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Protein Powder for Human Consumption

With Focus on Herring Roe, Salmon and
Whey Proteins

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Chemical Engineering and Biotechnology
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Preface

This Master`s thesis concludes my degree, Master of Science, in Chemical Engineering and Biotechnology at the Norwegian University of Science and Technology (NTNU) in Trondheim. The work was carried out in the spring 2017 at the Department of Biotechnology and Food Science, with Professor Turid Rustad as a supervisor and Associate Professor Grete Hansen Aas from NTNU Ålesund as a co-supervisor. This thesis is a continuation of the specialization project carried out in the fall 2016.

I would like to thank my supervisors, Turid Rustad and Grete Hansen Aas for good guidance and feedback. You have always been available for questions and the discussions we have had about my results have been very helpful.

I would also like to thank Siri Stavrum and Trude Johansen for helping me finding chemicals and laboratory equipment. I have really appreciated your good mood and your guidance in the lab.

At last, I will thank my family and friends, who have always supported me. The five years at NTNU have been hard work, but I am very grateful for getting to know so many wonderful people. The time we have had together have been great. A special thanks to Magnhild Seim Grøvlen, the many hours spent in the lab the last year have been so much more fun with you around, and to Tina Olaussen for proofreading my thesis.

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Marit Selbekk Liaklev

Summary

The fish industry in Norway produced 885 000 tonnes of rest raw material in 2014. Today, most of the rest raw material from fish, such as herring (*Clupea harengus*) and Atlantic salmon (*Salmo salar*), is used in silage or fishmeal and -oil production for animal and fish feed. With a growing world population, it is important that the fish material is used as sustainably as possible. Transforming rest raw material from the fish industry into products for human consumption will increase the profitability of the industry. Protein powders made from herring and salmon can be used as functional ingredients in food or as a nutritional supplement.

The aim of this study was to compare the nutritional, functional, bioactive and sensory properties of protein powders from herring roe, salmon and whey. The chemical composition varied between the different raw materials, and the protein content ranged from 64.0 to 81.5 %. The herring protein powder (HPP) and whey protein powder (WPP) had the highest nutritional values due to a high content of proteins and essential amino acids, which exceeded the requirements set by FAO/WHO for an adult human. A pepsin/HCl solution was used to simulate the digestion of proteins in the stomach. The digestibility of the HPP and WPP were above 90 %, while the digestibility of salmon meal was approximately 72 %.

The molecular weights of the soluble proteins in the WPP and salmon meal were investigated with gel filtration. The WPP consisted mainly of peptide fractions with molecular weights of 4700 and 13 000 Da, while the salmon meal consisted of peptide fractions below 900 Da. This correlated well with the higher amount of free amino acids (FAA) measured for salmon meal dissolved in distilled water and citric acid-phosphate buffer, compared to the whey protein powder dissolved in the same solvents.

From earlier studies, the solubility of the herring protein powder was reported to be below 2 % in distilled water. Enzymatic hydrolysis with trypsin was performed and the effect on different characteristics and properties were investigated. The minor change in molecular weight distribution, low amount of FAA and acid soluble peptides, together with low a solubility (less than 7 %), indicate that the herring protein powder hydrolysate mainly consisted of insoluble intact proteins similar to the native HPP. This makes it difficult to use the powder in liquid products, such as sports drinks or drinking yoghurts.

The solubility of the salmon meal and WPP were also investigated, in addition to other functional properties such as emulsion capacity, emulsion stability, water holding capacity (WHC) and swelling capacity. The solubility was determined both as a function of pH and temperature. At a pH between pH 3 and 7, the solubility of the WPP and salmon meal ranged from 65.7 to 95.9 % and 14.2 to 18.1 %, respectively. The effect from increasing the temperature on the solubility of HPP and salmon meal was minimal. Both the emulsion capacity and stability were higher for the WPP compared to the salmon meal. Although the salmon meal was able to form an emulsion, it was not able to make it stabile, and this would make it difficult to use the salmon meal as an emulsifier in multiple phase foods. WHC was the functional property where salmon meal showed significantly better properties than WPP. The WHC of minced cod filets increased with addition of salmon meal, while it decreased with addition of WPP. A positive correlation was observed between a high water holding capacity and a high swelling capacity.

The antioxidant properties of whey protein powder was investigated with the DPPH scavenging method. The scavenging of the DPPH radicals increased with increasing WPP concentration. The DPPH method was not suitable for measuring the antioxidant properties of salmon meal and no results were obtained for the bioactive properties of this powder.

Bread rolls were made from mixtures of wheat flour, wholemeal and 0, 5, 10, 15 and 20 % of WPP, HPP or salmon meal. The bread rolls mixed with WPP received the best acceptance. The low solubility of the HPP resulted in a grainy mouthfeel at high protein concentrations. A fishy taste and odour, probably due to the high fat content (17.3 %) were the major problems with the salmon meal bread rolls. Protein addition above 10 % is probably necessary in order to increase the protein intake from bread rolls significantly.

Among the three protein powders investigated in this study, WPP has most likely the best potential as a food additive due to the high nutritional value and sensory acceptance. If the sensory properties of HPP could be improved by modifications of the proteins or taste masking, this powder could also be used in enrichment products. This would be desirable due to a high nutritional quality of the herring roe proteins.

Sammendrag

I 2014 produserte fiskeindustrien i Norge 885 000 tonn marint restråstoff. I dag blir mesteparten av restråstoffet fra fisk som sild (*Clupea harengus*) og Atlantisk laks (*Salmo salar*) brukt til produksjon av ensilasje eller fiskemel og -olje, som igjen brukes i dyre- og fiskefôr. Verdens befolkning øker stadig og det er viktig at de marine råvarene brukes på en bærekraftig måte. Profitten til fiskeindustrien vil øke hvis restråstoffet omdannes til produkter for humant konsum. Proteinpulver basert på sild og laks kan bli brukt som funksjonelle ingredienser i mat eller som kosttilskudd.

Målet med denne oppgaven var å sammenligne de ernæringsmessige, funksjonelle, bioaktive og sensoriske egenskapene til proteinpulver produsert fra silderogn, laks og myse. Den kjemiske sammensetningen var varierte mellom de ulike råmaterialene, og proteininnholdet varierte fra 64,0 til 81,5 %. På grunn av et høyt innhold av proteiner og essensielle aminosyrer, hadde sildeproteinpulveret (HPP) og myseproteinpulveret (WPP) den høyeste ernæringsverdien. Innholdet av essensielle aminosyrer var høyere enn verdiene anbefalt av FAO/WHO for et voksent menneske. For å etterligne fordøyelsen av proteiner i magesekken ble det brukt en pepsin/HCl-løsning. Fordøyeligheten til HPP og WPP var begge over 90 %, mens fordøyeligheten til laksemelet var rundt 72 %.

Molekylvektsfordelingen til de løselige proteinene i WPP og laksemelet ble bestemt med gelfiltrering. WPP bestod hovedsakelig av peptidfraksjoner med molekylvekter rundt 4700 og 13 000 Da, mens laksemelet bestod av peptidfraksjoner med molekylvekt lavere enn 900 Da. Dette stemte godt overens med det høyere innholdet av frie aminosyrer (FAA) målt for laksemelet løst i destillert vann og sitronsyrefosfatbuffer, sammenlignet med myseproteinpulveret løst i de samme løsemidlene.

Tidligere studier har vist at sildeproteinpulveret har en løselighet lavere enn 2 % i destillert vann. Enzymatisk hydrolyse med trypsin ble utført og effekten på ulike karakteristika og egenskaper ble undersøkt. Endringen i molekylvekstfordeling var liten, løseligheten til proteinene var mindre enn 7 % og innholdet av FAA og syreløselige peptider var lavt. Dette tyder på at hydrolysatene fra sildeproteinpulveret hovedsakelig bestod av uløselige og intakte proteiner på samme måte som det opprinnelige HPP. Dette gjør det vanskelig å bruke pulveret i flytende produkter, som sportsdrikker eller drikkeyoghurter.

Løseligheten til laksemelet og WPP ble også undersøkt. Det samme ble andre funksjonelle egenskaper som emulsjonsevne, emulsjonsstabilitet, vannbindingsevne (WHC) og svelleevne. Løseligheten ble undersøkt både som en funksjon av pH og temperatur. Mellom pH 3 og 7 varierte løseligheten til WPP og laksemelet fra 65,7 til 95,9 % og 14,2 og 18,1 %, respektivt. Effekten av å øke temperaturen var liten på både løseligheten til HPP og laksemelet. Både emulsjonsevnen og emulsjonsstabiliteten var høyere for WPP sammenlignet med laksemelet. Selv om laksemelet var i stand til å danne en emulsjon, var stabiliteten lav, noe som gjør det vanskelig å benytte laksemelet som en emulgator i mat med både polare og upolare komponenter. WHC var den funksjonelle egenskapen hvor laksemelet viste vesentlige bedre egenskaper sammenlignet med myseproteinet. Laksemelet gjorde at WHCen til kvernet torskefileter økte, mens myseproteinet gjorde at WHCen minket. Det ble observert en positiv korrelasjon mellom en høy vannbindingsevne og høy svelleevne.

