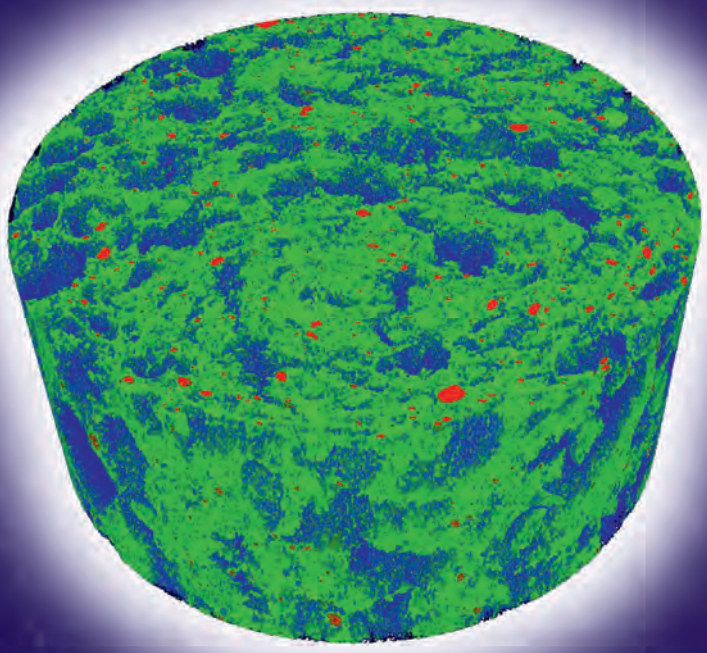
Towards Sustainable Fish Feed Production Using Novel Protein Sources



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Thesis

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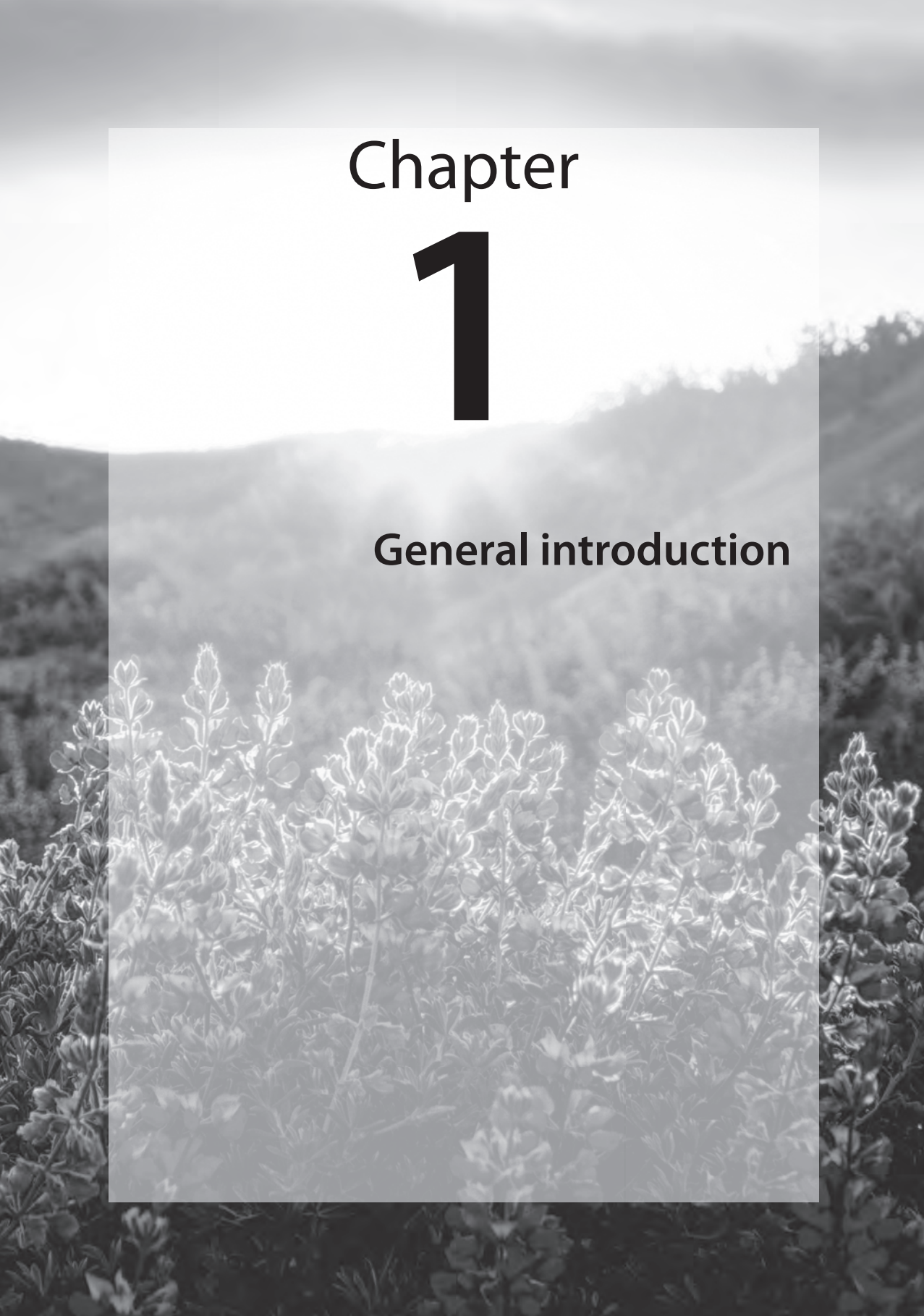

Chapter 1

General introduction

1. Introduction

Research on increasing the sustainability of aquafeeds is essential for aquaculture to fulfil its potential in providing high-quality protein for the global population in the coming decades. According to the Food and Agriculture Organization of the United Nations (FAO), fisheries and aquaculture are vital to global food security and poverty alleviation. Although the supply of fish from marine capture fisheries is stabilizing at around 90 million tons (FAO, 2010), the demand for fish and fish products is increasing. Production from world fisheries and aquaculture is projected to reach about 172 million tons in 2021, with most of the growth coming from aquaculture (FAO, 2012). Aquaculture has seen an average annual growth rate of 8% over the past two decades (FAO, 2008) making it the fastest growing food production sector in the world.

However, there is a major challenge facing aquaculture in continuing this growth. Fish need feed with high levels of protein and energy. Traditionally, for carnivorous or omnivorous fish, these are provided mainly as fish meal (FM) and fish oil, which also contributes to the health-promoting aspects of fish in the human diet, for example, with omega-3 fatty acids. The challenge lies in the need to increase feed production without increasing the demand for these marine raw material resources while maintaining the health benefits.

Aquaculture today consumes 60% of the FM and 85% of the fish oil produced (FAO, 2008), mainly from industrial coastal fisheries and from the trimmings produced during processing for human consumption. Therefore, the industry is heavily dependent on marine resources. Production from these resources cannot be increased without increasing the environmental impact. At best, sustainably managed fisheries may be able to yield the current harvest of 5 million tons of FM and 1 million tons of fish oil (IFFO, 2011), but this is far below the current and expected demand. Therefore, to meet the growing demand for fish, aquaculture must identify alternatives. The research reported in this thesis focuses on alternatives for the proteins in fish feed.

2. Fish feed production: the manufacturing challenges

The fish feed production process involves a series of unit operations such as particle size reduction, mixing, extrusion, and subsequent drying followed by

vacuum coating and cooling (Fig. 1). Extrusion, a central structuring operation, includes the conditioning of the feed (0.5–2 min; 70–90 °C) before it enters the extruder barrel. The feed mash is cooked in the extruder at 80–130 °C and 20–30 bar for a short time (0.3–1 min) and kneaded. The heat is mostly generated through the dissipation of mechanical (kneading) energy along the extruder barrel (Barron et al., 2002; Chevanan et al., 2007; Gatlin et al., 2007). The typical amounts of water added to the extrusion process are high (25–32% of the mash feed rate). To avoid product deterioration during storage, the extrudates have to be dried to a moisture level below 10%. Normally, hot air is used as a drying medium and the air temperature ranges from 80 to 140 °C. The drying time can vary from 20 to 50 min. Subsequently, vacuum coating is used to infuse the dry, porous pellets with oil (up to 40%).

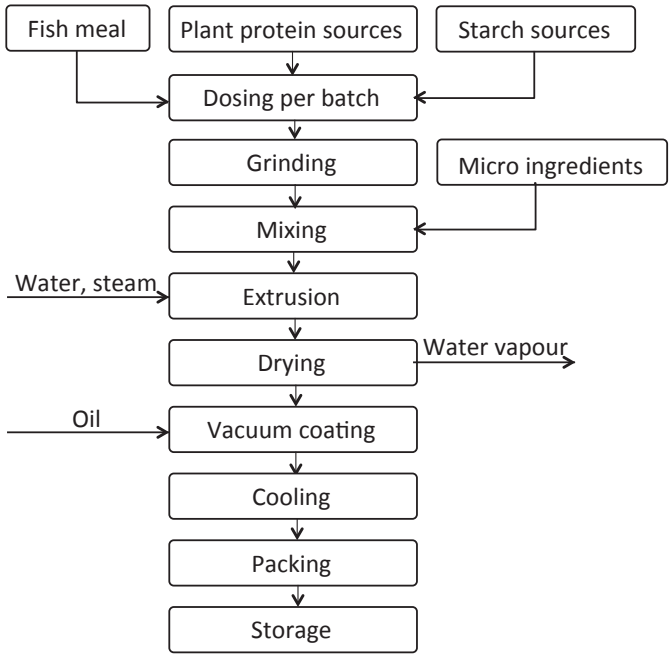

Fig. 1. Schematic overview of the current fish feed manufacturing process.

Feed pellets must meet a series of physical specifications. They must be sufficiently durable to withstand the stresses exerted during transport, handling and in pneumatic feeding devices (Aarseth, 2004; Aarseth et al., 2006). Intensification of aquaculture has been accompanied by more extensive feeding systems (plastic tubes) in which the pellets can be transported from a few hundred meters to up to

1400 m. Apart from economic loss, fines generated by pellet crumbling can also have an adverse effect on the water quality when suspended in water.

The pellets must be consistent in appearance, size and density, and the density must be controlled precisely in the extrusion process to give the required oil absorption and sinking speed characteristics. Pellets that stay afloat will not be eaten by the fish; pellets that sink too fast may escape being eaten. Starch is used in feeds for carnivorous fish to promote expansion of the product after extrusion, giving the pellets their porosity (Glencross et al., 2010). This porosity allows the pellet matrix to be vacuum infused with oil (Sopade et al., 2006; Øverland et al., 2007). In addition, the oil must stay inside the pellet during storage, especially at increased temperatures. This depends on the pore structure of the pellets; larger and well-connected pores have more leakage.

The final properties of the pellets are highly dependent on the type of raw materials used. A different protein source may change the viscosity of the mash (feed into the extruder). A mash with a high viscosity requires dilution with some water to reduce the viscosity to manageable levels. Water acts as a plasticizer of the materials during extrusion. Miller (1985) observed that extrusion of dry expanded dog food at low moisture leads to reduction in the plasticity and elasticity of the extrudate. Therefore, the feed particles need to be hydrated enough. Addition of water is undesirable; a high level of water leads to high water activity, which needs to be reduced to have a product that is shelf stable. Excess water has to be removed after extrusion by drying. Unfortunately, plant proteins in general need more moisture for extrusion than FM, and thus require more drying after extrusion. Drying is recognized as one of the most important unit operations with respect to energy consumption, odour emissions and plant safety. In commercial fish feed manufacturing, drying typically represents about 65% of the total energy consumption.

Therefore, it is clear that alternative protein sources must not only provide the right nutrient profile but must also have the techno-functional properties that allow the formation of fish pellets with good properties using the current process (Allan and Booth, 2004; Kim et al., 2006; Knudsen et al., 2006; Øverland et al., 2007).

3. Alternative protein sources

Some candidate protein sources have a lower crude protein content than FM, which suggests that the space in the diet formulation previously available for starch-rich ingredients is reduced in plant-based diets. This leaves fewer possibilities for adaptation of other properties, such as expansion of the extrudates after extrusion, to give the right porosity. The influence of plant-based materials as listed in this section on these types of properties is therefore important. In FM-based products, the structure of the pellet is known to be easily accessible for oil infusion during coating. Oil impregnation must not be hindered by changes in the microstructural parameters of the pellets when using plant proteins.

Overall, plant-derived feedstuffs all have some functional characteristics that can be positive or negative relative to FM for their suitability for use in aquafeeds. It is therefore likely that a combination of plant-derived feed ingredients is required to replace FM. The aim of this thesis is to investigate the most important techno-functional properties of plant protein-rich ingredients compared with FM.

4. Current plant proteins used in aquafeeds

4.1. Soy protein concentrate

Soybean, *Glycine max* Linnaeus, is the leading oilseed crop produced globally. Its production in 2009–2011 averaged 254 million tons per year. A large part of this production is used for the extraction of oil yielding a cake with high protein content. This cake is processed to yield a wide array of soybean products, such as soy flour, soybean meal, soy protein concentrate (SPC) and soy protein isolate. Soy products are regarded as economical and nutritious feedstuffs with a high crude protein content and a reasonably balanced amino acid profile (Gatlin et al., 2007). They have been used widely as functional ingredients in food (Rao et al., 2002). SPC is produced by aqueous-alcohol extraction of defatted soy flakes (Lusas and Riaz, 1995) and typically contains 60–65% crude protein. The extraction removes or deactivates anti-nutritional factors, soluble carbohydrates and fibre, but not phytic acid (Bureau et al., 1998; Storebakken et al., 2000), which chelates with minerals such as phosphorus

and zinc and reduces their availability in monogastric animals (Kumar et al., 2012). This most widely used method to produce SPC also reduces the protein solubility to less than 10%. However, the extraction can eliminate the bitter off-flavour (Morr and Ha, 1991). An added concern compared with FM is its high concentration of carbohydrates, largely present as oligosaccharides, of which some are not available to aquatic animals (Gatlin et al., 2007). Although SPC has a relatively balanced amino acid profile for fish, it is low in some essential amino acids, especially methionine (Storebakken et al., 2000), which need to be added in the diet in crystalline form (Kaushik et al., 1995; Mambrini et al., 1999; Refstie et al., 2001).

4.2. Wheat gluten

Wheat gluten (WG) is an interesting alternative dietary protein source to FM (Helland and Grisdale-Helland, 2006). Although most gluten is used in cattle feed, a small (<1%) but increasing quantity of WG produced is used by baking industries seeking greater control over the finished product either for quality control or nutritional marketing (Gatlin et al., 2007). A wet processing step is commonly used as an industrial separation technique for wheat flour, which suspends and washes away the starch while most of the gluten remains. Although energy and water intensive, it is considered to be a mild processing technique. This is reflected in the high digestibility of cysteine in diets containing WG (Storebakken et al., 2000) and the overall high protein digestibility of this product (Davies et al., 1997; Pfeffer et al., 1995; Robaina et al., 1999; Storebakken et al., 2000; Sugiura et al., 1998). Unlike soy products, WG contains less carbohydrates and anti-nutrients of concern that limit its use in feeds (Hardy, 2010; Storebakken et al., 2000). Among cereal proteins, WG is unique in its ability to form a dough that is viscoelastic and can act as a gas barrier (MacRitchie, 1992). However, it is not clear whether these properties are beneficial when making fish feed pellets. Wheat storage proteins are characterized by their high content of glutamine and proline and conversely, by their low content of charged amino acids. As a consequence, they are insoluble in water (Morel et al., 2002).

5. Candidate plant proteins for increased use in aquafeeds

5.1. Lupine protein concentrate

Lupines include several species of grain legumes with a high protein content (about 35% of the dry weight) and relatively low oil content (8–10%). Lupines are produced in significant quantities throughout the world. In 2004, 1 million tons of lupines were produced (FAOSTAT, 2005). Among the lupine seed species, the most cultivated, primarily in Australia, is blue lupine (*Lupinus angustifolius* L.), whereas the typical European and South American varieties are yellow (*Lupinus luteus* L.) and white (*Lupinus albus* L.) lupines. So-called sweet lupines are varieties of white lupine with a low content of alkaloids (Roemer, 1993). Because heat processing of sweet lupines is not required (Drew et al., 2007), they may be processed by air classification into a lupine protein concentrate (Cheftel et al., 1985) containing 61% crude protein (Booth et al., 2001). The amino acid pattern of lupine protein is comparable with that of soybeans (Cerletti and Duranti, 1979) with a good balance of essential amino acids. Based on the nutritional studies of sweet lupine fractions in finfish, this high-protein legume has great potential as an aquafeed ingredient (Drew et al., 2007). It has been previously reported that lupine proteins may strengthen the structure of a processed/cooked product (Kiosseoglou et al., 1999; Mavrakis et al., 2003).

5.2. Canola protein concentrate

Canola, *Brassica rapa* L., is produced from cultivars of rapeseed that have been bred to contain low levels of erucic acid and glucosinolates, a group of anti-nutrients that interfere with normal thyroid function (Gatlin et al., 2007). Canola seed is an oilseed, and canola oil is the primary product of its cultivation. According to the United States Department of Agriculture, canola production exceeds 40 million metric tons per year (USDA, 2004).

Canola meal is the product that results after oil extraction and removal of the solvent. It can be further processed by aqueous extraction of the protein to yield canola protein concentrate (CPC). This process removes most of the anti-nutritional factors present in canola meal, and thus is an excellent protein source for salmonids (Drew, 2004). CPC has a protein content that is similar to SPC and lupine protein concentrate. Canola protein has high biological

value compared with other protein sources. As the primary protein source in aquafeeds for salmon and trout, CPC supports growth rates similar to those of fish fed FM-based diets, as long as some amino acid supplements are used to overcome limiting amino acid levels (Gatlin et al., 2007).

With regard to important functional properties of canola proteins, it has been reported previously that the water absorption capacity of rapeseed flours was similar (exceeded 200%) to that of soybean flour (Sosulski et al., 1976). Canola proteins have been considered as potential ingredients for food products that require a gel-like structure (Aider and Barbana, 2011). Uruakpa and Amtfield (2004) found that canola protein isolate can serve as a structuring agent in mixed food systems.

At present, CPC is not widely available for use in aquafeeds but there is increasing interest in its production in larger quantities.

6. Sustainability assessment of different feed formulations using energy, classical exergy and eco-exergy

Once the possible alternatives to FM in feeds have been defined with respect to all the nutritional and technological aspects, a proper sustainability assessment of various formulations is needed. Pelletier and Tyedmers (2007) stated that understanding and improving the environmental performance of feed ingredients and alternative feed formulations is central to improving the sustainability of salmon farming as a whole.

In this thesis, the replacement of fish protein harvested from wild sea fish by cultivated plant-based alternatives is investigated. In this research, we use the concept of exergy to get an objective insight into the environmental impact of various protein sources. Exergy analysis is akin to life cycle analysis, with the addition that the value of each stream (and emission) is quantified with its total available potential to do work.

Cumulative industrial energy consumption has been calculated previously to evaluate the comparative environmental performance of salmonid feed ingredients and feed formulations (Boissy et al., 2011; Pelletier and Tyedmers, 2007). As energy is converted from one form to another, it is neither lost nor destroyed. It does, however, "lose a certain quality which can be described as

its ability to do work" (Torrie, 1981). This measure of the usefulness or value or quality of an energy form is called exergy. Technically, exergy is defined using thermodynamics principles as the maximum amount of work that can be produced by a system or a flow of matter or energy as it comes into equilibrium with a reference environment (Dewulf et al., 2008; Moran and Sciubba, 1994; Szargut et al., 1988). Exergy, therefore, is a measure of the total amount of usefulness in any stream or system. It would be beneficial to compare various feed formulations based on total exergy usage during their production.

Aquafeeds are composed of various ingredients originating from living organisms. Exergy only takes into account the inherent potential to do work in a specific system. The growth of a living organism, however, takes much more resources than is stored in its tissues after death, and thus, the expenditure of resources, in terms of exergy, to create these tissues is much larger than the amount of exergy left in them. This can be take into account with the concept of eco-exergy, which takes into account the quantity of information embedded in the biomass, estimated from the number of encoded amino acids in the genome of the species (Bendoricchio and Jorgensen, 1997; Jørgensen et al., 1995). Eco-exergy can quantify the (thermodynamic) value of a living organism and even in an ecosystem of living organisms, which may be sacrificed for the creation of, for example, fish feed. It is therefore a useful concept when comparing feed products made from plants with products made by removing fish, as part of a natural ecosystem, from the sea. This new concept of eco-exergy was therefore proposed to investigate the impact of different ingredients of feed formulations on the environment to capture effects that cannot be investigated from a classical exergy point of view.

7. Outline of the thesis

This thesis aims to develop scientific insight on how the functional properties of protein-rich ingredients interfere with processing and final product quality. A proper understanding of these relationships guides us towards more efficient processes and could further contribute to the development of sustainable aquaculture. The research described in the thesis covers four topics:

(I) the interactions between protein-rich ingredients and process parameters;

(II) the relationships between protein-rich ingredients, pellet structure formation and physical properties of feeds;
(III) the functional properties of the ideal protein source;
(IV) the sustainability of various compositions of salmon feeds.

A schematic outline of this thesis is shown in Fig. 2.

Chapter 2 explores the effects of replacing FM by SPC and WG on the extrusion process, moisture requirements and resulting physical properties of feeds. To minimize the number of experiments and cover a broad range of formulations, a mixture design was used. The optimal compositional window remaining after considering the required physical properties of feeds was reported.

Chapter 3 evaluates how the ingredients influence the structure of the fish feed pellet and relates these to changes in technological properties. The microstructure of pellets at various levels of SPC and WG was studied using scanning electron microscopy (SEM), cryo-SEM and X-ray microtomography. The microstructural parameters responsible for the mechanical properties of feeds are identified.

Chapter 4 introduces two new protein sources as alternatives to FM as a result of general necessity for diversification of plant proteins and potential intensification of the current process. The use of a shearing device for characterizing the techno-functional properties of ingredients is reported in this chapter. Guidelines are proposed for the functional properties of the ideal plant-based protein source.

Chapter 5 discusses the environmental impact of various protein sources using three different ecological indicators. The limitations of traditional thermodynamic analysis and the advantages of extending classical exergy analysis to eco-exergy are reported.

The thesis ends with a general discussion in **Chapter 6**, which focuses on the industrial consequences of the current work, the future outlook and further possibilities to increase the sustainability of aquaculture as a whole.

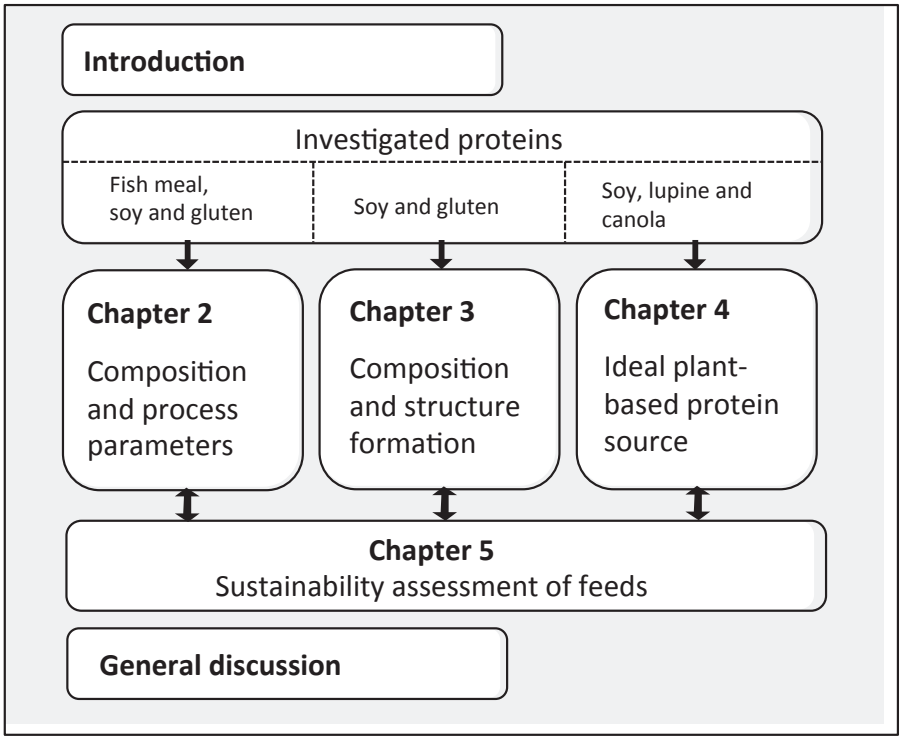

Fig. 2. Schematic overview of the thesis: towards sustainable fish feed production using novel protein sources.

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Chapter 2[1]

Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed

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Abstract

Evaluation of feed ingredient functionality plays a vital role in modern fish feed manufacturing practice. The aim of this study was to examine the extrusion behaviour of blends containing alternative protein sources from plant origin to fish meal (FM), such as wheat gluten (WG) and soy protein concentrate (SPC), and the consequences for the physical attributes of the resulting feed extrudates. A mixture design was applied, varying the levels of protein sources included in the formulation from 50 to 450 g kg^{-1}. Each diet was produced with added feed moisture content of 20, 26 and 32 g per 100 g (wet basis). The partial least squares regression models were fitted and their performance was evaluated on the basis of R^2 and the root mean squared error of cross-validation over the complete data set. A higher inclusion level of FM in the diet decreased the values of the extruder system parameters, such as torque, pressure at the die and melt temperature. In contrast, inclusion of SPC significantly increased the values of these extruder-related parameters. The viscoelastic properties of WG gave higher radial expansion; FM showed the opposite effect. The results show that the feed moisture was the dominant factor for extrudate density and oil absorption capacity. Products with higher breaking strength were observed with increasing levels of WG and SPC. Combining the product requirements for both extrudate density and hardness showed that the largest optimal compositional range is available at low feed moisture content. However, maximum FM replacement is possible at high feed moisture content.

1. Introduction

Fish meal (FM) is a scarce and increasingly expensive protein source for commercial salmon and trout feeds. This explains the need for the replacement of fish proteins by vegetable alternatives. Numerous studies have investigated the potential of alternative protein sources (Gatlin et al., 2007). Most of these studies showed that the partial replacement of FM can be successfully accomplished with respect to nutritional and health aspects (Bransden et al., 2001; Gomes et al., 1995; Kaushik et al., 1995; Refstie et al., 2001; Torstensen et al., 2008). A viable alternative feedstuff to FM must posses certain nutritional characteristics, such as low levels of fibre, starch and antinutrients, plus have a relatively high protein content, high nutrient digestibility and reasonable palatability (Gatlin et al., 2007). According to Glencross et al. (2007), ingredient functionality would be also one of the key parameters in ingredient assessment.

In addition to the nutritional aspects, fish feeds containing plant proteins should also meet physical characteristics. The extrudates should have sufficient porosity to allow good oil absorption and durability that remain upon product storage, transportation and pneumatic feeding. Typically, these parameters are greatly influenced by the raw material selection and therefore by the introduction of alternative protein sources. Vital wheat gluten (WG) and soy protein concentrate (SPC) are the two most prominent vegetable alternatives to FM with respect to their availability and nutritional value in grower salmon diets. In extrusion cooking, WG is significantly involved in the microstructural and textural formation of the extrudates (Faubion and Hoseney, 1982). SPC is already widely used in commercial salmon diets and its use is expected to increase further. In general, soy proteins are well known in the food and feed industries for a wide range of functional properties (Chen et al., 2010; Liu and Hsieh, 2008; Renkema et al., 2001; Yu et al., 2009).

In previous studies on the effect of plant protein sources and process parameters on extruder responses and product properties, the level of FM in the diets was high (≥30%) and within a relatively narrow range, often at lower added moisture content. Therefore, the present research was carried out to study the behaviour of various levels of protein source, with the aim to (almost) fully replace FM. We analysed the effects of protein composition and variation in feed moisture on the extruder system parameters (torque, pressure at the die and melt temperature) and the technical properties of fish feed. Feed moisture

content was included since it is recognized as one of the vital process variables and a significant factor affecting the properties of the final product (Chen et al., 2010; Chevanan et al., 2008; Chevanan et al., 2007; Wang et al., 2001). Based on the outcomes, an estimation of the compositional space available to replace fish protein is provided.

2. Materials and methods

2.1. Raw materials and production of extruded feeds

The chemical composition of the raw materials examined is given in Table 1. WG had the highest level of crude protein and starch compared with FM and SPC. The SPC had the lowest level of crude protein, and FM had the lowest level of total carbohydrates and starch.

Table 1. Chemical composition of fish meal (FM), wheat gluten (WG) and soy protein concentrate (SPC)

	FM[a]	WG[b]	SPC[c]
Composition g kg^{-1}			
Dry matter	921	922	916
Crude protein	697	776	604
Crude fat	110	50	17
Ash	132	12	60
Total carbohydrate	6	129	38
Starch	3	70	9

[a] Low temperature dried fish meal, Welcon, Måløy, Norway
[b] Vital wheat gluten powder, Cargill®, Barby, Germany
[c] IMCOSOY, Imcopa, Araucaria, Brazil

The formulations consisted of: commercial salmon grower diet (control diet), FM-based diet (FM diet), combination of FM and WG (FM×WG diet), combination of FM and SPC (FM×SPC diet), WG-based diet (WG diet), combination of WG and SPC (WG×SPC diet), SPC-based diet (SPC diet) and combination of FM, WG and SPC (centre diet). Table 2 shows formulations of dry mix, without oil. The evaluated protein sources were varied at levels of 50, 250 and 450 g kg^{-1} of complete diet. Simultaneously, the sum of FM, WG and SPC inclusion level was constant at 550 g kg^{-1} for all experimental diets.

Table 2. Formulation and chemical composition of the dry feed mixes

	Diets[a]							
	Control	FM	FM×WG	FM×SPC	WG	WG×SPC	SPC	Centre[d]
Formulation (g kg^{-1})								
Fishmeal	520	658	365.5	365.5	73.1	73.1	73.1	268
Wheat gluten	56.3	73.1	365.5	73.1	658	365.5	73.1	268
Soy protein concentrate	267	73.1	73.1	365.5	73.1	365.5	658	268
Wheat[b]	126	153	153	153	153	153	153	153
Sunflower meal	26.5	23.4	23.4	23.4	23.4	23.4	23.4	23.4
Vitamin and mineral premix[c]	3.8	19	19	19	19	19	19	19
Analysed composition								
Dry matter (DM)	917	919	911	917	920	916	912	913
In g kg^{-1} DM								
Protein	586	603	618	575	647	599	553	595
Fat	66	70	61	55	51	34	24	49
Starch	91	88	119	108	141	147	139	121
Ash	90	100	65	80	43	45	59	66

[a] Complete formulation of the diets contains 297and 316 g kg^{-1} oil mixture for the control and experimental diets, respectively. The inclusion level of examined protein sources in the dry mix of 73.1, 365.5 and 658 g kg^{-1} corresponds to 50, 250 and 450 g kg^{-1} in the complete formulation, respectively.
[b] Supplied by Skretting Norway, Stavanger, Norway.
[c] Vitamin levels according to NRC 93 specification (proprietary composition, Skretting ARC, Stavanger, Norway).
[d] Centre point in the mixture design.

All diets were processed at Skretting ARC Technology Plant (Stavanger, Norway). The dry ingredients were pre-mixed in a vertical mixer (custom designed, Skretting ARC, Norway) and ground in a Dinnissen hummer mill (drive capacity 30kW, Sevenum, The Netherlands), with a screen size of 1.0 mm. Subsequently, the ingredients were mixed in a Dinnissen horizontal ribbon mixer (500LTR, Sevenum, the Netherlands) for 7 min. The feed mash was conditioned in a differential diameter conditioner (custom designed, Skretting ARC). Extrusion experiments were carried out using a twin-screw extruder (TSE 36 HC Thermo Scientific, Thermo Fisher, UK) with barrel length of 1008 mm and a length/diameter ratio of 28:1. The extruder processing conditions were pre-determined for a control diet and applied throughout the study (Table 3). Slightly higher barrel temperature was used in zone 5 to facilitate

material flow in this barrel section. The ingredients were extruded as described, leading to extrudates having a diameter ranging from 6.8 to 9.2 and a length of 8 mm approximately. The knife rotation speed was adjusted according to the specified length of the extrudates.

Table 3. Constant extrusion process conditions

Process parameter	Dimension	Set point
Capacity feed mix	kg h^{-1}	75
Steam pre-conditioner	%	5
Oil pre-conditioner	%	0
Oil extruder	%	0
Set-point water temperature	°C	60
Pre-conditioner temp. down spot	°C	75
Set-temperature extruder barrel zone (2-7)	°C	100;100;100;110;100;100
Screw speed	rpm	650
Die diameter	mm	6
Die open area	mm^2	28.26

The configuration of the screws is shown in Fig. 1. The direction of the product flow was from left to right. The transport zones were built of either 1D or 1/2D conveying elements. The first forwarding kneading block consisted of 6 mixing elements at an orientation of 60 °; the two reversing kneading blocks consisted of 7 and 5 mixing elements, respectively, with a staggering angle of 30°.

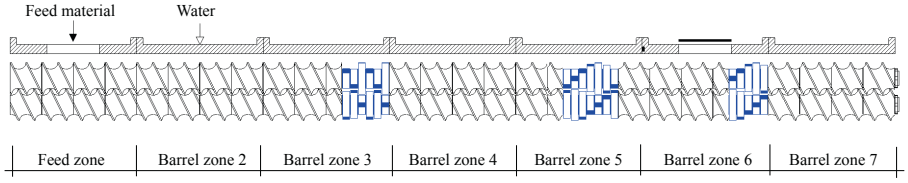

Fig. 1. A Side-view diagram of the screw configuration, from inlet (left) to die (right).

Extrudates were dried in a conventional, batch hot-air dryer (custom designed, Skretting ARC, Norway) at 90 °C to ~ 8% moisture content. The samples used to determine the technical properties were not coated with oil in order to avoid fat leakage when analysing the specific density and hardness of the feed.

2.2. Chemical analysis

Chemical analysis of the dry matter, protein, fat and ash was carried out by Skretting ARC laboratory (accredited analytical service provider). The total carbohydrate and starch analyses were carried out by Masterlab (Boxmeer, The Netherlands). Dry matter was calculated by gravimetric analysis after oven drying at 105 °C for 18 h. Protein levels were calculated from the determination of total nitrogen using the Kjeltec 2400 Auto System, based on N x 6.25. The fat concentration was measured by nuclear magnetic resonance (Maran Ultra NMR, Resonance Instruments, Witney, UK). Starch was analysed using an enzymatic method described by McCleary et al. (1994). Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550 °C for 17 h.

2.3. Determination of water holding capacity

The water holding capacities (WHC) of the protein sources were determined using methods modified from Heywood et al. (2002) and Lin and Zayas (1987). Five grams of total flour were dispersed in 25 mL of distilled water in a 50 mL centrifuge bottle. Bottles were agitated for 10 min, and then centrifuged at 4000 rpm for 30 min.

After decanting the supernatant, each bottle was weighed and the WHC (g of water per g flour) was calculated using Eqn. (1):

$$\mathrm{WHC} = \frac{\left[\left(\text{wt of bottle after decanting} - \text{wt of dry bottle}\right) - \text{total flour wt (g)}\right]}{\text{total flour wt (g)}} \quad (1)$$

The results were the average of three replicates.

2.4. Measurement of extrudate properties

Kernel morphology: The extrudates were photographed using a zoom digital camera (Olympus μ 780, 5x optical zoom) with 7.1 effective megapixels for high-definition pixel images.

Radial expansion: The ratio between the extrudate diameter and die nozzle orifice diameter was used to quantify the product expansion at the extruder die. The reported values were the average of 10 replicates from randomly selected extrudates.

Specific density: A volumetric displacement method using glass beads with a diameter of 0.1 mm as a displacement medium was used to determine

the specific density of the extrudates. The method was originally developed by Hwang and Hayakawa (1980). The specific density of the extrudates was calculated using Eqn. (2):

$$\rho_s = \frac{W_{ex}}{V_{eg} - \left(W_{gs} / \rho_{gs}\right)} \quad (2)$$

where ρ_s, is the specific density using the glass bead displacement method (g l^{-1}), W_{ex} is the extrudate mass (g), V_{eg} is the volume of the extrudates and glass powder, W_{gs} is the mass of glass beads displaced (g) and ρ_{gs} is the specific density of the glass beads (g l^{-1}). The values were obtained from an average of two measurements.

