common to plot the score vector of PC1 against the score vector of the PC2 (score plot) and the loading vector of PC1 against the loading vector of the PC2 (loading plot). The score plot gives a map of the relationships between the original objects and the loading plot the relationships between the original variables. The plots are excellent tools for studying correlation between variables, similarities or grouping of objects and the inter-relationships between objects and variables.

Detailed description of the principles behind and the use of PCA are given in Wold *et al*. (1987); Martens & Martens (2001) and Esbensen (2006).

7.4.2 Factorial and central composite design

In a factorial design the influences of all experimental variables, factors and interaction effects on the responses are studied. The simplest form of such a design is a 2^k factorial design where the factors or numbers of variables, k, are studied at two levels coded as -1 and 1. The variables can either be continuous (quantitative) or categorical (qualitative). As an example, a 2^2 factorial design requires a total of four experiments (corner points in Fig. 19) and can be described by the following mathematical model.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \varepsilon \quad (9)$$

Where y is the estimated response, x_1 and x_2 the predictor variables, β_0 the intercept, β_1, β_2 and β_{12} the regression coefficients of each factor and the interaction term between them and ε the residual (error). If the factors are quantitative, a zero level (centre point in Fig. 19) can also be added to the design. At least three to five replications of centre points should be added. A 2^k design assumes linearity in the factor effects. Adding centre points, it is possible to check for curvature and the repetition allows obtaining an independent estimate of error. If there is curvature in

the response function a second-order response surface model (quadratic model) should be considered. One of the most important designs for fitting a second-order response surface model is the central composite design (CCD). In this design axial points (star points) are added to the corner points and centre point. Fig. 19 illustrates a two factor rotatable central composite design where all the corner and star points are located on a circle around the centre.

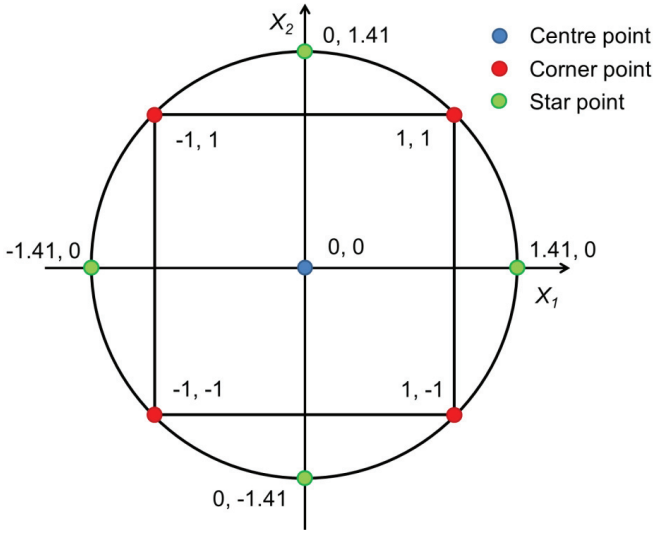


Figure 19: A graphical presentation of a two factor central composite design.

The design allows us to estimate the intercept, linear terms, the interaction between variables and quadratic terms according to the following equation:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon \quad (10)$$

The design gives us full rotatability, meaning that each experimental point contributes equally to the total information with the same precision in all directions from the centre. A two factor central composite design is used in Paper IV with three replications of centre points. In general, a rotatable CCD based on a full factorial design gives 2^k factorial points, $2k$ star points and n centre points. The star points are situated in a distance from the centre according to:

$$\alpha = \sqrt[4]{2^k} \tag{11}$$

In Paper III, a three factor CCD was used with 8 factorial points, 6 star points and with three replications of centre points (total of 17 experiments). With α equal to 1.68 the corner (or cube) points and star points are all located on a sphere around the centre points and described by the following mathematical model.

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \varepsilon \tag{12}$$

Detailed description of factorial and central composite design are given in Lundstedt *et al.* (1998) and Myers & Montgomery (2002).