De antioksidative egenskapene til myseproteinet ble undersøkt spektrofotometrisk, hvor inhiberingen av frie DPPH-radikaler ble målt. Inhiberingen av radikalene økte med økende konsentrasjon av WPP. Da denne metoden ikke var egnet for å måle DPPH-inhibering av laksemelet, var det ikke mulig å si noe om de antioksidative egenskapene til dette pulveret.

Rundstykker ble laget ved å blande hvetemel, sammalt hvete og 0, 5, 10, 15 og 20 % av WPP, HPP og laksemel. Rundstykkene bakt med myseprotein ble best mottatt. Ved høye konsentrasjoner av HPP, resulterte den lave løseligheten av HPP i en kornete følelse i munnen. Både smak og lukt av fisk ble rapportert av deltakerne som evaluerte rundstykkene bakt med laksemel. Dette var mest sannsynlig på grunn av det høye fettinnholdet (17,3 %) i laksemelet. Det er rimelig å anta at det er nødvendig å tilsette mer enn 10 % protein for at proteininntaket økes vesentlig ved å spise rundstykker tilsatt ekstra protein.

Blant proteinpulverne som ble undersøkt i denne studien er det myseproteinpulveret som har det største potensialet på grunn av den høye ernæringsverdien og samtidig gode sensoriske egenskaper. Hvis de sensoriske egenskapene til HPP kan forbedres, enten ved å modifisere proteinene elle skjule smaken med tilsats av andre komponenter, kan også dette pulveret brukes i proteinberikede produkter. Dette hadde vært ønskelig da den ernæringsmessige kvaliteten til silderognproteinene også var høye.

List of Abbreviations

Abbreviation	Stands for
ACE	Angiotensin-converting enzyme
AFSP	Arrowtooth flounder soluble protein
BSA	Bovine serum albumin
DH	Degree of hydrolysis
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	Dry weight
EAA	Essential amino acids
EC	Emulsion capacity
ES	Emulsion stability
E/S	Enzyme to substrate
FAA	Free amino acids
FAO	Food and Agriculture Organization of the United Nations
FDPH	Freeze dried protein hydrolysate
FPH	Fish protein hydrolysate
FPLC	Fast protein liquid chromatography
FPC	Fish protein concentrate
FPP	Fish protein powder
HBP	Herring body protein powder
HPLC	High pressure liquid chromatography
HPP	Herring protein powder
IMP	Inosine monophosphate
MSG	Monosodium glutamate
NSSH	Norwegian spring spawning herring
PPC	Protein powder concentration
PRT	Progressive resistant training
RAS	Renin-angiotensin system
RCT	Randomized controlled trials
ROS	Reactive oxygen species

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Abbreviation	Stands for
SD	Standard deviation
SSA	Sulphosalicyclic acid
TAA	Total amino acids
TCA	Trichloroacetic acid
TEAA	Total essential amino acids
TFPF	Tilapia fish protein flour
WHC	Water holding capacity
WHO	World Health Organization
WPC	Whey protein concentrate
WPP	Whey protein powder

Table of Content

1 Introduction

1.1 Marine Rest Raw Material

The world's population is growing fast and more than 9 billion people need to be fed by 2050. Fisheries and aquaculture will play an important role in securing this. In 2014 the global total catch and the marine capture production in Norway, was 93.4 and 2.3 million tonnes, respectively (FAO, 2016). Since the global catch cannot be expected to grow, it is important that the fish material is used as sustainably as possible including material that is considered as by-products. There is no single definition of what marine by-products or rest raw material consists of, but it usually includes heads, viscera, bone, skin and cut-offs (Rustad, 2003). Products and rest raw materials from the fish industry can be divided into four major groups; fish material used in fertilization, for animal feed, for human food, and for speciality products (Gildberg, 2002).

Different estimates for the amount of rest raw material are available. Some have estimated that discards from seafood processing can account for approximately 75 % of the total weight of the catch (Shahidi, 1994, Pastoriza et al., 2004), while others have estimated that the annual discard from the worlds fisheries constitutes 25 % of the catch (Rustad, 2007). However, most commonly it is estimated that up to 50 % of the fish is discarded during seafood processing (Guérard et al., 2005). The amount of by-products in fish depends on several factors such as size, species, season and fishing area (Falch et al., 2006). In Norway, 885 000 tonnes of rest raw material was produced in 2014 where 70 % was utilized (Richardsen et al., 2015). An overview of how the rest raw material was used in Norway in 2014 is given in Figure 1. Almost 50 % of the rest raw material from 2014 was used for silage production. The other main products were fishmeal and -oil products. Only 14 % was used for human consumption in one way or another (Richardsen et al., 2015). Transforming the rest raw material into products for human consumption will increase the profitability of the industry (Arason et al., 2009).

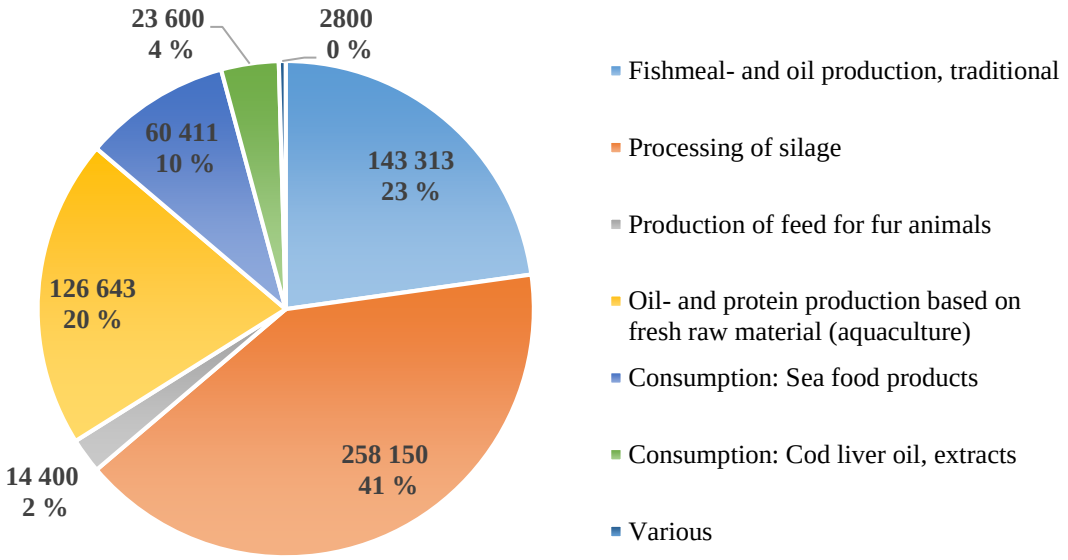

Figure 1. The product types produced from rest raw material in Norway in 2014. The values are given as both rest raw material weight (tonnes) and percentage (%). The values used in this illustration are collected from Richardsen et al. (2015).

1.2 Herring and Salmon as a Rest Raw Material

The top 10 fish species contributing to the marine capture production include 5 small pelagic fish species (FAO, 2016). The Norwegian Pelagic Industry has a low profitability, but a better utilization of the raw material would increase the income (Larssen et al., 2014). Most of the rest raw material of the pelagic sector comes from herring (*Clupea harengus*), mackerel (*Scomber scombrus*) and capelin (*Mallotus villosus*) (Richardsen et al., 2015). Mackerel is usually sold as round fish, while there is an increasing trend of fileting the herring before export, and this generates an increasing amount of rest raw material (Østvik et al., 2008). In 2014, 70 % of the Norwegian herring landings were filleted, which resulted in approximately 162 000 tonnes of rest raw material mainly processed to fishmeal and -oil. Other pelagic fishes, such as Norway pout (*Trisopterus esmarkii*) and sandeel (*Hyperoplus lanceolatus*), are used in the fishmeal and -oil industry where 100 % of the raw material is used (Richardsen et al., 2015).

The Norwegian production of farmed Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) reached 1.37 million tonnes in 2014. From that, 370 600 ton of rest raw material was generated and almost 90 % of the rest raw material was exploited. Viscera and trimmings are the major constituents of this material (Richardsen et al., 2015).