The breaking strength of extrudates was determined using a texture analyser TM2 DLX (Food Technology Corporate, USA) with a 13 kN load cell. Twenty grams of sample were placed in the cell and analysed using a cross head speed of 4.42 mm s^{-1}. The maximum force required to break the extrudates was expressed as the breaking force (kg). The reported values were the average of 10 replicates.

Oil absorption capacity: Samples of the extrudates (500 g) from each treatment were placed into a laboratory vacuum coater (custom designed, Skretting ARC, Norway) with an excess (400 g) of heated (60 °C) fish oil and thoroughly mixed throughout the whole coating cycle. Air was slowly evacuated from the vacuum chamber until all visible signs of air escaping from the extrudates ceased. This normally occurred at a reduced pressure of 0.85 bar. Once all visible signs of air escaping had ceased, the vacuum chamber was re-equilibrated to atmospheric pressure and the oil was allowed to infuse into the feed. The extrudates were then removed from the coater and any excess oil was removed by placing the feed between two absorbent paper towels. The final weight of the oil infused into the extrudates was then recorded and the relative oil uptake was calculated.

2.5. Experimental design and statistical analysis

A mixture design was applied to systematically vary the levels of FM, WG and SPC in the diets. In addition, a full factorial design was used for the added feed moisture content. Each experimental diet was produced at added feed moisture contents of 20, 26 and 32 g per 100 g (wet basis). Two additional centre points were included in the design to verify the stability and reproducibility of the extrusion process.

The study comprised 25 production runs. The results were analysed by multivariate data analysis, using Unscrambler computer software (Unscrambler® 9.7, 2009, Camo A/S, Oslo, Norway). The datasets were standardized because the variables had different units. The standardization was performed by dividing each parameter with its standard deviation. Partial least squares (PLS 1) was used as the regression method. The models were obtained using systematic cross-validation. The significance levels of each variable were determined on the basis of the jack-knife estimates of the uncertainties of the model parameters. Further information on the method can be found elsewhere (Esbensen, 2006).

The inter-relationships between the dependent variables were found using the correlation coefficients (*R*). The significance of the *R*-values was decided at a probability level of 0.01. The contour plots were performed using Statistica version 9.0 (Statsoft Inc., Tulsa, OK, USA). The contour plots were generated from the raw data using the quadratic fit.

3. Results

3.1. Feed extrusion and chemical composition of the diets

All the experimental diets were extruded using the constant extrusion parameters pre-determined for the control diet and presented in Table 3. The extruder system parameters and the product characteristics reproduced well when the production of the centre diet was repeated.

The chemical analysis revealed that the content of crude protein, fat and starch in the diets varied. These three changes are of important consideration, because the effects of examined protein sources might also be caused by differences in chemical composition between the diets. Especially, the polysaccharides present will influence the water absorption and the extrudate hardness as a result thereof. The crude protein level varied from 553 to 647 g kg $^{-1}$, while the fat level varied from 24 to 70 g kg $^{-1}$ (Table 2). Starch level changed notably with the inclusion of both vegetable protein sources. Starch levels ranged from a low of 88 g kg $^{-1}$ in the FM diet to a height of 147 g kg $^{-1}$ in the WG×SPC diet. Starch is commonly added to salmonid feed to improve binding of the matrix and facilitate expansion with a practical inclusion level of approximately 10% in the final feed (Sørensen et al., 2010).

Table 4. Extruder torque, pressure at the die, melt temperature, radial expansion, specific density, peak breaking force and oil absorption capacity of the extrudates

Diet formulation	Motor torque[a] (Nm)	Pressure at the die[b] (bar)	Melt temperature[c] - zone 5 (°C)	Radial expansion[d] (-)	Specific density[e] (g l^{-1})	Peak breaking force[f] (* 100 kg)	Oil absorption capacity (%)
Control	98	8	121	1.19 (0.03)	870	7.1 (0.2)	36.6
20 g per 100 g (wet basis) moisture							
FM	93	7	124	1.14 (0.02)	890	6.5 (0.2)	34.6
FM×WG	103	14	128	1.28 (0.02)	950	8.6 (0.2)	28.2
FM×SPC	111	12	131	1.19 (0.02)	920	7.8 (0.3)	32.0
WG	76	9	129	1.36 (0.02)	980	9.3 (0.2)	27.9
WG×SPC	134	20	135	1.29 (0.02)	960	9.4 (0.3)	28.4
SPC	145	40	138	1.30 (0.01)	750	9.2 (0.2)	51.4
26 g per 100 g (wet basis) moisture							
FM	94	8	118	1.18 (0.02)	800	6.3 (0.3)	43.0
FM×WG	87	10	120	1.39 (0.02)	750	8.3 (0.2)	48.2
FM×SPC	99	11	124	1.14 (0.01)	820	7.3 (0.3)	42.9
WG	110	15	124	1.40 (0.02)	910	9.8 (0.3)	32.1
WG×SPC	122	18	128	1.42 (0.03)	760	8.6 (0.2)	47.1
SPC	136	25	134	1.37 (0.03)	700	8.6 (0.2)	56.4
32 g per 100 g (wet basis) moisture							
FM	85	6	113	1.23 (0.03)	730	6.5 (0.2)	51.1
FM×WG	84	5	113	1.39 (0.02)	660	6.9 (0.3)	63.3
FM×SPC	96	7	118	1.23 (0.02)	740	6.5 (0.2)	53.8
WG	97	8	116	1.49 (0.03)	720	8.2 (0.3)	50.3
WG×SPC	106	12	119	1.53 (0.04)	690	6.9 (0.1)	55.3
SPC	119	18	123	1.36 (0.01)	710	7.8 (0.3)	57.5

[a] Mean of run-length measurement; [b] Mean of run-length measurement; [c] Mean of run-length measurement; [d] Mean (standard deviation) of 10 replicates; [e] Mean of duplicates; [f] Mean (standard deviation) of 10 replicates

3.2. Effects of protein source and feed moisture on extruder system parameters

The effects of the protein source and feed moisture on the extruder system parameters are presented in Table 4. The motor torque values varied between 76 and 145 Nm, depending on the protein source and feed moisture. The lowest motor torque (76 Nm) was obtained at a high level of WG (450 g kg^{-1}), and low feed moisture (20 g per 100 g, wet basis). The highest motor torque was observed at a high level of SPC (450 g kg^{-1}) and low feed moisture (Table 4). Except for the high WG diet, Table 4 also shows that motor torque decreased with increasing moisture. In this study, the pressure measured at the die ranged from 5 to 40 bar. When the feed moisture was increased from 20 to 32 g per 100 g (wet basis) at 450 g kg^{-1} inclusion of SPC, the pressure at the die decreased from 40 to 18 bar (Table 4).

Considering all treatment combinations, the highest recorded melt temperatures (t_m) were in the zone 5, which could be attributable to the set barrel temperature of 110 °C and the long reverse kneading block in this barrel head. The temperature t_m ranged from 113 to 138 °C. There was a wider range of t_m at low and medium added feed moisture (Table 4). The lowest t_m was observed for the FM and FM×WG diets at high added moisture. The highest melt temperature was recorded for the SPC diet at low added moisture. The weighted regression coefficients of the models and their quality analysis are presented in Table 5. All models were able to explain 57.8–96.5% of the variation (R^2). The PLS regression models for torque, pressure at the die and melt temperature showed R^2- values of 0.86, 0.58 and 0.96, respectively. The highest slope (0.98) was obtained for the melt temperature. The WG formulation at 20 g per 100 g (wet basis) feed moisture was detected as an outlier due to slight fluctuations in the motor torque and therefore it was not included in the model. The negative weighted regression coefficients in the model (Table 5) showed negative effects of FM, WG and feed moisture, and a positive effect of SPC on the motor torque. The results also indicated significant effects of FM, SPC and feed moisture on the motor torque.

Although a decrease of feed moisture resulted in increased die pressure, the effect was not significant (Table 5). However, there was a significant decrease in die pressure with an increase in FM and a decrease in SPC. The FM, SPC and feed moisture significantly influenced the melt temperature (Table 5). Increasing the level of SPC resulted in a higher t_m; FM and feed moisture showed the opposite effect. Increasing the level of WG did not have a significant effect (Table 5).

Table 5. Effect of the proportion of FM, WG, SPC and added feed moisture on the extruder system parameters and technical properties of the extrudates

Property	Equation	R^2	Slope[a]	RMSEP[b]
Torque (Nm)	–0.46·FM* – 0.02·WG + 0.49·SPC* – 0.42·MO*	0.86	0.90	6.82
Pressure at the die (bar)	–0.40·FM* – 0.10·WG + 0.52·SPC* – 0.26·MO	0.58	0.55	5.59
Melt temperature – zone 5 (°C)	–0.32·FM* – 0.05·WG + 0.38·SPC* – 0.78·MO*	0.96	0.98	1.39
Radial expansion (–)	–0.50·FM* + 0.47·WG* + 0.03·SPC + 0.27 ·MO	0.77	0.71	0.057
Specific density (g l^{-1})	0.05·FM + 0.15·WG – 0.20·SPC – 0.79·MO*	0.60	0.66	0.07
Peak breaking force (kg)	–0.49·FM* + 0.37·WG* + 0.13·SPC – 0.49·MO*	0.77	0.81	55.98
Oil absorption capacity (%)	–0.08·FM – 0.17·WG + 0.24·SPC + 0.78·MO*	0.63	0.68	7.06

In the equations, FM, WG and SPC are proportions of these ingredients and MO is added feed moisture.
FM, fish meal; WG, wheat gluten; SPC, soy protein concentrate; MO, feed moisture
[a] Slope of the regression line between abscissa (X) and ordinate (Y)
[b] Root mean square error of prediction.
* The significance of the weighted regression coefficient

3.3. Effects of the protein source and feed moisture on the technical properties of the feed

Fig. 2 shows the effect of the inclusion of FM, WG and SPC on the appearance of the extrudates. The products obtained from the various diets clearly differed in terms of the surface roughness and colour. However, at 32 g per 100 g (wet basis) feed moisture, the differences were much less pronounced.

The values for the radial expansion, specific density, peak breaking force and oil absorption capacity of the extrudates produced at different levels of added moisture are shown in Table 4. The product with the lowest radial expansion was produced for the FM×SPC diet at medium feed moisture (26 g per 100 g, wet basis). The highest radial expansion was observed for WG and SPC inclusion at 250 g kg^{-1} and high moisture level (32 g per 100 g, wet basis). When the level of FM increased from 50 to 450 g kg^{-1}, the radial expansion decreased progressively, whereas an increased level of WG resulted in higher radial expansion. Radial expansion increased only marginally with increasing levels of SPC and feed moisture (Table 5). The density of the feed varied from 660 to 980 g l^{-1} (Table 4) and was significantly affected by feed moisture (Table 5). The actual composition had a smaller effect. Increasing the level of FM and WG resulted in a slight increase in density, whereas increasing the level of SPC led to a decrease in extrudate density.

Fig. 2. Feed extrudate morphology at 26 g per 100 g (wet basis) added moisture. Difference in surface roughness of the control (a), FM, WG and SPC diets (b, c and d), respectively.

There were significant differences in extrudate hardness for the different protein sources (Table 4). The peak breaking force of the products ranged from 629 to 980 kg force; the maximum occurred with a WG level of 450 g kg^{-1} and medium moisture content. The lowest hardness was observed for the high level of FM and feed moisture of 26 g per 100 g (wet basis). With an increase in the level of FM and feed moisture, there was a decrease in extrudate hardness. A higher level of WG increased the peak breaking force significantly. Table 5 shows that the level of FM and feed moisture were dominant factors influencing the extrudate hardness, followed by the level of WG in the formulation. Inclusion of SPC did not show a statistically significant effect.

The lowest oil absorption capacity was observed for WG at 450 g kg^{-1} and low feed moisture; the highest was in diet containing 250 g kg^{-1} FM and WG at high added moisture. There were almost no differences in the effect on oil absorption capacity for the different protein sources.

The regression models for radial expansion, specific density, peak breaking force and oil absorption capacity showed R^2 values of 0.77, 0.60, 0.77 and 0.63, respectively (Table 5). The highest slope (0.81) was observed for the peak breaking force.

3.4. Correlation of the response variables

Correlation analysis of multivariate data provides valuable information about the relationship between the different properties in extrusion studies. Higher correlation between some unexpected variables and lower correlation between some expected variables can reveal important inferences (Nehru et al., 2007). The correlation coefficients obtained between the extruder system parameters and the technical properties of the feed are presented in Table 6.

Table 6. Correlation coefficients for extruder system parameters and technical properties of the extrudates

	TRQ	PRE	t_m	REX	SD	PBF	OAC
TRQ	1*						
PRE	0.89*	1*					
t_m	0.75*	0.79*	1*				
REX	0.13	0.16	–0.09	1*			
SD	–0.05	–0.05	0.46	–0.40	1*		
PBF	0.49	0.60	0.70*	0.41	0.41	1*	
OAC	0.07	0.08	–0.42	0.38	–0.99*	–0.39	1*

TRQ, torque; PRE, pressure at the die; t_m, melt temperature (zone 5); REX, radial expansion; SD, specific density; PBF, peak breaking force; OAC, oil absorption capacity.
* Denotes significant correlation coefficients.

The results show a strong linear correlation between the torque, pressure at the die and the t_m values. Ilo et al. (1999) observed that unit density is inversely related to the expansion ratio. This agrees with the results from the present research, although the correlation was not significant. In general, these findings imply the significance of axial expansion. The specific density was highly correlated with the oil absorption capacity. Peak breaking force and t_m gave a correlation coefficient of 0.70.

4. Discussion

To quantify the influence of the protein source and the feed moisture on extruder responses and the technical quality of the feed, we applied a mixture design in combination with the multivariate PLS regression technique. The levels of protein sources used are consistent with current and foreseeable future commercial guidelines. This experimental design minimized the required number of experiments and covered a wide range of ingredient contents. These methods allowed the original 133 data to be reduced to 28 weighted regression coefficients. None of the models showed signs of non-linearity as observed by normal probability plots of residuals (data not presented).

In current fish feed manufacturing practice, several technical properties of the constituent ingredients are required to meet the demands for efficient processing and properties of the final product. These properties include the ability to bind within the pellet matrix and allow expansion of the product (Glencross et al., 2010). This should result in a finely dispersed pore structure. The importance of porosity is manifested through the sinking rate of the extrudates, as well as the ability to infuse oil into the extrudate matrix in a vacuum (Øverland et al., 2007).

4.1. The behaviour of the mixtures in the extruder

The composition of raw materials is known to have a significant effect on extruder system parameters. The negative correlation between FM and motor torque (Table 5) may be explained by the higher fat content in this material compared with the vegetable alternatives.

The strong positive effect of SPC on the motor torque (Table 5) can be attributed to the high water holding capacity of this ingredient (2.63 g H_2O per g flour) compared with FM (1.47 g H_2O per g flour) and its lower fat content. The same mechanisms can also link WG inclusion and the motor torque because wheat gluten had the lowest water holding capacity (1.39 g H_2O per g flour). The dependence of motor torque on feed moisture is well known. This finding was expected and is in accordance with the results presented by Lin et al. (2000). Motor torque and pressure at the die are functions of the dough viscosity during extrusion cooking (Pansawat et al., 2008).

The metal-sensing temperatures differed widely (≤28°C) from the temperature continuously measured by the flush-mounted melt thermocouples (data not

presented). This was expected because heat was generated during extrusion through viscous dissipation of the mechanical energy (Meng et al., 2010). Therefore, the strong positive effect of SPC on t_m (Table 5) can be attributed to an indirect effect through the increase in motor torque and resulting viscous dissipation. Water acts as a plasticizer in the extruder, and a higher feed moisture reduces the melt viscosity and the viscous dissipation (Ilo et al., 1996). For this reason, the product temperature was lower when feed moisture was higher (Table 5).

4.2. Product properties

In general, inclusion of vegetable proteins led to increased hardness, rougher surface, and greater radial expansion of the extrudates. In addition, these parameters were greatly affected by the feed moisture added.

The level of expansion is an important factor in aquafeeds as it affects the density, fragility, hardness and oil holding capacity (Rosentrater et al., 2009). The inclusion of FM was negatively associated with the radial expansion (Table 5) and it may be explained by the lowest starch level among the protein sources evaluated (Faubion et al., 1982; Linko et al., 1981). WG tends to expand in the radial direction most likely due to the viscoelastic properties of glutenin proteins, while expansion of SPC was mainly in the axial direction (Table 5). Cheftel et al. (1992) stated that when soy protein was extruded at a moisture content higher than 50%, fibrous and non-expanded products were observed. Although the moisture content in this study was not higher than 32 g per 100 g (wet basis), the tendency of soy proteins to form a fibrous structure may be one possible explanation for the lack of radial expansion for SPC. Extrudate density is another important factor in fish feed and determines the floatability of the product (Chevanan et al., 2009). This parameter considers volumetric expansion in all directions (Phillips et al., 1984). Inclusion of WG was positively associated with the product density (Table 5).

Extrudate hardness essentially refers to the physical integrity of the finished product in terms of handling and transport. It relates to generation of broken extrudates. Our results show that extrudate hardness was hardly correlated with either radial expansion or specific density (Table 6), which suggests the importance of other aspects of the protein source and feed moisture. The difference in peak breaking force for feeds containing high FM compared to feeds containing high SPC and especially WG (Table 4), emphasize that protein sources differ in functionality.

It has been found that extrudate hardness is important for nutrition of animals (Thomas van der Poel, 1996), suggesting that the melt temperature or motor torque will influence the nutritional value of the feed produced due to the correlation found (Table 6). However, the effects might be limited as Barrows et al. (2007) reported no effect of extruder barrel temperature on feed intake and weight gain of rainbow trout (*Oncorhynchus mykiss*). On the contrast, extrudate hardness is related to water stability and Bæverfjord et al. (2006) found that the low water stability of the diet resulted in separation and accumulation of free oil in the stomach of rainbow trout. In the next section, we will define an optimal range of extrudate hardness which is also anticipated to result in satisfactory water stability of the feeds. Most likely, the observed hardness values above the upper limit may impact feed intake negatively. However, this needs further investigation.

4.3. Towards a compositional window of optimal technical properties of extrudates

The contour plots were made to visualize the potential interactions between components that were not included in the regression models. This section focuses on specific density and hardness as an example. Other properties could have been investigated using the same method. We expected to find possible interactions between SPC and WG in case of extrudate density. The contour plots (Fig. 3) revealed interactions of FM and SPC at 32 g per 100 g (wet basis) added water. No other notable interactions were observed. According to the requirements for sinking and oil absorption, the optimum specific density ranged from 740 to 920 g l^{-1}. This optimum was observed for FM feeds and combination of FM and SPC at 20 g per 100 g (wet basis), as well as combination of FM and SPC at 32 g per 100 g (wet basis) added moisture, as shown in Fig. 3.

According to the commercial guidelines, acceptable hardness (740–850 kg) was found under conditions of restricted moisture for feeds containing higher levels of FM, whereas higher moisture content was appropriate for feeds containing high levels of SPC and WG (Fig. 4). However, no notable interactions were observed among protein sources on extrudate hardness.

The contour plots can also be used to derive the optimal compositional range of the protein sources evaluated when the product requirements for density and hardness are combined. Fig. 5 shows the area in which the requirements for both technical properties are met. The largest overlapping

area was obtained at low moisture content. In that case, about 40–50% of the

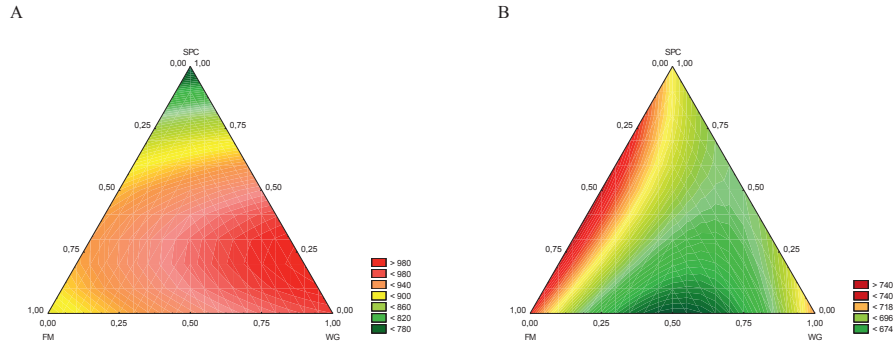

Fig. 3. Contour plots showing the effects of FM, WG, SPC and feed moisture (A- 20 and B-32 g per 100 g, wet basis) on specific density of extrudates.

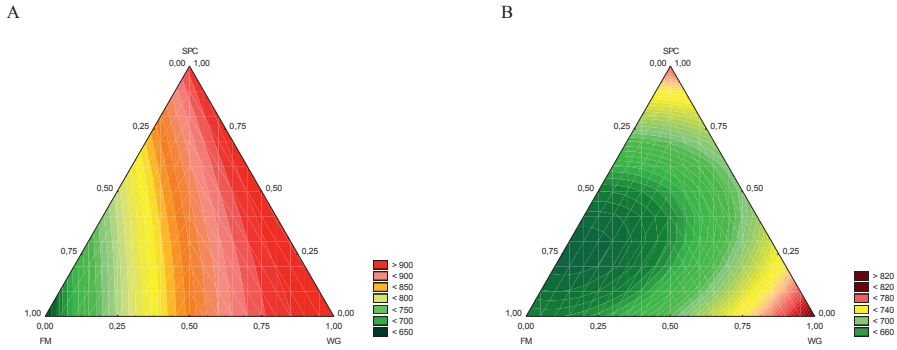

Fig. 4. Contour plots showing the effects of FM, WG, SPC and feed moisture (A- 20 and B-32 g per 100 g, wet basis) on peak breaking force of extrudates.

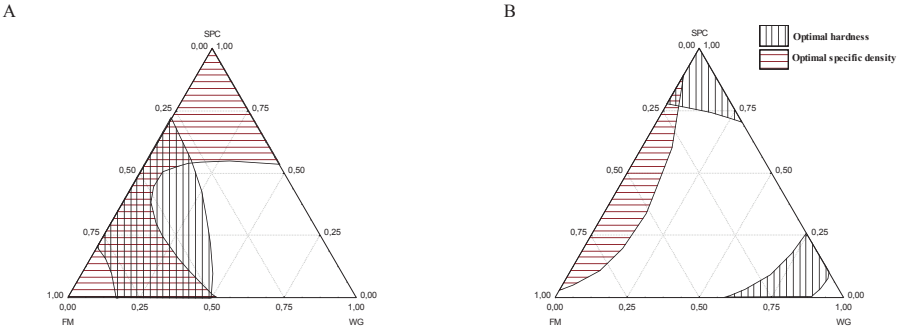

Fig. 5. Contour plots showing the optimal hardness and specific density of extrudates at moisture level of 20 and 32 g per 100 g, wet basis (A and B), respectively

FM could be replaced by rather arbitrary proportions of WG and SPC or slightly higher SPC content. With higher moisture contents, about 75% of FM could be replaced by SPC.

However, high levels of WG did not fulfil the requirements for both product properties, regardless of the feed moisture added. It could be postulated that the inclusion of the third relevant product parameter might lead to a further diminishing of the compositional window. For example, the extrudate surface became rather rough when high levels of SPC were included. Therefore, it can be concluded that FM has unique functional properties that affect the technical quality of fish feed. Future research should therefore focus on improving our understanding of the functional properties of FM and finding ways to adjust the properties of plant proteins accordingly.

5. Conclusions

The use of a mixture design in combination with multivariate PLS regression is a fast and efficient technique for quantifying the differences between the protein sources studied. The replacement of FM with plant proteins and changes in feed moisture affected the extruder system parameters and the technical properties of fish feed.

From the results, it can be concluded that FM has unique functional properties that are not naturally present in the vegetable protein sources evaluated. SPC and WG were positively associated with the strength of the extrudates. However, the compositional window remaining after considering two technical properties of feed was rather narrow. The greater part of FM could be replaced with SPC at higher moisture levels. High levels of WG did not fulfil the product requirements and WG is therefore less good alternative to FM.

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Chapter 3[2]

Wheat gluten in extruded fish feed: effects on morphology and on physical and functional properties

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Abstract

This article focuses on understanding the role of vital wheat gluten on the structural parameters of extruded fish feed and its correlation to the physical and functional properties. Gluten–soy protein concentrate blends with five gluten concentrations (0–200 g kg^{-1}) were produced. An abrupt reduction in oil uptake was observed with the 200 g gluten kg^{-1} blend. Inclusion of gluten from 100 to 200 g kg^{-1} resulted in unacceptable product properties. Sinking of feed pellets with 0 and 50 g gluten kg^{-1} was 100%, whereas only 36% of pellets with 200 g gluten kg^{-1} sank. We suspect that this is due to a relationship between morphological structure and oil impregnation during coating of feeds. The addition of gluten at 200 g kg^{-1} gave a smoother and non-porous outer surface. Pellets without gluten had a larger number of cells that were smaller than 200 µm ($P<0.05$) compared with pellets with 100 and 200 g gluten kg^{-1}. More spherical cell shapes ($P<0.01$) and a compact structure were favoured in the presence of gluten. The closed porosity increased ($P<0.05$), whereas interconnectivity between pores decreased ($P<0.01$), with increasing gluten content from 0 to 200 g kg^{-1}. The effects of the addition of gluten are probably related to the film-forming properties of gluten.

1. Introduction

In an effort to increase formulation flexibility in the production of modern salmonid feeds, and to enhance the sustainability of feeds by replacement of fish protein with plant protein, vital wheat gluten has been shown to have high potential as a feed ingredient (Gatlin et al., 2007). Among plant proteins, soy protein concentrate (SPC) is still the primary alternative ingredient to fish meal due to its availability and competitive prices, but the high concentration of carbohydrates in SPC remain a concern (Gatlin et al., 2007). In this respect, vital wheat gluten may have good potential due to its high protein content and nutrient digestibility (Robaina et al., 1999; Sugiura et al., 1998), its lower level of indigestible fibres and absence of anti-nutritional factors. Compared with fish meal, wheat gluten is low in methionine and especially low in lysine, whereas it is higher in cysteine content (Allan et al., 2000). It has been reported previously that in salmonids, supplementation with lysine (Cheng et al., 2003; Davies et al., 1997) or a combination of lysine and methionine (Pfeffer and Henrichfreise, 1994) is required for diets containing wheat gluten to maintain fish growth. Wheat gluten is also extensively used in food applications due to its functionality (Day et al., 2006) and availability in large quantities (Domenek et al., 2004).

Feed pellets obtained after extrusion should have a well-defined porosity that allows sufficient oil absorption capacity leading to specific sinking rates and durability (Glencross et al., 2010). In a commercial fish feed manufacturing operation, incorporation of gluten was shown to significantly influence the physical properties of feed, such as oil infusion. For example, in high-fat feeds (>300 g kg^{-1} added fat), gluten can be used only in small amounts (<100 g kg^{-1}) because of the negative effect on this and other related technical features of the feed. The underlying mechanism affecting the physical properties of these extrudates is as yet unclear.

A quantitative description of the microstructure of the extrudate helps in understanding its mechanical properties (Robin et al., 2010). The microstructural features of cellular products, such as average cell size, cell size distribution, cell wall thickness, control product attributes such as the void fraction and the interconnectivity of the cells (Gibson and Ashby, 1997). The infusion of liquids such as oil strongly depends on cell size distribution, the degree of interconnectivity between the cells and the average cell wall thickness (Trater

et al., 2005). The precise influence of using wheat gluten for partial or complete replacement of fish meal on the morphology has not been described yet.

Several techniques were used to study the microstructure of expanded extrudates, such as scanning electron microscopy (SEM) (Warburton et al., 1992), and light microscopy (Chanvrier et al., 2007) followed by digital imaging (Stojceska et al., 2008). However, these techniques only gave information about a surface or a fracture plane. X-ray microtomography (XMT) provides a non-invasive means of assessing the morphology in three dimensions. To the best of our knowledge, this technique has not yet been used to study the impact of plant proteins on the morphology of fish feed extrudate.

The specific objective of this study was to determine the links between the physical and microstructural characteristics of extruded feeds based on gluten–SPC blends and relate these to the properties of the respective components. The microstructure of the pellets was altered through inclusion of different levels of wheat gluten mainly.

2. Materials and methods

2.1. Raw materials and processing

All diets were processed at Skretting ARC Technology Plant (Stavanger, Norway). The formulations consisted of a commercial salmon grower diet (0 g gluten kg^{-1}) and four experimental diets containing gluten, in which SPC was partly replaced to give diets containing 50, 100, 150 and 200 g gluten kg^{-1} (50, 100, 150 and 200 g gluten kg^{-1} diets, respectively). Commercially available SPC (Imcosoy) and gluten (vital wheat gluten powder) were obtained from Imcopa (Imcopa SA, Araucaria, Brazil) and Cargill (Cargill Germany GmbH, Barby, Germany), respectively. Table 1 gives the formulations and chemical composition of the diets. The chemical composition of the raw materials is given in the footnotes of Table 1. Chemical analysis of the diets (Table 1) revealed that the inclusion of gluten led to a lower level of crude fibre and higher level of starch and fat. However, no notable differences in the content of crude protein and ash were observed between the feeds (Table 1).

Table 1 Formulation and chemical composition of the dry feed mixes.

	0 g gluten kg^{-1}	50 g gluten kg^{-1}	100 g gluten kg^{-1}	150 g gluten kg^{-1}	200 g gluten kg^{-1}
Formulation (g kg^{-1})[a]					
Whe[a]tb	108.9	108.9	108.9	108.9	108.9
Faba beans (dehulled)[c]	108.2	108.2	108.2	108.2	108.2
Fishmeal[d]	233.3	233.3	233.3	233.3	233.3
Soy protein concentrate[e]	466.6	388.9	311.1	233.3	155.6
Wheat gluten[f]	0	77.8	155.6	233.3	311.1
Sunflower meal[g]	46.7	46.7	46.7	46.7	46.7
Mineral and vitamin mix[h]	36.6	36.6	36.6	36.6	36.6
Analysed composition					
Dry matter (DM) (g kg^{-1})	922	920	922	921	914
In g kg^{-1} DM					
Protein	582	598	603	601	621
Fat	47	49	53	60	63
Starch	126	125	141	164	155
Crude fibre	42	40	36	31	31
Ash	81	79	75	67	66

[a]Complete formulation of the diets contains 345 g kg^{-1} oil mixture. The inclusion level of gluten in the dry mix of 77.8, 155.6, 233.3, and 311.1 corresponds to 50, 100, 150 and 200 g kg^{-1} in the complete formulation, respectively.
[b]Supplied by Skretting AS, Stavanger, Norway. Containing (g kg^{-1}): dry matter 869; In g kg^{-1} DM: protein 135; fat 32; starch 692; crude fibre 25; ash 18.
[c]Supplied by Skretting AS, Averøy, Norway. Containing (g kg^{-1}): dry matter 862; In g kg^{-1} DM: protein 304; fat 23; starch 534; crude fibre 21; ash 30.
[d]Low temperature dried fish meal, Welcon, Egersund, Norway. Containing (g kg^{-1}): dry matter 931; In g kg^{-1} DM: protein 723; fat 142; starch 5; crude fibre 4; ash 145.
[e]Containing (g kg^{-1}): dry matter 923; In g kg^{-1} DM: protein 673; fat 22; starch 62; crude fibre 49; ash 65.
[f]Containing (g kg^{-1}): dry matter 918; In g kg^{-1} DM: protein 821; fat 67; starch 87; crude fibre 6; ash 13.
[g]Supplied by Skretting AS, Stavanger, Norway. Containing (g kg^{-1}): dry matter 910; In g kg^{-1} DM: protein 404; fat 37; starch 45; crude fibre 182; ash 72.
[h]Vitamin levels according to NRC 93 specification (proprietary composition, Skretting ARC, Stavanger, Norway). Complete formulation of the diets contains 23.8 g kg^{-1} mineral and vitamin mix.

The dry ingredients were premixed in a vertical mixer (custom designed, Skretting ARC, Stavanger, Norway) and ground in a Dinnissen 30 kW hammer mill (Dinnissen, Sevenum, The Netherlands), with a screen size of 1.0 mm. Subsequently, the ingredients were mixed in a Dinnissen 500LTR horizontal ribbon mixer (Dinnissen, Sevenum, The Netherlands) for 7 min. The feed mash was conditioned in a differential diameter conditioner (DDC 2, Wenger Mfg.

Co., Sabetha, KS, USA) and extruded in a Wenger TX-57 twin-screw extruder. The barrel of the extruder was 57 mm in diameter and the length-to-diameter ratio (L/D) was 17.5:1. The screw configuration was composed of a series of intermeshing feed screws (FS), a forwarding kneading block (FK) and reversing kneading blocks (RK) arranged according to the defined barrel diameters (*D*) such that the overall configuration from the drive end was: 5*D* FS, 1*D* FK, 8*D* FS, 0.5*D* RK, 1*D* FS, 0.5*D* RK, 1.5*D* FS: to the die. The extruder barrel consisted of four head sections, with each section jacketed to permit either steam heating (sections 1–4) or water cooling (sections 2–4). Temperature control of the second, third and fourth section is achieved by balancing the heating and cooling power input.

The ingredients were processed as described, yielding pellets with a diameter of 8.7 mm and a length of approximately 10.0 mm. The feed was dried in a Wenger Series III horizontal 3-zones dryer (Wenger Mfg. Co., Sabetha, KS, USA) to approximately 920 g kg^{-1} dry matter. The allotted oil component of each diet was vacuum infused to the pellets in a Forberg 6-l vacuum coater (pilot-scale coater) (Forberg®, Larvik, Norway). The mixtures were extruded using the parameters presented in Table 2. Slight adjustments were made to the screw speed and barrel temperature to obtain similar expansion for each composition. The knife rotation speed was adjusted according to the specified length of the pellets. Other extruder operating conditions were constant for all the feeds produced. After at least 10 min of running, discrete samples of pellets ($n = 4 \times 1000$ g) were collected every 15 min to create a repeated measures assessment of each diet.

The specific mechanical energy (SME) and specific thermal energy (STE) were read directly from the control panel of the extruder. The data were collected by APIS real-time Process Explorer (version 4.1, Prediktor, Fredrikstad, Norway). The values presented are the means of 10 measurements taken at equal time intervals throughout the production run.

2.2. Chemical analysis

Chemical analyses of the dry matter, protein, fat and ash were carried out by Skretting ARC laboratory (an accredited analytical service provider). The starch and crude fibre analysis was carried out by Masterlab (Boxmeer, The Netherlands). The dry matter was calculated by gravimetric analysis after oven

Table 2 Extruder and dryer processing parameters during feed production.