7.4.3 Multivariate regression techniques

The first- and second-order response surface models described in Eq. 9, 10 and 12 is called multiple linear regression (MLR) models with two (Eq. 9, 10) or three (Eq. 12) independent variables (predictor variables). The models describe a plane in a two- or three-dimensional space. Regardless of the shape of the generated response surface (e.g. curvature) they are all MLR models because they are linear functions of the regression coefficients (β -values). Estimation of the β -values in MLR models is performed by use of the least squares methods. This method estimates the β-values so that the squares of the errors, $\sum \varepsilon_i^2$, is minimized. In matrix notations a MLR model can be written as:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{E} \tag{13}$$

Where **y** is the vector of the response variables, **X** the matrix of the independent variables, **b** the vector of the regression coefficients and **E** the residual matrix. The least squares estimator of the β-values can be found by the following equation:

$$\mathbf{b} = (\mathbf{X}^T\mathbf{X})^{-1}\mathbf{X}^T\mathbf{y} \tag{14}$$

The estimation of the β -values assumes that all the predictor variables are independent and rigorously controlled at a preselected level. The number of variables

must be less than the number of objects and all random variation is contained in the measured responses (i.e. the value of the predictor variables is assumed exact). The residuals must also be uncorrelated. In the case of non-designed experiments, covariance between the predictor variables or objects may give situations where $\mathbf{X}^T\mathbf{X}$ is not full rank and **b** will be poorly estimated. The solution to the above mention restrictions of MLR is to decompose into orthogonal latent variables (Kvalheim 1990). A latent variable technique called principal component regression (PCR) decomposes the **X**-matrix to a lower number of PCs. Because of the orthogonality, the PCs are uncorrelated and the score matrix can be used as variables in MLR. The partial least squares regression (PLSR) technique is a similar but a more powerful technique than PCR. In PLSR the latent variables are extracted by an algorithm that links the **X**-matrix and the **y**-vector and seeks to explain as much of the common variance between them. In Paper I and II the number of predictor variables was greater than the number of objects and the studies were not based on factorial experimental designs. PLSR was therefore the preferred regression technique. PLSR was also used for extrudate samples reported in Paper III because the studied variables were not rigorously controlled by the preselected levels in the factorial design. All other findings reported in Paper III and IV was based on CCD and MLR.

Variable selection is used to identify the best subset of predictor variables to be included in the model. By removing insignificant or unreliable variables these will improve prediction and often reduce the complexity of the model. In the MLR models reported in Paper III and IV the best subset of predictor variables included in the models were identified by use of backward elimination. First a model was built including all the predictor variables. By use of *F*-statistic, variables that contribute least to the predictions were then removed from the model one at a time. The stepwise removal of variables was guided by a threshold significance level set to $P > 0.05$. The quality of the fitted models was evaluated using ANOVA, *F*-statistics, and coefficient of multiple determinations (r^2).

The PLSR models reported in Paper I, II and III were evaluated by leave-one-out cross-validation or full cross validation. In full cross validation the dataset is

partitioned into subsets that are set equal to the numbers of objects (*N*). A single subset, which in this case equals to one object, is selected as validation and the model is then calibrated without this object. From the applied model the predicted response, $\hat{y}_i$ is calculated for the left out object. The procedure is repeated until all objects have been used as validation and the final model is evaluated from the residuals (y_i - $\hat{y}_i$) of all objects combined. The uncertainty of the regression coefficients was estimated from the cross-validation and used for backward elimination of insignificant ($P >$ 0.05) variables. Higher *P*-value was accepted if the removed variable significantly decreased the model quality. Prediction ability and the optimal number of partial least squares components of the regression models were validated from the root mean square error of prediction (RMSEP(Y)) defined by:

$$RMSEP(Y) = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - \hat{y}_i)^2} \qquad (15)$$

Root mean square error of calibration (RMSEC(Y)) was calculated by the same formula (Eq. 15) but on the calibration residuals. Influence plots (residuals vs. leverage) and normal probability plots of studentized *y*-residuals were used for outlier detection.