The amount of by-products used for human consumption varies between the different fisheries. In 2011, approximately 1.3 % of herring rest raw material was used for human consumption. The amount of salmon rest raw material used for production of protein isolates and hydrolysates are only approximately 5 %. Today most of the material is still used for the production of silage (~50 %) or oil (~25 %) (RUBIN, 2012). Silage can be processed into products such as oil and protein concentrate and used as feed ingredients for monogastric animals and farmed fish (Šližytė et al., 2016a). Herring roe is a rest raw material with a great potential and represents, depending on the season, between 5 and 20 % of the total raw material. Roe from Norwegian spring spawning herring (NSSH) (*Clupea harengus L.*) and Atlantic herring (*Clupea harengus*) have a high content of proteins, essential amino acids, ω-3 fatty acids and vitamins. The protein concentration varies through the season and increases with maturation (Larssen et al., 2014).

1.3 Production of Protein Powders

Proteins recovered from fish rest raw material can be used to produce fish protein powders (FPP). Several processing methods are used for developing FPP and some of them are described in this section.

1.3.1 pH-Shift Method

The pH-shift method was patented by Hultin and Kelleher (Hultin and Kelleher, 1999, Hultin and Kelleher, 2000). This method is especially useful for raw materials such as fatty, pelagic fish species and deboned muscle tissue since these materials have earlier experienced loss of protein functionality during processing (Hultin and Kelleher, 1999). The principle of this method is that muscle proteins in water are solubilized, either in acid or alkali, followed by centrifugation. The pH of the supernatant with the soluble proteins is then adjusted to the protein`s isoelectric point so they precipitate (Marmon and Undeland, 2010, Hultin and Kelleher, 1999). Whole proteins with retained functionality have been isolated from herring light muscle in a study by Undeland et al. (2002).

1.3.2 Solvent Extraction Method

In the solvent extraction method, chemical solvents are used to remove fat, water and fishy-tasting components from the raw material. The most commonly used solvents are ethanol and propanol (WINDSOR, 2001b). Solvent extraction is often the choice for fatty fishes such as

herring because the lipids are effectively separated from the proteins (Kristinsson and Rasco, 2000b). Fish protein concentrate (FPC) produced by solvent extraction (Type A FPC) gives a product with a high biological value. The advantage with this method is a colorless and odorless product with a low lipid content (<1 %), while the disadvantage is loss of functional properties. Type A FPC has very low solubility and poor emulsification properties (Kristinsson and Rasco, 2000b).

Arctic Nutrition, which is a Norwegian Biotechnology company, produces a protein powder from herring roe with ethanol extraction. The flow sheet in Figure 2 gives an overview of the production process for the protein powder Romega® HCP Virgin.

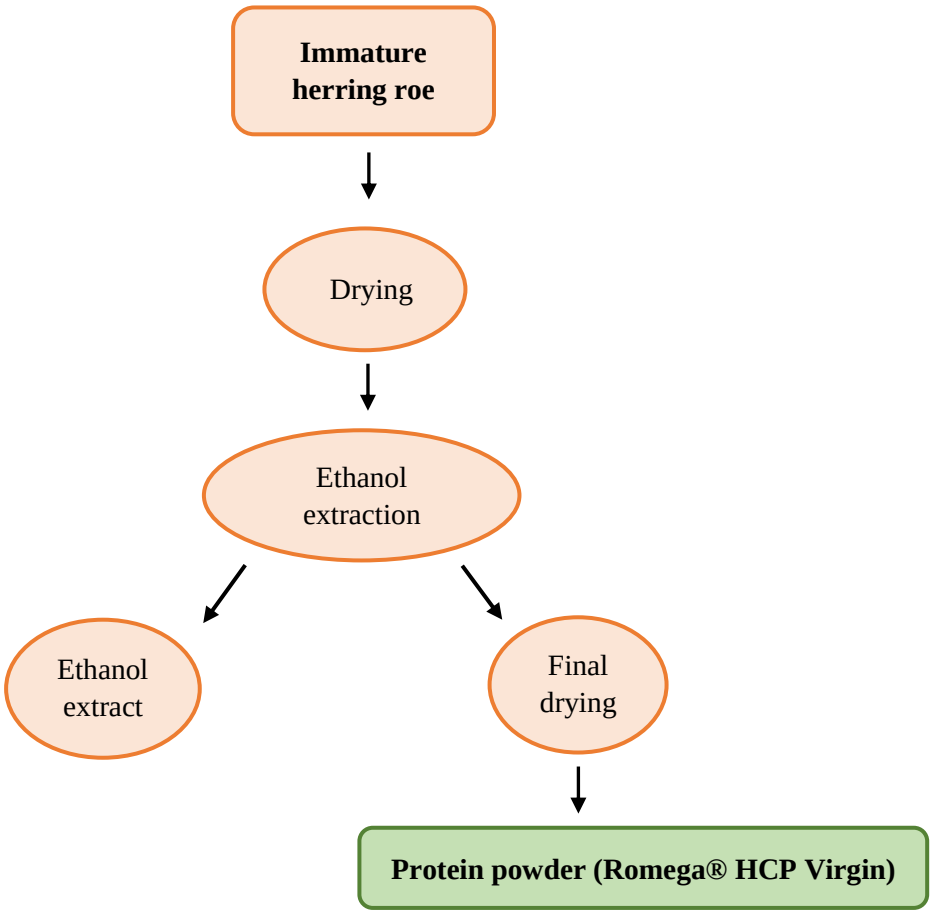

Figure 2. Overview of production method for Romega® HCP Virgin. The immature herring roe goes through an initial drying stage before the ethanol extraction. The ethanol extract is separated from the protein fraction which goes through another drying stage before final product is reached. (Adapted from material provided by Arctic Nutrition).

1.3.3 Production of Fish Protein Hydrolysates

Hydrolysis is the reaction in which chemical bonds in a molecule, such as a peptide bond in a protein, are split by addition of water (Nelson et al., 2013). Figure 3 shows hydrolysis of a dipeptide giving two amino acids.

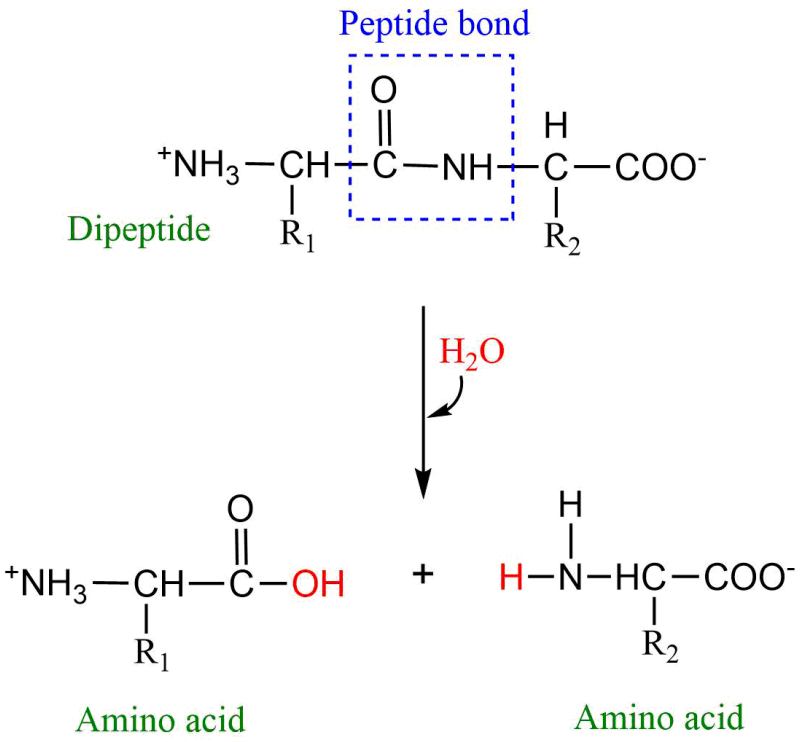

Figure 3. Hydrolysis of a dipeptide giving two amino acids. One water molecule is consumed in the process.

Hydrolysis of rest raw materials can be performed either chemically or enzymatically. Chemical hydrolysis of peptide bonds involves an acid or a base to catalyse the reaction, while enzymatic hydrolysis uses enzymes as catalysts. Enzymatic hydrolysis is the preferred method as it results in products with higher functionality and nutritive value compared to a chemical hydrolysis, which operates at extreme temperatures and pH (Kristinsson and Rasco, 2000b).