Diet formulation	0 g gluten kg^{-1}	50 g gluten kg^{-1}	100 g gluten kg^{-1}	150 g gluten kg^{-1}	200 g gluten kg^{-1}
Added moisture[a] (% of feed rate)	31	31	31	31	31
Extrusion					
Capacity feed mix, kg h^{-1}	150	150	150	150	150
Steam added to pre-conditioner, kg h^{-1}	10.5	10.5	10.5	10.5	10.5
Water added to pre-conditioner, kg h^{-1}	16.5	16.5	16.5	16.5	16.5
Temperature pre-conditioner[b], °C	74	72	72	72	70
Water added to extruder, kg h^{-1}	19.5	19.5	19.5	19.5	19.5
Temperature of extruder water, °C	60	60	60	60	60
Temperature section 1[c], °C	64	62	63	62	64
Temperature section 2[d], °C	63	60	62	60	62
Temperature section 3[e], °C	80	81	81	64	86
Temperature section 4[f], °C	80	79	81	70	81
Revolution of screws, rpm	372	378	366	372	403
Die orifice diameter, mm	6.5	6.5	6.5	6.5	6.5
Die open area (mm^2)	66.4	66.4	66.4	66.4	66.4
SME[g], kJ kg^{-1}	179	170	144	139	130
STE[h], kJ kg^{-1}	233	232	216	207	225
Drying					
Temperature section 1, °C	90	95	100	100	100
Temperature section 2, °C	85	90	95	100	100
Temperature section 3, °C	75	90	85	95	95
Total drying time (min)	13	13	13	13	13

[a]Calculated on the basis of raw materials fed to the pre-conditioner; weight of raw materials on 'as is' basis.
[b]Temperature pre-conditioner, temperature at the outlet of the pre-conditioner.
[c-f]Actual extruder barrel temperature at sections 1–4.
[g]SME, specific mechanical energy.
[h]STE, specific thermal energy.

drying at 105 °C for 18 h. The protein levels were calculated by determining the total nitrogen content using the Kjeltec 2400 Auto System, based on N × 6.25. The fat concentration was measured by Maran Ultra NMR nuclear magnetic resonance (Resonance Instruments Ltd, Witney, UK). NMR measures the number of hydrogen protons of some character in a sample. This is done by

letting the protons induce a current in a closed coil. The sample was placed in an external magnetic field, which aligns the protons, creating a net magnetic field. An RF pulse is applied to the sample, creating a dynamic change in the field. This induces a current in a coil surrounding the sample. The induced current is then related to the fat content by a simple two-point calibration. The gross ash content was determined gravimetrically as the mass remaining after combustion of a sample in a muffle furnace at 550 °C for 17 h. Starch was analysed using an enzymatic method described by McCleary et al. (1994). The crude fibre content was determined as the loss in mass resulting from ashing of the dried residue obtained after acid and alkaline digestion of the sample according to ISO 6865 (ISO, 2000).

2.3. Structure characterization

2.3.1. Digital imaging

The pellets were photographed using a zoom digital camera (Canon IXUS 210, Canon Inc., Headquarters, Tokyo, Japan) with 14.1 effective megapixels for high-definition pixel images.

2.3.2. Light microscopy analysis

Light microscopy was performed using a Stereo Discovery V12 stereomicroscope (Carl Zeiss, Goettingen, Germany). Pellets coated (impregnated) with oil were cut in half along the cross section with a razor blade. The pictures of the samples were taken with the cutting side facing up.

2.3.3. Scanning electron microscopy (SEM)

Vacuum-dried samples were cut with a razor blade and glued onto a sample holder using carbon adhesive tabs (Electron Microscopy Sciences, Hatfield, PA, USA) or Leit-C (Neubauer Chemikalien, Münster, Germany), air dried for 2 h and subsequently stored overnight under vacuum before analyses. The samples were sputter coated with 20 nm of iridium in SCD 500 (Leica, Vienna, Austria). Samples were analysed at 2 kV at room temperature in a Magellan 400 field emission SEM (FEI company, Eindhoven, The Netherlands). The images were digitally recorded. The raw micrographs of the pellet surfaces were reconstructed using Visiopharm image analysis software (VisioMorph – Visiopharm Integrator System®, Visiopharm, Hørsholm, Denmark) and analysed

for porosity. The program calculated the percentage of the image containing black pixels. The porosity is reported as the area of pores as a percentage of the total area.

2.3.4. Cryo-Scanning electron microscopy

The frozen (–20 °C) sample was cut in half and glued onto a brass Leica sample holder using carbon glue Leit-C (Neubauer Chemicalien, Münster, Germany), directly frozen in liquid nitrogen and simultaneously fitted into the cryo-sample loading system (VCT 100). The Leica sample holder was transferred to a MED 020/VCT 100 non-dedicated cryo-preparation system (Leica, Vienna, Austria) on a sample stage at –93 °C. The sample was freeze dried in this cryo-preparation chamber for 5 min at –93 °C and 1.3×10^{-6} mbar to remove water vapour contamination from the surface of the sample. The sample was sputter coated with a 15-nm layer of tungsten at the same temperature. The sample was then transferred into the Magellan 400 field emission SEM (FEI company, Eindhoven, The Netherlands) on the sample stage at –122°C and 4×10^{-7} mbar. The analysis was performed with a secondary electron voltage of 2 kV and probe current of 25 pA. All images were recorded digitally.

2.3.5. X-ray microtomography

The 0, 100 and 200 g gluten kg^{-1} feed pellets were scanned using a SkyScan 1172 desktop X-ray microtomography imaging system (SkyScan, Kontich, Belgium) with a pixel size of 5.4 µm, operating at a voltage of 50 kV and current of 160 µA (to obtain optimum contrast between the solid and gaseous phases). A 12-bit, 11-megapixel, cooled CCD camera was used to collect the X-ray data. The images were acquired with a rotation step of 0.4 degrees over a total rotation of 180 degrees. Image reconstruction was accomplished using the Volumetric Reconstruction for Micro CT Instruments (SkyScan, Kontich, Belgium) software (Version 2.1, SkyScan, Kontich, Belgium). This reconstruction software uses a filtered back-projection algorithm with a modified cone-beam reconstruction (Feldkamp et al., 1984). The measurements were limited to an appropriate volume of interest (VOI), that is, a cylinder measuring 5.4 mm in diameter and 2.7 mm in height located in the centre of each pellet. The structural parameters were calculated by SkyScan CTAn software, version 1.5.13 (SkyScan, Kontich, Belgium) after applying a global thresholding to segment the solid and gaseous phases. The porosity of the pellets was calculated as the

ratio of the volume of the pores to the total volume of the pellet, where the pellet volume is equal to the VOI previously defined. The structure separation function yielded the cell size distribution, and the thickness distribution function yielded the distribution in thickness of the cell walls. In addition, the open and closed porosity are reported. A closed pore in 3D is characterized by a connected assemblage of space voxels that is fully surrounded on all sides in 3D by solid voxels. An open pore is defined as any space located within a solid object or between solid objects, that has any connection in 3D to the space outside the object or objects. Percent open porosity is the volume of open pores as a percentage of the total VOI volume. The microstructure of the pellets was also described in terms of average cell size, structure surface per volume ratio, degree of anisotropy and connectivity density. The analyses were done on 3D image. All experiments were done in four replicates.

2.4. Measurement of the physical and functional parameters of feeds

2.4.1. Specific density

A volumetric displacement method using glass beads with a diameter of 0.1 mm as a displacement medium was used to determine the specific density of the pellets including pores. The method was originally developed by Hwang and Hayakawa (1980). The specific density of the pellets was calculated using the following equation:

$$\rho_s = \frac{W_{pl}}{V_{pg} - \left(W_{gs} / \rho_{gs}\right)} \tag{1}$$

where ρ_s is the specific density using the glass bead displacement method (g l^{-1}), W_{pl} is the pellet mass (g), V_{pg} is the volume of the pellets and glass powder, W_{gs} is the mass of glass beads displaced (g) and ρ_{gs} is the specific density of the glass beads (g l^{-1}). The values were obtained from an average of three measurements.

2.4.2. Oil absorption capacity

The oil absorption capacity (OAC) was measured according to a modified method of Lin et al. (1974). Approximately 0.5 g of the ground pellets and 10.0 ml of rapeseed oil were added to a 15-ml conical graduated centrifuge tube. The

contents in the tube were mixed for 3 min with a vortex mixer to disperse the sample into the oil. After a holding period of 30 min, the tube was centrifuged for 25 min at 3050 × *g*. The separated oil was then removed with a pipette, and the tube was inverted for 25 min to drain the oil prior to reweighing. The OAC was expressed as grams of rapeseed oil bound per gram of ground pellet. Triplicate measurements were performed on each sample.

2.4.3. Maximum oil infusion into pellets

Unlike the OAC, the maximum oil infusion test was performed under vacuum conditions, using the whole pellets. The pellets (500 g) from each treatment were placed in a laboratory vacuum coater (custom designed, Skretting ARC, Norway) and the air was slowly evacuated from the vacuum chamber down to a reduced pressure of 0.15 bar. A mixture of heated (80 °C) fish and rapeseed oil (50:50) was sprayed through a nozzle in an excess amount (approximately 350 g) and thoroughly mixed with the pellets throughout the coating cycle. The vacuum chamber was then re-equilibrated to atmospheric pressure and the oil was allowed to infuse into the feed. The pellets were then removed from the coater and any excess oil was removed by placing the feed between two absorbent paper towels. The final weight of the oil infused into the pellets was then recorded and the relative oil uptake was calculated. The reported values were the average of three replicates.

2.4.4. Fat leakage analysis

Coated pellets from the previous analysis were placed in a plastic bucket with blotting paper in the bottom and stored at 40 °C for 24 h. The leakage of fat was measured as the loss of fat from 100 g of feed.

2.4.5. Holmen durability index

This feed tester has recently been introduced in the fish feed industry. It uses air to rapidly circulate the feed and simulates a combination of mechanical and pneumatic stresses (Kaliyan and Vance Morey, 2009). One hundred grams of coated product were placed into the New Holmen Portable Pellet Tester (NHP 100, Borregaard Lignotech, Sarpsborg, Norway), for 120 s. The samples were then collected and weighed. Three replicates were tested to calculate the Holmen durability index for each diet. The chamber of the NHP 100 was cleaned and the filter paper was changed between each replicate. The measurements are

in line with the internal quality control guidelines for coated pellets (Skretting AS, Stavanger, Norway), which ensures the industrial relevance of the results.

2.4.6. Percentage of sinking pellets

A 3000-ml cylinder was filled with 40 g l^{-1} salt water. One hundred pellets from each treatment were randomly selected and individually dropped into the cold water (6 °C) from 5 cm above the water surface. The pellets that did not sink within 3 min were given a zero score and percentage of sinking pellets was calculated. The results were the average of three replicates.

2.4.7. Water absorption index

The water absorption index (WAI) was determined by the method of Anderson et al. (1970). The pellets were first milled to a mean particle size of approximately 270 µm, determined by laser diffraction analysis (Mastersizer 2000, Malvern Instruments Ltd., Malvern, UK). A 2.5-g sample was dispersed in 25 g of distilled water. After stirring for 30 min, the dispersions were rinsed into tarred centrifuge tubes, made up to 32.5 g and then centrifuged at 3050 × *g* for 10 min. The supernatant was decanted and the sediment was weighed. The WAI was calculated using the following equation: WAI = weight of sediment/ weight of dry solids. All determinations were conducted in triplicate.

2.5. Statistical analysis

The results were submitted to one-way analysis of variance (ANOVA) and a least significant difference test (LSD); a confidence interval of 95% was used to compare the means. Statistical analyses were carried out using UNISTAT (Unistat Computer Software Ltd, London, UK).

3. Results

3.1. Macrostructure, physical and functional characteristics of feeds

Fig. 1 shows the effect of the inclusion of 200 g gluten kg^{-1} on the appearance of the final product. The appearance of the products with 50, 100 and 150 g gluten kg^{-1} was similar to the sample without gluten (results not shown).

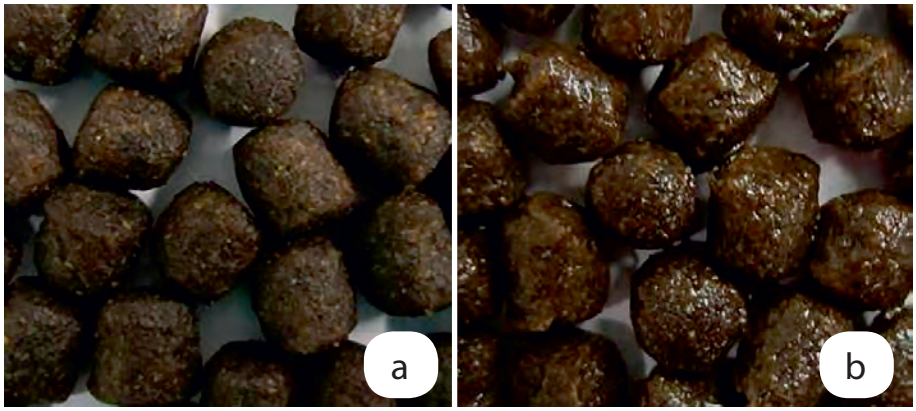

Fig. 1. Oil-coated pellet morphology for feed with 0 (a) and 200 g gluten kg^{-1} (b). Samples were coated using pilot-scale coater.

When the 0 and 200 g gluten kg^{-1} samples were compared, a clear difference could be observed in terms of oil uptake. The surface of the sample with 0 g gluten kg^{-1} was practically oil free, implying that most of the oil was captured inside the pellet. Stereo light microscopy was used to evaluate the macroscopic morphology of the cross sections of the 0 and 200 g gluten kg^{-1} samples. Fig. 2 clearly shows the region in the 200 g gluten kg^{-1} sample that is not loaded with oil.

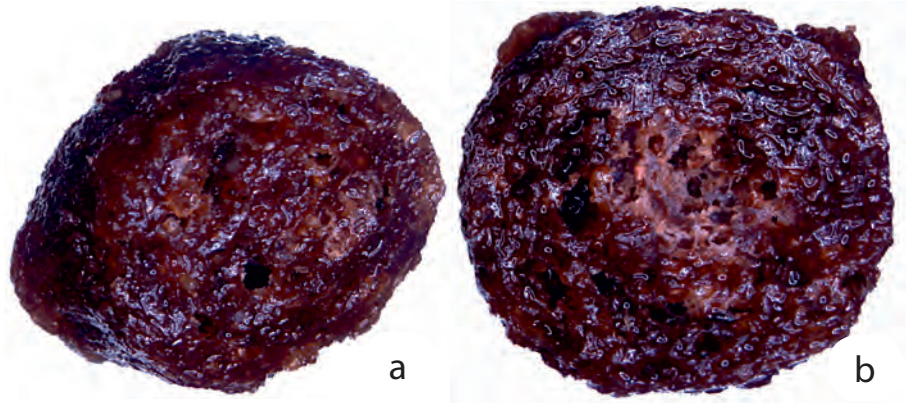

Fig. 2. Stereomicroscopy images of cross sections for feed with 0 (a) and 200 g gluten kg^{-1} (b). Samples were coated using the pilot-scale coater. Magnification is ×10.2.

Table 3 lists the effects of gluten on the physical and functional properties of the feeds. The density of the feed ranged from 683 to 758 g l^{-1}. Except for 150 g gluten kg^{-1}, all the feeds had very similar density. Although statistically significant difference was observed, these differences are not considered to be

of technical relevance for the effects explained in this study. It is not completely clear why the feed with 150 g gluten kg^{-1} behaved differently. Obviously, this composition resulted in more expansion compared with the other pellets. The trends for the maximum infusion of oil are known to closely follow density (Draganovic et al., 2011). It was reported that the density and maximum oil infusion had a correlation coefficient of –0.99. Here, except for 200 g gluten kg^{-1}, we found that the maximum oil infusion trend was similar to that for the density for 0 to 150 g gluten kg^{-1}.

Table 3 Effects of gluten on the physical and functional characteristics of the feed.

Diet formulation	Specific density ($g\ l^{-1}$)	Maximum oil infusion (%)	Sinking (%)	Fat leakage (%)	Holmen durability (%)	WAI[a] ($g\ g^{-1}$)	OAC[b] ($g\ g^{-1}$)
0 g gluten kg^{-1}	758^{c}	51.1^{a}	100.0^{c}	6.8^{c}	97.2^{b}	2.42^{a}	0.82^{a}
50 g gluten kg^{-1}	750^{c}	53.4^{b}	100.0^{c}	5.4^{b}	96.9^{ab}	2.44^{a}	0.87^{b}
100 g gluten kg^{-1}	738^{b}	54.7^{c}	94.7^{c}	4.7^{ab}	97.0^{ab}	2.45^{a}	0.82^{a}
150 g gluten kg^{-1}	683^{a}	58.2^{d}	70.7^{b}	3.5^{a}	98.2^{c}	2.44^{a}	0.84^{ab}
200 g gluten kg^{-1}	735^{b}	53.9^{bc}	35.7^{a}	3.7^{a}	96.8^{a}	2.80^{b}	0.83^{a}
MSE*	25.23	0.47	21.00	0.62	0.05	0.023	0.000
P value	0.0000	0.0000	0.0000	0.0023	0.0001	0.0517	0.0850

Means (n = 3) without a common superscript within a column are significantly different in an LSD test; $P<0.05$.
[a] Water absorption index
[b] Oil absorption capacity
* Mean square error from analysis of variance (df = 14).

It can be seen from Table 3 that the percentage of sinking feed decreased ($P<0.01$) with increasing gluten content. The lowest percentage sinking (35.7%) was obtained at 200 g gluten kg^{-1}; the highest (100%) was observed at 0 and 50 g gluten kg^{-1} and it was followed by 100 g gluten kg^{-1} (94.7%).

Fat leakage varied from 3.5% to 6.8%. It decreased as the gluten level increased from 0 to 150 g kg^{-1}; however, with 200 g gluten kg^{-1}, a slightly higher value was again found (Table 3). In general, the changes observed in fat leakage also suggest differences in the morphology of the products.

Durability is typically manifested by retention of the integrity of the feed during storage, transportation and pneumatic feeding (Aas et al., 2011). There were statistically significant differences in durability among the feeds (Table

3). The highest durability value was found for 150 g gluten kg^{-1}, followed by 0 g gluten kg^{-1}. Overall, except for 200 g gluten kg^{-1}, the feeds with lower starch content tended to have lower durability compared with the feeds with higher starch content.

Gluten was not found to have a significant effect on the WAI. The WAI has been correlated with the microstructural features of the extrudates (Badrie and Mellowes, 1991). In the current work, the WAI values varied between 2.4 and 2.8 (g g^{-1}). Adding 200 g gluten kg^{-1} increased the WAI significantly (Table 3), suggesting a structural change.

As shown in Table 3, the OAC of ground pellets increased significantly when 50 g gluten kg^{-1} was added, and then decreased to a level similar to 0 g gluten kg^{-1} as the added gluten was further increased to 200 g kg^{-1}. In general, no significant effect of gluten was observed. It was previously shown that the OAC of ground extrudates is mostly influenced by protein–oil hydrophobic interactions (Li and Lee, 1996). Due to grinding, the effects of the oil uptake rate and pore availability are not included in this measurement. In contrast, it provided information about the interaction between the oil and the pellet matrix. Obviously, the addition of gluten did not change the interaction between the matrix and the oil after grinding.

3.2. Pellet microstructure

SEM micrographs of the outer surfaces and cross sections of extruded pellets are shown in Figs. 3 and 4, respectively. Fig. 3a clearly shows differences in roughness between the samples. The product with 200 g gluten kg^{-1} shows a rather smooth surface (Fig. 3a). The green areas in Fig. 3b represent the pores and it is obvious that there are fewer pores on the surface of the pellets with 200 g gluten kg^{-1} compared with the other treatments. Image analysis revealed lower surface porosity for 200 g gluten kg^{-1} (1.8%) compared with the values of 6.9, 8.6 and 6.7% for 0, 50 and 100 g gluten kg^{-1}, respectively.

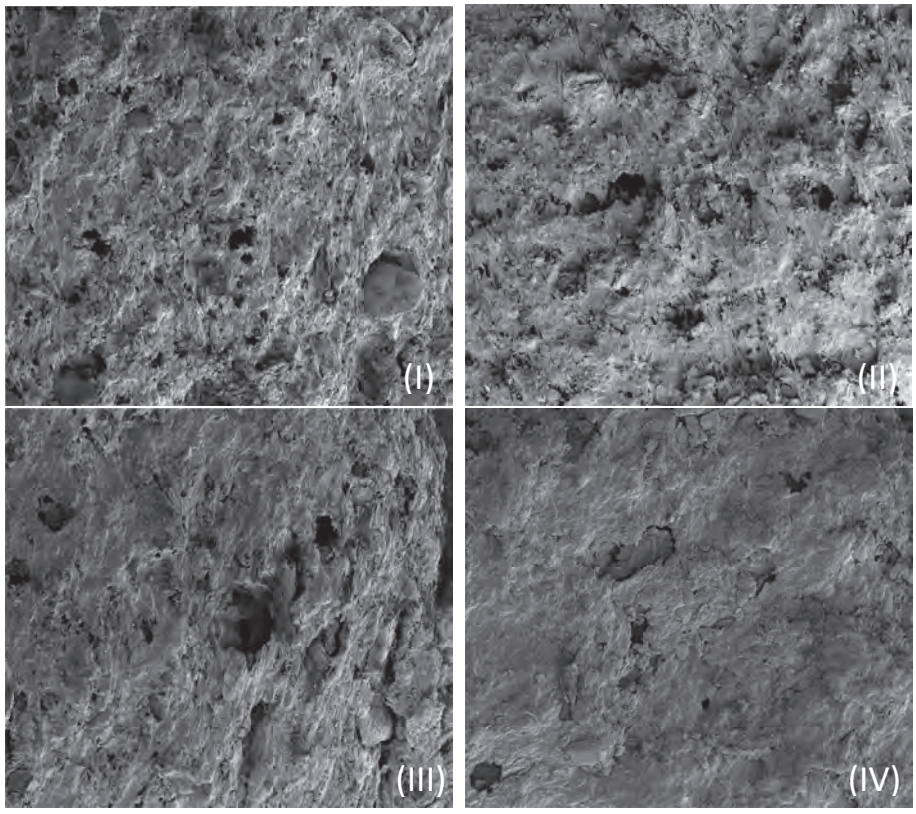

Fig. 3a. Surface porosity of pellets with 0 (I), 50 (II), 100 (III) and 200 g gluten kg^{-1} (IV), imaged using SEM. Each image is a compilation of a series of representative micrographs. Magnification is ×300.

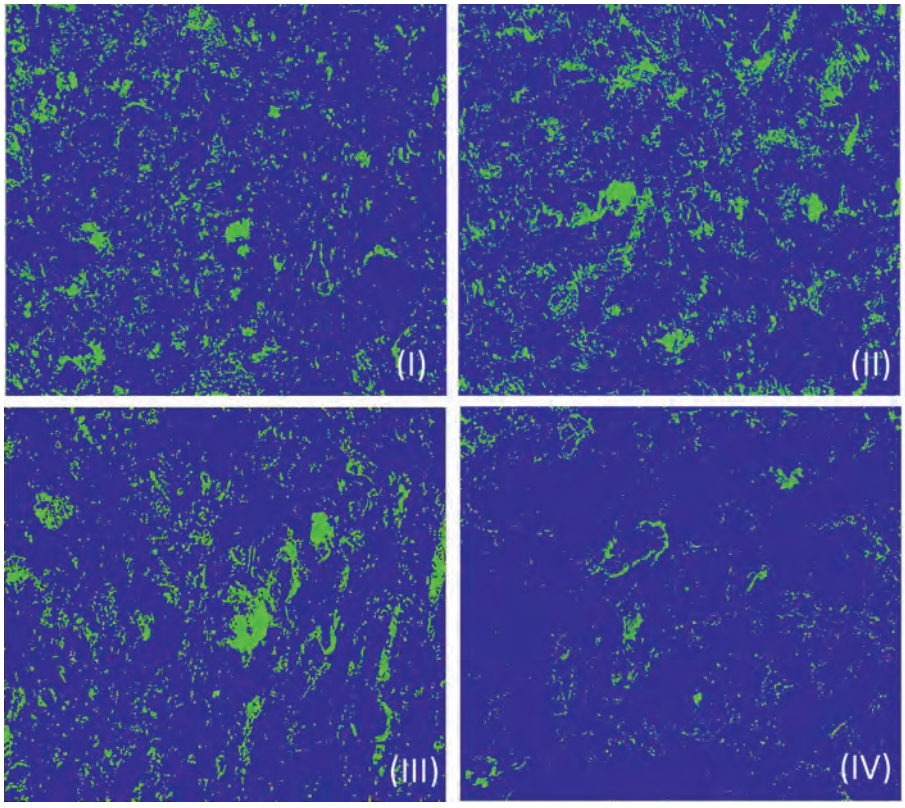

Fig. 3b. Reconstructed micrographs of surface porosity of pellets with 0 (I), 50 (II), 100 (III) and 200 g gluten kg^{-1} (IV).

Fig. 4 also shows that the solid matrix is more compartmentalized or less compact in the sample without gluten.

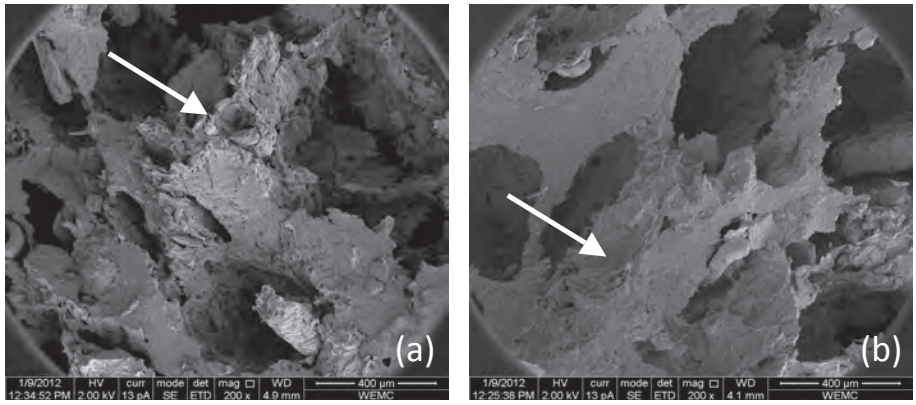

Fig. 4. SEMs of the cross section of pellets with 0 (a) and 200 g gluten kg^{-1} (b), showing a more fractured (a) or a more compact structure (b). Magnification is ×200.

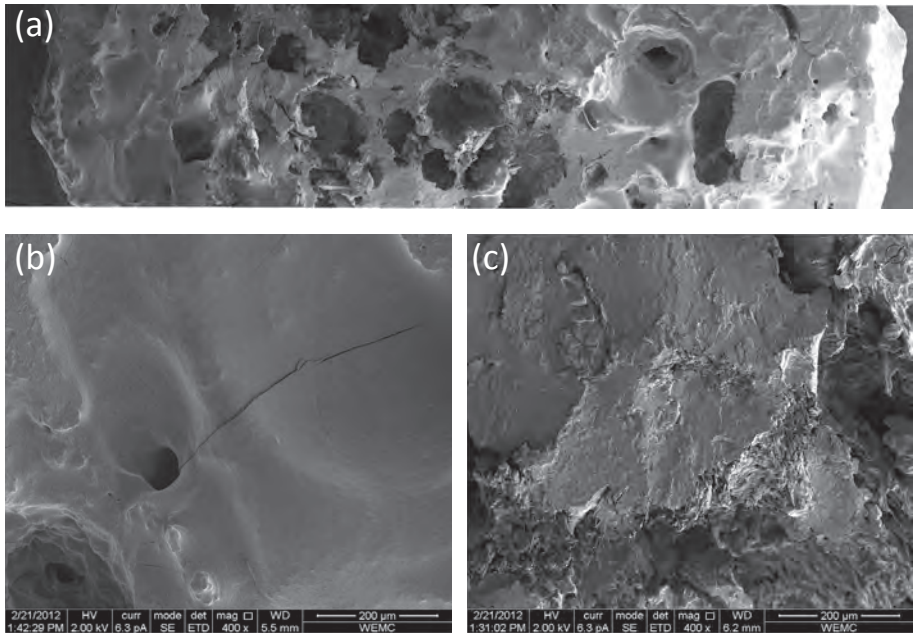

Fig. 5. Cryo-SEM image of a sample with 200 g gluten kg^{-1}. Sample was coated using the pilot-scale coater. (a) the image of cross-section is a compilation of a series of representative micrographs (×35 magnification); (b) and (c) representative images of the periphery and the inner part of the sample, respectively (×400 magnification).

The cryo-SEM micrographs (Fig. 5) of the fraction planes reveal the internal structure of the oil-coated pellet containing 200 g gluten kg^{-1}. Most of the pores at the periphery are completely filled with oil, but towards the core of the pellets, larger voids are not yet filled (Fig. 5a). Unlike the inner part of the pellet (Fig. 5c), ridges and fracture points are not visible at the periphery where the contours are smoother (Fig. 5b), which we contribute to the overlying lipid.

The quantitative microstructural data extracted from the XMT analysis are presented in Table 4. No significant differences in porosity were observed. The average air-cell diameter (D_{cell}) ranged between 319 and 375 µm and was significantly affected by gluten. The lowest D_{cell} was observed for pellets without any gluten and it increased by 15% with 200 g gluten kg^{-1}.

Table 4 Three-dimensional structural parameters obtained with XMT.

Diet	Porosity (air-cell volume fraction) (volume %)	Average air-cell diameter, D_{cell} (µm)	Structure surface/ volume ratio, SV ($\times 10^{-2}$ μm^{-1})	DA[a] (unitless)	Open porosity (%)	Closed porosity (%)	Connectivity density ($\times 10^{-7}$ μm^{-3})
0 g gluten kg^{-1}	50.9±2.1[a]	318.7±35.1[a]	2.44±0.16[a]	1.53±0.04[b]	50.6±2.0[a]	0.6±0.1[a]	4.24±0.45[b]
100 g gluten kg^{-1}	54.1±3.2[a]	364.9±25.8[b]	2.37±0.15[a]	1.43±0.02[a]	53.7±3.2[a]	0.8±0.1[b]	3.04±0.48[a]
200 g gluten kg^{-1}	53.2±2.3[a]	374.9±19.0[b]	2.27±0.22[a]	1.42±0.03[a]	52.7±2.3[a]	0.9±0.2[b]	2.95±0.46[a]
MSE*	6.69	753.17	0.00	0.00	6.55	0.02	0.00
P value	0.2580	0.0387	0.4521	0.0016	0.2754	0.0243	0.0000

Data shown are mean ± standard deviation. Means ($n = 4$) without common superscript within a column are significantly different in an LSD test; $P<0.05$. The sum of open and closed porosities corresponds to the total porosity values (air-cell volume fraction).

[a] Degree of anisotropy

*Mean square error from analysis of variance (df = 9).

Fig. 6 shows that most pore sizes were between 11 and 330 µm. Porosity smaller than 11 µm could not be detected due to limitations in resolution. The inclusion of gluten led to

(1) less cells smaller than 200 µm,
(2) more cells in the range from 550 to 700 µm, and
(3) higher volumetric frequency of structures between 48 and 177 µm thick and lower frequency between 200 and 344 µm (Fig. 7).

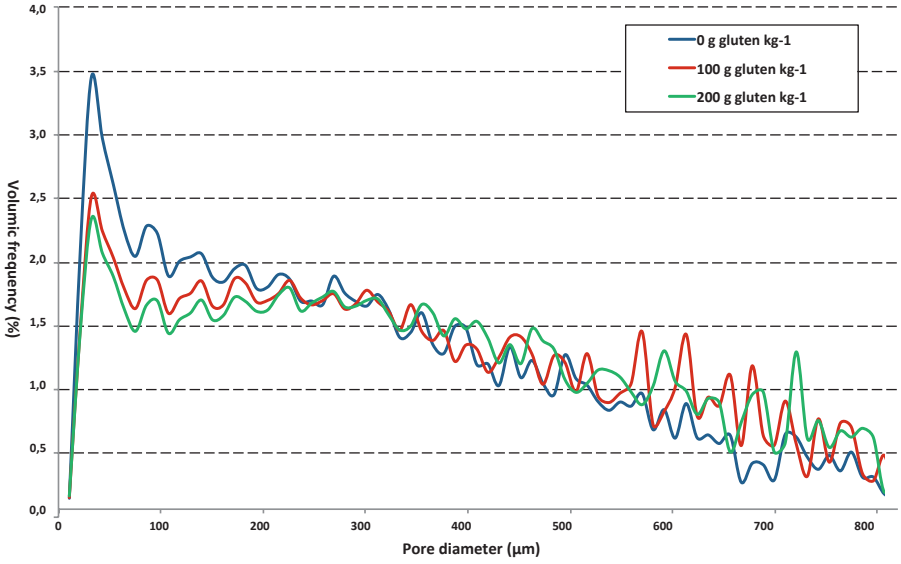

Fig. 6. Cell size distribution in the pellets (average from four replicates).

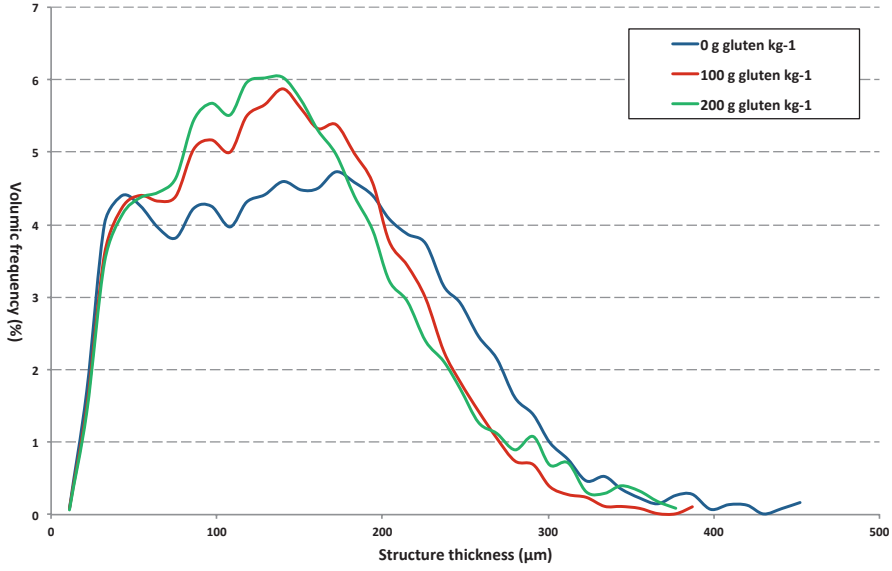

Fig. 7. Cell wall thickness distribution in the pellets (average from four replicates).

When cells expand just after emergence from the die, their walls stretch and thus become thinner until they rupture (Trater et al., 2005). This explains how an increase in cell size is accompanied by thinner lamellae between the cells (Fig. 7). Obviously, inclusion of gluten delays rupturing.

With the addition of gluten, the surface-to-volume ratio (SV) tended to decrease, but the differences were not statistically significant (Table 4). The highest SV (0.024 μm^{-1}) was observed for the feed without gluten. Even though there is a (non-significant) trend of slightly higher porosity with more gluten, smaller cells imply more surface area, and therefore it is logical that the samples without gluten are spread over a greater cell surface area.

The degree of anisotropy (DA) is a measure of the preferential alignment of structures along a particular directional axis in three dimensions (Bellido et al., 2006). There is a trend of decreasing ($P<0.01$) anisotropy with more gluten. The DA values ranged from 1.42 to 1.53 and they were lower with the inclusion of gluten, indicating that cells were more spherically shaped (Table 4). Cell anisotropy was more evident at 0 g gluten kg^{-1}.

Table 4 shows that all products have an open porous structure; about 98-99% by volume of all cells is open. This is in the normal range for extruded products (Bhatnagar and Hanna, 1997; Hicsasmaz and Clayton, 1992). Although low in absolute magnitude, the percentages of closed pores increased ($P<0.05$) as the gluten increased from 0 to 200 g kg^{-1}.

Table 4 shows that the connectivity density between pores decreased ($P<0.01$) with increasing gluten level, although no statistically significant difference was detected between 100 and 200 g gluten kg^{-1} feeds.

A series of XMT reconstructed images for the 0, 100 and 200 g gluten kg^{-1} treatments is given in Fig. 8. The dark areas represent the void cells, whereas the continuous solid matrix, mostly the cell walls, is in orange or white (denser material). A change in the pellet microstructure can be observed with the addition of gluten (Fig. 8). The microstructure of the 0 g gluten kg^{-1} pellet shows elongated cells at the periphery, caused by axial expansion after emergence from the die. The centre of the pellet without gluten has an irregular structure.