Prior to PCA and multivariate regression it is common to mean-centering and weighting the original variables. Mean-centering is performed by subtracting the mean from each entry of the variable. Weighting is important when the variables are represented in different units and some have large variance compared to others. An example is loose bulk density (standard deviation (SD) = 71) and pH (SD = 0.17) reported in Paper I. Without weighting, loose bulk density will dominate and pH will be poorly explained. An often used weighting method is standardization where each entry is divided by the standard deviation of the variable. With this treatment each variable will have the same SD equal to one.

Detailed description of the different regression techniques are given in Geladi & Kowalski (1986); Kvalheim 1990; Martens & Martens (2001); Wold *et al.* (2001);

Myers & Montgomery (2002) and Esbensen (2006). A tutorial of variable selection in regression is found in Andersen & Bro (2010).

8. Summary and discussion of the main results

8.1 Impact of herring meal properties on physical feed quality (Paper I)

The objective of this study was to identify physical and chemical properties of herring meal (FMH) with significant impact on SME, starch gelatinization, pellet durability and pellet hardness. The aim was to span the range in fishmeal physicochemical properties possible to obtain in commercial fishmeal processing of herring raw material. Several analyses were performed on the produced FMH batches. In addition to standard chemical analyses such as dry matter, protein, fat, ash and salt, the batches were also analysed for pH, oil adsorption and water-holding capacity, degree of protein hydrolysis and WSP. The peptide size distribution in WSP fraction was also quantified. The non-soluble protein content was defined as the difference between crude protein and the WSP. Physical measurements such as particle size distribution, dust fraction, bulk density and flow-figure were also performed.

Fifteen feed mixes were prepared, each containing one of the FMH batches as the only protein source. A standardized level of whole wheat flour was also added. The feed mixes also contained standardized level of fish oil, vitamin- and mineral mixtures and astaxanthin.

The effects of variation in 18 physicochemical properties were assessed based on standardized extrusion, drying and coating conditions and resulted in a large span in the measured extruder and pellet response variables. SME varied from 9.0 to 21.0 Wh kg^{-1} and degree of starch gelatinization from 420 to 850 g kg^{-1} of total starch content. The correlated responses durability and hardness ($P < 0.01$) varied from 0 to 100% and 4.9 to 133.4 N, respectively. They were both uncorrelated to degree of starch gelatinization indicating that the applied glucoamylase methodology was not adequate for quantification of the binding properties of the starch molecules.

The correlation between the FMH variables and responses were studied by use of PCA, and PLSR models with high explained variance were established for SME, starch gelatinization and pellet hardness (r^2 = 0.907 to 0.970). Several physicochemical fishmeal properties with significant ($P < 0.05$) impact on the response variables were identified and used to discuss the underlying physical and chemical mechanisms.

Reduced fishmeal particle size combined with a higher value of flow-figure affected SME positively. Higher value of flow-figure indicates an increase in the content of fibrous and/or fine particles in the fish meal samples. Increased level of small and fibrous particles in the feed mixture will increase the particle to particle contact area within the extruder barrel and thereby the shear stress and SME. Reduced water-holding capacity gave positive effect for both SME and starch gelatinization. Water-holding capacity is the ability of the fishmeal to adsorb and retain water. Fishmeal particles will compete with starch particles regarding moisture uptake in the extrusion process. A fishmeal with low water-holding capacity will adsorb less moisture, and more water will thereby be available for the starch particles. This will result in improved gelatinization. Increased degree of protein hydrolysis gave positive effect for both SME and starch gelatinization. Degree of protein hydrolysis reflects the degree of protein breakdown. Small peptides and amino acids may have an additive plasticizing effect together with water and increased level in the feed mass may result in higher starch gelatinization. Combined, the water-holding capacity and degree of protein hydrolysis will improve the starch gelatinization (starch pasting) and thereby also increase the melt viscosity, with resulting positive contribution to SME.