Enzymatic hydrolysis can be performed with enzymes already present in the fish, called autolytic hydrolysis or by addition of commercial enzymes (Kristinsson and Rasco, 2000b). Enzymes used to catalyze the hydrolytic cleavage of peptide bonds are called proteases (Nelson et al., 2013). Such enzymes can either cleave the peptide bond from the N-terminus or the

C-terminus (exoproteinases), or within the protein molecules (endoproteinases). Some proteases will only cleave the peptide bond when it is adjacent to particular amino acids. The digestive enzyme trypsin, which is a serine protease, catalyzes only the hydrolysis of peptide bonds on the C-terminal side of Arg and Lys residues (Nelson et al., 2013, Sigma-Aldrich). Some commercial enzymes used to produce fish protein hydrolysates (FPH) are Alacase (Liceaga-Gesualdo and Li-Chan, 1999), Flavourzyme (Kristinsson and Rasco, 2000a), Papain, Bromelain and Protamex (Šližytė et al., 2016a).

Since enzymes have different specificity to the substrate, the peptides formed during hydrolysis and their functionality would depend on the enzyme (Kristinsson and Rasco, 2000a). In addition, the choice of substrate and the degree of hydrolysis (DH) is also of great importance for the properties of a hydrolysate (Mullally et al., 1995). The DH indicates the percentage of peptide bonds cleaved in the protein (Adler-Nissen, 1979).

1.4 Production of Fish Meal

Fish meal is a protein product not intended for human consumption, since it is not normally produced under sufficient hygienic conditions, which is required to remove contamination of disease-causing bacteria. Fish meal produced under sufficiently hygienic conditions, is called FPC C and usually contains at least 65 % protein (Geirsdóttir, 2005, WINDSOR, 2001b).

Most fish types can be used to produce fish meal, but species with a high fat content are more profitable (WINDSOR, 2001a). Both the type of raw material used and the production process affect the composition of the final product (Jensen and Keller, 1990). The production steps of fish meal usually include cooking, pressing, drying and grinding of the fish raw material. Antioxidants, such as ethoxyquin, are often added to oily meals to prevent the oil to react with the oxygen in the atmosphere and hence damage the meal quality (WINDSOR, 2001a).

Vital Seafood AS produces fish meals based on by-products (trimmings and guts) from the slaughterhouse and processing of Norwegian farmed salmon at Marine Harvest. Figure 4 gives an overview of the production process. The quality requirements are fulfilled if the salmon meal contains approximately 8 % water, more than 66 % protein, less than 14 % fat and a digestibility of more than 85 % (mink).

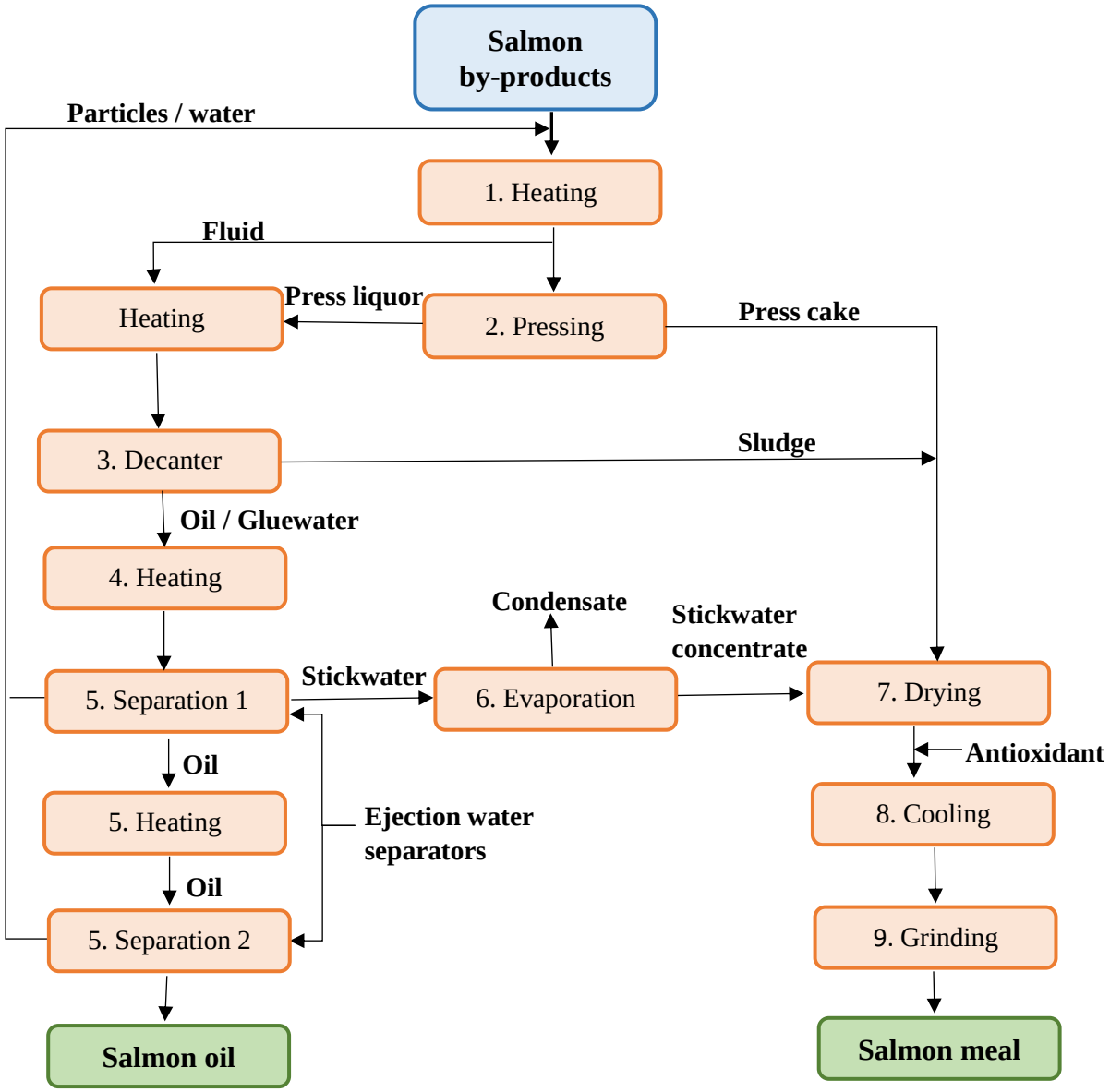

Figure 4. Overview of the production process of salmon oil and meal. ***Step 1***: Raw materials are heated (min. 85 °C > 25 min) to release the fat in the fish and prevent microbial growth. ***Step 2:*** The fish material is lead into a press which removes most of the remaining liquid. This stage separates the material into two fractions: press liquor and the press cake. The press cake is transported to the drying process. ***Step 3:*** The press liquor is separated in a decanter and the dry matter (sludge) is mixed with the press cake. ***Step 4:*** The decanter fluid is pumped into a glue water separator to extract more fat. ***Step 5:*** The oil continues through two oil separation steps to obtain less than 0.15 % protein in the final oil product. ***Step 6:*** Stickwater (remaining fluid after separation) is concentrated and pumped into the drying process for the solids. ***Step 7:*** Drying of press cake, sludge and stickwater concentrate as gently as possible. ***Step 8:*** The raw fish meal is cooled down to approximately 25 °C. ***Step 9:*** The meal is grinded in a mill to break down any lumps or bigger particles. (Adapted from material provided by Marine Harvest).

1.5 Properties of Proteins

Several requirements must be fulfilled for a food protein. It must be nutritionally adequate, be digestible, have appropriate functional properties, be nontoxic, have high availability and be sustainable (Ustunol, 2014a). This section will describe the functional, bioactive and nutritional properties of proteins with focus on marine and whey proteins. Whey proteins are one of the two major groups of proteins found in milk and constitute approximately 20 % of the total protein content (Coultate, 2016). In the production of cheese, whey proteins are the ones remaining soluble after lowering the pH to around 4.6. At this pH, the casein proteins precipitate and can be processed further into cheese. Whey proteins were originally considered a by-product from cheese production, but have several applications today due to their good functional properties (Farkye and Shah, 2014).

1.5.1 Functional Properties

The functional properties of food proteins have been defined as "those functional and chemical properties which affect the behaviour of proteins in food systems during processing, storage, preparation and consumption" (Kinsella and Melachouris, 1976). Some of the functional properties of proteins in food applications are solubility, gelation, water-binding capacity and emulsifying properties. These properties are influenced by protein source, environmental and processing parameters (Thorkelsson et al., 2009).

One of the most important functional properties of proteins is the solubility, which affects properties such as emulsification and foaming. The molecular weight of the proteins also influences the solubility, together with ionic strength and hydrophobic interactions (Wilding et al., 1984). Hydrophobic interactions promote protein-protein interactions and have a negative effect on the solubility, while ionic interactions promote water-protein interactions, which has a positive effect on the solubility (Kristinsson and Rasco, 2000b). The solubility of intact myofibrillar proteins in water over a wide pH range is poor (Spinelli et al., 1972), but may be increased by enzymatic hydrolysis (Chobert et al., 1988). Hydrolysates are more soluble than intact proteins due to the newly exposed ionizable carboxyl and amino groups of the amino acids, which gives a higher hydrophilicity (Kristinsson and Rasco, 2000b).