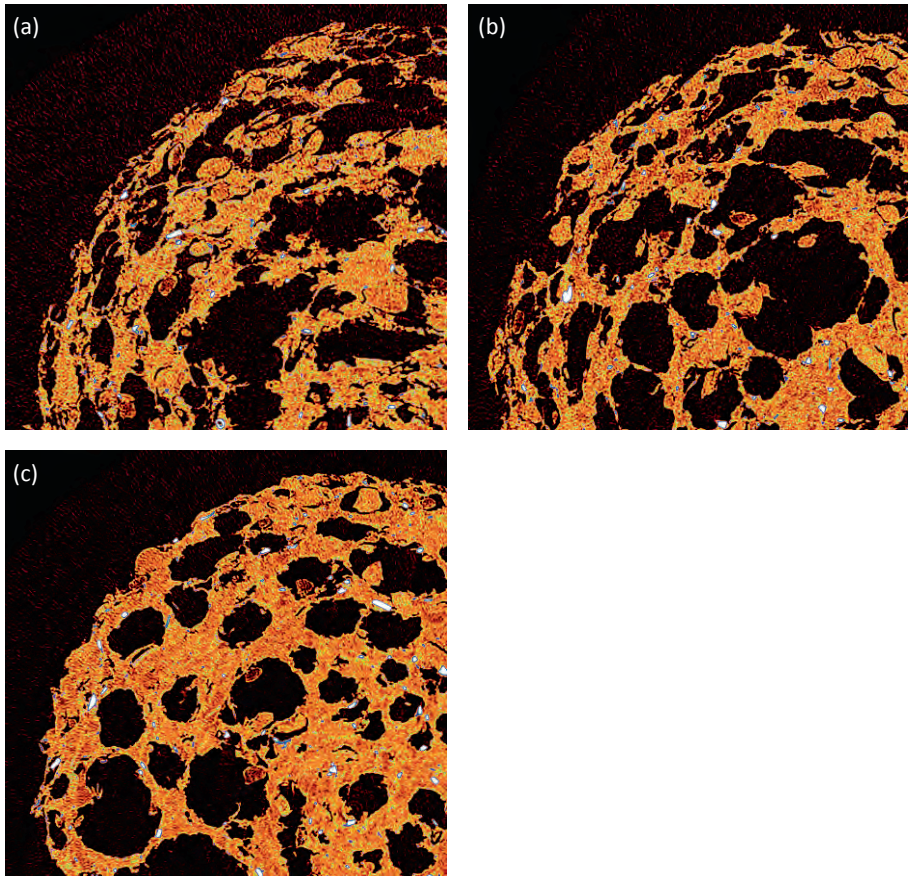

Fig. 8. Reconstructed two-dimensional horizontal X-ray slice images of pellets with 0 (a), 100 (b) and 200 g gluten kg^{-1} (c). The parts of the radial sections presented are of the whole pellet, not the VOI.

4. Discussion

In this work, the microstructure of feed pellets has been found to be highly influenced by the replacement of SPC by vital wheat gluten. The changes in microstructure might be a response to changes in the mechanical behaviour of the melt due to changes in the concentration of the two plant protein sources. The overall effect is discussed and the origin of the differences is explained based on the structure formation properties of both plant proteins.

4.1. Effects of chemical composition on the physical characteristics of feeds

The increased inclusion of wheat gluten in the pellets resulted in a marginal increase of protein and a significant increase of starch. The starch level was targeted at 120 g kg^{-1} DM. However, actual measured starch was between 125 and 164 g kg^{-1} for the feed with 50 and 150 g gluten kg^{-1}, respectively (Table 1). The increased starch level of the feed with 150 g gluten kg^{-1} might be the reason for the greater expansion of the pellets and therefore the highest oil infusion (Table 3), despite the changes made to the process parameters (Table 2). This confirms the findings of Glencross et al. (2010), who found that a higher level of starch results in greater oil uptake due to greater pellet expansion. A similar trend was observed when the gluten level increased from 0 or 50 to 100 g kg^{-1} (Table 3). However, this result was not repeated for the 200 g gluten kg^{-1} inclusion level in the current experiment. Based on the high correlation between the density and maximum oil infusion, as mentioned earlier, we would have expected a higher maximum oil infusion at 200 g gluten kg^{-1}. This indicates hindered oil infusion. In the case of 100 and 200 g gluten kg^{-1}, the reduction in sinking is an indicator that there was no uniform infusion of oil into the product. Besides this effect, the reduced sinking for 150 g gluten kg^{-1} could also be attributed to the lower density caused by the high inclusion level of starch compared with the other feeds.

Øverland et al. (2009) reported previously that starch from wheat is a primary component responsible for the binding properties in extruded diets for salmonids. Therefore, the improved durability of the 150 g gluten kg^{-1} diet (Table 3) in this study could be associated with the higher starch level in this diet compared with other feeds. In general, the numeric durability values were high for all the feeds and well within acceptable quality criteria according to commercial guidelines.

4.2. Effect of addition of gluten on the morphology and functional characteristics of feeds and oil infusion

The changes in the morphology of the pellets are probably related to the film-forming properties of gluten (Moore et al., 2004; Park and Chinnan, 1995). Parker et al. (1990) and Moss (1974) reported that mixing in bread making causes the gluten to form a highly extensible network, which stretches into thin film walls around the growing gas cells during leavening. The same effect

takes place after exiting the die, during expansion of the matrix, due to flashing off of the excess steam. The higher temperatures used during extrusion might allow gluten having the same effect as in dough development. The network formation of hydrated gluten during mechanical deformation was previously reported by Bugusu et al. (2002). Li and Lee (1996) correlated the polymerization of gluten through disulfide bond crosslinking with higher WAI values, and the formation of a compact, solid structure. Hashimoto et al. (2002) reported higher WAI values with increased gluten concentration in cassava starch/gluten blends. The increase in WAI with the addition of 200 g gluten kg^{-1} in our study is therefore an indicator of network formation by the gluten.

The irregular structure of the pellets without gluten (Fig. 8) is probably caused by rupture of smaller cells due to insufficient strength of the surrounding lamellae. Parada et al. (2011) concluded that the solid matrix is more fragmented with the addition of fibre by using the average number of objects per unit length. The compartmentalisation observed in this study (Fig. 4) could be cause by the higher fibre present in the diets with more SPC (i.e. less gluten). The structure around the bubbles at the rim is solidified quickly, as this part cools off quickest. This is in line with the anisotropy; there was simply not enough time for relaxation. The centre, however, remains mobile for some time, as it cools more slowly, and if the matrix material is not elastic enough to be able to accommodate the expanding bubbles, it will rupture there, leading to partly fused smaller cells and large, irregular cells. The gluten is well deformable compared with other types of proteins with the same level of moisture; thus the expansion can be better accommodated. Not only does the gluten provide a highly adaptive environment for the bubbles to grow during expansion but the gluten network remains flexible for some time, which allows the bubbles to attain a more or less spherical shape.

Rosenquest et al. (1975) attributed the small cell size to a reduction in the strength and stretchability of the dough. When the moisture in the dough flashed to the gaseous state on extrusion, the walls of the cells broke while the cells were still small. In contrast, as gluten is known to be an excellent film former, the addition of gluten results in the lamellae remaining stable over more expansion, thus leading to larger bubbles and thinner lamellae. This confirms some of the observations regarding macrostructure that were made in our previous work (Draganovic et al., 2011).

Based on images shown in Fig. 2, the greater difference in maximum oil

infusion was expected for the 200 g gluten kg^{-1} pellets compared with the other treatments (Table 3). Besides differences in morphology, the rate of oil infusion is also important here. Greater amounts of oil were used in the infusion tests compared with pilot-scale coating, which in turn can lead to better oil impregnation. This factor can affect the speed at which the two materials are intermixed, as well as the distance that one material can diffuse into another (Ellis et al., 1993). Therefore, for 200 g gluten kg^{-1}, we would expect even lower values for maximum oil infusion in the case of pilot-scale coating (Fig. 2).

4.3. Relationship between physical properties and cellular structure

The results from the current study suggest that oil impregnation is hindered by the cell structure. Although the density would certainly influence these parameters (Barrett and Ross, 1990), the density is kept constant in this study, and therefore this effect should be attributed to the microstructure. The effects of gluten seen here are also found with some other additions. Some natural ingredients can reduce oil uptake because of their film-forming capability (Pedro, 2009). In the work done by Bouchon and Pyle (2004), it was shown that a more elastic network in a restructured potato chip may result in a less permeable outer layer, which is an effective barrier against oil absorption during frying.

The micrographs used in this study are useful for estimating the size and position of the pores on the extruded samples (Reitz et al., 2008) and allow qualitative observations (Bouchon and Pyle, 2004).The outer layer influences different properties. The smooth and regular outer surface obtained after extrusion with 200 g gluten kg^{-1} (Fig. 3a), will give a certain resistance to oil impregnation; the oil will stay on the surface and simply drain off. Bouchon and Pyle (2004) concluded that the evenness of the outer surface and the permeability of the outer layer play a fundamental role in the oil infiltration pattern.

The influence of gluten can be also seen from the results for the diet without gluten. Soy protein concentrate is exposed to intolerable levels of stretching during the expansion stage, which cannot be accommodated. The mechanical properties and their timescales do not match the timescale of the expansion process. Thus, a highly irregular and partially ruptured structure results, which allows very easy infusion of oil. The gluten introduces better deformability at processing time scales, and thus a matrix with gluten can adapt to the expansion, resulting in a structure that is highly cellular. But, slightly more cells are either closed or almost closed (Table 4). These findings are in line

with the results presented by Ghorpade et al. (1997). They reported that soy protein isolate did not affect the open pore volume of starch-based extrudates; inclusion of gluten above 200 g kg^{-1} resulted in a decreased open pore volume. Even though the absolute volume of closed cells for three feeds in this study count for less than 1.7% of the total porosity (Table 4), they contribute to the overall density of the pellets. In general, we believe that the potential effect of closed pores on the reduced oil uptake is minor compared with the effects of surface porosity (Fig. 3b) and other microstructural parameters reported in Table 4. In accordance with the results from the present study, it has been shown for tortilla chips that due to capillary pressure, small narrow pores lead to more fat uptake than wide pores (Moreira et al., 1997). Moreover, the whole pore pathway has to be considered. It has been stated by Saguy and Pinthus (1995) that long continuous channels lead to increased fat uptake. Both interconnectivity density and anisotropy give an indication of the pore pathway; less spherical pores and better interconnectedness between pores suggest the presence of longer, continuous channels. The values of those two parameters decreased with 200 g gluten kg^{-1} (Table 4) and it can be expected that, the oil will penetrate more slowly during oil impregnation, which will result in more pores (even somewhat open ones) that are not filled with oil.

The observed reduction in fat leakage with 200 g gluten kg^{-1} (Table 3) could also be attributed to the differences in skin porosity. Once the oil is impregnated in the pellet with the use of a vacuum, the surface acts as an effective barrier against its diffusion in the opposite direction, towards the outside. Moreover, the leakage of fat could be also hindered by the lower pore interconnectivity and less fragmented structure with the 200 g gluten kg^{-1} pellets. For the duration of the test (24 h) there was limited time for oil diffusion and the differences in fat leakage would be more pronounced with a longer time.

An ideal pellet seems to benefit from inclusion of both, SPC and gluten. Soy protein concentrate provides high interconnectedness, while gluten provides stronger pellets. When these two components are the main sources of protein in the diet besides fish meal, their optimization may lead to an optimal pellet.

4.4. The role of the extrusion process

As stated in Section 2, a slight modification in extruder settings was necessary to obtain pellets with similar density. The 150 g gluten kg^{-1} sample needed an adjustment in the barrel temperature (sections 3 and 4), whereas the 200 g

gluten kg^{-1} sample was extruded with a slightly higher rotation speed (Table 2). The question now is whether the changes in product properties discussed above could be attributed to the changes in the extrusion conditions rather than compositional changes.

To check for this and the influence of the scale of the process, a second twin-screw extruder (TSE 36 HC, Thermo scientific, Staffordshire, UK) with a screw diameter of 36 mm and an L/D of 28:1 was used to produce 0 and 200 g gluten kg^{-1} formulations (results not shown). These diets were processed under comparable operating conditions. Here, the inclusion of gluten led to even greater differences in oil uptake using the pilot-scale coater for pellets with similar density. Moreover, the percentage sinking of the pellets, fat leakage and the WAI followed exactly the same trend as with the TX-57 extruder. Therefore, we are confident that the changes in the properties of the final product described in this article are mainly caused by differences in the technological properties of SPC and gluten.

5. Conclusions

This study shows the relationship between the pellet microstructure and its techno-functional properties. Most of the changes in techno-functional properties can be explained by the changes in microstructure. Changes in microstructure were induced by altering the composition through the inclusion of wheat gluten.

The inclusion of wheat gluten in fish feed pellets leads to a reduction in oil impregnation and oil uptake during coating, but yields strong, highly porous pellets. The pores are still highly interconnected, albeit slightly less than without gluten.

Pellets without gluten have an irregular, very open internal structure, while those with gluten have a more regular cellular structure. In the radial direction, a larger number of cells of smaller diameter were observed close to the surface of the pellets without gluten, indicating a relatively porous skin, while gluten yielded a rather smooth, non-porous surface.

These effects seem to be related to the film-forming properties of gluten, which are still effectively present at the high temperatures used during the extrusion process.

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Chapter 4[3]

Lupine and canola protein concentrate in fish feed: a comparative assessment of the techno-functional properties using a shear cell device and an extruder

3 This chapter has been submitted for publication.

Abstract

The techno-functional properties of soy, lupine and canola protein concentrates (SPC, LPC and CPC, respectively) in fish feed were evaluated relative to fish meal (FM). The effects were studied using a shear cell device and an extruder with emphasis on the added moisture content. Six diets were formulated: an SPC-based diet with 300 g SPC kg^{-1}, diets containing 100 and 200 g LPC kg^{-1} or 100 and 200 g CPC kg^{-1} and an FM-based diet with 450 g FM kg^{-1}. Each diet was extruded with an added moisture content of 29%, 25% and 22% of the mash feed rate. The results of the extrusion trials confirmed the observations made from the shear cell device. Thus, the shear cell device can be used to study processing conditions that are close to extrusion conditions. The technological properties of LPC closely resembles FM: high solubility, low water-holding capacity (WHC) and low paste viscosity. The LPC 100 and 200 g kg^{-1} diets could be extruded at 22% moisture, which gives an extrudate with reduced drying requirements. In addition, less specific mechanical energy was needed for extrusion. In contrast, both SPC and CPC have high WHC and paste viscosity. This explains the higher feed moisture required during extrusion. The properties of the feeds containing CPC could be well within the ranges acceptable for commercial fish feed use at even higher moisture content compared with SPC.

1. Introduction

The global demand for seafood has made aquaculture one of the fastest growing food production activities in the world (FAO, 2009). The expansion of aquaculture production has been accompanied by rapid growth of aquafeed production (Gatlin et al., 2007). The challenge for the aquaculture industry is to identify economically viable and more environmentally benign alternatives to fish meal (FM) and fish oil, on which many present aquafeeds are still based.

Soy protein concentrate (SPC) possesses most of the nutritional characteristics required for an alternative feedstuff to FM (Gatlin et al., 2007). Currently, SPC is the protein source of choice among plant ingredients due to its relatively high protein content, high nutrient digestibility, low level of anti-nutritional factors, and excellent availability. In addition, the price is relatively low. However, regardless of these benefits of SPC, sustainability also implies the use of plant proteins originating from different sources and locations. Proteins from vegetables other than soy are a valuable alternative to fish protein due to the renewability of the raw material and the variety of sources (especially legumes, cereals and oilseeds) (Moure et al., 2006). Aubin et al. (2009) stated that the increasing production of aquaculture products makes diversification of protein and lipid sources an important challenge.

From a technological viewpoint, SPC is easy to store, handle and blend with other ingredients. Furthermore, it possesses good expansion capabilities during extrusion and contributes to the strength of the pellets. However, the inclusion of SPC in the feed also requires the addition of more moisture for the extrusion process due to its high water-holding capacity (WHC) (Bhattacharya et al., 1986; Draganovic et al., 2011; Zayas, 1997). The extra water has to be removed subsequently after extrusion through drying. This is undesirable because it is very energy intensive, emits odour to the environment and may compromise plant safety (dust generation). It is therefore interesting to evaluate other plant materials. Recent advances in fractionation technologies have provided more fractions, such as LPC and CPC, with higher protein and lower carbohydrate contents relative to their unprocessed raw materials.

From the point of view of reduced addition of moisture during extrusion, LPC might be interesting because of its low viscosity as demonstrated by Chew et al. (2003). In addition, the nutrient profile of lupines shows their potential to replace significant proportions of FM in aquafeeds (Allan and Booth, 2004;

Burel et al., 1998; Gatlin et al., 2007; Glencross et al., 2005; Glencross et al., 2004). Although some work has been done on the technological aspects of lupine kernel meals in fish feed (Glencross et al., 2010), we are not aware of such studies on the use of LPC. LPC is used in food due to its functionality, for example, as bread improvers (Drakos et al., 2007; Lqari et al., 2002).

Canola is one of the most abundant protein meals and it represents 12.4% of the world protein meal production (Ash and Dohlman, 2006), ranked second behind soy (Drew, 2004). The high biological value of canola protein products has already been confirmed in feeding trials with salmonids (Higgs et al., 1994; Mwachireya et al., 1999). Its apparent protein digestibility coefficient is the highest for any protein source ever assessed including FM. It thus has a high potential for use in fish feed and may contribute to the diversification of proteins. It is therefore remarkable that the techno-functional characteristics of CPC in fish feed have not yet been reported.

The current article presents a compilation of the most interesting techno-functional properties of three plant protein-rich ingredients in comparison with FM. In this study, we show that LPC allows the addition of less moisture during extrusion of low FM diets, probably by reducing the WHC of the total mash. This offers opportunities for mild drying after extrusion while still yielding good feed product properties. In contrast, when replacing SPC with CPC, product properties can be maintained only with greater addition of moisture. Although the main objective of this research was to investigate the effects of partial replacement of SPC with CPC and LPC on the specific mechanical energy usage during extrusion, the feed moisture requirements and the properties of the fish feed products, we also evaluated the usefulness of the pilot-scale shearing cell device as a fast and simple method to investigate the techno-functional properties of the feed ingredients.

2. Materials and methods

2.1. Feed ingredients and diet formulations

The formulations consisted of a commercial salmon grower diet (SPC diet), based on SPC, and four experimental diets in which SPC was partly replaced by either LPC or CPC to give diets containing 100 and 200 g LPC kg^{-1} or 100 and 200 g CPC kg^{-1}. In addition, the composition of the sixth feed (FM diet)

was similar to that of some feeds still available on the market (with FM as the major constituent). All diets were formulated to be roughly isonitrogenous and isoenergetic and contained approximately 110 g starch kg^{-1}. Table 1 gives the formulations and chemical composition of the diets. The chemical composition of the raw materials is given in the footnotes to Table 1.

Table 1 Formulation and chemical composition of the dry feed mixes.

	SPC	100 g LPC kg^{-1}	200 g LPC kg^{-1}	100 g CPC kg^{-1}	200 g CPC kg^{-1}	FM
Formulation (g kg^{-1})[a]						
Wheat[b]	108.9	108.9	108.9	108.9	108.9	108.9
Faba beans (dehulled)[c]	108.2	108.2	108.2	108.2	108.2	108.2
FM[d]	233.3	233.3	233.3	233.3	233.3	699.9
SPC[e]	466.6	311.1	155.6	311.1	155.6	0
LPC[f]	0	155.6	311.1	0	0	0
CPC[g]	0	0	0	155.6	311.1	0
Sunflower meal[h]	46.7	46.7	46.7	46.7	46.7	46.7
Mineral and vitamin mix[i]	36.6	36.6	36.6	36.6	36.6	36.6
Analysed composition (g kg^{-1} dry matter)						
Dry matter (g kg^{-1})	933	933	930	932	936	943
Protein	587	573	567	556	569	614
Fat	53	66	81	56	56	105
Starch	115	114	122	131	115	106
Crude fibre	38	33	25	44	46	19
Ash	84	80	74	84	88	120

[a]Complete formulation of the diets contains 345 g kg^{-1} oil mixture. The amount of LPC and CPC included in the dry mix (155.6 and 311.1 g kg^{-1}) corresponds to 100 and 200 g kg^{-1} in the complete formulation, respectively; the amount of FM included in the dry mix (699.9 g kg^{-1}) in the FM-based diet corresponds to 450 g kg^{-1} in the complete formulation.
[b]Supplied by Skretting AS, Stavanger, Norway. Containing (g kg^{-1}): dry matter 869; in g kg^{-1} dry matter: protein 142; fat 32; starch 688; crude fibre 25; ash 17.
[c]Supplied by Skretting AS, Averøy, Norway. Containing (g kg^{-1}): dry matter 886; in g kg^{-1} dry matter: protein 303; fat 33; starch 525; crude fibre 38; ash 31.
[d]Low temperature dried FM, Welcon, Egersund, Norway. Containing (g kg^{-1}): dry matter 932; in g kg^{-1} dry matter: protein 724; fat 137; starch 5; crude fibre 0; ash 142.
[e]Supplied by Imcopa SA, Araucaria, Brazil. Containing (g kg^{-1}): dry matter 922; in g kg^{-1} dry matter: protein 652; fat 17; starch 64; crude fibre 57; ash 62.
[f]Supplied by L.I. Frank, Twello, The Netherlands. Containing (g kg^{-1}): dry matter 935; in g kg^{-1} dry matter: protein 630; fat 119; starch 4; crude fibre 5; ash 36.
[g]Supplied by Bunge, St. Louis, MO, USA. Containing (g kg^{-1}): dry matter 958; in g kg^{-1} dry matter: protein 665; fat 50; starch 2; crude fibre 63; ash 74.

[h]Supplied by Skretting AS, Stavanger, Norway. Containing (g kg^{-1}): dry matter 914; in g kg^{-1} dry matter: protein 384; fat 31; starch 2; crude fibre 191; ash 76.
[i]Vitamin levels according to NRC 93 specification (proprietary composition, Skretting ARC, Stavanger, Norway). Complete formulation of the diets contains 23.8 g kg^{-1} mineral and vitamin mix.

2.2. Shear cell trials

2.2.1. Shearing device

Shear cell trials were performed at the Laboratory of Food Process Engineering at Wageningen University (Wageningen, The Netherlands). The shearing device was developed to study the influence of simple shear deformation on breakage and structure development in a number of biopolymer systems (Manski et al., 2007; Peighambardoust et al., 2004; van der Zalm et al., 2012). The device allows processing under conditions that are relevant to extrusion (Emin et al., 2012; van den Einde et al., 2005). The device consists of stationary and rotating cones, which are both jacketed; the temperature is regulated by a circulating water flow. The shearing device is connected to a Thermo drive unit (Thermo Scientific, Staffordshire, UK) with an interface and controlling unit for on-line measurement of temperature and torque values. The contact surface of the cone and plate is grooved to avoid slippage of the material during shear processing. A schematic configuration of the shear cell is shown in Fig. 1.

2.2.2. Sample preparation and shearing process

Before the shear processing, the sample material was blended with water in a Philips kitchen blender (type HR 7744, Amsterdam, The Netherlands) for 1 min. The mass ratios between the dry flour and water was 40:60 for feed ingredients; ratios of 65:35, 60:40 and 50:50 were used for each sample of meal mix. After blending with water, the total amount that was placed in the shear cell was 54 g and 82 g in the case of feed ingredients and meal mixes, respectively. After filling the shear cell zone with the material, the cone-plate cell was closed hydraulically with a vertical compression force of 3500 N onto the material, which was kept constant during all experiments. The pressure inside the chamber was adjusted to 2 bar to prevent evaporation of water during the experiments. All samples were sheared at 90 °C and 50 rpm for 20 min.

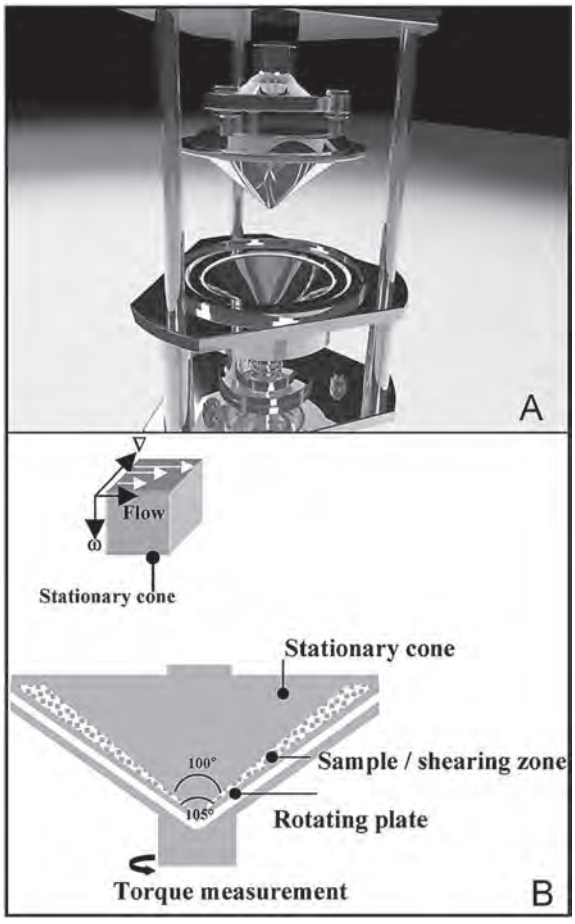

Fig. 1. The shear cell device. (A) Animation and (B) schematic overview of the shear cell device. Cone angle=100°, angle between cone and plate (shearing zone), θ=2.5°.

2.3. Extrusion trials

All diets were processed at Skretting ARC Technology Plant (Stavanger, Norway). The dry ingredients were pre-mixed in a vertical mixer (custom designed; Skretting ARC, Stavanger, Norway) and ground in a Dinnissen 30 kW hammer mill (Dinnissen, Sevenum, The Netherlands), with a screen size of 0.75 mm. The ingredients were then mixed in a Dinnissen horizontal ribbon mixer (500LTR, Sevenum, The Netherlands) for 7 min. The feed mash was conditioned in a differential diameter conditioner (DDC 2; Wenger Manufacturing, Sabetha, KS, USA) and extruded in a Wenger TX-57 twin screw extruder. The barrel of the extruder was 57 mm in diameter and the length-to-diameter ratio was 17.5:1.

The extruder barrel consisted of four head sections, with each section jacketed to permit either steam heating (sections 1–4) or water cooling (sections 2–4). Temperature control of the second, third and fourth sections was achieved by balancing the heating and cooling power input.

The ingredients were extruded as described, yielding extrudates with a diameter of approximately 9 mm and a length of approximately 10 mm. The knife rotation speed was adjusted according to the specified length of the extrudates. The feed was dried in a Wenger Series III horizontal 3-zones dryer (Wenger Manufacturing, Sabetha, KS, USA) to approximately 900 g kg^{-1} dry matter. Subsequently, the pellets obtained were coated with oil in a Forberg 6-l vacuum coater (Forberg, Oslo, Norway). After running for at least 10 min, discrete samples of pellets (n=3; ×3000 g) were collected every 15 min to create a repeated measures assessment of each diet.

2.4. Chemical analysis

Chemical analyses of the dry matter, protein, fat and ash were carried out by Skretting ARC Laboratory (an accredited analytical service provider). The starch and crude fibre analyses were carried out by Masterlab (Boxmeer, The Netherlands). The dry matter content was assessed with gravimetric analysis after oven drying at 105 °C for 18 h. The protein levels were calculated from the determination of total nitrogen using the Kjeltec 2400 Auto System, based on N×6.25. The fat concentration of meal mixes and FM was measured by nuclear magnetic resonance (Resonance Instruments Ltd, Witney, UK). This method was described previously by Draganovic et al. (2013). The fat content of other feed ingredients used in this study was determined using a solvent extraction system (Soxtec Avanti 2050 Auto System, Foss Tecator AB, Höganas, Sweden). The gross ash content was determined gravimetrically following the loss of mass after combustion of a sample in a muffle furnace at 550 °C for 17 h. The starch content was analysed using an enzymatic method described by McCleary et al. (1994). The crude fibre content was determined as the loss of mass resulting from ashing of the dried residue obtained after acid and alkaline digestion of the sample according to ISO 6865 (ISO, 2000).

2.5. Determination of the WHC

The WHC is the ability of a biopolymer to absorb and bind water against gravity (Porter and Skarra, 1999). The WHC of the protein fractions was

determined using methods modified from Heywood et al. (2002) and Lin and Zayas (1987). Four grams of total flour were dispersed in 25 ml of distilled water in a 50-ml centrifuge bottle. The bottles were shaken for 20 min, and then centrifuged at 3050×*g* for an additional 15 min. After decanting the supernatant, each bottle was weighed and the WHC (grams of water per gram of flour) was calculated using Eqn. (1):

$$\text{WHC} = \frac{[(\text{wt of bottle after decanting} - \text{wt of dry bottle}) - \text{total wt of flour (g)}]}{\text{total wt of flour (g)}} \quad (1)$$

The results were based on the average of three replicates.

2.6. Rapid visco-analysis

Each diet mash was evaluated for its pasting characteristics using a Rapid Visco Analyzer (RVA; Newport Scientific, Warriewood, NSW, Australia). Approximately 22 g of distilled water was dispensed into the clean canister. This amount was corrected for the moisture content of the sample such that the solid-to-water ratio was kept constant for the diet mashes tested. Six grams of sample were poured into the water in the canister before testing in the RVA. The mixtures were subjected to the following heat treatment: 1 min at 50 °C, ramping to 95 °C in 4 min, held at 95 °C for 3 min, and cooling to 50 °C in 4 min. The total test time was thus 12 min. Key features to be examined were the peak viscosity (maximum viscosity reached) and the final viscosity. Three replicates were analysed for each sample.

2.7. Protein solubility

To determine the solubility of the protein, 4 g of sample was dispersed into 25 ml of distilled water and shaken at ambient temperature for 20 min. The dispersion was then centrifuged in an Eppendorf 5810 centrifuge (Hamburg, Germany) at 3050×*g* and 20 °C for 15 min. After appropriate dilution, the protein content of the supernatant was determined by the Kjeldahl method (N×6.25). The protein solubility was expressed as the amount of the soluble substance (in percentage) released from the total amount of protein in the sample material. All determinations were conducted in triplicate.

2.8. System parameters

The specific mechanical energy (SME) was read directly from the control panel of the extruder. The data were collected by APIS real-time Process

Explorer (version 4.1; Prediktor, Fredrikstad, Norway). The values presented are the means of 10 measurements taken at equal time intervals throughout the production run.

2.9. Measurement of the physical quality parameters of feeds

2.9.1. Pellet morphology

The pellets were photographed using a zoom digital camera (Nikon D3s) with 12.1 effective megapixels for high-definition pixel images.

2.9.2. Specific density

A volumetric displacement method using glass beads with a diameter of 0.1 mm as a displacement medium was used to determine the specific density of the pellets. The method was originally developed by Hwang and Hayakawa (1980). The specific density of the pellets was calculated using Eqn. (2):

$$\rho_s = \frac{W_{pl}}{V_{pg} - \left(W_{gs} / \rho_{gs}\right)} \quad (2)$$

where ρ_s is the specific density using the glass bead displacement method (g l^{-1}), W_{pl} is the pellet mass (g), V_{pg} is the volume of the pellets and glass powder, W_{gs} is the mass of glass beads displaced (g) and ρ_{gs} is the specific density of the glass beads (g l^{-1}). The values were obtained from an average of three measurements.

2.9.3. Maximum oil infusion

Samples of the pellets (500 g) from each treatment were placed in a laboratory vacuum coater (custom designed; Skretting ARC, Norway) with an excess (400 g) of heated (60 °C) fish oil and mixed thoroughly throughout the coating cycle. The air pressure was slowly decreased inside the vacuum chamber until all visible signs of air bubbles escaping from the pellets ceased. This normally takes about 1–1.5 min and happens at an air pressure of around 0.15 bar. Once all visible signs of air bubbles escaping had ceased, the vacuum chamber was re-equilibrated to atmospheric pressure, which leads to infusion of the oil into the pellets. The product was then removed from the coater and any excess oil was removed by placing the feed between two absorbent paper towels. The final weight of the oil infused into the pellets was then recorded and the relative oil uptake was calculated.

2.9.4. Percentage of sinking pellets

A 3-l cylinder was filled with water containing 40 g l^{-1} salt (sodium chloride). One hundred pellets from each treatment were randomly selected and individually dropped into cold water (6 °C) from 5 cm above the water surface. The pellets that did not sink within 3 min were given a zero score and the percentage of sinking pellets was calculated. The results were the average of three replicates.

2.9.5. Fat leakage analysis

Coated pellets from the maximum oil infusion analysis were placed in a plastic bucket with blotting paper in the bottom and stored at 40 °C for 24 h. The leakage of fat was measured as the loss of fat from 100 g of feed.

2.9.6. DORIS durability

The DORIS test was performed by placing 300 g of coated product into the DORIS tester (Akvasmart; AKVA Group, Bryne, Norway.). The DORIS tester comprises an Archimedean screw that feeds product into a vane, simulating the stresses experienced by feed during pneumatic conveyance in automated feeding systems. The sample was then sieved. A shaker (HAVER EML 200 Digital plus; T. HAVER & BOECKER, Germany) with amplitude 2.3 (level 7 vibration intensity of the shaker; the maximum setting is level 9) was used to sieve the samples through a rack of sieving screens with mesh sizes of 7.1 mm and 2.36 mm for 2 min. After sieving, the weight of the materials on the 2.36-mm screen and on the bottom pan was determined. The DORIS values are presented as the percentage of fractured product (product size 2.36–7.1 mm) and the fines (product size <2.36 mm) of the starting weight. The test was carried out in triplicate for each feed.

2.9.7. Holmen durability index

One hundred grams of coated product were placed into the New Holmen Portable Pellet Tester (NHP 100; Borregaard LignoTech, Sarpsborg, Norway), for 120 s. The samples were then collected and weighed. Three replicates were tested to calculate the Holmen durability index (HDI) for each treatment combination. This feed tester has recently been introduced in the fish feed industry. It uses air to rapidly circulate the feed and simulates a combination of mechanical and pneumatic stresses (Kaliyan and Vance Morey, 2009).

Table 2 Extruder and dryer processing parameters during feed production.

Diet formulation	SPC				100 g LPC kg^{-1}			200 g LPC kg^{-1}			
Added moisture[a] (% of feed rate)	16	22	25	29	22	25	29	16	22	25	29
Extrusion											
Capacity feed mix, kg h^{-1}	150	150	150	150	150	150	150	150	150	150	150
Steam added to pre-conditioner, kg h^{-1}	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Water added to pre-conditioner, kg h^{-1}	0	0	0	16.5	22.5	0	22.5	0	0	0	16.5
Temperature pre-conditioner[b], °C	82	82	82	82	82	82	82	82	82	82	82
Water added to extruder, kg h^{-1}	13.5	22.5	27	16.5	0	27	10.5	13.5	22.5	27	16.5
Temperature of extruder water, °C	35	35	35	35	35	35	35	35	35	35	35
Temperature section 1[c], °C	75	75	80	90	80	90	80	75	80	90	90
Temperature section 2[c], °C	75	75	80	90	80	90	80	75	80	90	90
Temperature section 3[c], °C	75	75	80	90	80	90	80	75	80	90	90
Temperature section 4[c], °C	75	75	80	90	80	90	80	75	80	90	90
Revolution of screws, rpm	496	279	403	279	484	434	428	496	477	434	440
Die orifice diameter, mm	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Die open area (mm^2)	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2
Drying											
Temperature section 1, °C	75	80	95	95	83	100	90	75	85	100	102
Temperature section 2, °C	65	75	90	93	65	95	90	65	67	95	102
Temperature section 3, °C	65	70	85	90	60	95	90	65	62	93	102
Total drying time (min)	11.3	10.8	10.4	10.8	11.3	10.8	13.8	10.8	10.8	10.8	11.3

Table 2 (continued)

Diet formulation	100 g CPC kg^{-1}			200 g CPC kg^{-1}				FM			
Added moisture[a] (% of feed rate)	22	25	29	16	22	25	29	16	22	25	29
Extrusion											
Capacity feed mix, kg h^{-1}	150	150	150	150	150	150	150	150	150	150	150
Steam added to pre-conditioner, kg h^{-1}	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5	10.5
Water added to pre-conditioner, kg h^{-1}	0	0	16.5	0	0	0	16.5	0	0	0	16.5
Temperature pre-conditioner[b], °C	82	82	82	82	82	82	82	82	82	82	82
Water added to extruder, kg h^{-1}	22.5	27	16.5	13.5	22.5	27	16.5	13.5	22.5	27	16.5
Temperature of extruder water, °C	35	35	35	35	35	35	35	35	35	35	35
Temperature section 1[c], °C	80	80	80	75	80	80	80	75	80	80	80
Temperature section 2[c], °C	80	80	80	75	80	80	80	75	80	80	80
Temperature section 3[c], °C	80	80	80	75	80	80	80	75	80	80	80
Temperature section 4[c], °C	80	80	80	75	80	80	80	75	80	80	80
Revolution of screws, rpm	291	298	322	496	329	285	285	496	527	515	527
Die orifice diameter, mm	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.5
Die open area (mm^2)	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2	33.2
Drying											
Temperature section 1, °C	90	95	95	70	90	95	100	75	90	95	100
Temperature section 2, °C	80	85	95	60	80	90	95	65	85	90	90
Temperature section 3, °C	80	85	90	60	80	90	90	65	85	85	90
Total drying time (min)	11.3	10.8	11.3	10.4	11.3	10.8	10.4	10.8	10.8	10.8	10.8

[a]Calculated on the basis of raw materials fed to the pre-conditioner; weight of raw materials on an as-is basis.
[b]Temperature pre-conditioner, temperature at the outlet of the pre-conditioner.
[c]Actual extruder barrel temperature at each section, 1–4.