A positive effect of WSP on durability and hardness was observed in this study and may be explained by two different mechanisms 1) crosslinking of large water soluble polypeptides and 2) a plasticizing effect of smaller peptides and amino acids: 1) Stickwater from fresh herring has high gelatine content and the measured high molecular weight water soluble fractions in this study corresponded to fish gelatine. Gelatine readily swells and dissolves in the presence of water and heat. Upon cooling, gelatine cross-links and enhances texture formation. 2) Small water soluble peptides

and amino acids present in fishmeal contain both hydrophobic and hydrophilic residues and may interact with both the polar and nonpolar protein components. They may therefore, like water, be effective plasticizers. In contradiction, increased level of the non-soluble protein phase gave reduced durability and hardness. Increased level of non-soluble proteins is a result of reduced level of WSP and the observed negative effect may be attributed to the lack of efficient plasticizers (i.e., lack of small water soluble peptides and amino acids) in the feed mix. A high non-soluble protein level gives high amount of solid particles suspended in the fluid-like melt with a negative impact on durability and hardness.

In addition it was observed that an increase in salt level combined with reduced pH value improved pellet durability and hardness. Proteins are charged molecules. In the low water content environment within the extruder barrel, electrostatic interactions will be important and influenced by soluble salts and pH.

8.2 Influence of replacing herring meal with fishmeal from sand eel (Paper II)

The purpose of this study was to investigate the impact of two types of fish raw material, i.e. herring and sand eel, on fishmeal physicochemical properties, SME, starch gelatinization and physical feed quality. Five independent fishmeal batches from sand eel (FMSE) were produced at one of the three factories reported in Paper I, and studied together with the six FMH batches produced at the same factory. The same analyses as for the FMH (confer chapter 8.1) were performed for the FMSE. The feed mix composition and the standardized extrusion, drying and coating conditions were similar for the FMSE as for the reported FMH. For one of the feed mixes based on a FMSE, an additional experiment was performed on a high shear screw profile, but with other conditions similar to the standardized experiments.

The input variables were studied by use of PCA to identify the impact of fish species on fishmeal physicochemical properties. A clear difference in properties was documented with the FMSE and FMH groups separated by principal component 1.

The FMSE batches contained more hydrolysed WSP, more salt and fines (dust fraction), and were denser than the FMH batches. The FMH had higher level of large water soluble polypeptides, higher content of non-soluble protein and higher pH than the FMSE.

A larger variation in both SME and starch gelatinization was observed for FMH (9.2 to 19.3 Wh kg^{-1} and 560 to 840 g kg^{-1}, respectively) compared to FMSE (11.8 to 15.1 Wh kg^{-1} and 910 to 970 g kg^{-1}, respectively), with SME positively correlated to starch gelatinization for both the FMH ($P < 0.001$) and FMSE ($P < 0.053$) samples. With the inclusion of type of raw material in the PLSR analysis a model could be established for starch gelatinization ($r^2 = 0.637$). Improved degree of starch gelatinization was associated with increased degree of protein hydrolysis ($P = 0.011$) and by replacement of FMH with FMSE (category variables) in the feed mix (P = 0.012). The positive effect of degree of protein hydrolysis can be attributed to the possible plasticizing effect of small peptides and amino acids. The category variables contain information related to starch gelatinization not uncovered by the applied analytical approach.