Sathivel et al. have studied the functional properties of freeze-dried protein powders from different parts of herring. Protein powders made from whole herring, herring body and herring

head all had nitrogen solubility values higher than 78 % (Sathivel et al., 2004). Several researchers have studied the properties of hydrolysates from Atlantic salmon (He et al., 2012, Kristinsson and Rasco, 2000a, Gbogouri et al., 2004). Muscle proteins from Atlantic salmon hydrolysed with various proteases all showed nitrogen solubility above 90 % (Kristinsson and Rasco, 2000a). Several studies have reported high solubility of whey protein isolates or concentrates (Smith et al., 2016, Luck et al., 2013) and that the solubility increase with increasing degree of hydrolysis (Chobert et al., 1988).

Food consisting of multiple phases, such as foams and emulsions, can be stabilized using proteins since they have both hydrophobic and hydrophilic moieties. Two of the most important environmental factors that affect the interfacial properties of proteins are pH and ionic strength. The emulsifying properties of most proteins are low at pH near the isoelectric point due to charge neutralization and poor solubility (Kristo and Corredig, 2014). For a peptide to possess good emulsifying and interfacial properties, the peptide should have a minimum length of 20 residues (Lee et al., 1987). The work of Turgeon et al. also confirms that there is an optimum mean molecular peptide size that gives better emulsification properties than others. Short peptides are less effective in stabilizing emulsions compared to larger ones (Turgeon et al., 1991). He et al. investigated the effect of different processing conditions on the emulsion capacity of Atlantic salmon hydrolysates, such as hydrolysis time, enzyme to substrate (E/S) ratio and choice of enzyme. They found that emulsion capacity (EC) decreased with increasing hydrolysis time, and the highest EC (53 m^2/g) was obtained using Neutrase with a hydrolysis time of 30 min and 0.5 % and 1.75 % E/S ratio (He et al., 2012). Gagné and Adambounou investigated the emulsion capacity and emulsion stability (ES) of autumn-harvested herring roe at different stages of maturity (Gagné and Adambounou, 1994). The emulsion capacity for acidic whey protein concentrates (WPC) is usually in the range of 38-52 mL oil per gram of protein, when the solubility ranges from 25 to 82 % (Farkye and Shah, 2014). This correlates well with the EC for the WPC described by Sinha et al. (2007).

The ability to absorb and retain water against gravitational force within a protein matrix, such as a fish filet, can be described as the water holding capacity (WHC) (Kristinsson and Rasco, 2000b). Proteins can be used to retain water when added to different food products and hence increase the WHC of the protein matrix.

Freeze dried protein hydrolysates (FDPH) from Atlantic salmon have been mixed with Atlantic salmon muscle mince (patties with 1.5 % FDPH) by Kristinsson and Rasco (2000a). FDPH was more effective than the reference proteins (egg albumin and soya protein concentrate) in binding water in the minced patties. Šližytė et al found that adding fish hydrolysate powder (2 % of minced muscle mass) to cod mince, increased the WHC by 16 % for fish protein hydrolysate from fresh backbones (Šližytė et al., 2009). Peptides may interact with the water directly or with proteins in the food products, which may increase their ability to hold water (Kristinsson, 2007). Freezing affects the water holding capacity of fish mince, and Arason et al. (2009) found that fresh mince had a higher water holding capacity compared to frozen.

Swelling is another important property of proteins that involves water and is the expansion that results from uptake of water. In several food products, such as wheat flour doughs and sausages, imbibition of water in proteins is important. Protein source, protein particle size, ionic strength, temperature and pH are factors influencing the swelling. Today, soy and milk products are used to enhance swelling and hold water in food (Pomeranz, 1985). Food consistency, such as the consistency of meat, is connected to protein swelling and water holding capacity. A protein network consists of cross-links between the peptide chains, and a decrease in the number of cross-links leads to swelling. An increase will have the opposite effect and result in shrinkage (syneresis) of the protein gel (Belitz et al., 2009).

1.5.2 Bioactive Properties

Proteins can contain amino acid sequences with biological activities, meaning they can have a positive impact on the body`s function after gastrointestinal digestion. Such sequences are called bioactive peptides and hydrolysis of the protein is often necessary in order to make the bioactive peptide available. The hydrolysis can be performed both in vivo and in vitro. The length of bioactive peptides is usually between 2 and 20 amino acids. Some of the physiological properties of bioactive peptides that have been reported are antihypertensive, antioxidative, antimicrobial, immunomodulative and anticancer (Shahidi and Li, 2014, Undeland et al., 2009).

The most extensive studied bioactive peptides from food sources are antihypertensive peptides. They inhibit the angiotensin-converting enzyme (ACE) and/or renin, which are two key enzymes in the renin-angiotensine system (RAS), which are involved in blood pressure regulation. Inhibition of these enzymes can prevent and/or treat hypertension (Shahidi and Li,

2014). Peptides from Atlantic salmon (*Salmo salar*) and wild caught cod (*Gadus morhua*) have shown ACE inhibition (Dragnes et al., 2009). Ewart et al. produced ACE inhibitory peptides by digestion of by-products (backbone and tail) from salmon aquaculture industry with a neutral protease. The salmon protein hydrolysate lowered the blood pressure significantly in spontaneously hypertensive rats (Ewart et al., 2009). ACE inhibitory effect has also been identified for different peptides derived from whey proteins (alpha- and beta lactoglobulin) hydrolysed with different enzymes (Pihlanto-Leppala et al., 2000).

The antioxidant effect of bioactive proteins can be explained due to their radical scavenging activities and efficiency in metal ion chelation. Several enzymatic and non-enzymatic antioxidant mechanisms can be used to eliminate reactive oxygen species (ROS) which can damage lipids, proteins and DNA in the body (Johansen et al., 2005). In vitro antioxidant activity can be measured with the DPPH scavenging method. 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical with a maximum absorbance at 517 nm in methanol. When a proton-donating substance such as a protein hydrolysate encounter DPPH, the radical is scavenged and the absorbance is reduced (Wu et al., 2003, Galla et al., 2012). Fish hydrolysates made from herring by-products (Sathivel et al., 2003, Šližytė et al., 2014), defatted salmon backbones (Šližytė et al., 2016b), cod backbones (Šližytė et al., 2009) and mackerel (Wu et al., 2003) have all shown antioxidative properties. Several authors have also reported an antioxidant effect from hydrolysed whey proteins (Elias et al., 2005, Hernández-Ledesma et al., 2005, Peña-Ramos and Xiong, 2003).

1.5.3 Nutritional Properties

It is desirable for a protein powder or a fish meal to have high protein content. The chemical composition of a protein powder or a fish meal is influenced by raw material, production method and different processing conditions (Šližytė et al., 2016a, Jensen and Keller, 1990). Table 1 gives the proximate composition of protein powders made from different raw materials.

Table 1. Proximate composition of protein powder from herring body (HBP), hydrolysate from salmon by-products (viscera, head and frames) and hydrolysis with Protamex and endogenous enzymes, herring meal and whey protein concentrate (WPC). The values are given as g/100 g material.

Raw material	**HBP[a]**	**Salmon hydrolysate[b]**	**Herring meal[c]**	**WPC[d]**
Moisture content	5.5	7	7.1	nd
Crude protein	73.4	77	74.5	78.2
Lipids	3.6	4.2	10.1	4.9
Ash	17.7	8	10.4	6.5

[a](Sathivel et al., 2004), [b](Šližytė et al., 2016a), [c](Tibbetts et al., 2006), [d](Sinha et al., 2007). nd: not determined.

The amino acid composition of a protein determines the nutritional value. Generally, animal proteins have a higher nutritional value compared to proteins from plants, as plant proteins often are deficient of some essential amino acids (Ustunol, 2014a). The amino acid composition of fish muscle is a very good source of easily digestible and nutritive proteins (Kristinsson and Rasco, 2000b). Whey proteins have a high biological value, which exceed most other proteins (Sinha et al., 2007). In Table 2, the amino acid composition of protein powder from herring body, hydrolysate from salmon by-products, herring meal and whey protein concentrate are given. Requirements made by Food and Agriculture Organization of the United Nations/World Health organization (FAO/WHO) for the essential amino acids for infants and adults are also added.