It simulates severe treatment of the feed by pneumatic handling (Van der Poel, 1996) and gives a wide range of results due to the long time span over which the stresses are applied.

2.10. Experimental design and statistical analysis

The effects of inclusion of LPC and CPC (100 and 200 g kg^{-1}) and the added feed moisture (29%, 25% and 22% of the mash feed rate) were investigated with respect to the pasting properties of the mash, extruder SME, specific density of feed, maximum oil infusion, sinking, fat leakage, DORIS and Holmen durability. In addition, the SPC-based diet, 200 g LPC kg^{-1}, 200g CPC kg^{-1}, and the FM-based diet were processed at 16% added moisture using exactly the same process parameters. However, those feeds were not used in the further analyses.

The results from the 18 experiments (Table 2) were submitted to a one-way analysis of variance (ANOVA) and a Tukey honestly significant difference (HSD) test; a confidence interval of 95% was used to compare the means. Statistical analyses were carried out using UNISTAT computer software (Unistat, 2011).

3. Results and discussion

The aim of this work was to understand the effects of various protein sources on SME consumption, addition of moisture during extrusion and the overall properties of fish feed products. We demonstrated that the shear cell device can be used efficiently to study the behaviour of feed ingredients under thermo-mechanical treatments, which can be used to predict the behaviour of material during extrusion.

3.1. Feed extrusion and chemical composition of the diets

The mixtures were extruded using the parameters presented in Table 2. The screw speed and barrel temperature were used to optimize expansion of the extrudate and maximum oil infusion, but these changes did not significantly influence the effects reported in this study, as shown later.

The SPC-based diet processed with 16% added moisture resulted in high dust formation at the extruder discharge and a fragile product. Consequently, these kernels were not used in further analysis. Dust formation is a clear sign

of insufficient hydration of the mash particles. To check if the properties of the product could be attributed to the changes in extrusion conditions rather than compositional changes, we decided to process 200 g LPC kg^{-1}, 200 g CPC kg^{-1} and the FM-based diet at this moisture level, using exactly the same process parameters. As a result, the morphology of the pellets containing either LPC or FM was improved significantly and no dust formation was observed. In contrast, addition of 200 g CPC kg^{-1} did not result in any improvement.

It has been reported previously that the effects of protein sources might also be caused by differences in chemical composition between the diets (Draganovic et al., 2011). Chemical analysis revealed that the crude protein level varied from 556 to 614 g kg^{-1}, and the fat level varied from a low of 53 g kg^{-1} in the SPC-based diet to a high of 105 g kg^{-1} in the FM-based diet (Table 1). The starch in the feed facilitates expansion and binding of the pellet matrix (Sørensen et al., 2010). The actual starch measured was between 106 and 131 g kg^{-1} for the feed based on FM and the 100 g CPC kg^{-1} diet, respectively (Table 1). The inclusion of CPC in the feed resulted in a significant increase in crude fibre. No notable differences were observed in the ash content between the diets, except for the FM-based diet, which showed a higher value (Table 1).

3.2. Effects of the protein source and the addition of moisture on the shear cell torque and extruder specific mechanical energy consumption

To mimic the extrusion conditions, we tried to use similar moisture levels during the shearing experiments as used in extrusion. However, this was unsuccessful because of difficulties in closing the shear cell device in the case of the SPC-based diet and the 200 g CPC kg^{-1} diet at 30% water content or lower, when 82 g of material was used; the cell could not be closed because the mash was too solid. Therefore, it was decided to use higher water content than typically used in extrusion; namely 35%, 40% and 50% (of the total water and material mass).

Fig. 2A shows the torque curves of the shear runs for different protein sources. LPC behaves similar to FM; both show low and constant torque values over time. The highest torque was observed with CPC, followed by SPC. The curve of the CPC-based feed tends to decrease slightly with time, which suggests weakening of the melt. The torque curve for SPC, however, increases with time and then levels off (Fig. 2A).

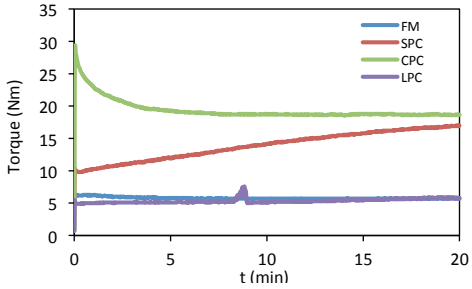

Fig. 2. (A) Effects of feed ingredients on torque at 60% water content.

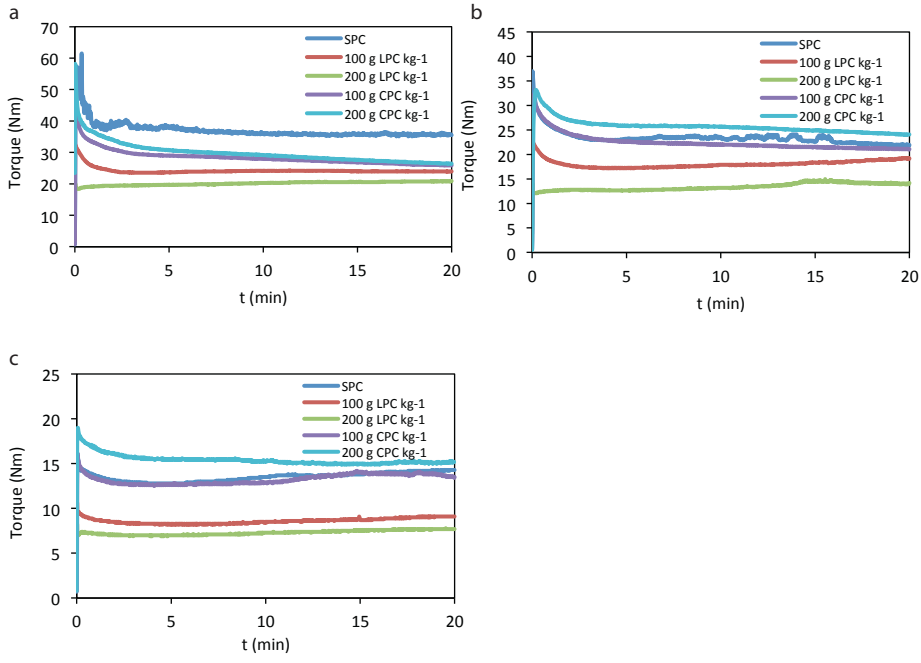

Fig. 2. (B) Effects of formulation on torque at 35% (a), 40% (b) and 50% (c) water content.

Fig. 2B shows the torque curves during shearing of the meal mixes. The higher moisture content led to lower torque. Inclusion of LPC led to a decrease in the torque at all three moisture levels. Remarkably, the torque values were not much different between 100 and 200 g LPC kg^{-1}. Inclusion of 100 g CPC kg^{-1} gave torque values similar to those for the SPC-based diet at 40% and 50% water content; 200 g CPC kg^{-1} resulted in an increase in the torque at the same water content (Fig. 2B). The highest torque value at 35% water content was obtained for the SPC-based diet.

All the curves were relatively constant over time except for the 100 and 200

g CPC kg^{-1} meals at 35% and 40% water content, in which a slight decrease was observed (Fig. 2B).

The effects of the formulation and moisture content on the extruder SME responses are presented in Fig. 3. There were statistically significant differences in SME consumption among the feeds ($P<0.01$). The SME was highest for the 200 g CPC kg^{-1} diet with low moisture content (16%). The lowest SME was observed for the 200 g LPC kg^{-1} diet at 29% added moisture (Fig. 3). Generally, for all feeds, the SME decreased as the moisture level increased (Fig. 3) because water reduces the viscosity of the melt and thus results in decreased shear stress, which is in agreement with the study of Lam and Flores (2003).

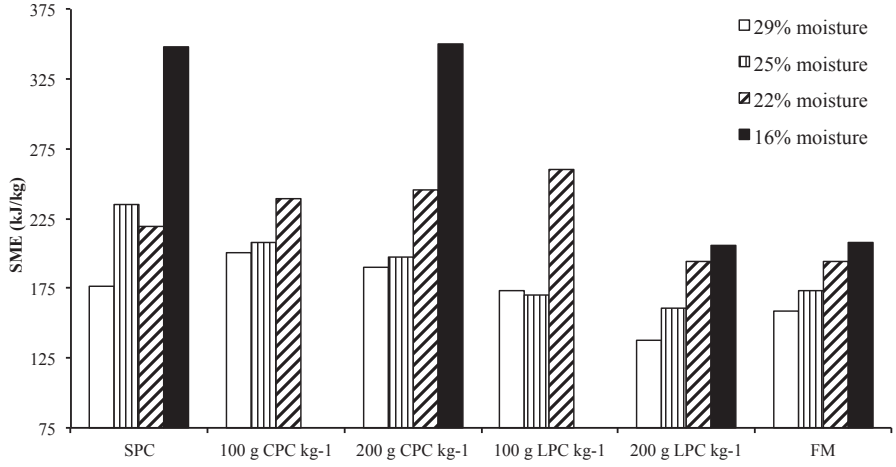

Fig. 3. Effects of the protein source in the diet and added feed moisture on SME. Means (n=10) with the same letter are not significantly different.

At 16%, 22%, 25% and 29% moisture, the SME decreased by 41%, 11%, 31% and 22%, respectively, when SPC was partially replaced by 200 g LPC kg^{-1} (Fig. 3). Replacing SPC with LPC had a pronounced effect on the SME despite the fact that both diets containing LPC were extruded at notably higher screw speeds. The effect of LPC was so great that the SME for the 200 g LPC kg^{-1} diet at 16% moisture was even lower than the SME of the SPC-based diet at 25% moisture (Fig. 3).

The low WHC of LPC plays a role in this, because more water is available for plasticizing other ingredients when LPC is added. The SPC showed a WHC of 2.4 g H_2O (g flour)$^{-1}$; which for LPC was only 0.9 g H_2O (g flour)$^{-1}$. Fig. 3 shows

that the SME values for the 200 g LPC kg^{-1} diet were very similar to those of the FM-based diet at all values of moisture content. However, the WHC of FM was somewhat higher (1.8 g H_2O (g flour)$^{-1}$). In addition, the increased fat level in the feeds containing LPC and the FM-based feed (Table 1) may have an effect on the lower SME.

No substantial differences in SME were observed when SPC was replaced with CPC (Fig. 3). For the 200 g CPC kg^{-1} diet, slightly higher values were found compared with the SPC-based diet at 16%, 22% and 29% added moisture (0.6%, 10.6% and 7.4% change, respectively); a lower value was found at 25% moisture (16% change). As with the LPC, the high SME values for diets containing CPC can be attributed mostly to the high WHC of this ingredient (3.6 g H_2O (g flour)$^{-1}$) plus the low fat level (Table 1).

The RVA assessment was used to examine the effects of the protein source on the pasting properties of the feed mash. Fig. 4 shows the RVA pasting profiles of the six diets. The inclusion of LPC significantly decreased the peak viscosity ($P<0.05$) and the final viscosity ($P<0.05$), whereas the inclusion of CPC had the opposite effect. The final viscosity of the 200 g LPC kg^{-1} diet was the lowest (0.50 Pa s) and it was followed by the FM-based diet (0.55 Pa s) and the 100 g LPC kg^{-1} (0.63 Pa s) diet (Fig. 4). The low viscosity of LPCs has been reported previously by Chew et al. (2003). In contrast, the highest viscosity by far was recorded for the 200 g CPC kg^{-1} diet (3.19 Pa s) (Fig. 4), which is probably related to its high WHC. In general, the viscosity results from this study corroborate the findings observed for the torque and SME in the shear cell and extrusion trials, respectively.

3.3. Effects of LPC and CPC and added moisture on the physical characteristics of the feed

Fig. 5 shows the materials formed by shearing. Visual examination of the sheared materials revealed that CPC formed a more consistent structure with a smoother surface compared with SPC, which crumbled during handling and gave off fines. In contrast, shearing of LPC and FM resulted in paste-like structures; with LPC, the resulting material was stickier.

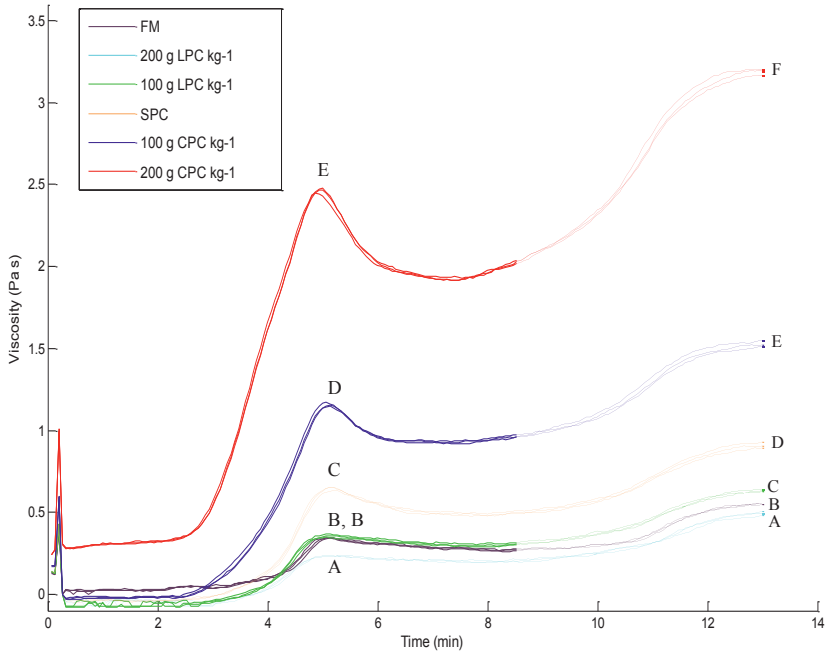

Fig. 4. Rapid viscosity analysis profiles of SPC-based, 100 g LPC kg^{-1}, 200 g LPC kg^{-1}, 100 g CPC kg^{-1}, 200 g CPC kg^{-1} and FM-based mash. $^{A-F}$ Different letters denote a significant (P<0.05) difference among the means for the peak and final viscosity (from left to right, respectively) in a Tukey HSD test.

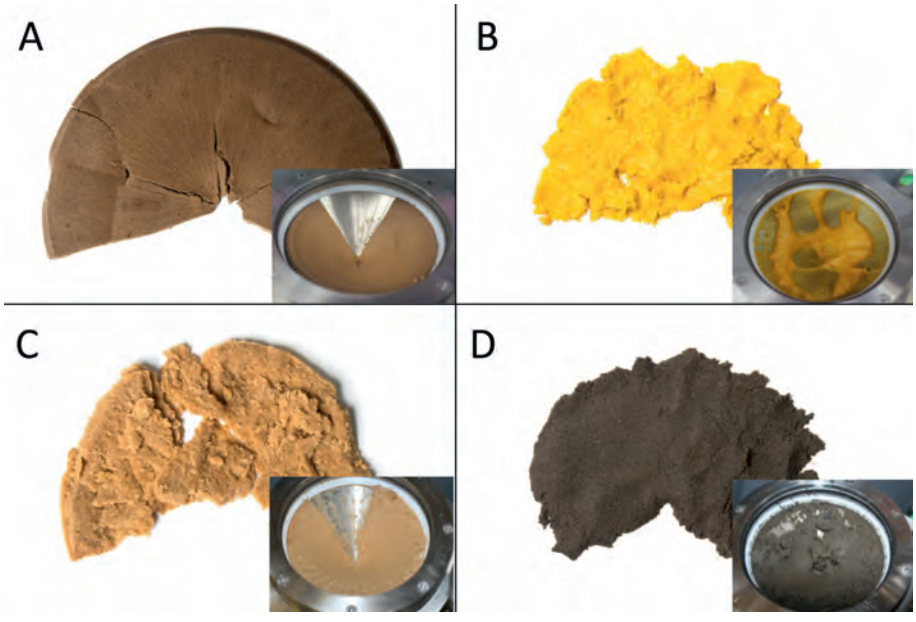

Fig. 5. Structures of CPC (A), LPC (B), SPC (C) and FM (D) after 20 min of shearing at 50 rpm and 90 °C. The water content of all materials is 60%.

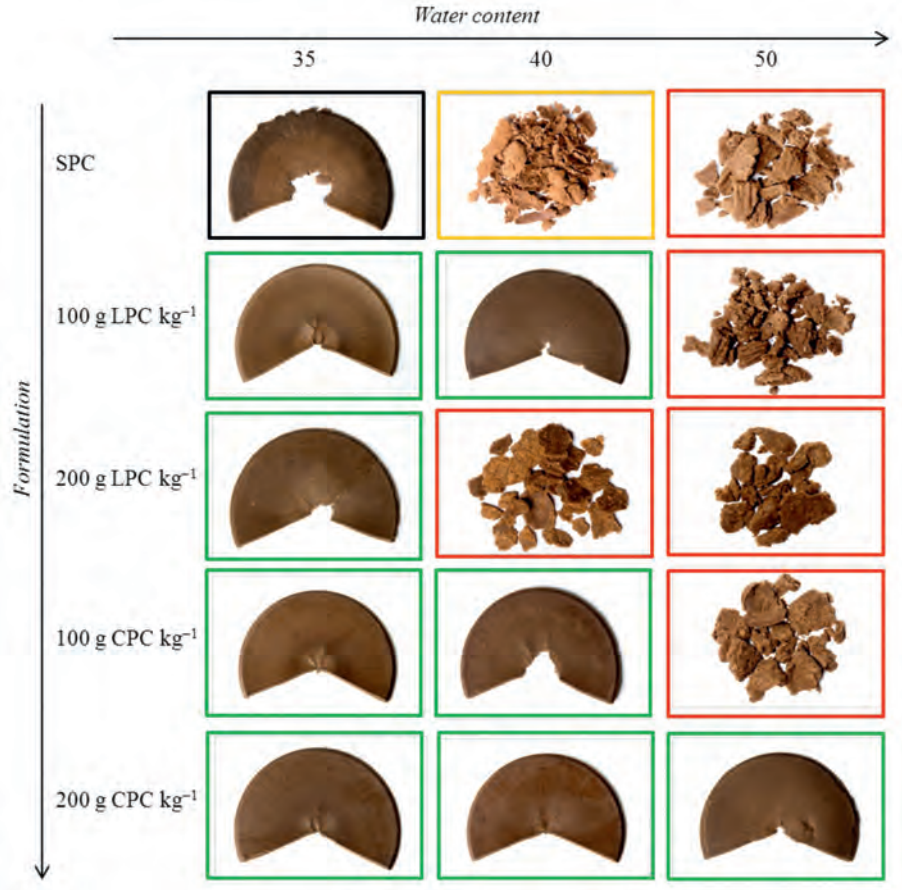

Fig. 6. Overview of all meal mix materials after shearing. The meal mixes were sheared for 20 min at 50 rpm and 90 °C. Red square, crumbled material; yellow square, crumbled material with a greater proportion of fines; black square, structured material with a torn up appearance and still some fines present; green square, cohesive, structured material with a smooth surface. SPC, soy protein concentrate; LPC, lupine protein concentrate; CPC, canola protein concentrate.

The effect of including protein-rich ingredients in the diet on the structure formation in the shear cell is visualized in Fig. 6. Except for the 200 g CPC kg^{-1} diet, all diets at 50% water resulted in material that was too moist and crumbled during handling. At this moisture content, the material was solidified in the case of canola protein, which obviously better tolerates higher moisture levels.

Although the moisture levels used here were higher than those normally used in extrusion, the consistency of the structures shown in the green squares was similar to the common dough melt at the discharge of the extruder die,

and we thus anticipate that these mixtures will produce elastic extrudates with a smooth outer surface. The SPC-based diet at 35% water content gave a structure with a rougher surface compared with those in the green squares and less uniform appearance, with some fines present. For diets containing SPC, it would be more difficult to obtain a completely smooth outer surface, even though a 35% water content provides sufficient hydration of particles.

In general, results from the extrusion trials showed that the inclusion of LPC leads to acceptable product characteristics at restricted moisture content. The implications for reduction in energy use and improvements in process efficiency are evident. In contrast, SPC could be partly replaced with CPC only with addition of more moisture.

The appearance of the pellets in the SPC-based diet followed an expected pattern. The pellets in the SPC-based diet produced at 16%, 22% and 25% added moisture had torn edges and a rough cutting surface with marked protrusions and grooves. This also led to the generation of fines during handling that need to be removed. These effects were more pronounced at lower moisture contents. Similar effects were observed when SPC was replaced with either 100 or 200 g CPC kg^{-1} at all moisture contents. Figs. 7 and 8 show the differences in pellet morphology at 16% and 22% moisture content, respectively. At 29% added moisture, the SPC-based diet had a smoother surface, but still had some grooves. Similarly, after shearing the same diet at even higher moisture content than in extrusion, the surface was not completely smooth (Fig. 6). Remarkably, the poor appearance of pellets observed during extrusion of diets containing canola disappeared at higher moisture levels as can be seen from the shear cell results with all three water levels tested (Fig. 6). It is therefore expected that at added moisture levels higher than 29%, CPC will contribute more to the binding of the extrudate matrix and formation of adequate product quality compared with SPC.

In contrast, adding 100 and especially 200 g LPC kg^{-1} improved the appearance of the pellet notably (Figs. 7 and 8). A smooth surface and shiny appearance was observed for these two diets at all three moisture levels. When 22% moisture was used, the 100 and 200 g LPC kg^{-1} diets had even better appearance than the SPC-based diet at 29% moisture.

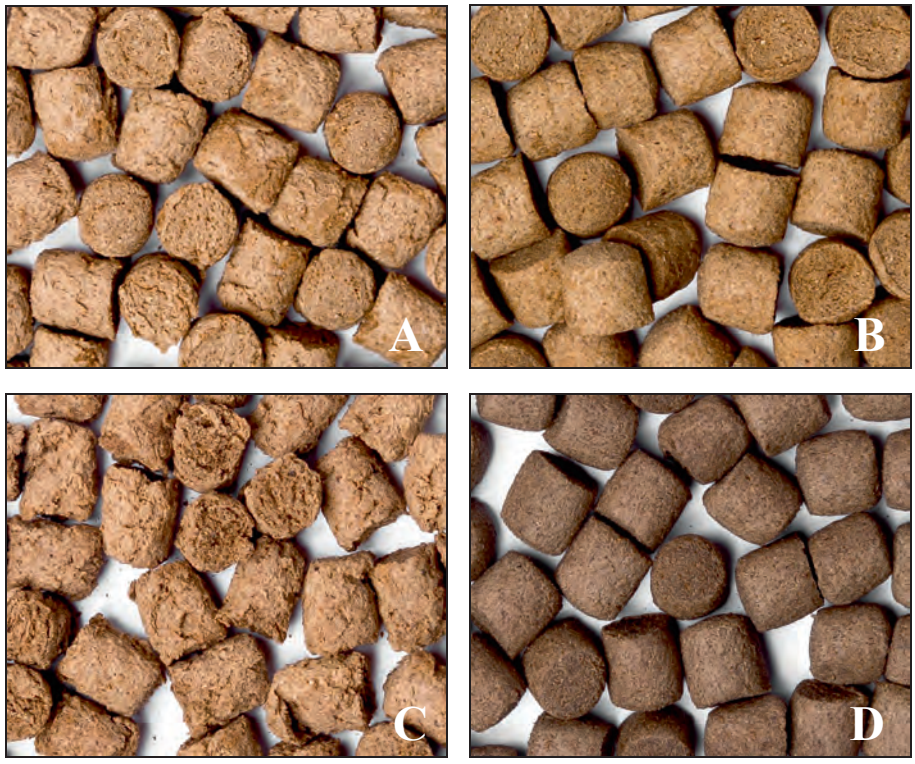

Fig. 7. Feed pellet morphology at 16% added moisture for (A) SPC-based, (B) 200 g LPC kg^{-1}, (C) 200 g CPC kg^{-1}, (D) FM-based diets.

The production of modern extruded aquafeeds for finfish is closely linked to the feeding behaviour of the target species, therefore the sinking rate of the pellets, and hence the control of the pellet bulk density, is of critical importance (Glencross et al., 2010). Although replacement of SPC with LPC did not notably change the starch content, it did result in lower overall expansion, which was compensated with higher screw speed (Table 2). This is in agreement with Glencross et al. (2010) who found a significant decrease in oil uptake and radial expansion with increasing levels of lupin kernel meals. The same authors suggest further increases in the level of starch in the diet to overcome these limitations. The density of the feed without oil varied from 684 to 777 g l^{-1} (Table 3); it was lower for the 200 g LPC kg^{-1} diet at 29% and 22% added moisture and the FM-based diet at 29% moisture than for the other treatments. The trends for maximum infusion of oil are known to closely follow the density (Draganovic et al., 2011); it was reported that the density and the maximum oil infusion had a correlation coefficient of –0.99. Although the specific densities

differ significantly in statistical terms ($P<0.01$) (Table 3), the differences are not considered of technical importance because all the feeds showed adequate oil infusion. Furthermore, the percentage sinking was high (≥98%) in all cases (Table 3).

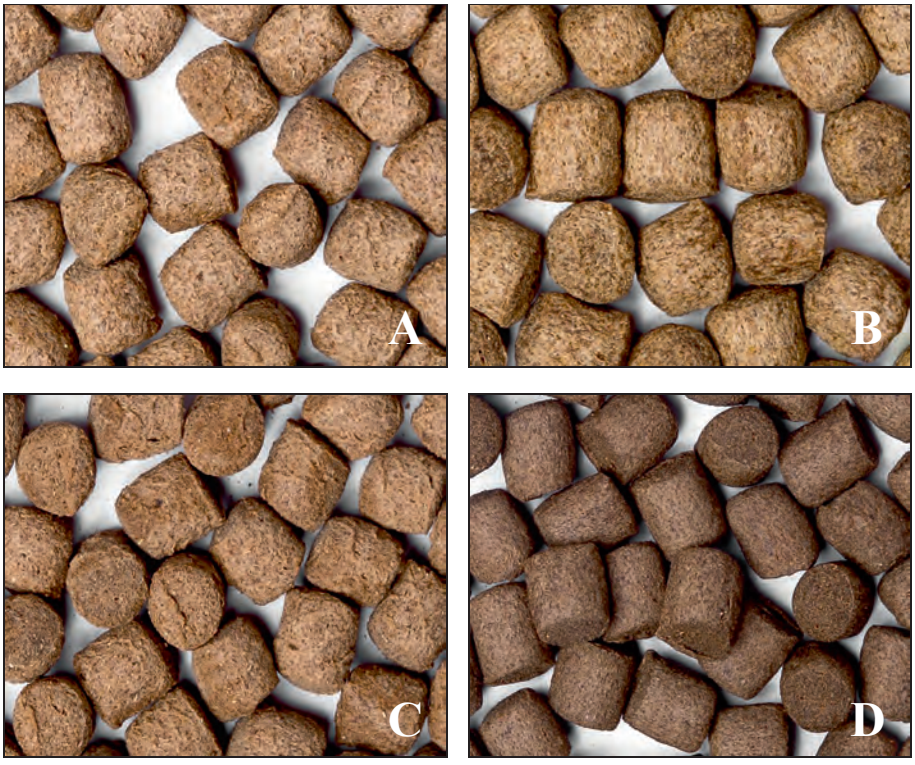

Fig. 8. Feed pellet morphology at 22% added moisture for (A) SPC-based, (B) 200 g LPC kg^{-1}, (C) 200 g CPC kg^{-1}, (D) FM-based diets.

Fat leakage varied from 4.5% to 12.5%. The lowest fat leakage was obtained at 200 g LPC kg^{-1} at 25% moisture; the highest was observed for the FM-based diet at 29% moisture, followed by the same diet at 25% moisture (Table 3). In general, diets containing LPC showed lower fat leakage compared with other feeds, which is probably related to the microstructure of the pellet (Draganovic et al., 2013).

The durability of the product during handling is a strong indicator of feed integrity during storage, transportation and pneumatic feeding. Although there were significant ($P<0.01$) differences in DORIS durability among the feeds, the inclusion of both LPC and CPC did not result in notable differences from a

Table 3 Effects of LPC and CPC on the physical characteristics of the feed.

Diet formulation	Specific density (g l^{-1})	Maximum oil infusion (%)	Sinking (%)	Fat leakage (%)	DORIS durability (%)	Holmen durability (%)
29% feed moisture						
SPC	729^{efg}	53.1^{bcde}	100.0^{b}	7.7^{cd}	93.8^{c}	98.0^{bc}
100 g LPC kg^{-1}	710^{bc}	53.8^{cdef}	100.0^{ab}	5.8^{b}	97.3^{cd}	99.6^{e}
200 g LPC kg^{-1}	684^{a}	55.8^{fg}	99.0^{ab}	5.2^{ab}	96.8^{cd}	99.2^{e}
100 g CPC kg^{-1}	746^{i}	51.6^{bcd}	100.0^{b}	7.7^{cd}	96.8^{cd}	98.4^{cd}
200 g CPC kg^{-1}	744^{hi}	50.7^{b}	100.0^{b}	7.5^{c}	98.6^{d}	98.7^{d}
FM	693^{a}	57.3^{gh}	100.0^{b}	12.5^{h}	70.3^{a}	97.9^{b}
25% feed moisture						
SPC	706^{b}	57.0^{gh}	98.7^{ab}	7.56^{c}	96.9^{cd}	98.7^{d}
100 g LPC kg^{-1}	718^{cde}	54.1^{def}	98.7^{ab}	5.3^{ab}	95.8^{cd}	99.7^{e}
200 g LPC kg^{-1}	723^{def}	50.7^{b}	99.0^{ab}	4.5^{a}	97.0^{cd}	99.5^{e}
100 g CPC kg^{-1}	746^{i}	51.4^{bc}	100.0^{b}	8.3^{cdef}	98.0^{d}	98.3^{bcd}
200 g CPC kg^{-1}	729^{efg}	53.1^{bcde}	99.7^{b}	7.9^{cde}	97.0^{cd}	98.3^{bcd}
FM	717^{bcd}	55.4^{efg}	100.0^{b}	10.5^{g}	71.2^{a}	98.5^{cd}
22% feed moisture						
SPC	777^{j}	48.1^{a}	100.0^{b}	5.3^{ab}	98.9^{d}	98.2^{bcd}
100 g LPC kg^{-1}	718^{cde}	56.1^{fg}	99.3^{ab}	8.8^{def}	98.1^{d}	99.3^{e}
200 g LPC kg^{-1}	694^{a}	58.9^{h}	97.7^{a}	4.9^{ab}	96.7^{cd}	97.4^{a}
100 g CPC kg^{-1}	739^{ghi}	53.0^{bcde}	100.0^{b}	9.3^{f}	97.2^{cd}	98.1^{bc}
200 g CPC kg^{-1}	727^{def}	55.5^{efg}	99.3^{ab}	9.0^{ef}	94.1^{c}	98.6^{d}
FM	734^{fgh}	54.1^{def}	100.0^{b}	9.4^{fg}	79.1^{b}	98.1^{bc}
MSE[a]	13.850	0.677	0.333	0.160	1.403	0.024
P value	0.0000	0.0000	0.0002	0.0000	0.0000	0.0000

Means (n=3) without a common superscript within a column are significantly different in a Tukey HSD test; $P<0.05$.
[a]Mean square error from analysis of variance (df=36).

technical point of view (Table 3). In contrast, the durability was greatly reduced compared with other feeds when FM was used as a main protein source. Decreasing the moisture content from 29% to 22% significantly improved the durability of the SPC-based diet and the FM-based diet; the opposite effect was observed for the 200 g CPC kg^{-1} diet (Table 3). Thus, addition of CPC leads to

the most durable product at 29% added moisture, which confirms that canola proteins strengthen the structure at higher moisture levels and is in line with the results obtained with the shearing device. In general, the numeric durability values were high for all the feeds analysed and well within the acceptable range according to the commercial guidelines, except for the FM-based diet (Table 3).

Even smaller variations were observed with the HDI. The highest HDI was found for the 100 g LPC kg^{-1} diet at 25% moisture, followed by the same diet at 29% and the 200 g LPC kg^{-1} at 25% moisture (Table 3). With the exception of the 200 g LPC kg^{-1} diet at 22% moisture, replacing SPC with LPC led to a statistically significantly higher HDI. Furthermore, the value for the HDI was not very responsive to changes in added moisture (Table 3). In commercial feed manufacturing practice, HDI values ≥93% are recommended. Thus, all the treatment combinations showed adequate HDI values.

3.4. Technological requirements for plant-based materials used in fish feed

The materials examined show large differences in the amount of water required for good material plasticization. Even though lupine and canola seed proteins are mainly globulins like soy proteins, they behave differently. SPC and CPC showed poor solubility of 2.7 and 1.7 %, respectively, whereas the protein solubility of LPC and FM was much higher (9.7 and 15.1 %, respectively). According to Chen et al. (2011), solubility is one of the most important properties of protein, as it is an indication of good interaction with water, which is necessary for strong plasticization by water; good solubility is an indication that the glass transition temperature of the material will drop fast with the addition of water. Therefore, water solubility is also directly related to other useful functional properties.

Table 4 summarizes the results found in this study. FM has unique technological properties (high solubility, low WHC and low paste viscosity) and is thermostable. SPC is often used to replace FM. Given its much higher WHC, this is only possible when the dough is extruded at a higher moisture content. A higher moisture content leads to more plasticization and thereby reduces the viscosity of the mash. Additional moisture is required to improve the deformability and the formation of a coherent mass.

Table 4 Overview of the technological properties of the protein sources examined

Feed ingredient	Solubility	WHC	Gelation on heating	Viscosity	Pellet quality low moisture content	Pellet quality high moisture content
FM	High	Low	No	Low	Good	Fair
LPC	High	Low	No	Low	Good	Good
SPC	Low	High	Yes	High	Poor	Good
CPC	Low	High	Yes	High	Poor	Good

CPC also has low solubility and high WHC. In addition, the melt viscosity is high as can be seen from the shearing device results. In contrast to SPC, CPC is able to form a coherent mass with a smooth surface at high water content (Fig. 6).

The technological properties of LPC resemble those of FM. It has high solubility and low WHC. As a result, most of the water is available to plasticize the other components in the dough at low moisture levels. In addition, its paste viscosity is low and not much influenced by thermal treatment. The similarity of LPC to FM is reflected in similar extrusion behaviour. It may even be possible to reduce the moisture level even further, which would lower the amount of drying required. The changes in the properties of the pellets from SPC and from LPC can be explained by a lower glass transition temperature (T_g) of the dough mass based on LPC. LPC did not become a hard gel. Although the T_g values were not measured here, the diets containing SPC and CPC will be either close to their glass transition or even be glassy at low moisture content, which prevents the formation of a smooth pellet. The limited moisture available might be absorbed by the plant material, leaving less water available for the other materials. These might be in the glassy stage at lower overall moisture content. Fan et al. (1996) stated that the extrudate structure is strongly dependent on T_g. Barrett and Kaletunc (1998) used T_g as a parameter to evaluate the effects of processing conditions on the textural attributes of extrudates. They and Champion et al. (2000) stated that water is the major plasticizing component that affects T_g.