The correlated responses durability and hardness ($P < 0.001$) varied from 0 to 100% and 4.9 to 94.1 N, respectively. A PLSR model with high explained variance were established for hardness ($r^2 = 0.929$). Improved hardness was associated with decreased level of non-soluble protein (P < 0.001) and by replacement of FMSE with FMH (P < 0.001). Hardness was also found to be negatively correlated with the level of non-soluble protein for feeds containing FMH ($P = 0.007$) and FMSE ($P < 0.001$) respectively, with similar slopes but different intercepts. This means that at an equal level of WSP, extruded feed containing FMSE have significant lower physical quality than feed containing FMH. This can be attributed to differences in thermal and rheological properties between the two studied groups and improper cooking in the extruder barrel for FMSE based feed mixes. Improper cooking or transformation may result in increased level of particles in the extrudates and poor physical feed quality. Proper transformation of such feed mixes will demand that more mechanical energy (shear) is needed to reach the critical melt transition temperatures at a given moisture

level. An increase in durability (0 to 96%) and hardness (28.4 to 92.2 N) observed when replacing a low shear screw profile with a high shear screw profile for one of the FMSE supports this theory. For both raw materials, reduced level of non-soluble protein increased hardness. This can be attributed to the positive binding effects of increased level of WSP, which is in agreement with the findings in Paper I.

8.3 Impact of water-soluble protein on extrusion behaviour (Paper III)

The aim of this research was to document the effect of WSP as a plasticizer in the fish feed extrusion process and to assess effects of WSP level and steam and water addition on physical pellet quality. Five experimental feed mixes with increasing WSP level were prepared. The level of WSP was predetermined by the chosen range in the applied 3-factor central composite design (63.3 to 225 g kg^{-1} DM). The feed mixes were standardized to an equal level of dry matter and starch. The feed mixes were extruded according to the steam and water levels determined by the experimental design (0 to 111.5 g kg^{-1} DM), giving a total of 15 experimental settings with 3 replications of the center point (17 trials in total).

MLR models with r^2 in the range of 0.862 to 0.976 ($P < 0.001$) were established for the responses SME, hardness, cutting strength, SEI, length, bulk density and oil adsorption capacity. The T_g and T_f in the extrudates were measured by use of the PTA. Based on the analysed WSP and moisture level in the extrudates, PLSR models for T_g and T_f were established with r^2 at 0.981 and 0.930, respectively.

The measured SME values varied between 59.1 and 135 kJ kg^{-1}. The addition of WSP had a positive effect on SME while moisture had negative effect. This could be explained by a much higher viscosity of WSP compared to water, and by differences in biopolymer interactions depending on type of plasticizer. Increased levels of water and steam caused a decline in SME, with steam explaining more than 75% of the variance in SME. This indicates that moisture and temperature were the two most important factors that reduce melt viscosity and SME.

No loss of WSP during the extrusion process could be observed and the measured T_g and T_f in the extrudates varied between <3.6 and 14.9 °C and 33.1 and 83.0 °C, respectively. The two predictor variables, i.e. the analysed WSP and moisture level in the extrudates (g kg^{-1} wet), had negative regression coefficients in both the T_g and T_f models giving the lowest transition temperatures with a combination of a high WSP and a high moisture level. For the T_f model the two predictor variables showed equal effect on T_f per mass unit, which documents the potential of WSP as plasticizer in fish feed production.

Hardness and cutting strength (55.1 to 173.5 N and 17.4 to 28.1 N, respectively) gave similar information regarding physical pellet quality. There were an increase in hardness and cutting strength at increased WSP levels with the highest effect at low water and steam levels. Due to a possible softening effect caused by an excess amount of plasticizer reduced hardness and cutting strength were observed at the highest WSP level. Reduced hardness and cutting strength were observed with increasing levels of water and steam, due to the observed decrease in SME. The results show the potential for the use of WSP as a binder. The WSP reduces T_f but still have a positive effect on SME and viscous dissipation. This will improve the cooking efficiency, melt homogeneity and final product properties compared to the addition of moisture. WSP will also influence the viscoelastic properties of the final product and have a positive effect on physical pellet quality by establishment of intermolecular binding network through hydrogen- and ionic bond and hydrophobic interactions.