Table 2. Amino acid composition of protein powder from herring body (HBP), hydrolysate from salmon by-products (viscera, head and frames) and hydrolysis with Protamex and endogenous enzymes, herring meal and whey protein concentrate (WPC) together with the FAO/WHO suggested requirements for essential amino acids. The values are given as g/100 g

Amino acid	HBP[a]	Salmon hydrolysate[b]	Herring meal[c]	WPC[d]	FAO[e] (infant)	FAO[e] (adult)
Alanine	9.46	5.70	6.3	4.50		
Arginine'	7.33	5.62	5.8	2.32		
Aspartic acid	7.50	7.78	9.1	10.42		
Cysteine	0.64	0.94	1.0	0.60		
Glutamic acid	13.01	11.31	12.8	19.45		
Glycine	14.41	8.58	6.0	1.74		
Histidine'	2.8	2.63	2.4	2.20	2.6	1.6
Isoleucine*	2.20	3.60	4.5	5.56	4.6	1.3
Leucine*	5.78	5.79	7.5	13.29	9.3	1.9
Lysine*	9.17	7.12	7.7	8.98	6.6	1.6
Methionine*	2.88	2.43	2.9	3.41	4.2 $^{\alpha}$	1.7 $^{\alpha}$
Phenylalanine*	3.77	3.12	3.9	3.62	7.2$^{\beta}$	1.9$^{\beta}$
Proline	5.60	4.92	4.2	5.76		
Serine	4.03	3.92	3.8	4.68		
Threonine*	2.93	3.93	4.3	4.80	4.3	0.9
Tryptophan*	nd	0.82	1.2	nd	1.7	0.5
Tyrosine	1.43	1.89	3.1	1.52		
Valine*	2.93	4.26	5.4	6.54	5.5	1.3

* Essential amino acids. ' Essential for infants and children in growth. $^{\alpha}$methionine + cysteine, $^{\beta}$phenylalanine + tyrosine. nd: Not determined. [a](Sathivel et al., 2004), [b](Opheim et al., 2015), [c](WINDSOR, 2001a), [d](Sinha et al., 2007), [e](FAO/WHO, 1991).

1.5.4 Digestibility

Amino acid composition and digestibility are the main factors determining the quality of a protein source (World Health Organization, 2007). The digestibility of proteins can be measured in several ways. It can be determined at the faecal or at the ileal level. The latter is considered the most accurate due to that the faecal digestibility includes microbial metabolism of the nutrients which can either over- or underestimate the digestibility (Trottier and Walker, 2014). Deglaire et al. have studied ileal digestibility in humans by sampling through a naso-ileal tube after intake of ^{15}N-labeled amino acids and with a post valve T-caecum cannula in pigs (Deglaire et al., 2009). Since measuring ileal digestion in humans is unpractical for rou-

tine studies and it is desirable to decrease the use of animal studies, in vitro enzymatic methods can be used instead to simulate gastrointestinal digestion processes. In vitro digestibility methods can be one step procedures where the food is mixed with pepsin and HCl (Boisen, 2000, Butts et al., 2012). It can also be more complex, simulating both gastric and intestinal digestion using a pepsin-HCl mixture before neutralization and digestion with pancreatin (Akeson and Stahmann, 1964), trypsin (Saunders et al., 1973) or intestinal fluid from pigs (Furuya et al., 2008).

The TNO Nutrition and Food Research in Netherlands has developed a computer-controlled, dynamic, multi-compartmental model (TIM) which simulates the stomach and the small intestine of humans and monogastric animals. It include peristaltic movement, gastric emptying and secretion of gastric, biliary and pancreatic fluids (Minekus et al., 1995). Havenaar et al. have used this model to determine the ileal protein and amino acid digestibility of immature herring roe protein and they found that the digestibility of immature herring egg proteins ranged from 71 to 92 % (Havenaar et al., 2016). The digestibility of salmon and whey protein hydrolysates have also been analysed using the TIM-1 model, and Framroze et al. found that salmon protein hydrolysate had a higher proportion of bioaccessible nitrogen compared to the different types of whey proteins used in this study (Framroze et al., 2014).

1.6 Addition of Protein Powders to Food Products

Today, whey proteins are used in a wide range of food applications due to their high nutritional value, functional properties and cost effectiveness (Jeewanthi et al., 2015). WPC can be used in acidic food and beverages since it is soluble at low pH, which is a unique property (Pelegrine and Gasparetto, 2005, Kumar et al., 2010). Whey proteins can also be used to reduce water loss during cooking, as demonstrated by Barbut (2007) who mixed poultry with whole milk proteins and whey hydrolysates. In the food industry, protein hydrolysates can also be used as milk replacers, protein supplements and flavor enhancers in confectionary products (Kristinsson and Rasco, 2000b). It is desirable to add fish protein powders into food due to its functional and nutritional properties. Sathivel et al. have demonstrated that arrowtooth flounder soluble protein (AFSP) powder can be used to produce a more stable emulsion in mayonnaise. The AFSP powder could also be used in enrichment products (Sathivel et al., 2005b). Vareltzis et al. produced hamburgers from minced beef mixed with ethanol extracted

FPC from sardines. They found that the functional properties such as WHC increased with increasing concentration of FPC (Vareltzis et al., 1990).

Bread exists in various forms and plays a significant role in the diet for many people. Unfortunately, the nutritional quality of protein in wheat is low due to the low content of the essential amino acid lysine (Coultate, 2016). The nutritional quality of bread can thus be increased by addition of lysine or animal protein, such as a fish protein concentrate (Stillings et al., 1971). This could help to improve the nutritional health of people suffering from insufficient protein or essential amino acid intake. Several studies where bread have been enriched with either animal protein or lysine have been performed. Adeleke and Odedeji have studied the acceptability of bread fortified with tilapia fish protein flour (TFPF), while Bastos et al. have looked at wheat bread enriched with Red-tailed Brycon (*Brycon cephalus*). The bread made with both TFPF and Red-tailed Brycon resulted in products with a higher nutritional value and received good sensory acceptance compared to the control (Adeleke and Odedeji, 2010, Bastos et al., 2014).

Changes in physical and sensory characteristics of doughs and of bread enriched with different amounts of FPC from red hake (*Urophycis chuss*) and lysine have been assessed by Sidwell and Hammerle (1970). An increasing amount of FPC affected physical characteristics, such as loaf volume and crumb structure much more than an increasing content of lysine. Bread with 5 or 10 % FPC were accepted by the participants, while higher amounts were less accepted. Using the same FPC as above, Stillings et al. (1971) compared the nutritional effectiveness of the FPC and lysine mixed in different amounts with wheat flour before and after processing into bread. When fed to rats, the weight gain was generally higher for FPC than for lysine.

1.7 Sensory Properties

The sensory properties are important for a product made for human consumption. Protein powders added food products may have a negative impact on the sensory quality.
Peptides with molecular weight greater than 6 kDa are tasteless, since the peptides cannot reach the action site of the receptor (Lin et al., 2012). Fish protein hydrolysates produced with enzymatic hydrolysis have the disadvantage of generally having a bitter taste (Kristinsson and Rasco, 2000b). Aspevik et al. investigated the sensory properties of hydrolysates produced

from Atlantic salmon by-products. Peptides smaller than 2k Da with a high hydrophobic peptide fraction and a high degree of hydrolysis were found to have a positive correlation with a significant bitter taste. The choice of protease can also influence the bitterness, and Alacase 2.4L produced significantly more bitter peptides than Protex 7L and Promod 671L (Aspevik et al., 2016).

In protein rich foods where hydrolytic processes occur (e.g. fish, meat), free amino acids can also contribute to the flavour. The taste depends on the configuration of the amino acid. L-amino acids generally taste bitter, while the D-form of the amino acid generally tastes sweet. The taste intensity varies, and L-tryptophan and L-tyrosine are the most bitter amino acids. In high concentrations, L-glutamic acids have a meat broth flavour (Belitz et al., 2009).

Due to their high molecular weights, proteins are often tasteless, but may bind aroma compounds. Ketones, alcohols, phenols, aldehydes and lipid oxidation products can bind to proteins and produce beany flavour, rancidity, bitterness and harsh taste (Lin et al., 2012). The main unsaturated fatty acids found in fish oil are linolenic acid, arachidonic acid and docosahexaenoic acid. Oxidation of these fatty acids can give an unpleasant odour, especially a fishy odour (Zeng and Huang, 2012). Lipid oxidation and development of fishy odour can be reduced by using fresh fish together with an antioxidant (Yarnpakdee et al., 2012b). Different pre-treatments of mince from Indian mackerel (*Rastrelliger kanagurta*) have proven to be effective for reducing the total lipid content and hence reducing lipid oxidation and fishy odour and taste (Yarnpakdee et al., 2012a). The pre-treatment resulted in a successful fortification of milk with fish protein hydrolysate at a level of 0.2 %.