3.5. Usefulness of the shearing device

The results from this study show that the shear cell device is adequate for assessing processing conditions that are close to the conditions in an extruder during the production of fish feed.

SME is an important parameter as it indicates the melt behaviour during extrusion, which in turn determines the product qualities (Yuliani et al., 2009). Although not statistically tested, the shearing torque values in this study correlate well with the SME in an extruder. Furthermore, the cohesiveness and viscosity of the material under extruder-like processing conditions can be readily studied.

Compared with pilot-scale extrusion, shear cell trials consume less time and materials. The latter can be important for feed ingredients that are at the early stage of development, when a very small amount of material is available. Results from shear cell trials are a solid basis for the development of formulations and processes before moving to a larger scale. Shear cell trials can help in revealing the functional properties of FM, and therefore can be used to set design criteria for various materials, such as other plant-based materials or microalgae, which may lead to lower viscosity, higher solubility and stronger gel formation.

4. Conclusions

The potential use of SPC, LPC and CPC in fish feeds was evaluated by two processing methods: shearing in a cone-plate shearing device and extrusion. The behaviour of these three materials during processing was compared with FM.

The shear cell device is a good, simple and fast apparatus for characterizing the techno-functional properties of raw materials, and for determining the influence of processing conditions.

Both shearing and extrusion showed that FM and soy have different technological properties; soy is normally used as a replacer for FM. SPC has a higher WHC and higher paste viscosity, which explain the higher feed moisture required during extrusion. CPC has similar properties to SPC and also requires more moisture. The properties of the feeds containing CPC could be well within the ranges that are acceptable for commercial fish feed use at higher moisture contents compared with SPC, but the higher moisture levels would require more drying, and thus higher energy consumption.

LPC had similar or even better properties than FM. For formulations with 100 and 200 g LPC kg^{-1}, the moisture level during extrusion could be reduced without negatively influencing the final properties of the product. Clearly, this

results in a notably decreased drying requirement, leading to reduced energy consumption and better process efficiency.

The effect of adding LPC can be explained by considering that LPC reduces the viscosity of the feed mash through a plasticizing effect and reduced WHC. This reduced viscosity lowers the SME input, which leads to energy savings additional to the reduced drying.

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Chapter 5

Sustainability assessment of salmonid feed using energy, classical exergy and eco-exergy analysis

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Abstract

Reduction of the environmental impact of feed products is of paramount importance for salmon farming. This article explores the potential to compare three thermodynamically based ecological indicators. The environmental impact of partial replacement of fish meal (FM) and fish oil with alternative ingredients was investigated using energy, classical exergy and eco-exergy analysis. Seven hypothetical feeds were formulated: one with high levels of FM and fish oil, four feeds based on plant ingredients, one containing krill meal, and one based on algae-derived products. Analysis included cultivation of crops and algae, fishing for fish and krill, industrial processing of these ingredients and production of complete fish feed. Because most harvested products are refined in multiple product outputs that have good value to society, two scenarios were compared. In the base case scenario, no allocation of co-products was used and all the environmental costs were ascribed to one specific co-product. Co-product allocation by mass was used in the second scenario; this is considered to be the preferred scenario because it accurately reflects the individual contributions of the co-products to the environmental impact of the feed products. For this scenario, the total energy consumption for a fish-based diet was 14,500 MJ, which was similar to a krill diet (15,600 MJ), about 15–31% higher than plant-based diets, and 9% higher than an algae diet. Substituting FM and fish oil with alternative ingredients resulted in minor changes in total classical exergy degradation (2–16% difference). The calculations based on energy only consider the energy conservation based on the First Law of Thermodynamics, whereas those based on classical exergy also takes the Second Law of Thermodynamics into account; energy that can do work is distinguished from energy that is lost as heat to the environment. The calculations based on eco-exergy consider the total loss of work energy in the environment including the work energy associated with the information embodied in the genomes of organisms. The diet based on fishery-derived ingredients was the highest total work energy consumer compared with plant-based diets (24–30% greater), the diet containing krill meal (25% greater), and the algae diet (four times higher). Thus, reducing FM and fish oil levels in fish feed can contribute significantly to more sustainable aquaculture. In particular, algae-derived products in aquafeeds could drastically decrease environmental costs in the future.

1. Introduction

As the aquaculture industry continues to expand globally, access to key feedstuffs, such as fish meal (FM) and fish oil, will become increasingly limited because of the finite resources available for wild harvesting (Gatlin et al., 2007). Aquaculture that relies on FM as a dominant protein ingredient is another source of pressure on populations of wild fish (Pauly et al., 2002). To reduce the effect of aquaculture on the ecosystem, enhanced efforts are needed to thoroughly evaluate reasonable alternatives, such as feedstuffs from plant origin (Gatlin et al., 2007). Wheat gluten and soy protein concentrate (SPC) have shown high potential as alternative proteins to FM with respect to their availability and nutritional value. However, it is not straightforward to conclude that plant proteins inherently contribute to sustainability if we take into account the renewable and nonrenewable resources and waste emissions related to the production of these feed ingredients. The theoretical impact of replacing FM and fish oil in rainbow trout feeds was investigated using nutritional modeling and life cycle assessment by Papatryphon et al. (2004). They showed that completely replacing FM and fish oil with plant sources did not decrease the environmental impact when use of energy is considered. In addition, it was previously reported (Draganovic et al., 2011) that replacing FM with gluten, and particularly SPC, increases the quantity of water added during fish feed production to compensate for differences in the technological properties of fish and plant protein. Consequently, energy consumption for drying has to be increased. It can be concluded that there is a need for a comprehensive analysis of different protein sources in fish feeds.

We proposed using three thermodynamic analyses to provide an ecological evaluation of the differences in sustainability of various salmon feed compositions.

(A) Energy analysis has been applied traditionally to compare the energy consumption per kilogram of production of different salmon feeds. In this study, not only the energy of processing of feed ingredients is considered but also the direct energy inputs related to agricultural production and the processing systems from which the feed ingredients were derived (Pelletier and Tyedmers, 2007).

(B) Energy can, however, be energy that can do work or energy that cannot do work but is lost to the environment as heat by the temperature of the

environment. Energy is conserved (the First Law of Thermodynamics), whereas energy that can do work (often named exergy) is lost inevitably by all processes (the Second Law of Thermodynamics). Humans are interested in the work energy, not in the heat energy lost to the environment. It would therefore be beneficial to compare different salmon feeds by the amount of exergy that is consumed in their production. The exergy content of the feed itself would be equal to the free energy (chemical work energy) of the various components. But the work energy related to production, such as electricity and fossil fuel, has to be included as well (Balkan et al., 2005; Dewulf and Van Langenhove, 2002; Kotas, 1986; Tekin and Bayramoğlu, 1998). Szargut (1989) described a method to calculate the exergy for a given chemical composition. Exergy is defined as the amount of work the system under consideration can perform when brought into equilibrium with the environment (room temperature and 1 atm). Exergy is therefore calculated slightly differently than the free energy because exergy has the environment as a reference.

(C) Classical exergy analysis does not consider that living organisms carry a lot of information. Information is a form of free energy according to Boltzmann (1905), which implies that living organisms contain more work energy than just the chemical energy of their components (proteins, lipids, carbohydrates, etc.) (often named eco-exergy). The information in organisms is embodied in the genome and is used to determine the amino acid sequence in the enzymes that are controlling the biochemical processes in living organism.

By calculating eco-exergy and including other exergy degradation, the total embodied work energy capacity can be calculated; this is the chemical work energy (free energy) of the chemical components, the work energy used for production, and the work energy embodied in the information that the living organisms carry. It means that the salmon feed with the lowest total work energy capacity or degradation is the preferred salmon feed from an ecological or sustainability point of view.

The primary aim of this paper is to estimate the impact of alternative ingredients to fish meal and fish oil on the environment, regardless of the geographic region of fish feed production. As a secondary aim, we want to introduce eco-exergy as a key driver for sustainability of aquaculture.

The diets considered in this work were formulated to be nutritionally

equivalent or biochemically optimal to ensure maximum growth of salmon. Therefore, identical growth performance could be expected in fish fed different diets for this study.

2. Materials and methods

2.1. Methods

Fig. 1 shows the system chosen for the analysis. The system boundaries include the agriculture of wheat, grain legumes, oil seeds and microalgae, fishing for fish and krill, industrial processes to extract oils and produce protein concentrates, and the production of fish feeds made from them. Pesticides, fertilizers, manure, seed, electricity and diesel consumed during the agriculture are accounted for. Transportation stages are important. It has been reported previously that transportation may contribute from about 5 to 12% to the total energy use for the production of 1 ton of salmonid feeds (Papatryphon et al., 2004; Pelletier and Tyedmers, 2007). Transportation expenditure could potentially be substantial depending on the distances travelled and, more importantly, the mode of transportation used. Fish feed factories are located all around the world, and therefore general figures for transportation do not exist. The transportation expenditure for SPC, for instance, would be different for a feed plant in Norway compare with a plant in Brazil where soybeans are sourced locally. The same is true with FM. There are several FM manufacturers in Norway, located close to fish feed plants. In other countries, this ingredient may need to be transported longer distances, possibly including transportation by truck or rail freight, which is more energy intensive than shipping by sea. In general, local sourcing of ingredients is important. Because the aim of this study is to provide a global perspective on the sustainability of aquaculture, the transportation is not included in the analysis. In agreement with Apaiah et al. (2006), chains in the manufacture of machines are not accounted for. Inputs from feed additives (minerals, pigments, etc.) were not included in the calculations. Typically, more than 10 different micro-ingredients are used in the fish feed formulation and as they are applied in very small but varying amounts they are difficult to account for and are therefore not considered. In general, plant protein sources are deficient in some nutrients (e.g. lysine and methionine) compared with FM. Thus, it is anticipated that addition of those

nutrients in the diet as micro-ingredients will slightly increase the total energy/exergy expenditure of the plant-based diets, although they are added in small amounts.

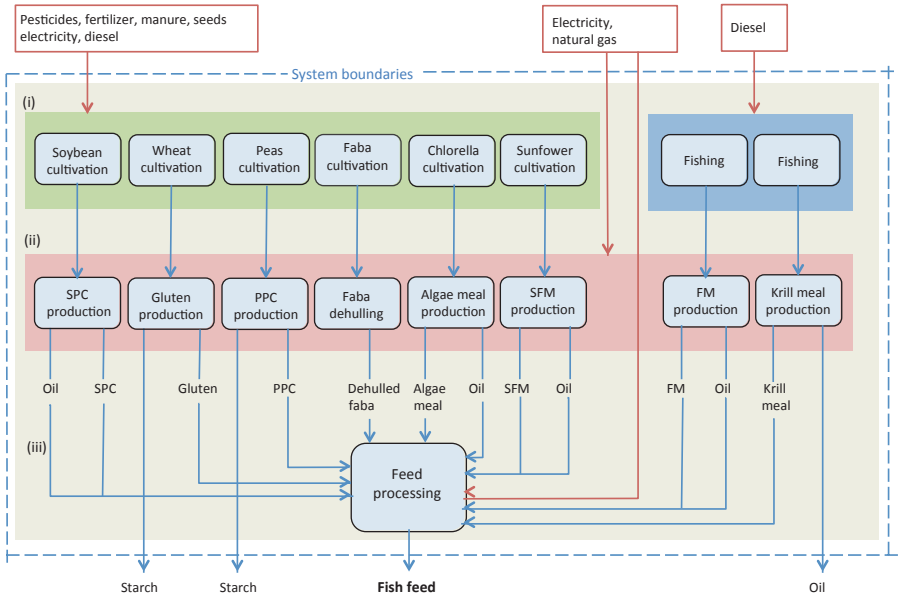


Fig. 1. System boundaries for the production of fish feed representing: (i) primary production/fishing, (ii) industrial stage ingredient preparation, and (iii) industrial stage mixing of ingredients and processing. The streams of all ingredients included in this study are shown. SPC, soy protein concentrate; PPC, pea protein concentrate; SFM, sunflower meal; FM, fish meal.

As stated in the Introduction, three different methodologies are used in this work to consider the production of 1 metric ton (1000 kg) of complete fish feed. The total energy consumption corresponds to the sum of all contributions, namely energy for crop cultivation/fishing, industrial preparation of ingredients and feed processing. The total classical exergy degradation corresponds to the sum of all contributions, namely chemical exergy of the biomass, work energy for crop cultivation/fishing, industrial preparation of ingredients and feed processing. The total work energy including eco-exergy (work energy^{iee}) degradation relates to the sum of all contributions, namely eco-exergy of the components originating from living organisms, work energy for crop cultivation/fishing, industrial preparation of ingredients and feed processing.

The mass and energy balances and the classical exergy dissipation profile were determined for each feed ingredient. The governing equations for a

steady-state system are:

Mass balance:

$$\sum m_{\text{in}} - \sum m_{\text{out}} = 0 \tag{1}$$

Energy balance:

$$\sum \left(mh\right)_{\text{out}} - \sum \left(mh\right)_{\text{in}} = Q - W \tag{2}$$

Exergy dissipation:

$$\sum \left(mb\right)_{\text{in}} - \sum \left(mb\right)_{\text{out}} - \sum_{k} Q_k \left(1 - \frac{T_0}{T_k}\right) - W = \text{Ex}_{\text{loss}} \tag{3}$$

where m is mass (kg), h is enthalpy (kJ mol^{-1}), Q is heat (kJ), W is work performed by the system, b is steam availability (kJ mol^{-1}), T is temperature (K) and Ex is exergy (kJ). Subscript k denotes the index of the heat source, in denotes inlet, and out denotes outlet.

The intrinsic chemical exergies (kJ kg^{-1}) of wheat, grain legumes, oil seeds, wet fish, krill, and algae (composition given in the Appendix, Table A1) were calculated based on their heating values given by Szargut et al. (1988):

$$\text{Ex}_{\text{Chem}} = \beta_{\text{Chem}} \times \text{LHV} \tag{4}$$

In contrast to the β factor mentioned earlier that accounts for the information that an organism contains, here β_{Chem} is the ratio of the chemical exergy to the lower heating value of the fuel (LHV) of the organic fraction of the biomass. Therefore, we have specified this factor as β_{Chem}. It is calculated from statistical correlations developed by Szargut and Styrylska (1964). The following correlation is used:

- for solid biofuels

$$\beta_{\text{Chem}} = \frac{1.044 + 0.0160\text{H/C} - 0.3493\text{O/C}\left[1 + 0.0531\text{H/C}\right] + 0.0493\text{N/C}}{1 - 0.4124\text{O/C}} \tag{5}$$

- for liquid vegetable oils:

$$\beta_{\text{Chem}} = 1.0374 + 0.0159\frac{\text{H}}{\text{C}} + 0.0567\frac{\text{O}}{\text{C}} \quad (6)$$

where H/C, O/C, and N/C represent atomic ratios in the fuel.

The chemical exergy of the biomass components is presented in the Appendix (Table A2).

The sum of a component's chemical exergy was weighted by their mass fraction in the biomass (this implies that mixed exergies are neglected; they are generally small compared with the chemical exergies of the individual macromolecular components) and it yields the chemical exergy of the biomass (kJ kg^{-1}) (Bosch et al., 2012):

$$\text{Ex}_{\text{Chem,Biomass}_i} = \sum \text{Ex}_{\text{Chem},i} x_i \quad (7)$$

where x_i is the mass fraction (kg kg^{-1}).

2.1.1. Eco-exergy calculation

Jørgensen et al. (1995) and Bendoricchio and Jørgensen (1997) found the information that different organisms are carrying on the basis of the information embodied in the genome and thereby also in the amino acid sequence of the life-controlling enzymes and, on this basis, they calculated the exergy using the following equation:

$$\text{Ex} - \text{total density} = \sum_{i=1}^{N} \beta_i c_i \quad (8)$$

(as detritus equivalents at temperature T = 300 K in g l^{-1}) where β_i is a weighting factor accounting for how much information is contained in the *i*th organism, c_i is the concentration in, for example, grams per litre of the *i*th organism. Notice that the equation considers exergy density expressed, for example, as grams of detritus equivalents per litre. If we want to obtain the exergy of an organism, for example, the concentration is replaced by the biomass and if we want to obtain the exergy in kilojoules, it is necessary to multiply by the chemical exergy content of average detritus, which is 18.7 kJ g^{-1}.

It can be shown that the beta value is a direct measure of the free energy of the amino acid sequence determined by the genome (Jørgensen, 2012;

Jørgensen et al., 2010), which makes the interpretation very clear. Furthermore, the exergy calculation for organisms uses a reference other than the classical exergy, because the environment in nature or an ecosystem in which an organism is living is another ecosystem, but we would like to determine the work energy capacity of the organisms or the entire ecosystem. Therefore, it is necessary to use the same ecosystem or organism but at thermodynamic equilibrium as reference. All gradients are eliminated at thermodynamic equilibrium and the system is an inorganic system containing no exergy or free energy. Therefore, the exergy calculated by this method is denoted eco-exergy to underline that it includes information and uses another reference to be able to express the work energy capacity of living organisms.

The following formula was used to calculate the eco-exergy (kJ kg^{-1}) of seven fish feeds:

$$\mathrm{Ex} = \sum_{i=0}^{n} \mathrm{Ex}_{\mathrm{Chem,Biomass}_i} \beta_i \tag{9}$$

The latest β values proposed by Jørgensen et al. (2005) have been used in this work (Table 1).

Table 1 β values used in this work.

Organism	Conversion factor (β)
Microalgae	20
Crustaceans	232
Plant	275
Fish	499

Eco-exergy, which includes the information carried by living organisms, has been applied for all components that originate from living organisms, because it expresses nature's loss of work energy in delivering the living organisms (microalgae, crustaceans, plants and fish) as raw material for the fish feed. It is calculated as the chemical exergy of the organism's biomass multiplied by the beta value. The chemical exergy represents a more exact value than the 18.7 kJ g^{-1} for biomass, mentioned above. The calculated eco-exergy is added than to the other contributions of work energy to obtain the total work energy.

2.2. Data collection

Data on the agriculture of wheat (Oren and Ozturk, 2006), faba beans (Petkova and Pavlov, 2010), peas (Gerdzhikova et al., 2009; Zhelyazkova, 2010), cultivation and processing of algae (Xu et al., 2011), and sunflower (Keller, 2011; Ozilgen and Sorguven, 2011) were obtained from the literature. Mass balance data on dehulling of faba beans were obtained from Agrimarin AS (Stavanger, Norway). The data on the agriculture and processing of soybeans was derived from Ozilgen and Sorguven (2011), Qaim and Traxler (2005), and personal communication with the industry (Imcopa SA, Araucaria, Brazil). Data on the energy use in fisheries were taken from Tyedmers (2000); data for krill was obtained from Parker and Tyedmers (2012). Energy input for fisheries can vary widely (20–3400 l of fuel per ton of fish landed) (Tyedmers, 2004). We used a value of 32 l in this study (Tyedmers, 2000) because reduction fisheries are much more efficient than fisheries for human consumption (Tyedmers, 2004). FM and fish oil processing yields and energy inputs were calculated from a technical report from a fish meal producer (Welcon, 2010) and confirmed by personal communication with experts in the same industry (Norsildmel AS, Fyllingsdalen, Norway). With respect to wheat processing, inventory data were taken from the literature (Narayanaswamy et al., 2003; van der Zalm, 2011); inventories of inputs used for processing peas were obtained from Apaiah et al. (2006) and confirmed by personal communication with the industry (Agrimarin AS, Stavanger, Norway). Feed processing inventories were based on data from the fish feed factory (Skretting, 2009).

2.3. Sensitivity and scenario analyses

The sensitivity of the results to the choice of the allocation procedure was assessed by comparing the energy, classical exergy, and eco-exergy results for fish feeds using two allocation scenarios: (a) a base case scenario, whereby the ingredients used in fish feeds were considered as the only products from primary production and industrial stage processing, and therefore, no allocation was used; and (b) the mass allocation scenario in which the energy consumption, classical exergy, and eco-exergy degradation for the main product (e.g. wheat starch) and the resulting co-products (e.g. gluten) was allocated a share according to the relative mass of these products.

2.4. Composition of fish feeds

To compare feeds on the basis of equal performance potential (hypothetical), we selected feed pellets with a diameter of 9 mm and with similar nutrient profiles (Table 2). These feeds correspond to the so-called energy and nutrient dense category, representing the state of the art, also known as low pollution fish feeds. The hypothetical composition of the diets was based on information on the major ingredients in current use and ingredients that may be used potentially to ensure comparable feed quality (Table 2). All diets were formulated to be iso-energetic, to have the minimum required amount of digestible protein, and to contain at least 60 g kg^{-1} starch to give the pellets good binding properties. The diets were also formulated to meet or exceed the requirements for all essential amino acids and other nutrients (National Research Council, 2011). The composition of the first feed (FM diet) is similar to that of some feeds still available on the market (FM is the major constituent). In the diets with protein-rich plant ingredients, FM was mainly replaced with either SPC (SPC diet), a combination of SPC and wheat gluten (SPC×gluten diet), sunflower meal and gluten (SFM×gluten diet) or pea protein concentrate (PPC diet). The sixth formulation contained krill meal in addition to SPC (SPC×krill). The last feed (algae diet) is a prospective feed in which FM was mainly substituted by SPC and algae meal.

Table 2. Formulation and chemical composition of the feeds

	FM	SPC	SPC×gluten	SFM×gluten	PPC	SPC×krill	Algae
Formulation (g kg^{-1})							
Fish meal	600.0	150.0	150.0	150.0	150.0	50.0	0.0
SPC	0.0	370.0	290.6	52.6	83.7	360.1	211.1
Wheat gluten	0.0	0.0	150.0	150.0	50.0	0.0	60.0
Sunflower meal	0.0	0.0	0.0	190.0	0.0	0.0	0.0
Peas concentrate	0.0	0.0	0.0	0.0	265.0	0.0	0.0
Krill meal	0.0	0.0	0.0	0.0	0.0	100.0	0.0
Algae meal	0.0	0.0	0.0	0.0	0.0	0.0	243.9
Wheat	88.8	86.5	60.8	78	58.7	86.3	70.3
Dehulled faba beans	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Fish oil	288.7	107.1	93.7	100.9	105.9	108.3	0.0
Soya oil	0.0	249.8	218.7	0.0	247.2	252.6	0.0
Sunflower oil	0.0	0.0	0.0	235.5	0.0	0.0	0.0
Algae oil	0.0	0.0	0.0	0.0	0.0	0.0	381.3
Mineral and vitamin mix (not used in the analysis)	2.5	16.7	16.2	23.5	19.5	22.7	13.3
Calculated composition (g kg^{-1})							
Dry matter	935	944	943	945	945	945	955
Protein	418	353	416	342	343	344	330
Fat	370	385	346	387	390	393	393
Crude fibre	2	23	20	30	12	23	14
Starch	67	68	60	70	78	68	60
Digestible energy (MJ kg^{-1})	22	22	22	22	22	22	22

FM, fish meal based diet; SPC, soy protein concentrate based diet; SPC×gluten, soy protein concentrate and wheat gluten based diet; SFM×gluten, sunflower meal and wheat gluten based diet; PPC, pea protein concentrate based diet; SPC×krill, soy protein concentrate and krill meal based diet.

The FM content was relatively low in the plant-based diets (150 g kg^{-1}) and the diet containing krill (50 g kg^{-1}). As shown in Table 2, fish oil and algae oil were the only sources of oil in the FM and algae diets, respectively. For five diets with relatively low amounts of FM, vegetable oil was added at 70% of the total oil added; the reminder was fish oil.

2.5. Assumptions

Energy used for dehulling of faba beans was assumed to be the same as for dehulling of peas. In accordance with the study by Lechon et al. (2005), the energy and exergy input for production of the seeds was considered to be the same as the production of the crop. With respect to thermal drying of algae, Hassebrauck and Ermel (1996) reported that typical thermal dryers use from 3.3 to 3.9 MJ kg^{-1} of evaporated water. In this study, we used an average value (3.6 MJ kg^{-1}). Based on the results from our previous study (Draganovic et al., 2011), it was assumed that the FM diet requires less drying (about 30%) during processing compared with other diets.

2.6. Statistical analysis

Due to potential variability in the chemical composition of the ingredients, different practices between factories and the fact that the β-coefficients are estimations and not the absolute values, we presumed a standard deviation (SD) of ±10% of the total energy consumption, classical exergy and work energy^{iee} degradation for various feeds when the mass allocation scenario was used.

To generate three data points, the original value (considered as the mean) ±10% SD was used. The results were subjected to a one-way analysis of variance (ANOVA), and a Tukey honestly significant difference (HSD) test and a confidence interval of 95% was used to compare the means (n=3). Statistical analyses were carried out using UNISTAT computer software (UNISTAT, 2011).

Table 3 Energy and classical exergy used to produce 1 ton of feed ingredient (base case scenario)

Inputs (MJ ton^{-1})	Fish meal	SPC	Gluten	Sunflower meal	PPC	Krill meal	Algae meal	Wheat	Faba beans	Fish oil	Soy oil	Sun-flower oil	Algae oil
(a) ENERGY													
Crop cultivation/fishing													
Agrochemicals													
Insecticides	0	0	139.8	0	608.7	0	0	15.7	116.5	0	0	0	0
Herbicides	0	1705.7	642.5	1280	0	0	0	72.2	446.8	0	2845.1	1357.7	0
Fungicides	0	0	530.5	0	0	0	0	59.6	481.6	0	0	0	0
Growth regulators	0	0	0	0	26.4	0	0	0	0	0	0	0	0
Chemical fertilizer													
Nitrogen	0	4364.6	33251.1	5735.1	5880.5	0	5230.1	3735.1	1668.6	0	7280.2	6083.2	34048
Phosphorus	0	974.5	4838.6	640.4	3793.5	0	281.8	543.5	745.2	0	1625.5	679.3	1834.5
Potassium	0	0	0	0	0	0	0	0	0	0	0	0	0
Manure	0	2165.7	0	0	0	0	0	0	0	0	3612.4	0	0
Seeds	0	1067.7	5961.8	0	1260.2	0	0	669.7	328	0	1780.9	0	0
Electricity	0	1071.4	0	0	3.8	0	1797.1	0	0.7	0	1787.2	0	11699.3
Diesel	8186.2	5845.8	23135.0	4227.3	5426.9	34518.7	0	2598.7	1970.1	28242.3	9750.8	4483.9	0
Industrial stage ingredient preparation													
Electricity	912.3	615.2	11146	423.4	973.7	0	7514.8	0	101.2	3147.4	562.8	449.1	48921.3
Natural gas	9776	9651.4	19430	0	0	18565.9	0	0	0	33727.3	4555.6	0	0
Total	18874.5	27462.1	99075.3	12306.2	17973.7	53084.6	14823.8	7694.5	5858.7	65117	33800.4	13053.2	96503.1

Inputs (MJ ton^{-1})	Fish meal	SPC	Gluten	Sunflower meal	PPC	Krill meal	Algae meal	Wheat	Faba beans	Fish oil	Soy oil	Sun-flower oil	Algae oil
(b) CLASSICAL EXERGY													
$Ex_{Chem, Biomass}$	26920.0	68056.1	157310.9	57916.9	73693.4	50800.8	29235.6	17671.3	19564.7	92584	113502.2	61518.6	190900
Crop cultivation/fishing													
Agrochemicals													
Insecticides	0	0	132.2	0	575.6	0	0	14.8	110.1	0	0	0	0.0
Herbicides	0	1501.1	565.4	1126	0	0	0	63.5	393.1	0	2503.8	1194.3	0
Fungicides	0	0	492	0	0	0	0	55.3	446.7	0	0	0	0
Growth regulators	0	0	0	0	2.1	0	0	0	0	0	0	0	0
Chemical fertilizer													
Nitrogen	0	1827.2	13923.6	2401.3	2462.1	0	2189.8	1563.8	698.6	0	3047.8	2547.0	14255.6
Phosphorus	0	420.3	2086.4	276	1635.7	0	121.5	234.3	321.3	0	701.1	292.8	790.9
Potassium	0	0	0	0	0	0	0	0	0	0	0	0	0
Manure	0	1537.5	0	0	0	0	0	0	0	0	2564.5	0	0
Seeds	0	1318.8	3933.2	0	826.4	0	0	441.8	242.9	0	2200.3	0	0
Electricity	0	1071.4	0	0	3.8	0	1797.1	0	0.7	0	1787.2	0	11699.3
Diesel	8513.6	6079.6	24062.7	4396.5	5643.9	35899.4	0	2702.6	2049.3	29372.0	10140.8	4663.4	0
Industrial stage ingredient preparation													
Electricity	912.3	615.2	11146	423.4	973.7	0	7514.8	0	101.2	3147.4	562.8	449.1	48921.3
Natural gas	10167.1	10037.5	20207.2	0	0	19308.5	0	0	0	35076.4	4737.8	0	0
Total	46513	92464.7	233859.6	66540.1	85816.8	106008.8	40858.9	22747.4	23928.6	160179.8	141748.2	70665.3	266567

SPC, soy protein concentrate; PPC pea protein concentrate.

3. Results

3.1. Feed ingredients

Table 3 shows the energy necessary to produce the different feed ingredients. No mass allocation is applied, which means that all the energy needed to produce a certain feed ingredient is attributed to that ingredient. It basically assumes that a certain source does not deliver other useful ingredients, making it a kind of worse-case scenario.

In total, the production of 1 ton of FM requires an energy input of 18,874 MJ, of which 43% is attributable to the use of fuel for fishing. On an equivalent mass basis, the production of wheat gluten costs five times more energy than FM, mainly due to the large amounts of wheat required (1 kg of gluten requires 9.2 kg of wheat). Of the protein sources, the energy required for gluten production was the highest, followed by the production of krill meal and SPC (Table 3a). The high overall energy consumption is a direct reflection of the low yield from whole krill (1 kg of krill meal requires 6.9 kg of freshly caught krill). The combustion of natural gas for steam generation is the main contributor in SPC production (35% of total energy use); diesel use on the land is also substantial (21%) (Table 3a). Chemical fertilizers are the major energy users in the case of gluten, SFM, and PPC (38%, 52%, and 54%, respectively); for algae meal, the main energy use is electricity for microalgae processing (51%). Of the all feed ingredients considered here, faba beans had the lowest energy use.

Oil produced by sunflower required the lowest amount of energy followed by soy, fish and algae oil (Table 3a).

Energy consumption for producing feed ingredients using mass allocation is summarized in Table 4. The energy consumption in the production of krill meal was the highest (Table 4). The energy consumption for FM was higher than that for gluten and for SPC, and substantially higher than that for SFM and PPC. Of the protein-rich ingredients, the percentage difference from the base case scenario is highest for gluten and lowest for krill meal (Table 4), due to the fact that wheat is relatively low in protein. Using the mass allocation scenario, energy use for the production of the oils varied from 56.4% to 86.7% of the base case; fish oil had the highest and sunflower oil the lowest value.

Table 4 Energy and classical exergy to produce 1 ton of feed ingredient (mass allocation scenario)

Feed ingredient	Value in ×100 MJ ton^{-1} and % change from the base case			
	Energy	% change	Classical exergy	% change
FM	146.2	–22.5	360.3	–22.5
SPC	127.3	–53.6	350.3	–62.1
Gluten	136.4	–86.2	288.5	–87.7
Sunflower meal	56.9	–53.7	307.9	–53.7
PPC	45.8	–74.5	218.9	–74.5
Krill meal	503.1	–5.2	1004.6	–5.2
Algae meal	128.5	–13.3	354.2	–13.3
Wheat	74.6	–3.0	220.6	–3.0
Dehulled faba beans	52.7	–10.0	215.3	–10.0
Fish oil	146.7	–77.5	360.9	–77.5
Soy oil	69.1	–79.6	289.7	–79.6
Sunflower oil	56.9	–56.4	307.8	–56.4
Algae oil	128.3	–86.7	354.5	–86.7

FM, fish meal; SPC, soy protein concentrate; PPC pea protein concentrate.

The results of the classical exergy analysis for cultivation/fishing and industrial conversion of fish, krill, wheat, oilseeds, legumes, and algae into feed ingredients are presented in Table 3b. The share of chemical exergy of the wet biomass in the total input varied from 48% to 87%; FM and algae meal had the lowest values of all protein-rich ingredients (Table 3b). This was largely due to the high water content of the raw materials for these two protein sources (Appendix, Table A1). To convert 4.7 kg of raw fish into 1 kg of FM with exergy content of 26.9 MJ kg^{-1}, nonrenewable input of 8514 and 10,167 MJ for diesel and natural gas, respectively, and 912 MJ of electricity are required. Furthermore, 1 kg of algae meal requires 6.9 kg of raw algae. When the plant protein sources are compared, the total exergy degradation values are in the same order as the energy use. Gluten has the highest exergy input requirement of all protein sources; 67% of exergy consumed by gluten production originates from the chemical exergy of the wet biomass and 19% from fossil fuels; the rest is mainly due to the use of other nonrenewable chemicals (fertilizers and pesticides). For the protein-rich ingredients, the lowest classical exergy degradation is required for algae meal (Table 3b).

With regard to the four oils, algae oil had the most significant exergy degradation, mainly driven by the low oil yield. Fish oil had the second most significant degradation of exergy and sunflower oil had the lowest (Table 3b).

Table 4 shows the classical exergy loss when mass allocation is applied. A trend similar to that for the use of energy was observed; the values for the protein sources were between 5.2% and 88% lower compared with the base case scenario; and were 56.4–86.7% lower for the oils.

Eco-exergy was used in this study to describe the differences between four types of living organisms: fish, plants, crustaceans, and microalgae. The results calculated for the work energy^{iee} for feed ingredients are shown in Table 5. Given that classical exergy analysis does not distinguish between living and dead organisms, the value of the classical exergy cost for FM was considerably lower relative to other ingredients (Table 3b), whereas this difference is reduced in the case of work energy^{iee} (Table 5).

Table 5 Work energy including eco-exergy degradation to produce 1 ton of feed ingredient (base case and mass allocation scenarios)

Feed ingredient	Value in ×100 GJ ton^{-1}	
	Base case scenario	Mass allocation scenario
FM	134.5	104.2
SPC	187.4	64
Gluten	433.4	48.7
Sunflower meal	159.3	73.7
PPC	202.8	51.7
Krill meal	118.4	112.2
Algae meal	6	5.2
Wheat	48.6	47.2
Dehulled faba beans	53.8	48.5
Fish oil	462.7	104.3
Soy oil	312.4	63.9
Sunflower oil	169.3	73.7
Algae oil	38.9	5.2

FM, fish meal; SPC, soy protein concentrate; PPC pea protein concentrate.

In the base case scenario, FM requires less work energy^{iee} relative to almost all alternatives, except for krill meal and algae meal (Table 5). However, this

is mainly due to the total assignment of all work energy[iee] costs to these ingredients; in reality, the raw materials are refined in a range of ingredients. Thus, using the mass allocation scenario, it becomes clear that FM is more expensive than all alternatives, except krill meal. Algae meal is about 20 times less costly than FM, implying that although plant proteins from wheat, soy, or peas give an immediate and significant improvement, there is scope for even larger savings by using algae.

For production of the oils, the highest work energy[iee] input was observed for fish oil (base case scenario); soybean oil, sunflower oil, and algae oil all needed significantly less (67%, 37% and 8%, respectively) (Table 5).

After co-product allocation, the work energy[iee] cost for the four oils ranged between 518 and 10,426 GJ. Similar to the base case scenario, the highest value was observed for fish oil and the lowest for algae oil.

3.2. Feed formulations

3.2.1. Total energy consumption

In the base case scenario, the total energy consumption is smallest for SPC and the conventional fish-based product (Fig. 2a). All other products cost more energy to prepare. The formulation of the product itself only uses a minor amount of energy; by far the largest energy expenditure is in the preparation of the ingredients. Therefore, as the production of gluten is energy intensive, the SPC×gluten and SFM×gluten products require more energy. SPC accounts for most of the total energy consumption for the SPC and SPC×krill products, whereas soy oil and gluten account for most of the total energy consumption for the PPC product (Fig. 2a). For the product based on algae, algae oil is by far the single most important contributor. Thus, replacing FM with plant-based ingredients actually requires more energy for the production chain. The fish, growing in the sea without any human effort, are caught with little energy expenditure for the fishing itself, whereas all plants need to be cultured, harvested, and processed.