Bulk density (323 to 506 g L^{-1}) was negatively correlated to SEI ($P < 0.001$), length ($P < 0.001$) and oil adsorption capacity ($P < 0.001$) and the MLR model for bulk density showed a negative effect of increasing WSP, steam and water. Three mechanisms can explain the observed effects: 1) An increase in steam level increases the driving force for diametrical expansion (SEI) due to adiabatic flash, 2) an increase in steam and/or water level increases the throughput and, at constant knife speed, giving a longer pellet and lower bulk density and 3) an increase in WSP and moisture

level (steam and/or water) lowers the resistance for expansion due to a decrease in T_f and melt viscosity.

The stickwater concentrate used to vary the WSP level also contains most of the water soluble nitrogen compounds (protein, peptides, amino acids, putrefaction products etc.), vitamins and minerals in the fish raw material. It is not possible to differentiate between the plasticizing effects of the different groups of constituents. Solubles compounds with possible plasticization effect are discussed in relation to literature data in Paper IV.

8.4 Plasticization effect of solubles in fishmeal (Paper IV)

This study was designed to assess the combined effects of solubles and moisture level on T_{gMid} and T_f in a fishmeal model system, and to quantify soluble constituents with possible plasticization effect.

Five experimental fishmeal samples with increasing level of water solubles (water soluble dry matter, WSDM) were prepared. The WSDM levels (94.8 to 379.8 g kg^{-1} DM) were chosen to span the range of WSP content observed in commercial fishmeal processing. The fishmeal samples were moistened to the levels determined by a 2-factor central composite design (85.2 to 222.6 g kg^{-1} wet), giving a total of 9 experimental settings with 3 replications of the center point (11 trials). From the PTA displacement versus temperature curve, $T_{gMid,}$ T_{gEnd} and T_f were quantified and MLR models with $r^2 > 0.951$ were established for the responses T_{gMid}, T_{gEnd}, T_{gEnd} -T_{gMid}, T_f, T_f -T_{gMid} and T_f -T_{gEnd}.

The measured T_{gMid} values varied between 6.2 and 25.7 °C and the established response surface model shows a significant negative squared effect of WSDM and a negative linear effect of moisture. Based on the main effects, each percent increase in moisture content had 3.1 times higher effect on T_{gMid} compared to the corresponding increase in WSDM. However, on a molar basis the effect of solubles addition will be higher compared to moisture. The T_{gMid} levels observed for fishmeal was significantly lower than values reported for other food proteins based on PTA analysis, i.e. in the

70-80 °C range for casein, soya and gluten at 10% moisture content (Bengoechea *et al.* 2007) compared to a predicted range of 17-25 °C in fishmeal. The effect of WSDM on T_{gMid} could be modelled based on the Gordon-Taylor equation.

The T_{gEnd} model showed negative main, and positive squared and interaction effects with a clearly reduced effect of WSDM at high moisture levels compared to low. The observed half transition range (T_{gEnd} - T_{gMid}) varied between 10.7 to 47.6 °C, which is consistent with the maximum transition range of 100 °C reported for food polymers (Yildiz & Kokini 2001). The glass transition range is reflecting the homogeneity of the biopolymer and a broad temperature range is characteristic for a multicomponent system with a large span in molecular weight. The observed broader effect of moisture compared to WSDM content on the glass transition range may be attributed to the lower molecular weight and more polar nature of water compared to the soluble constituents in the WSDM which is fully compatible with the press cake fishmeal.

The model for T_f showed a negative effect of solubles level and moisture content and a squared positive effect of both variables giving a decreasing effect on T_f at high levels of the two variables. The measured levels varied from 42.3 to 171.3 °C. Based on the main effects, each percent increase in moisture content had 1.2 times higher effect on T_f compared to WSDM, in good agreement with the similar effect reported for extrudates in Paper III.