1.8 Protein Requirements and Nutritional Health Aspects in Elderly People

Age, body size and physiological state are factors influencing the requirements of proteins and amino acids in humans (Gropper and Smith, 2013). The protein requirements have been defined by WHO as: "*the lowest level of dietary protein intake that will balance the losses of nitrogen from the body, and thus maintain the body protein mass, in persons at energy balance with modest levels of physical activity, plus, in children or in pregnant or lactating women, the needs associated with the deposition of tissues or the secretion of milk at rates consistent with good health*" (World Health Organization, 2007). Based on nitrogen balance studies, the average nitrogen (N) requirement for a healthy adult is 105 mg N/kg per day,

which equals 0.66 g protein/kg per day. *A safe level* of 133 mg N/kg per day or 0.83 g protein/kg per day has also been suggested. This is the 97.5th percentile of the population distribution of requirement and is expected to cover the requirements of most (97.5 %) of the healthy adult population (World Health Organization, 2007). For a typical European, there is usually no problem to reach these levels, since the diet normally provides enough total protein and the individually essential amino acids (Coultate, 2016).

Elderly adults is one group that need more dietary protein than younger adults, and age related changes in the metabolism, such as declining anabolic responses to the ingested protein is one of the reasons (Bauer et al., 2013). The protein intake in hospitalized elderly people is usually insufficient, and this group could benefit from products enriched with proteins (van Bokhorst–de van der Schueren et al., 2012, Leistra et al., 2011). The The PROT-AGE study group, which is an international study group appointed by the The European Union Geriatric Medicine Society in cooperation with other organizations, has evaluated the dietary protein needs with aging. This group has recommended at least 1.0-1.2 g protein/kg per day for older adults and even more for those suffering from acute or chronic diseases (1.2-1.5g/kg per day) (Bauer et al., 2013). The nutritional effect from bread and drinking yoghurt enriched with whey protein concentrate in older adults in a rehabilitation center and in acute hospitalized older adults have been investigated in two different studies with promising results (van Til et al., 2015, Stelten et al., 2015). Adding proteins into familiar and frequently consumed products may therefore be a successful strategy to increase the protein intake in elderly people.

The progression of sarcopenia, age-related loss in skeletal muscle mass and hence strength may be prevented or delayed by increasing the protein intake together with exercise (Fox, 2016, Daly, 2016). However, results obtained from randomized controlled trials (RCT) that have examined additional protein intake and progressive resistant training (PRT) are not consistent. Where some have managed to prove that additional protein intake combined with PRT led to a gain in muscle mass or muscle strength (Tieland et al., 2012, Daly et al., 2014), others have failed (Arnarson et al., 2013, Leenders et al., 2013)

1.9 Sensory Analysis

Together with the functional properties, nutritional properties and shelf life, the sensory properties determine the quality of a food product. Sensory properties, such as smell, taste, colour, texture and appearance, are perceived by our senses and sensory analysis is the measurement of such properties by human senses (Sensorisk studiegruppe, 1997). The Sensory Evaluation of the Institute of Food Technologists, have described sensory evaluation with the following definition: "*Sensory evaluation is a scientific discipline used to evoke, measure, analyze, and interpret reactions to those characteristics of foods and materials as they are perceived by the senses of sight, smell, taste, touch, and hearing*" (Stone et al., 2012). Sensory analysis can be used for several purposes, and the most important application areas are in quality control, product development, research, mapping and surveillance in public affairs (Sensorisk studiegruppe, 1997).

1.9.1 The Human Senses

Vision, audition, taste, smell and touch are the five major senses of the human body (Stone et al., 2012). It is important to note that even if there exists a distinct sensory organ for the different senses, information from each organ is often integrated into a complete experience in the brain. A change in one sensory property may therefore affect the other properties, giving a new perception of the food or object under observation (Kemp et al., 2009).

1.9.1.1 Vision

The sense of vision determines the appearance of any object. The retina, which covers ¾ of the backside of the inner eye ball, contains light sensitive receptor cells, known as rods and cones. The rods are responsible for the night vision, while the cones are used to see colours in daylight. These receptors convert the energy from the light waves into neural impulses, which enter the brain via the optic nerve. After interpretation of these signals by the brain, the appearance (colour, shape, size, surface texture etc.) of the object can be perceived (Store medisinske leksikon, 2016, Kemp et al., 2009). Surface characteristics such as roughness, greasiness, smoothness and uniformity can be assessed by visual texture evaluation (Lawless and Heymann, 2010).

1.9.1.2 Gustation – The Sense of Taste

The surface of the tongue and other areas of the mouth contain receptors, which detect non-volatile substances when dissolved in water, oil or saliva (Kemp et al., 2009). Between 50 to 100 specialized epithelial cells (taste cell) with long microvilli facing the external environment are located together in a barrel shaped ball, called a taste bud (Fox, 2016).

Salty, sour, sweet and bitter are the four classic taste qualities. In addition, there are three other tastes, namely umami (meatiness), astringency and pungency (hotness). Umami is associated with the two substances: inosine monophosphate (IMP) and monosodium glutamate (MSG). These compounds do not have a particular strong taste by themselves, but together they create a taste 20 times stronger than they would alone (Coultate, 2016). Table 3 shows examples of compounds that elicit particular tastes (Kemp et al., 2009).

Table 3. Examples of substances that elicit particular tastes (Kemp et al., 2009).

Taste	Substance
Salty	NaCl, KCl
Sweet	Sucrose, glucose, aspartame
Sour	Citric acid, phosphoric acid
Bitter	Quinine, caffeine
Umami	Monosodium glutamate

1.9.1.3 Olfaction – The Sense of Smell

The nasal epithelium is covered by millions of cilia which contain olfactory receptors which sense volatile molecules. During breathing/sniffing or retronasally during eating, volatile molecules enter the nose and the receptors send a signal to the brain to interpret the smell (Kemp et al., 2009). In addition to the receptor cells, the olfactory apparatus, consist of supporting cells and basal stem cells (Fox, 2016). The sensitivity of the olfaction is greatly affected by age and gender, but also individually differences. Women can for example be more sensitive to certain odours compared to men (Sensorisk studiegruppe, 1997).

Olfaction, provides information about the taste, and thus whether the food is edible or not. Smell (odour) and taste, collectively called flavour are two senses that interact and greatly affect each other. Together they will influence whether the food will be eaten or thrown (Sensorisk studiegruppe, 1997). The number of different odours available is enormous, and there have been some attempts to classify them. In 1972, seven primary odours were described by Amoore: camphoraceous, ethereal, floral, minty, musky, pungent and putrid (Coultate, 2016).

1.9.1.4 Touch

The skin has several types of sensory receptors that is activated when the given area is stimulated. Free nerve endings, Meissner`s corpuscles and Pacinian corpuscles are examples of receptors found in the skin. These receptors can detect sensations such as warmth, cold and texture (Fox, 2016). Tactile texture of food is divided into tactile hand feel (by hand or with utensils), oral-tactile texture, mouth feel and phase change (melting) in the oral cavity. Hardness, springiness and graininess are attributes which may be perceived by the skin and mouth. Some substances can stimulate trigeminal nerves in the mouth and give a cooling (menthol), burning, stinging (capsaicin), tingling, tickling (carbonated beverages) or astringent sensation, collectively known as mouth feel attributes when ingested (Lawless and Heymann, 2010, Kemp et al., 2009).

1.9.1.5 Audition

The ear consists of the outer, middle and the inner ear. The spiral organ in the inner ear is filled with thousands of tiny hair cells which are stimulated by the vibration of air from sound waves (Fox, 2016). The sound created while eating different food products can say something about the texture of the food and hence its quality. Sounds associated with eating certain food products (auditory texture) can have both a positive and negative influence on the perception of the product (Lawless and Heymann, 2010). A newly opened carbonated drink, will sound different than a drink that has been opened before (Kemp et al., 2009).

1.9.2 Test Panels Used in a Sensory Analysis

The group of people participating in a sensory analysis will vary depending on the purpose of the analysis. There are three different types of panel used in a sensory analysis. An *expert panel* consists of highly trained people with good knowledge of the test product. It is necessary with training to be part of such a panel, and the spectrum of products they cover is relatively narrow (Sensorisk studiegruppe, 1997). A *laboratory panel* is the most common and is a panel where the assessors can be either internal or external. The usual number of assessors in such a panel is between 6 and 10 people. The last type of panel is the *consumer panel*. It consists of a large number of untrained assessors, which represent a well defined group of consumers. The numbers of assessors vary, but the certainty of the results increased with increasing number of participants (Sensorisk studiegruppe, 1997).

1.9.3 Test Methods in a Sensory Analysis

The different sensory test methods can be divided into three major groups; discrimination tests, descriptive test and affective tests.