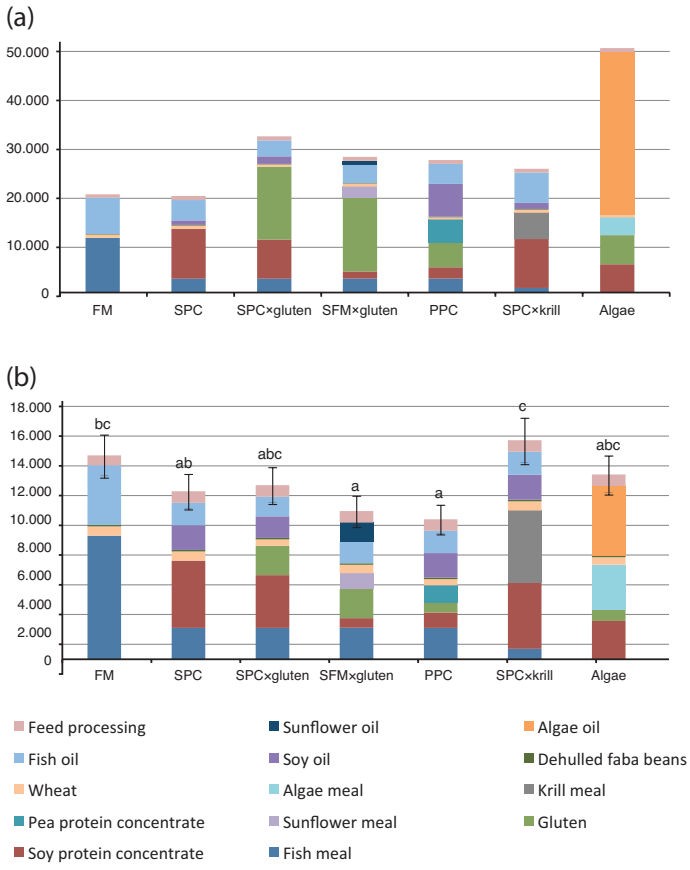


Fig. 2. The influence of feed formulation on total energy consumption (MJ equiv.) of 1 ton of fish feed. (a) Base case scenario; (b) mass allocation scenario. Means with same letter are not significantly different.

The situation is different with the mass allocation scenario (Fig. 2b). Most products now show less total energy consumption than the FM product, with the exception of SPC×krill. There were significant ($P<0.01$) differences in total energy consumption between the feeds. Even though krill meal is used in relatively small quantities (10% inclusion rate), it contributes a disproportionate share of the total energy consumption of the SPC×krill diet (Fig. 2b).

3.2.2. Total classical exergy degradation

Fig. 3a shows that for the base case scenario, the total classical exergy degradation follows the same pattern as the total energy use, the exception being the FM and PPC products. The exergy degradation of FM product was

almost three times lower than the corresponding value for the algae product and 17–46% lower than the other products.

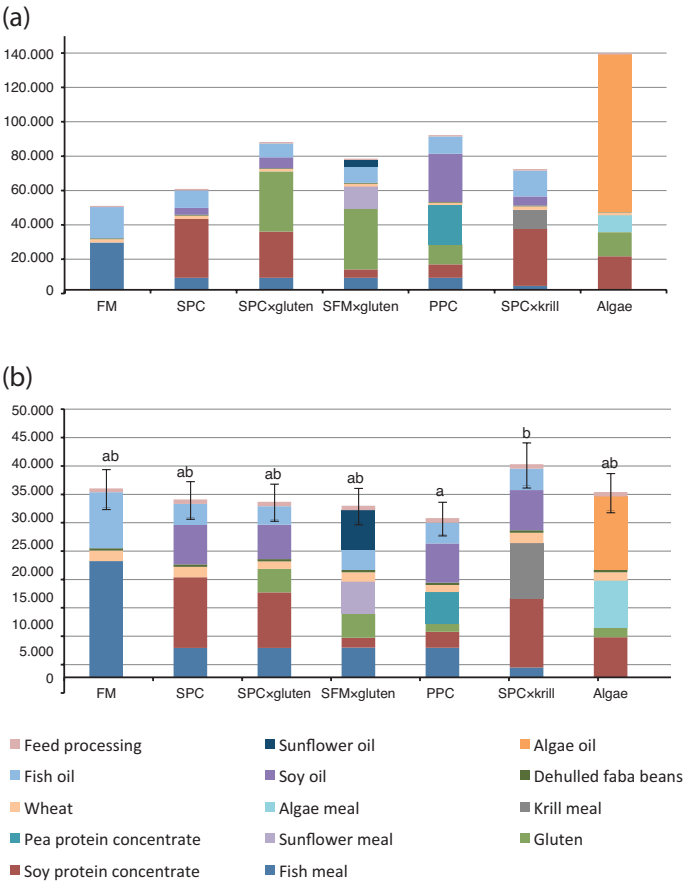


Fig. 3. The influence of feed formulation on the total classical exergy degradation (MJ equiv.) of 1 ton of fish feed. (a) Base case scenario; (b) mass allocation scenario. Means with same letter are not significantly different.

For the mass allocation scenario (Fig, 3b), all products require similar amounts of total exergy degradation, except for the SPC×krill and PPC diets, which showed somewhat higher and lower values, respectively. No significant differences were observed among the different feeds. Generally, co-product allocation by mass greatly reduces the contribution of gluten in all diets that contain this ingredient. For the algae diet, the contribution of algae oil was 27% smaller than in the base case scenario.

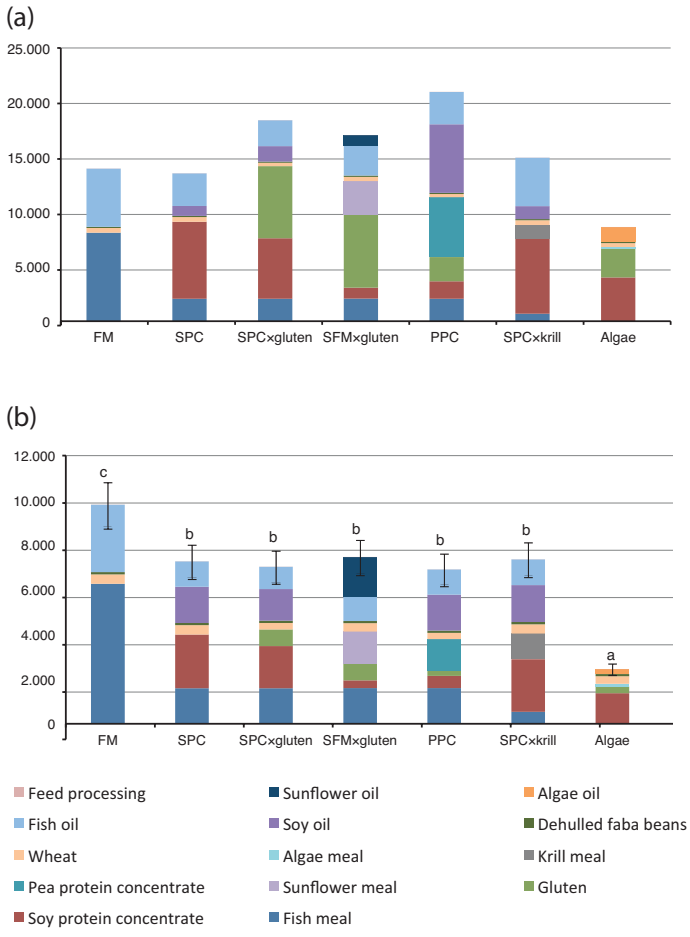


Fig. 4. The influence of feed formulation on the total work energy including eco-exergy degradation (GJ equiv.) of 1 ton of fish feed. (a) Base case scenario; (b) mass allocation scenario. Means with same letter are not significantly different.

3.2.3. Total work energy including eco-exergy degradation

Fig. 4a shows the impact of feed formulation on the total work energy^{iee} degradation for the base case scenario. Using this scenario, the highest work energy^{iee} input is found for the PPC product (20,975 GJ), followed by the SPC×gluten (18,386 GJ) and SFM×gluten (17,026 GJ) diets. Except for SPC product, the total work energy^{iee} values were higher for plant-based products than for conventional FM product, despite the fact that the trophic level of the plant species providing these ingredients is lower than that for fish. Thus, the difference in β value between fish and plants does not compensate for the energy input needed during the production of some plant protein sources

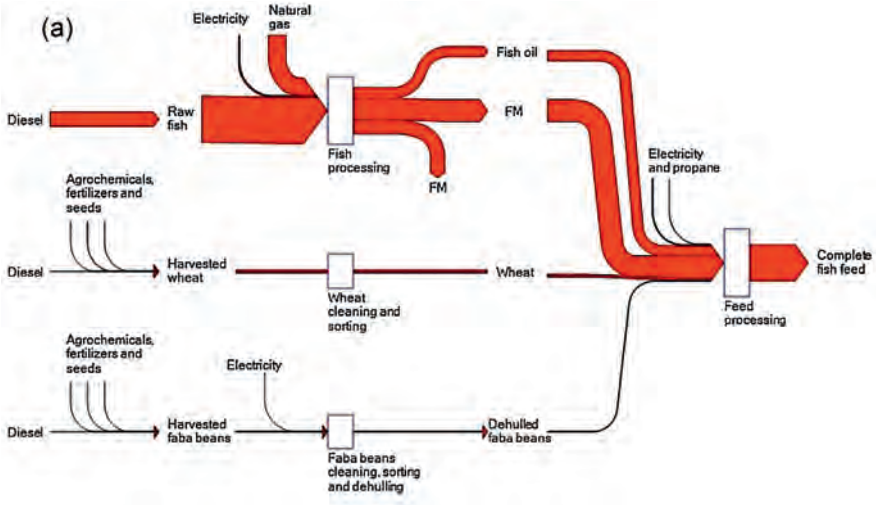
in this scenario. The SPC diet showed slightly lower total work energy^{iee} degradation compared with the FM diet (–3.1%).

Using mass allocation (Fig. 4b), replacement of FM and fish oil with alternative ingredients resulted in decreased (P<0.01) total work energy^{iee} degradation. The FM diet has by far the largest value (9805 GJ); all plant-based products have similar values (from 6905 GJ for the PPC product to 7458 GJ for the SFM×gluten product). The total work energy^{iee} degradation for algae product is much lower (2428 GJ).

For both scenarios here, the work energy needed to process the ingredients into the formulated product was negligible (i.e. <0.04% of the total work energy used).

4. Discussion

The results show that the energy, classical exergy, and work energy^{iee} requirements are strongly dependent on how the costs are ascribed to specific products. To illustrate these effects, Grassmann diagrams for FM and SPC×gluten diets are shown in Fig. 5. In a Grassmann diagram, the exergy flow for the different streams between the process components is shown as an arrow; the width of the arrow is proportional to the size of the exergy flow. Thus, it is easy to identify the components with high exergy levels. From Fig. 5, it is clear that ascribing all the costs to one specific co-product is arbitrary and does not reflect the value of all the co-products produced.



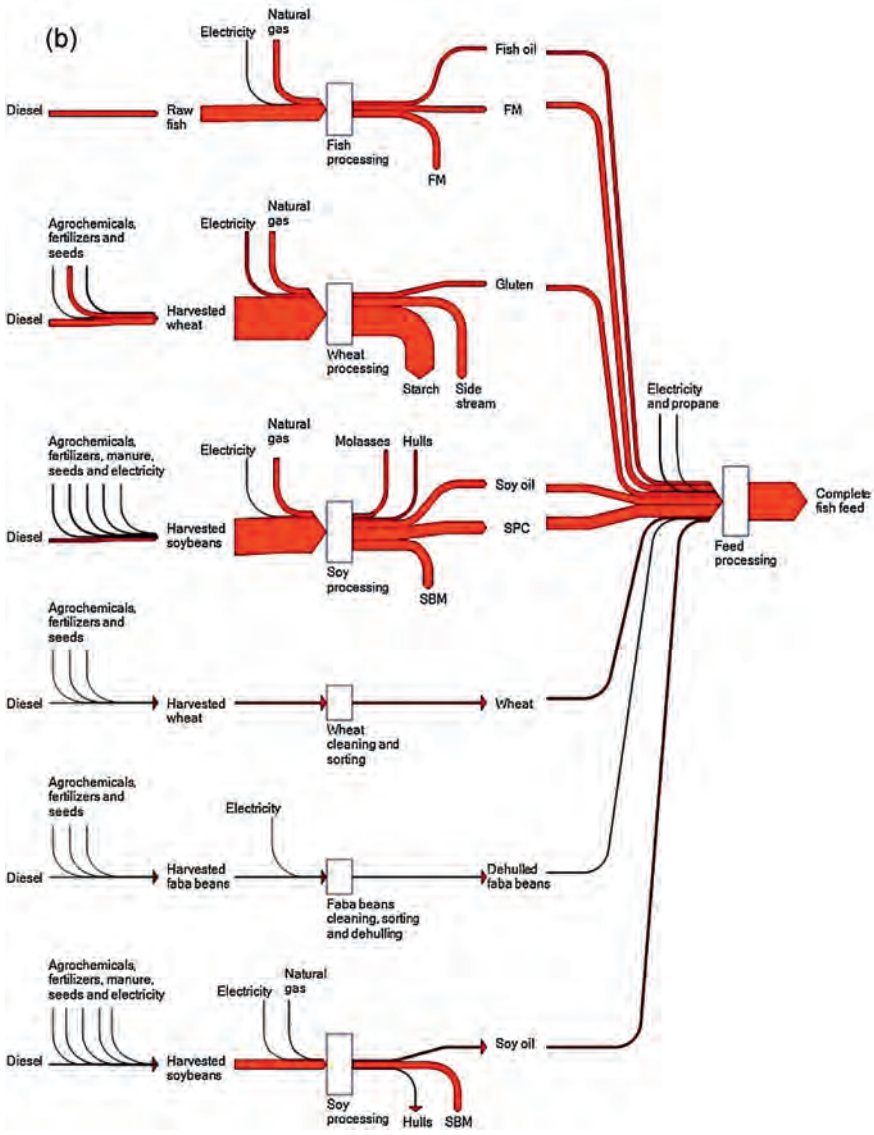


Fig. 5. Grassmann diagrams for FM (a) and the SPC×gluten diet (b). All the streams are expressed as MJ exergy per 1 ton of complete diet. FM, fish meal; SPC, soy protein concentrate; SBM, soybean meal.

4.1. Impact of allocation method on study outcomes

As stated by Ayer et al. (2007), the method of allocation for environmental interventions has a great influence on the results. In our study, we chose mass allocation to take into account co-products that have good value to society (Pelletier and Tyedmers, 2007). In general, the conversion of the starting

materials (from cultivation or fishing) results in a significant number of co-products that can be marketed. For example, soy refining produces hulls, molasses, oil, soybean meal, and SPC. Although criteria such as exergy content and economic value can be chosen, we believe that allocation by mass content provides the simplest and most realistic representation of actual biophysical flows for different feed ingredients (Ayer et al., 2007). Allocation on the basis of economic value is dependent on the economic valuation, which will vary over time and even by location (the value may be different in Norway from that in Asia or in Latin America), and is thus not a stable allocation key. Allocation on exergy content raises the question of how this value relates to the actual value to society. Mass, however, is a simple objective criterion.

The calculations reported in this article include the total energy consumption, classical exergy and work energy[iee] degradation of co-products (i.e. starch, hulls, molasses, gluten, meal, oil, or protein concentrate) of fish, krill, wheat, legumes, oilseeds, and algae. In the next section, the overall effects are discussed and the differences are explained, focusing mainly on the mass allocation scenario.

4.2. Impacts of feed ingredients and formulations

Pelletier and Tyedmers (2007) reported total industrial energy use for conventional feed production of 18,100 MJ ton^{-1}. In the current study, the energy use varies from about 20,000 MJ to about 32,400 MJ, apart from the algae diet (Fig. 2a). A much higher values was reported by Tyedmers (2000). They calculated direct energy use of approximately 43,500 MJ for fossil fuel and electrical energy for 1 ton of feed for conventional salmon aquaculture in British Columbia. The differences can probably be attributed to changes in the chemical composition of feed, origin of the ingredients, and the precise formulation of the product and the processing conditions.

Although the degree of intervention for growth of crops is much higher compared with fisheries (Pelletier and Tyedmers, 2007), the mass allocation scenario showed that it is not necessarily as energy intensive as many fisheries. When composing Table 4, we found that the underlying values for primary production of fish (6340 MJ), crop-derived feed ingredients (6010 MJ in average), and algae (6340 MJ) showed no major differences. Besides primary production, processing of raw materials is important and it strongly depends on the technology required. For example, although no substantial differences exist between wheat and pea cultivation, wet separation of wheat starch and

gluten requires considerably more energy (5950 MJ) than the air classification used in PPC production (250 MJ). Steam cooking and drying of minced fish, aqueous alcohol treatment of soybean meal, and subsequent drying or thermal drying of harvested algae consume significantly more energy than mechanical separation processes. For this reason, the SFM×gluten and PPC diets use the least energy (Fig. 2b). It has been reported previously that wheat gluten is more resource intensive than the most efficient fisheries products (e.g. menhaden meal and oil) (Pelletier et al., 2009), which is in accordance with our study for the base case scenario (Table 3a). However, after co-product allocation, energy consumption for gluten was even lower compared with FM (Table 4). This is due to a significant energy cost for the starch stream. Fishing contributes significantly to the overall energy consumption for krill meal because the greatest abundance of krill is found between the Antarctic continent and the polar front (Parker and Tyedmers, 2012).

The energy required to produce oil depends greatly on the oil source and is lower for plant oils than for fish and algae oils, despite intensive crop production (Table 4). As a result, apart from proteins, this low energy use for plant oils contributes to the overall lower total energy consumption of plant-based diets compared with the FM diet (Fig. 2b).

Opportunities for reducing classical exergy degradation by using plant-derived products or algae are poor (Fig. 3b). Processes with the highest fuel consumption have the lowest exergy efficiency (Sorguven and Ozilgen, 2012). In our study, krill meal, fish oil and FM had the highest fossil fuel consumption (52,300, 14,520 and 14,470 MJ, respectively), followed by SPC and gluten (8800 and 6700 MJ, respectively). The fact that there was no statistically significant difference in total classical exergy degradation between the FM diet and plant-based diets (Fig. 3b) was also a result of similar exergy costs for oils originating from these sources (Table 4). The SPC×krill was the most exergy inefficient diet (Fig. 3b); this can also be ascribed to the intensive use of energy sourced from fossil fuels, in addition to the inefficient conversion of harvested krill to krill meal.

From Table 1, the β value for fish is higher than for plants, crustaceans, and especially algae. This explains why all the diets containing alternatives to fish-based ingredients showed significantly lower total work energy^{iee} use compared with the FM diet when mass allocation was used. The algae diet uses the least total work energy^{iee}, which is mainly due to the low trophic level of the microalgae (Fig. 4b). Among the plant-based diets, the most successful

combination of ingredients was found in the PPC diet although the differences from other plant-based products are minor (Fig. 4b).

The work energy^{iee} results from this study indicate that algae oil is the best substitute for fish oil (Table 5). Besides the environmental factors, it is also important to consider that inclusion of omega-3 is one of the main reasons for including fish oil. Although the inclusion of vegetable oils does not compromise growth (Glencross and Turchini, 2010), tissue composition may be affected (Dosanjh et al., 1998; Greene and Selivonchick, 1990). In general, oils extracted from different algae species are rich in eicasopentanoic and docosahexaenoic acids (Miller et al., 2011) and thus the fatty acid profile of fish flesh may be maintained. Thus, there may be both nutritional and environmental reasons for using algae oil.

4.3. Sustainability of aquaculture as a function of feed and eco-exergy destruction

It has been reported previously that the production of fish feed accounts for most of the energy and material input and the environmental impact of farmed salmon production (Ellingsen and Aanondsen, 2006; Folke, 1988; Tyedmers, 2000). Pelletier and Tyedmers (2007) stated that the use of fish feed is a logical unit of analysis for evaluating the environmental performance of salmon aquaculture. According to Aubin et al. (2009), the increasing production of aquaculture products makes diversification of protein and lipid sources an important challenge. For that reason, the current study provides information on the total work energy^{iee} use of several salmonid feeds. A unique feature of this research is that the different origin of some feed ingredients (e.g. fish and crops) has been identified as a factor in environmental performance.

As already mentioned earlier, the use of feeds considered here would potentially result in the same growth performance of fish, which in turn would not affect the environmental performance of the overall farming system. Thus, the comparison made in this study meets the scope of the investigations.

Cornelissen (1997) stated that an important element for sustainable development is the use of exergy analysis. The same study suggests that exergy losses should be minimized to obtain sustainable development, particularly due to the use of nonrenewable energy forms. Fig. 6 represents the relationship between exergy, sustainability, and environmental impact.

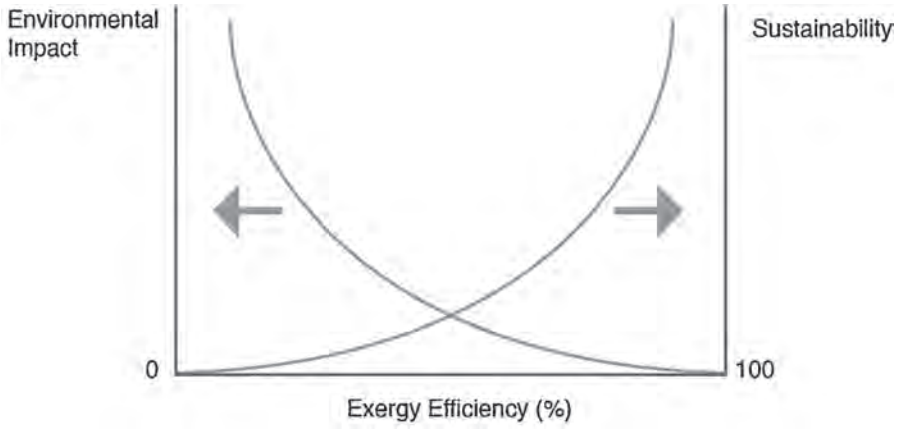


Fig. 6. Qualitative illustration of the relation between the environmental impact and the sustainability of a process, and its exergy efficiency (Rosen and Dincer, 2001).

When the exergy efficiency approaches 100%, the environmental impact approaches zero, whereas the sustainability becomes very high because the process approaches reversibility (i.e., no work energy or gradients are lost). In that context, eco-exergy reflects the quality in terms of information content (or in terms of ecological value) of what we extract from the environment and gives a weight to each species. As fisheries and aquaculture have very strong ecological impact on their (maritime) environment, it is therefore logical to compare the magnitude of the impact and potential lower total work energy^{iee} degradation associated with growing salmon using alternative feeds, originating from different species.

This study shows that (based on mass allocation to take into account the effects of multiproduct processing), the total work energy^{iee} degradation can be notably decreased and thus the sustainability of aquaculture can be increased (Fig. 4b). For the products considered here, even though we could not achieve complete replacement of all FM and fish oil, a significant reduction in total work energy^{iee} degradation can be achieved.

Considering future trends, substitution of FM and fish oil with algae-derived products offers an even further reduction in total work energy^{iee} degradation than plant-based products, and will meet new environmental constraints, particularly regarding the use of finite marine fishery resources.

Based on the results from the present study, feed formulators can and should strive to use the least environmentally costly formulations (and at the least economic cost). Analysis of the total work energy^{iee} is an insightful and

comprehensive method to evaluate environmental costs; it has a sound and objective basis in thermodynamics but also includes the ecological value of each biological resource.

5. Conclusions

This study shows that the environmental performance of salmon aquaculture could be greatly influenced by the nature of the complete feeds used. Within the scope of this research, we have shown that allocation by mass is a preferable scenario because processing of materials from cultivation and fishing results in many valuable co-products that often represent a significant portion of the mass flow.

Calculations based on energy consider only energy conservation based on the First Law of Thermodynamics, whereas calculations based on classical exergy also take the Second Law of Thermodynamics into account, because we distinguish between energy that can do work and energy that is lost as heat to the environment. This means that classical exergy considers energy efficiency in the production of the feed.

The role of information and structure associated with living species is fundamental for the study of complex systems, such as aquafeeds, that are composed of various ingredients originating from different species. Classical exergy does not take into account information content and does not allow us to consider the loss of usefulness of living organisms directly related to structures and information (Susani et al., 2006). From the results, the use of plant-based ingredients and krill meal instead of fish-derived products could significantly decrease total work energy^{iee} degradation. Moreover, complete substitution of FM and oil with algae-derived ingredients offers substantial opportunities to reduce the total work energy^{iee} and thus the environmental costs of salmon feed.

Because the use of total work energy^{iee} as an ecological index considers the complete loss of work energy, including the loss of the genetic information, it provides a useful measure of the impact on nature. Therefore, this approach is recommended for other animal feed types.

Appendix A

The chemical composition of the raw materials is shown in Table A1 and the chemical exergy of the biomass components is shown in Table A2. The specific energy and exergy values of input materials used in this study are listed in Table A3. All the data were taken from the literature. Brehmer (as cited in Ozilgen and Sorguven, 2011) draw attention to the noticeable variation in the cumulative exergy values for pesticide production based on the active ingredients and showed that those values are between 21 and 667 MJ kg^{-1} for the insecticides, 172–564 MJ kg^{-1} for the herbicides, and 38–474 MJ kg^{-1} for the fungicides. In this article, the averages of these ranges are used.

Table A1 Chemical composition of the raw materials.

Raw material	Composition (g kg^{-1} as is)					Reference
	Dry matter	Protein	Fat	Carbohydrate	Ash	
Wheat	850	136.2	24.9	673.6	15.3	McCance et al. (1945)
Faba beans	865	251	18	564	32	Skretting (2010)
Soybeans	877	354	210	267	46	Skretting (2010)
Sunflower	932.6	182.3	361.4	350.1	38.8	Rosa et al. (2009)
Peas	884.2	223.2	11.7	615.8	33.5	Marte et al. (2002)
Fish (Sand eel)	221	154	30	11	26	Norita (2003)
Krill	280	136.9	61.3	52.2	29.6	Sidhu et al. (1970)
Chlorella vulgaris	160	79.59	22.2	47.7	10.48	Tokusoglu and Unal (2003)

Table A2 Chemical exergy of biomass components.

Component	LHV (kJ kg^{-1})	β_{Chem}	Ex_{chem}
Water			530
Protein	22,230	1.10	24,488
Lipids	39,140	1.07	41,954
Carbohydrate polymer	16,340	1.15	18,808
Ash			1006

Table A3 Specific energy and exergy consumption for each input.

Input	Specific energy (MJ kg^{-1})	Reference	β_{Chem} value	Specific exergy (MJ kg^{-1})	Reference
Pesticides					
Insecticides	363.8	Polychronaki (2007)	–	344	Brehmer (as cited in Ozilgen and Sorguven, 2011)
Herbicides	418.2	Polychronaki (2007)	–	368	Brehmer (as cited in Ozilgen and Sorguven, 2011)
Fungicides	276	Unakitan et al. (2010)	–	256	Brehmer (as cited in Ozilgen and Sorguven, 2011)
Fertilizers					
Nitrogenous	78.1	Pimentel (2003)	–	32.7	Szargut et al. (1988)
Phosphorus	17.4	Pimentel (2003)	–	7.52	Wittmuss et al. (1975)
Potassium	13.8	Pimentel (2003)	-	4.6	Pimentel (1991)
Electricity	–	–	1.00	–	Szargut et al. (1988)
Diesel	54	Shapouri (2002)	1.04	56.2	Rosen and Dincer (2003)
Natural gas	55	Patzek (2004)	1.04	57.2	Ayres et al. (2006)

The values for specific energy and exergy of pesticides and fertilizers are reported in the literature as cumulative energy and exergy consumption, respectively.

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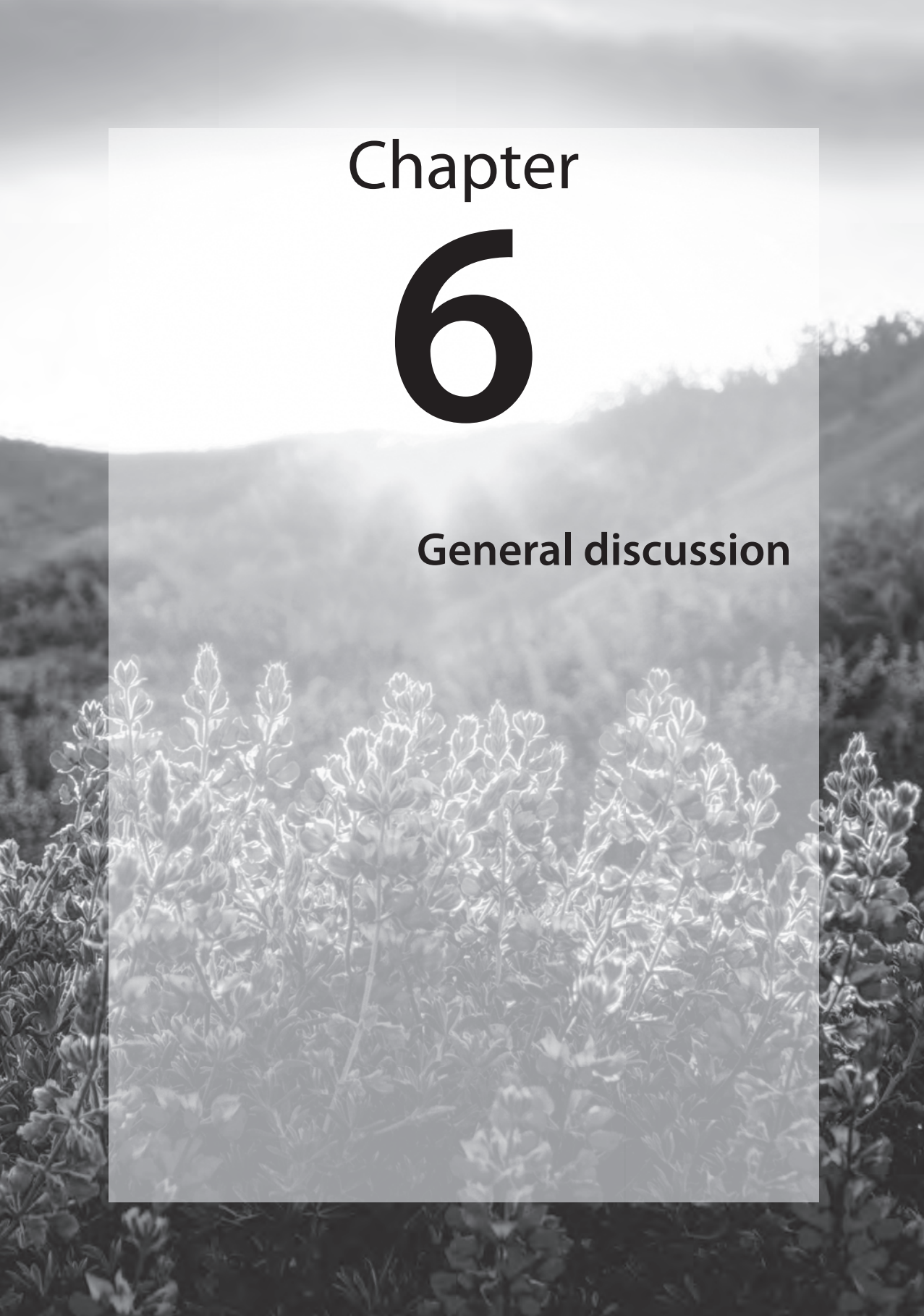

Chapter 6

General discussion

1. Introduction

The aim of this thesis is to explore the technological aspects of fish meal (FM) replacement with alternative ingredients without significant changes to the current process and with the ultimate intention of creating more sustainable fish feed products. The initial perspective was to understand the differences in techno-functional properties between FM and various plant protein sources. In this chapter, the conclusions from the previous chapters are combined into the overall conclusions. The environmental implications of the different methodologies used in sustainability assessment are also discussed. The industrial relevance of the work is analysed by considering reductions in water and energy use in relation to the choice of plant protein source.

2. Development of plant protein-based diets

Plant-based proteins are considered to be promising alternatives for the replacement of FM because of their nutritional value, availability and relatively low cost (up to now). However, the replacement of FM with plant ingredients does not just involve substitution of one ingredient for the other; because these new ingredients have different properties, the properties of the final products also change. Therefore, it is therefore necessary to acquire knowledge about the relationships between these ingredients, the conditions applied during processing and the resulting quality of the end product.

Therefore, we described the use of the two most prominent alternatives to FM, soy protein concentrate (SPC) and wheat gluten (WG) in **Chapter 2**. It was demonstrated that most of the FM can be replaced with SPC provided that additional moisture is used during extrusion. Consequently, more water needs to be removed after extrusion by drying, which implies that using SPC requires much more energy, contrary to our target of a more sustainable fish feed product.

WG did not fulfil the product requirements and cannot be the main protein substitute for FM, but can complement other protein sources. Because WG is a valuable feed ingredient from many different aspects, increased use in the diet would be important. For that reason, **Chapter 3** explores the links between the physical and microstructural characteristics of feeds in relation to the

addition of WG to understand the effect of the use of this ingredient on the morphology of the product in more detail. Most of the changes in the physical properties of the feeds, such as reduced oil impregnation, can be related to changes in the microstructure. In the case of WG, its film-forming properties, which are still effective at the high temperatures used during the extrusion process, negatively influence the pellet properties; the surface is smooth and non-porous, which besides other properties, does not allow good infusion with oil after extrusion and drying.

Due to the limitations in the use of SPC and WG, as described above, other plant protein sources were investigated in **Chapter 4**: lupine protein concentrate (LPC) and canola protein concentrate (CPC). The technological properties of LPC resemble those of FM. Replacing part of SPC with LPC was possible with reduced moisture levels during extrusion while still yielding good properties in the final product. This allows less drying after extrusion, and thus less energy consumption. In contrast, with the addition of CPC, the properties of the product could be maintained although at even higher moisture levels than was necessary with SPC. This means that use of CPC would require even higher energy consumption than SPC.

Chapter 4 introduces the shearing device as a tool to investigate the functional properties of the various protein sources and the behaviour of the full diets. We concluded that this device can be used efficiently to characterize the techno-functional properties of raw materials as a simple and a fast method. In the case of lupine, it was shown that inclusion of this material led to the lowest viscosity of the mash during processing, which explains the potential to work at low moisture levels.

Table 1 presents the relationships between the proteinaceous ingredients, process parameters and product properties. Although not quantitative, this table gives a general impression on how to proceed towards development of plant protein-based feed with optimal pellet quality and higher process efficiency.

Table 1. Influences of protein-rich ingredients on the extruder parameters and the final product properties (+ means that the functionality increases with increasing levels of the ingredient).

Ingredient / Target functionality	FM	SPC	WG	LPC	CPC
Required moisture content	--	++	+	--	++
Extruder SME	--	++	0 No direct influence	--	++
Density	0 No direct influence	-	0 No direct influence	+	-
Structure accesible for oil infusion	++	+	--	+	+
Strength	0 No direct influence	+	++ Compact structure formation	+	+

$^{+}$ Denotes a positive correlation between addition of ingredient and target functionality; $^{++}$ denotes significant correlation.
$^{-}$ Denotes negative correlation between addition of ingredient and target functionality; $^{--}$ denotes significant correlation.

3. Methodology improvement

3.1. Visualization techniques

The results described in **Chapter 3** show that a range of different pellet microstructures can be formed depending on the proteinaceous ingredients used and the overall chemical composition of the feed. In this project, visualization of the macrostructure was done using conventional photography and light microscopy. X-ray microtomography (XMT) was used to obtain a quantitative description of the inner microstructure, which helps in understanding the properties of feed.

Although not reported in this thesis, we also tried to apply this imaging technique to the pellets coated with oil, with the knowledge that the differences in density between the solid matrix, oil and air (void cells) can be detected by X-rays. From Fig. 1 (right-hand picture), it can be seen that some pores are still not yet filled with oil, although both products have the same

density and are coated with the same amount of oil. These results indicate that oil infusion could be studied further with this technique. For example, the effect of different protein sources could be visualized in future research.