The region between T_g and T_f can be defined as the rubbery phase. This region, dependent on both WSDM and moisture level, spanned a range of 36.1 to 148.6 °C for the fish meal samples. This differentiates fishmeal from other protein ingredients characterized in the literature, where a constant difference between T_f and T_{gMid} that is independent of moisture content were found (e.g. ~37 °C for gluten, ~39 °C of casein and ~75 °C for soya; Bengoechea *et al.* 2007).

During heating of the fishmeal samples in the PTA, the apparent viscosity was reduced from approximately 10^{12} at T_{gMid} to 10^5 Pa s at T_f, a viscosity below the critical level for extrudate bubble growth (10^7 to 10^8 Pa s). The temperature dependence of viscosity above T_g can be described by the WLF equation. The large

$T_f - T_{gMid}$ span shows that the WLF parameters (C_1 and C_2) are not universal for fishmeal and that they depend on both moisture- and WSDM content. For the studied fishmeal model system there is a large composition region of WSP and moisture with a higher difference between T_f and T_{gMid} compared to casein, gluten and soya protein isolate. This indicates a reduced temperature effect on viscosity reduction in the rubbery phase for fishmeal in this region. The constant difference between T_f and T_{gMid} for casein, gluten and soya protein isolate also indicates that the WLF parameters do not change with moisture content in these protein ingredients.

The groups quantified by chemical analysis in the fish solubles include: 1) the α-amino acids normally found in proteins; 2) non-protein α-, β- and γ-amino acids and taurine; 3) peptides; 4) putrefaction compounds including biogenic amines and volatile nitrogen (i.e. ammonia and trimethylamine); 5) ash and sodium chloride. Based on literature data (Stein & Green 1997; Stein *et al.* 1999; Farahnaky *et al.* 2009; Moreau *et al.* 2009) the most effective plasticizers in the water solubles will be the low molecular N-compounds (amino acids, peptides, putrefaction products etc.).

9. Conclusions

The experimental work included in this thesis has improved the understanding of how the variability in fishmeal physicochemical properties affect the extrusion cooking process and physical quality of fish feed. The work has also documented the plasticizing effect of water solubles in fishmeal applied in the fish feed extrusion process and elucidated the underlying mechanisms for the process and resulting effects on pellet quality. The main conclusions are summarized in the following (water solubles used as common designation for WSP and WSDM).

- Fishmeal is a complex protein ingredient with significant effect on the extrusion process, starch gelatinization and physical pellet quality, and with large differences in physicochemical properties within and between the studied fishmeal types (i.e. herring and sand eel).
- Fishmeal is purchased on the world commodity market based on a limited set of chemical and biological specifications. These specifications inadequately describe the technical properties of a fishmeal.
- Improved starch gelatinization (starch pasting) increases the melt viscosity in the extruder barrel, with resulting positive contribution to SME. However, the applied glucoamylase methodology for measuring starch gelatinization is not adequate for quantification of the pellet binding properties of the starch molecules.
- A fine-grained fishmeal with a fibrous structure may improve SME and cooking efficiency within the extruder barrel due to increased particle to particle contact (shear). Fishmeal structure is dependent on both drying conditions and fish species.
- Water solubles in fishmeal improve the physical feed quality. The effect can be explained by the following underlying mechanisms: 1) water solubles contain large water soluble polypeptides corresponding to gelatine. The content depends on species, and FMH contain more gelatine than FMSE. Gelatine cross-links and enhances texture formation. 2) Peptides, amino acids and other N-containing compounds in the water solubles contribute to intermolecular binding networks

through hydrogen-, ionic bond and hydrophobic interactions. 3) Water solubles improve cooking efficiency due to the plasticizing effect of low molecular N-compounds.