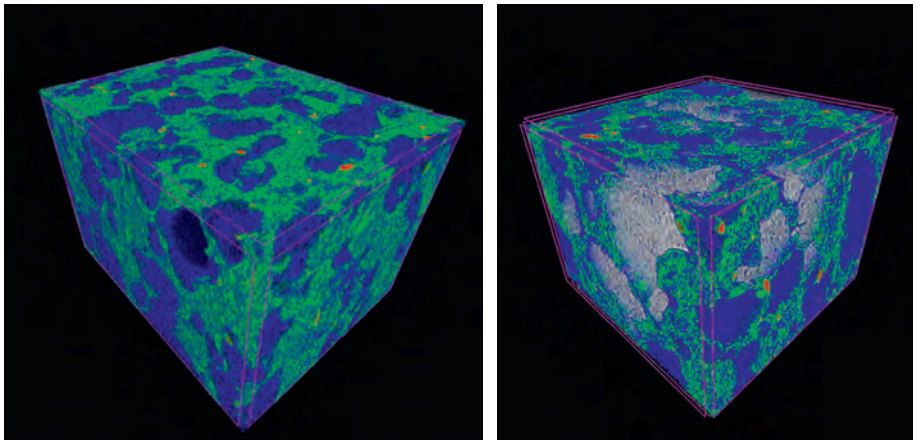

Fig. 1. Three-dimensional models of two coated products obtained with XMT. The objects presented are the volume of interest, not the whole pellet. The blue areas represent oil, white areas are void cells, and the continuous solid matrix is in green or red (dense material).

SEM and cryo-SEM are less suitable imaging techniques for these purposes because of preparation of the sample may alter the structure of the product.

Other techniques are also useful. The interconnectivity of the pore network plays a role in the overall physical properties of feed. Long continuous channels lead to increased fat uptake (Saguy and Pinthus, 1995). Mercury intrusion porosimetry can therefore offer opportunities.

Here, we did not focus on the changes in product durability during storage when using different protein-rich ingredients. To the best of our knowledge, the causes of reduction in durability over time are unexplored. To improve this, better characterization methods are important. XMT might also be a useful tool to gain more insight into oil migration in the pellet over time and its relation to pellet structure and the functional properties of ingredients. These visualization techniques can contribute greatly to further understanding of the relationships between the physical properties of the feeds and the microstructure in addition to the mechanisms involved in structure formation.

3.2. Eco-exergy as sustainability

Although FM is a finite resource, its replacement with a new ingredient does not necessarily imply more sustainable aquaculture as a whole; the use of fish is substituted with the use of plants; both have ecological value. It has been previously reported that replacing FM and fish oil with plant sources did not decrease the environmental impacts (Boissy et al., 2011; Papatryphon et al., 2004). Our study confirms this using traditional thermodynamic analysis for an ecological evaluation of the sustainability of various feed compositions, such as energy and classical exergy. We showed in **Chapter 5** that fishery-product substitution with alternative ingredients in general caused minor changes in these two parameters, although classical exergy considers energy efficiency in the production of the feed. This work establishes that these analyses might not include all relevant aspects when comparing FM with plant-based alternatives. That is why the exergy analysis was extended with the concept of eco-exergy. This concept includes the information carried by living organisms embodied in the genome and used to determine the amino acid sequence in the enzymes, which are controlling the biochemical processes in the living organism (Bendoricchio and Jørgensen, 1997; Jorgensen et al., 1995). It has been shown that it can also quantify the value of ecosystems. In **Chapter 5,** this information has been applied to all components that originate from living organisms because it expresses nature's loss of work when living organisms (microalgae, crustaceans, plants and fish) are used as raw material for the fish feed. After considering the genetic patrimony of the organism, the conclusions were different from those obtained with traditional methods. It became evident that a plant-based protein source has less consequence for the environment than the use of FM. We can therefore say that the thermodynamic analysis based on eco-exergy unambiguously shows that plant-based fish feed is more sustainable that fish feed based on FM and fish oil.

4. Future prospective

Chapter 4 shows that the properties of a plant protein material determine the amount of moisture used during extrusion. Fig. 2 gives an overview of the material and energy flows involved with a production of SPC- and LPC-based diets. The data on the energy consumption during extrusion and drying were

derived from a technical report from a fish feed producer (Skretting, 2009); the mass balance data were obtained from **Chapter 4**.

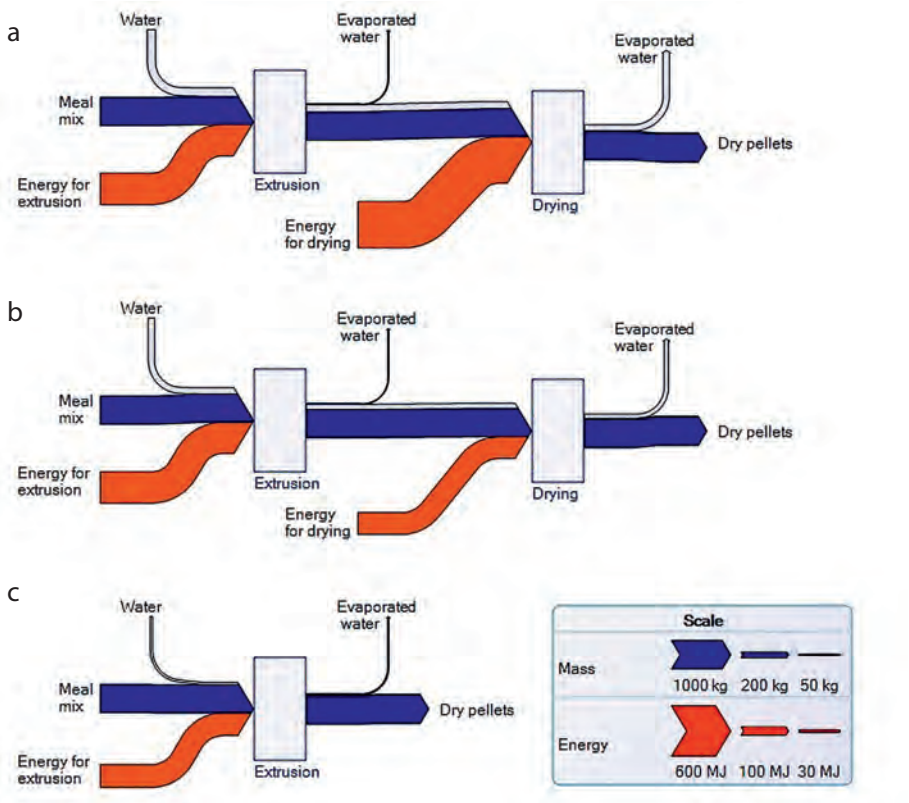

Fig. 2. Production schemes for SPC- (a) and LPC-based (b) diets and one prospective (c) diet. The SPC-based diet contains 300 g SPC kg^{-1}, while LPC-based diet contains 200 g LPC kg^{-1}, partially replacing SPC. The thickness of the arrows represents the size of the stream.

It is interesting to further investigate the property that determines the amount of moisture used. An ideal ingredient would be one with techno-functional properties such that (almost) no drying is necessary. This is indicated schematically in Fig. 2. The desirable properties for such an ingredient during extrusion seem to be low water-holding capacity and low gelling properties. Fig. 2 shows that the conventional process (for an SPC-based diet) requires the addition a substantial amount of water (290 g kg^{-1} meal mix). About 40 g kg^{-1} of the water added evaporates at the discharge of the extruder die, while the rest joins the extrudate stream. The amount of water added to the system in the case of an LPC-based diet is reduced to 220 g kg^{-1} meal mix. As a

consequence, the drying load is reduced by 53% compared with an SPC-based diet. Furthermore, based on the results from **Chapter 4**, it was assumed that the same amount of energy for extrusion is needed for both diets. The differences in the amounts of water required between protein-rich ingredients are such that the third diet has to be extruded at 80 g added water per kg of meal mix, which gives an extrudate that does not need to be dried. Because of the very low anticipated viscosity of this feed, we assumed lower extruded energy consumption (27% change) compared with the other two feeds. Although the LPC-based diet gives an immediate improvement, moving towards such a prospective ingredient may lead to significant reduction of energy use and even better process efficiency.

In general, the mash needs to be plastic inside the extruder (i.e. at 80– 130 °C), becoming glassy by the loss of moisture and reduction in temperature after expansion. The best mash thus needs careful tuning of the glass transition between these two conditions, which can be done by adding more or less water. A mash that by itself has a low glass transition will need less water to reduce the glass transition. Components (such as SPC or CPC) that have a strong interaction with moisture may remove that moisture from the other materials, and hence induce a higher glass transition than would be present without these components. The ideal component in Fig. 2c would be a component with a low intrinsic glass transition temperature and a relatively low interaction with moisture (thus leaving the moisture available for other components). This low interaction will be evident from a low water-holding capacity. Here we postulate that the quest for even more sustainable components should be aimed at identifying components with both a low glass transition temperature and a low water-holding capacity.

The environmental impact of large-scale drying operations has become a critical issue in recent years as a result of the consumption of fossil fuels and the carbon footprint (Jangam, 2011). The results from **Chapter 5** show that plant-based diets are more sustainable than diets based on fishery-derived products, even with the higher drying requirements necessary. Although drying was minimally involved in overall sustainability, good use of the material streams is of the greatest importance. The developments presented in Fig. 2 are needed for the following reasons:

- to improve capacity (dryers are often bottlenecks in a production line)
- to control of the process better
- to reduce fire risks, explosions; safer operation
- to minimize the capital costs and maintenance costs; horizontal belt dryers are standard in the fish feed industry and in principle they occupy large spaces in the factory
- to improve overall efficiency (cost optimization)
- to provide higher nutrient recovery (e.g. colours and vitamins) and digestibility
- to reduce odour emissions to the environment.

In addition, hot air dryers often have several other limitations, such as non-uniform product quality due to overdrying or underdrying caused by long, inadequate or non-uniform exposure of the product to the drying medium. Long drying times are the result of poor contact between the drying medium and the extrudates being dried (Jangam, 2011). In general, potential improvements in all these areas are possible by proper selection of plant proteins as described in this thesis.

In conclusion, any aspect that contributes to sustainability, including reduced drying, is of extreme importance. Alternative candidates to FM should have two essential characteristics: (1) be of plant origin and (2) capable of meeting the requirements for an energy efficient process. **Chapter 5** showed that after the next generation of fish feed based on plant ingredients, there is further potential in moving towards algae-derived products. Although FM can be replaced from both the nutritional and technological points of view, fish oil remains a challenge. As oils from algae are rich in polyunsaturated fatty acids, future research should focus on this oil source. Overall, algae-derived products seem to be the most credible alternative to respond to the growing aquaculture feed sector and to meet new environmental constraints.

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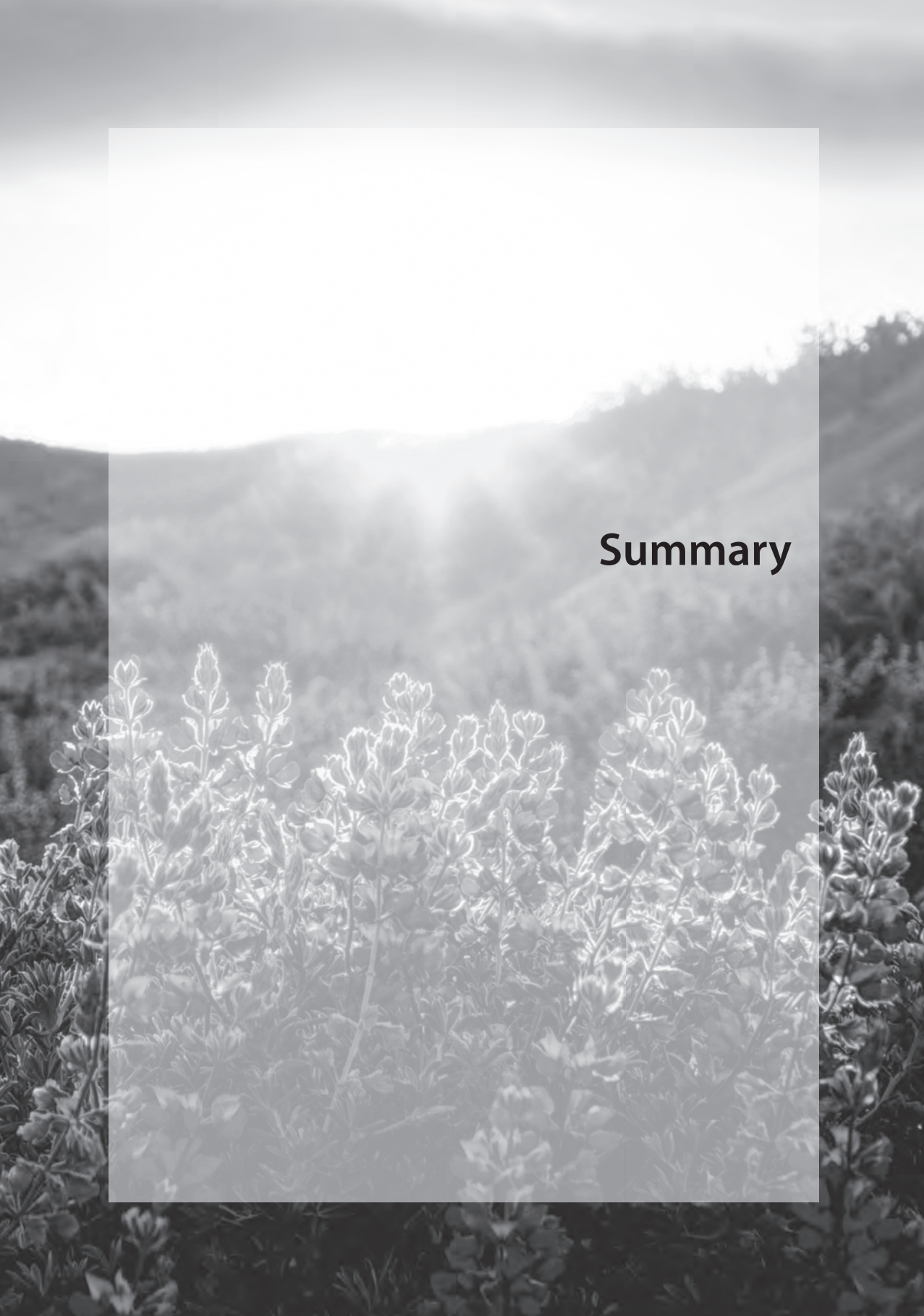

Summary

The consumption of fish and fish-related products is increasing. Due to improved welfare and suggested health benefits, consumers are now eating more fish. In 2008, global fisheries supplied the world with about 142 million tons of fish, of which 115 million tons was used as human food, which is an all-time high (FAO, 2010. The State of World Fisheries and Aquaculture. FAO, Rome, Italy). Fish for consumption can be harvested directly from the wild (capture fisheries), but a growing proportion of the fish comes from worldwide aquaculture (FAO, 2010). As a result, there is an increased need for feed for this growing industry.

Salmon is one species for which the whole life cycle can be controlled in aquaculture systems. By inclusion of ingredients derived from fish (i.e. fish protein, often called fish meal (FM) and fish oil), diets for farmed salmon aim to reflect the natural diet of wild salmon, which consists of smaller fish among others. This explains why FM has been the most important protein source in commercial feeds for finfish historically. Some of the diets available today may contain up to 50% FM. The oil content in grower diets for carnivorous fish may vary from 15% to 40%. Of the macro-ingredients, the rest of the diet is typically composed of plant proteins and starch sources. Starch is added for technological reasons; it provides binding properties and expands the pellet matrix.

However, FM and fish oil are finite resources and alternative ingredients are needed. This requires supplementation with feed additives to provide any essential micro-nutrients still required for complete nutrition.

Fish feed pellets are produced using the following steps: various ingredients are ground and mixed together; the material then is processed through a pre-conditioner and an extruder with the addition of steam and water; the resulting extrudates are dried to obtain the desired consistency and storage properties; oil is then rapidly infused into hot, dry pellets during the coating process before the finished product is cooled.

Any change in composition is not trivial because fish feed pellets need to fulfil strict technological requirements. When feeding to salmon, the pellet should sink slowly. In addition, the porosity of the pellet directly after extrusion should be such that it can capture sufficient oil. The pellets should not break or produce dust during transport and further handling. This makes the production of feed pellets a delicate process in which the techno-functional properties of the raw materials play an important role.

It is not a simple matter to conclude that plant proteins are more sustainable per se if all the renewable and nonrenewable resources and waste emissions related to the production of these ingredients are taken into account. For example, the use of plant-based ingredients might require additional water during extrusion leading to increased drying costs.

This thesis describes the relationships between the techno-functional properties of protein-rich ingredients and processing. Criteria have been developed for the use of plant-based materials in existing fish feed processes to allow the production of feed pellets that meet all the product requirements. Feed sustainability is assessed using three different methodologies and sustainable feed compositions are proposed.

Chapter 2 describes how the extrusion process, moisture requirements and final product quality were affected by replacement of FM with soy protein concentrate (SPC) and vital wheat gluten (WG). Considering future perspectives, the overall goal was to (almost) fully replace FM. Therefore, up to 40% of the FM in the complete formulation was replaced by plant proteins. Addition of plant proteins resulted in higher strength of the products, but, when more product properties were considered, we found that the operational window became very small. With soy and gluten, it was not possible to make a fish feed pellet that fulfilled all the requirements, indicating that the techno-functional properties of FM are unique. Generally, FM-based diets can be processed with very low added moisture, mainly due to the low water holding capacity (WHC) of this ingredient in addition to fat present in FM. In contrast, to fulfil product requirements, most of the FM can be replaced with SPC with high added moisture. WG, however, showed limitations as the main alternative to FM, but it could complement other plant proteins.

To understand why WG was not a good alternative for FM, we studied the effect of adding WG on the physical and functional properties of feed pellets in **Chapter 3**. Partial replacement of SPC with WG resulted in reduced oil impregnation and insufficient oil uptake during coating, even though the pellets were sufficiently strong and had the desired density. Scanning electron microscopy showed that addition of WG resulted in a smooth, non-porous surface. X-ray microtomography revealed that pellets without gluten had a more fragmented structure with a larger number of small cells. Most likely, the higher fibre content originating from the plant cell wall material present in SPC is responsible for this observation. Spherical shaped cells were favoured in the

presence of WG, suggesting the presence of closed pores. Interconnectivity between pores decreased with the addition of WG. These effects may be caused by the film-forming properties of WG, i.e. it forms a highly extensible network that stretches into thin film walls around the growing gas cells at discharge of the extruder die.

Chapter 4 presents the effects of partial replacement of SPC with two new protein sources for fish feed, lupine protein concentrate (LPC) and canola protein concentrate (CPC), on the extrusion process, moisture requirements and final product quality. In addition, the value of using the shear cell device was explored to help in understanding the techno-functional properties of raw materials in a simple and fast way. This apparatus was found to be efficient for characterizing the properties of raw material and their correlation with process conditions. Both shearing and extrusion showed that LPC closely resembles FM. Addition of LPC led to a reduction in moisture added to the process and decreased drying requirements, while yielding even stronger pellets compared with FM. In contrast, the properties of CPC and SPC were similar, becoming plasticized at high moisture content. Remarkably, acceptable product properties of feeds containing CPC required even higher added moisture than SPC. The effects may be related to differences in the WHC of the ingredients and resulting changes in the paste viscosity of the blends.

Chapter 5 compares the impact of alternative ingredients to FM and fish oil on the environment using three ecologic indicators: total energy consumption, classical exergy (work energy) and work energy including eco-exergy degradation. Replacement of fishery-derived products with alternatives reduced the values of the first two indicators only to a minor extent. However, both energy and classical exergy analysis ignore the fact that living organisms carry a lot of information, which is why the latter analysis was extended with the concept of eco-exergy. Eco-exergy includes the information carried by living organisms embodied in the genome and it gives a weight to each species. Human beings have the highest weighting factor (2149), whereas algae have one of the lowest (20). Fish have a determined weighting factor of 499, and plants have a factor of 275. As a result, eco-exergy analysis showed that removing fish from nature has a significantly higher impact on the environment than removing plants, crustaceans or algae.

The results and methodologies applied are discussed in **Chapter 6.** An overview of the techno-functional properties of all the protein-rich ingredients

examined is presented. This is followed by a description of the methodologies used in this thesis, with emphasis on the development of (1) the imaging techniques for characterization of pellet structure and physical properties; and (2) a new methodology for sustainability assessment of feed. The guidelines for desirable properties of plant protein developed in **Chapter 4** are used to evaluate the impact on reduction of energy use and better process efficiency.

Future trends in the development of sustainable feeds are described based on the insights obtained from this thesis. These include the use of plant proteins that contribute to a more intensified process with the ultimate goal of no drying and future exploration of the use of algae-derived products with special focus on the replacement of fish oil with algae oil.

Samenvatting

De consumptie van vis en aan vis gerelateerde producten neemt toe. Door de toegenomen welvaart en veronderstelde voordelen voor de gezondheid, eten consumenten nu meer vis. In 2008 voorzag de visserij wereldwijd in ongeveer 142 miljoen ton vis, waarvan 115 miljoen ton als menselijk voedsel werd gebruikt, een record ("The State of World Fisheries and Aquaculture" ["De staat van de Visserij en de Aquacultuur in de wereld"]. 2010, FAO, Rome, Italië). Vis voor consumptie kan rechtstreeks in de wilde natuur worden gevangen (vangstvisserij), maar steeds meer vis wordt verkregen uit wereldwijde aquacultuur (FAO, 2010). Hierdoor is er een verhoogde behoefte aan voeder voor deze groeiende industrie.

Zalm is een soort waarvan de gehele levenscyclus in aquacultuursystemen kan worden gecontroleerd. Door van vis afkomstige bestanddelen op te nemen (dat wil zeggen viseiwit, vaak vismeel genoemd, en visolie), wordt gepoogd om de voeding voor gekweekte zalm een afspiegeling te laten zijn van het natuurlijke voedingspatroon van wilde zalm, dat uit onder meer kleinere vis bestaat. Dit verklaart waarom vismeel traditioneel de belangrijkste eiwitbron in commercieel voeder voor vinvis is geweest. Sommige huidige visvoeders bevatten nog steeds tot 50% vismeel. Het aandeel olie in kweekvoeding voor vleesetende vis kan variëren van 15% tot 40%. Wat de macrobestanddelen betreft is de overige voeding meestal samengesteld uit plantaardige eiwitten en zetmeelbronnen. Zetmeel wordt toegevoegd om technische redenen; het versterkt bindende eigenschappen en maakt uitzetting van de korrel mogelijk.

Vismeel en visolie zijn echter eindige hulpbronnen en dus zijn alternatieve bestanddelen nodig. Vervanging van deze grondstoffen vereist aanvulling met andere ingrediënten in het diervoeder, teneinde te voorzien in de essentiële micronutriënten die nog nodig zijn voor een volledige voeding.

De productie van visvoerkorrels bestaat uit drie stappen. In de eerste stap worden diverse ingrediënten worden samen gemalen en gemengd. Daarop wordt het mengsel door een preconditioner en een extruder verwerkt, waarbij stoom en water worden toegevoegd; de resulterende mengsels worden gedroogd om de gewenste consistentie en opslageigenschappen te verkrijgen. Vervolgens worden de droge hete korrels tijdens het coatingproces snel in de olie gedrenkt, alvorens koeling van het afgewerkte product plaatsvindt.

Het veranderen van de samenstelling is niet triviaal, aangezien visvoerkorrels aan strikte technische eisen moeten voldoen. Bij het voeren van zalm moeten de korrels langzaam dalen. Bovendien dient de porositeit van de korrels

onmiddellijk na extrusie dusdanig te zijn dat ze voldoende olie kunnen opnemen. De korrels mogen niet breken of stof ontwikkelen tijdens het vervoer en verdere hantering. De productie van korrels is dus een gevoelig proces waarbij de technisch-functionele eigenschappen van de grondstoffen een belangrijke rol spelen.

De conclusie dat plantaardige eiwitten hoe dan ook duurzamer zijn is niet eenvoudig te trekken, indien rekening wordt gehouden met alle hernieuwbare en niet-hernieuwbare hulpbronnen en afvaluitstoot die verband houden met de productie van deze bestanddelen. Zo kan het gebruik van plantaardige bestanddelen het nodig maken dat er extra water tijdens de extrusie wordt gebruikt, waardoor het drogen meer kosten met zich meebrengt.

In dit proefschrift wordt het verband tussen technisch-functionele eigenschappen van eiwitrijke ingrediënten en de verwerking beschreven. Er zijn criteria ontwikkeld voor het gebruik van plantaardige materialen in de bestaande proces van visvoeder om de productie van korrels die aan alle productvereisten voldoen mogelijk te maken. De duurzaamheid van het voer wordt beoordeeld aan de hand van drie verschillende methodologieën en er worden duurzame voedersamenstellingen voorgesteld.

In **Hoofdstuk 2** wordt beschreven hoe het extrusieproces, het benodigde vochtgehalte en de definitieve productkwaliteit werden beïnvloed door de vervanging van vismeel door soja-eiwitconcentraat en vitaal tarwegluten. Gezien het toekomstperspectief was het doel om vismeel (bijna) volledig te vervangen. Daartoe werd tot 40% vismeel in de volledige formulering vervangen door plantaardige eiwitten. De toevoeging van plantaardige eiwitten leidde tot een grotere sterkte van de producten, de operationele mogelijkheden zeer gering werden als andere producteisen werden meegenomen. Met soja en gluten was het niet mogelijk visvoerkorrels te maken die aan alle eisen voldeden, wat erop wijst dat de technisch-functionele eigenschappen van vismeel uniek zijn. Over het algemeen kan op vismeel gebaseerde voeding met een zeer lage toegevoegde vocht worden verwerkt, voornamelijk vanwege de lage waterhoudende capaciteit van dit bestanddeel, naast het in vismeel aanwezige vet. Daarentegen kan, om aan de producteisen te voldoen, het meeste vismeel worden vervangen door soja-eiwitconcentraat met een hoge toegevoegde vochtigheid. Als voornaamste alternatief voor vismeel vertoonde vitaal tarwegluten echter beperkingen. Wel zou het andere plantaardige eiwitbronnen kunnen aanvullen.

Om te begrijpen waarom tarwegluten geen goed alternatief voor vismeel is, bestudeerden wij in **Hoofdstuk 3** het effect van de toevoeging van dit tarwegluten op de fysische en functionele eigenschappen van voerkorrels. De gedeeltelijke vervanging van soja-eiwitconcentraat door tarwegluten leidde tot verminderde olie-impregnatie en ontoereikende olieopname tijdens de coating, hoewel de korrels sterk genoeg waren en de gewenste dichtheid bezaten. Scans met een elektronenmicroscoop toonden aan dat de toevoeging van tarwegluten leidde tot een gladde, niet-poreuze oppervlakte. Microtomografie met röntgenstralen liet zien dat korrels zonder gluten een meer gefragmenteerde structuur met een groter aantal kleine cellen hadden. Waarschijnlijkst is het hogere gehalte aan ruwe celwandmateriaal, in het soja-eiwitconcentraat, de oorzaak van deze structuur. Na toevoegen van tarwegluten werden meer ronde cellen gevormd, wat de aanwezigheid van gesloten poriën verklaart. De interconnectiviteit tussen poriën nam af met de hoeveelheid toegevoegd tarwegluten. Deze effecten kunnen door de filmvormende eigenschappen van vitaal tarwegluten worden veroorzaakt: het vormt een zeer rekbaar netwerk dat zich uitstrekt tot dunne filmlagen rond de groeiende gascellen bij het verlaten van de extruderspuitkop.

In **Hoofdstuk 4** worden de gevolgen uiteengezet van gedeeltelijke vervanging van soja-eiwitconcentraat door twee nieuwe eiwitbronnen voor visvoeder – lupine-eiwitconcentraat en raapzaad-eiwitconcentraat – op de extrusie, het benodigde vochtgehalte en de definitieve productkwaliteit. Daarnaast werd de waarde van het gebruik van het shear cell-apparaat onderzocht, om tot een beter begrip te komen van de technisch-functionele eigenschappen van grondstoffen. Dit bleek een efficiënt apparaat te zijn om een beeld te krijgen van de eigenschappen van grondstoffen en hun correlatie met verwerkingsomstandigheden. Zowel shearing als extrusie toonde aan dat lupine-eiwitconcentraat sterk op vismeel lijkt. De toevoeging van lupine-eiwitconcentraat maakte het mogelijk om minder vocht te gebruiken tijdens extrusie, en dus minder droging, terwijl de voortgebrachte korrels zelfs sterker waren dan de met vismeel verkregen korrels. Daarentegen waren de eigenschappen van raapdzaad-eiwitconcentraat en soja-eiwitconcentraat vergelijkbaar: zij verweekten bij hoge vochgehalte. Voor aanvaardbare producteigenschappen van voeder met raapzaad-eiwitconcentraat was zelfs meer vocht vereist dan soja-eiwitconcentraat. De effecten kunnen te maken hebben met verschillen in waterhoudend vermogen van de bestanddelen en

hieruit voortvloeiende veranderingen in de viscositeit van de mengsels.

In **Hoofdstuk 5** wordt het effect op het milieu van alternatieve bestanddelen voor vismeel en visolie vergeleken, waarbij gebruik wordt gemaakt van drie ecologische indicatoren: totaal energieverbruik, klassieke exergie en exergie met inbegrip van degradatie van eco-exergie. De vervanging van visserijproducten door alternatieven verminderde de waarden van de eerste twee indicatoren slechts in geringe mate. Zowel de energieanalyse als de klassieke exergieanalyse gaat echter voorbij aan het feit dat levende organismen grote informatiedragers zijn, wat de reden is dat de laatstgenoemde analyse met het begrip eco-exergie werd uitgebreid. In eco-exergie zit door levende organismen gedragen informatie, belichaamd in het genoom, en vertaald in een wegingsfactor. Menselijke wezens hebben de hoogste wegingsfactor (2149), terwijl algen één van de laagste hebben (20). Vissen hebben een vastgestelde wegingsfactor van 499, en planten een factor van 275. Dientengevolge toonde de eco-exergieanalyse aan dat het onttrekken van vis aan de natuur een beduidend sterker effect heeft op het milieu dan het verwijderen van planten, schaaldieren of algen.

De toegepaste resultaten en methodologieën worden besproken in **Hoofdstuk 6.** Er wordt een overzicht gegeven van de technisch-functionele eigenschappen van alle onderzochte eiwitrijke bestanddelen. Hierop volgt een beschrijving van de in deze thesis gevolgde methodologieën, met nadruk op de ontwikkeling van (1) de beeldvormingstechnieken voor de karakterisering van de korrelstructuur en de fysische eigenschappen; en (2) een nieuwe methodologie voor de beoordeling op duurzaamheid van voeder. De in **Hoofdstuk 4** uiteengezette richtsnoeren voor wenselijke eigenschappen van plantaardig eiwit worden gebruikt om het effect te evalueren op de vermindering van energieverbruik en een betere verwerkingsefficiency.

Toekomstige ontwikkelingen van duurzaam voeder worden beschreven op basis van de in dit proefschrift verkregen inzichten. Deze omvatten het gebruik van plantaardige eiwitten die tot een intensievere verwerking leiden. Naast het vermijden van drogen na extrusie zullen in de toekomst mogelijkheden verkend worden van het gebruik van ingrediënten uit algen, met speciale aandacht voor de vervanging van visolie door algenolie.

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Curriculum Vitae

Vukasin Draganovic was born in Novi Sad, Serbia, on the June 12, 1980. He went to school in Novi Sad and received his high school diploma in 1999. He then began his studies in Agricultural Engineering at the University of Novi Sad. His BSc thesis was performed at the Department of Phytomedicine focusing on the biological control of stored-product pests. He did an internship at the R&D Centre at Uniroyal Chemical, Evesham, UK. In September 2004, he left Serbia to pursue a Masters degree in Feed Manufacturing Technology at the University of Life Sciences in Ås, Norway. He accomplished his MSc in 2006 with a thesis exploring the thermal properties of soy protein concentrates. Since 2007, he has been working for Skretting ARC as industrial researcher and research project leader. His research interests include fractionation of agri-feedstock into functional fractions, extrusion processing and microstructural characteristics of feed. In 2009, he started his industrial PhD project entitled "Towards sustainable fish feed production using novel protein sources" at the Food Process Engineering Department at Wageningen University, The Netherlands. Being an industry-oriented doctoral research fellowship, the project was funded by The Research Council of Norway. The results of this research are described in this thesis.

Publication list

Peer reviewed journals

Draganovic, V., Boom, R., Jonkers, J., van der Goot, A.J., 2013. Lupine and canola protein concentrate in fish feed: a comparative assessment of the techno-functional properties using a shear cell device and an extruder. Submitted for publication.

Draganovic, V., Jørgensen, S.E., Boom, R., Jonkers, J., Riesen, G., van der Goot, A.J., 2013. Sustainability assessment of salmonid feed using energy, classical exergy and eco-exergy analysis. Ecol. Indicators 34, 277–289.

Draganovic, V., van der Goot, A.J., Boom, R., Jonkers, J., 2013. Wheat gluten in extruded fish feed: effects on morphology and on physical and functional properties. Aquacult. Nutr. (in press).

Draganovic, V., van der Goot, A.J., Boom, R., Jonkers, J., 2011. Assessment of the effects of fish meal, wheat gluten, soy protein concentrate and feed moisture on extruder system parameters and the technical quality of fish feed. Animal Feed Sci. Technol. 165, 238–250.

Conference proceedings

Draganovic, V., van der Goot, A.J., Boom, R., Jonkers, J., 2012. Towards sustainable fish feed production using novel protein sources. 6th Central European Congress on Food. May 23-26, Novi Sad, Serbia.

Draganovic, V., van der Goot, A.J., Boom, R., Jonkers, J., 2012. Towards sustainable fish feed production using novel protein sources. 6th International Symposium on Food Rheology and Structure. April 10-13, Zurich, Switzerland.

Overview of Completed Training Activities

Discipline specific activities

Courses

Food structure and rheology (VLAG, 2012)
Sustainability analysis in food production (VLAG, 2011)
Process economics and cost engineering (OSPT, 2010)
Workshop on fractional separation of peas (Agrimarin, Norway, 2009)
Course in texture analysis (Stable Micro Systems, Norway, 2009)
Extrusion workshop (Clextral, France, 2009)
Mathcad training (Engineering Data Recourses, Norway, 2008)
Multivariate data analysis - Level 1 (CAMO, Norway, 2008)
Multivariate analysis of spectroscopic data (CAMO, Denmark, 2008)

Conferences

The 6th Central European Congress on Food (Cefood, Serbia, 2012) (oral presentation)
The 6th International Symposium on Food Rheology and Structure (ISFRS, Switzerland, 2012) (poster)
International Congress on Engineering and Food (IAEF & ICEF, Greece, 2011)
Aquatic Feed Processing Seminar (Wenger Co., USA, 2010)
Extrusion in the Future Seminar (Graintec Co., Denmark, 2010)
The 5th International Symposium on Food Rheology and Structure (ISFRS, Switzerland, 2009)

General courses

Course in Lync communication tool (Skretting ARC, 2012)
Nutreco project management course (Norway, 2012)
Industrial PhD seminars (three modules, The Research Council of Norway, Norway, 2010-2012)
Writing patent application (Skretting ARC, 2011)
Course in presentation and communication (Vivaldi, Norway, 2010)
Nutreco intercultural management course (Norway, 2009)
Project management course (Skretting ARC, 2008)

Optional courses and activities

Formulators Meeting (Skretting ARC, Germany, 2012)
Technical Network Meeting (Skretting ARC, Germany, 2011)
Formulators Meeting (Skretting ARC, Norway, 2011)
PhD study tour Food Process Engineering Group (USA, 2010)
Formulators Meeting (Skretting ARC, Norway, 2010)
Technical Network Meeting (Skretting ARC, Denmark, 2010)
Technical Network Meeting (Skretting ARC, Canada, 2010)
Formulators Meeting (Skretting ARC, Norway, 2009)
Technical Network Meeting (Skretting ARC, France, 2009)
Preparation of the PhD project proposal (2008-2009)