- Increased level of non-soluble protein gives reduced physical feed quality. This can be a result of incomplete cooking or transformation due to lack of efficient plasticizers (i.e. lack of low molecular N-compounds) in the feed mix.
- More mechanical energy (shear) is needed to transform FMSE compared to FMH based feed mixes under similar extrusion conditions.
- The documented plasticizing effect of water solubles is comparable to moisture addition. The uses of such plasticizers open up the possibility to obtain a satisfactory transformation at reduced moisture level with a potential for significant reduction of the energy consumption during drying of the extrudate.
- In contrast to moisture, addition of water solubles has a positive effect on SME and physical pellet quality. Non-volatile plasticizers like water solubles, will not be removed in the drying process and therefore also influence the viscoelastic properties of the final product.
- The effect of water solubles on T_{gMid} could be modelled based on the Gordon-Taylor equation. The T_{gMid} levels observed for fishmeal are significantly lower than values reported for other food proteins based on PTA analysis.
- A reduced temperature effect on viscosity reduction in the rubbery phase for fishmeal compared to plant based proteins and casein was oberved. Combined with significantly lower T_{gMid}, such differences in physicochemical properties may contribute to explain the unique functional properties of fishmeal.
- The apparent viscosity at T_f for fishmeal (10^5 Pa s) is below the critical viscosity level for extrudate bubble growth (10^7 to 10^8 Pa s) and a negative relationship between bulk density and the temperature difference between T_{die} and T_f was found for fish feed extrudates. Measurements performed on a PTA can therefore be a valuable tool for prediction of pellet expansion and oil adsorption capacity.
- Water solubles can be used as processing aids for the fish feed industry, serving multiple purposes as nutrient, plasticizer and binder in extruded fish feed.

10. Future outlooks

During the last decades there have been large changes in feed composition for salmon with fishmeal reduced from approximately 65% in 1990 to 18% in 2013 and replaced by plant based proteins. The new protein ingredients are not a uniform group and large variability in extrusion and binding properties can be expected. To better understand and control the extrusion process and physical pellet quality there is a need to improve the knowledge on technical properties of these ingredients and to study possible interactions between them.

Reported measurements of physical feed properties are inconsistent in type of equipment used, methodology and in the implementation of methods. In addition, there are also conflicting results on how these properties affect nutrient digestibility and the biological response of the fish. There is a need to standardize the different methods used to evaluate physical fish feed quality and to further investigate how these properties interact with feed intake and feed utilization.

This PhD project has confirmed that fishmeal has unique technical properties compared to casein and plant derived proteins. The work has documented the need for new analytical approaches to better characterize and understand the extrusion behaviour and binding properties of feed ingredients. Further research should focus on:

- Establish a methodology for measuring starch gelatinization that quantifies the binding properties of the starch molecules.
- The different feed ingredients will have different water-binding capacity and will compete regarding moisture uptake in the extrusion process. This will impact the thermomechanical transformation of the different ingredients. There is a lack of data in the literature comparing water-binding capacity of different feed ingredients.

- Particle structure may impact SME. Proper methods for quantification of powder friction and adhesion forces in powdery ingredients should be developed and studied in relation to SME and viscous dissipation in the extruder barrel.
- Lack of homogeneity is one of the major reasons for the difficulty in understanding the rheology of biopolymer melts and may explain the large variation in physical feed quality documented in this thesis. Proper measuring techniques to study feed melt homogeneity should be developed.
- The PTA is a useful tool for determining of the T_g and T_f, however, further studies are needed to validate the use of the technique related to pellet expansion and collapse over the die.
- Based on literature data the dominating plasticising effect in fishmeal can be attributed to the content of low molecular N-compounds. Further studies are needed to characterize the plasticization effect of low molecular organic and inorganic constituents and rheological properties of different feed ingredients.
- Pellet binding forces consist of a combination of covalent disulphide bridging and hydrogen-, ionic bonds and hydrophobic interactions. Techniques for describing the biopolymer binding structure and quantification of intra- and intermolecular forces in the feed pellet should be explored.

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