1.9.3.1 Discrimination Tests

Discrimination tests are used to test whether there is a sensorial difference between two very similar samples (Lawless and Heymann, 2010). In such tests, the assessors have to point out one or several samples that deviate from the rest. It is often not an option to answer "no difference", and therefore such methods are called "forced choice methods", which actually makes it easier to detect differences between the samples (Sensorisk studiegruppe, 1997). The most common discrimination tests are paired comparison test, triangle test, duo-trio-test and 2-out-of-5 test (Lawless and Heymann, 2010). Discrimination tests are also used to rank different samples after intensity or size decided by a given property or category (Kemp et al., 2009).

1.9.3.2 Descriptive Tests

Descriptive tests are methods used to describe and quantify sensory differences between products. Quality control tests and profiling tests are the most common tests (Sensorisk studiegruppe, 1997). A numerical scale is often used when assessing the quality of a product, and training of the judges is usually necessary to get a correct evaluation (Lawless and Heymann, 2010). One common problem is that the judges do not use the whole scale. Profiling tests are useful when it is desirable to investigate one or more of the sensory properties of a product. They can be used in development of new products, improvement of existing

products and study the effect of process changes (Sensorisk studiegruppe, 1997). Flavour profiling, texture profiling and sensory profiling are examples of different profiling techniques used to evaluate different properties of food (Lawless and Heymann, 2010).

1.9.3.3 Affective Tests

Affective tests, also called consumer tests, measure the degree of liking or disliking of a product (Lawless and Heymann, 2010). They can gain information about consumer preferences, opinions, attitudes, behaviour and perceptions about the product. Consumer tests are an important part of a product development process. The preference for a product may vary with geographic location, sex, age, lifestyle, values and product usage. The number and type of assessors are thus of great importance in affective testing (Kemp et al., 2009).

In order to quantify the degree of liking or disliking more easily, a hedonic scale was developed at the U.S Army Food and Container Institute in the late 1940s (Jones et al., 1955, Lawless and Heymann, 2010). The 9-point hedonic scale shown in Table 4 is the most common, but 5- and 7-point hedonic scales are alternatives used when less options are preferred (Lawless and Heymann, 2010).

Table 4. The 9-point hedonic scale used to assess the liking or disliking of a product. It is a balanced scale with equal amount of positive and negative intervals with a neutral category in the middle.

Score	Descriptive phrase
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike moderately
1	Dislike extremely

Table 5 summarizes the test methods mentioned in section 1.9.3, together with the questions the different methods can answer.

Table 5. Summary of different test methods used in a sensory evaluation of food and what type of questions they can answer.

Method	Question of Interest	Test
Analytical methods	Is there any difference between the products?	Paired comparison test Triangle test Duo-trio-test 2-out-of-5 test
	Can the product be ranked after increasing degree of …?	Ranking test
	What is the difference, and how large is it?	Descriptive test Quality control test
Affective methods	Has the difference any significance?	Preference test Acceptance test

1.10 Specialization Project

In the specialization project, performed in the autumn of 2016, the chemical composition and the functional properties of a herring roe protein powder from Norwegian spring spawning herring was investigated (Liaklev, 2016). Solubility, water holding capacity, emulsifying properties and antioxidant properties were evaluated. For comparison, the same experiments were performed with a commercial whey protein powder with chocolate taste. The results obtained for the functional properties for the whey protein powder were generally much better compared to the herring protein powder. The low solubility of the herring protein powder was the major problem and hydrolysis was proposed as a possible solution. Addition of the powders to food was suggested as future work since this was not performed.

1.11 The Aim of the Thesis

This master thesis is a continuation of the specialization project briefly described above. The aim was to compare the nutritional, functional, bioactive and sensory properties of different protein powders from herring roe, salmon and whey. To achieve this, the chemical composition, solubility, emulsifying properties, water holding capacity, swelling capacity, antioxidant properties and digestibility were investigated. The effect of different pre-treatments, such as hydrolysis and different temperatures on the functional and sensory properties of the fish powders were also studied. In search for a good model product, the powders were added into different food products and the effect on the nutritional, functional and the sensory properties were investigated.

2 Materials and Methods

2.1 Raw Materials

Three different protein powders have been analysed. Romega®HCP Premium is a protein powder made exclusively from Norwegian spring spawning herring (*Clupea harengus L.*) roe and is produced by the Norwegian company Arctic Nutrition by ethanol extraction. A certificate of analysis for the Romega®HCP Premium protein powder was provided by the producer and is given in Appendix A.1. The amino acid composition analysed by Eurofins Steins Laboratorium, also provided by the producer, is found in Appendix A.1.

Whey Professional Protein Powder (Life) with a neutral taste was bought at a local health nutrition store (Life). Ingredients, nutritional facts and the amino acid composition provided by the producer are given in Appendix A.2.

The third powder investigated was a fish meal produced by Vital Seafood AS and made from by-products (trimmings and guts) from the slaughterhouse and processing of Norwegian farmed salmon at Marine Harvest. The salmon meal was stored in a zip bag in a cold room (4 ºC), while the other two were stored at room temperature in plastic boxes.

The Romega®HCP Premium protein powder and the Whey Professional Protein Powder will from now on be referred to as herring protein powder (HPP) and whey protein powder (WPP) respectively. Figure 5 shows the three different powders.

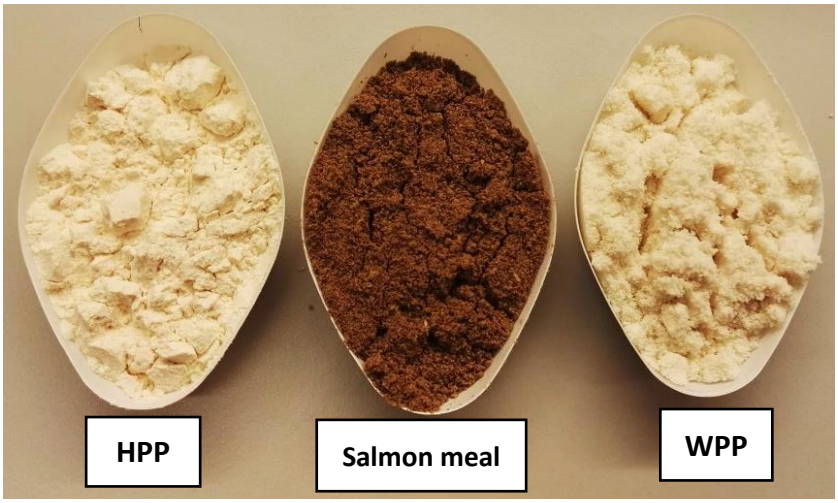

Figure 5. The herring protein powder (HPP) is to the left, salmon meal is in the middle and the whey protein powder (WPP) is to the right.

2.2 Methods

2.2.1 Enzymatic Hydrolysis of Herring Protein Powder

10 g of herring protein powder was weighed into Erlenmeyer flasks and distilled water was added until the total weight was 100 g. The experiment was performed in duplicates. In order to dissolve the water soluble components, the flasks were shaken at room temperature at 250 rpm for approximately 20 min. Trypsin from bovine pancreas (10 400 units/mg solids, Sigma Aldrich) was used for the hydrolysis. 0.1 % of the enzyme (w/w, dry weight HPP) was added into each flask, which was placed in a water bath (Comfort Heto Master Shake) with a temperature of 55 ºC. The flasks were gently shaken at 100 rpm. Since the protein powder sedimented on the bottom, the flasks were taken out of the water bath every 15 min and given a proper shake. No adjustment of the pH was performed. A sample (30 mL) was taken after 30, 60 and 125 min and transferred into 50 mL centrifuge tubes. The enzyme was inactivated by placing the samples in boiling water for 10 min. Finally, the samples were centrifuged at 4500 × g at 15 ºC for 20 min in an Eppendorf Centrifuge 5804R. The supernatant was transferred into new 50 mL centrifuge tubes and stored in a freezer (-18 °C) until further analyses.

2.2.2 Solubility of Herring Protein Powder Hydrolysates

The solubility of the herring protein powder hydrolysates was determined by the Lowry method (Lowry et al., 1951). A stock solution (1000 µg/mL) of bovine serum albumin (BSA) was used as a protein standard. The stock solution was diluted to the following concentrations: 12.5, 25, 50, 100, 150, 200 and 300 µg/mL. The hydrolysates (supernatants from hydrolysis) were diluted 1:25 with distilled H_2O. The absorbance was read at 750 nm with a spectrophotometer (Ultrospec 2000, UV/Visible spectrophotometer, Pharmacia Biotech). The absorbance measurements were performed in triplicates.

2.2.3 Solubility in Water and Citric Acid-Phosphate Buffer

Citric acid-phosphate buffer with pH 3, 5 and 7 was made as described by McIlvaine (1921). The pH was determined with a pH-meter (Radiometer Copenhagen PHM210 Standard pH meter) and adjusted if not correct. 0.2 g of salmon meal and whey protein powder was added to 10 mL volumetric flasks. The flasks were filled with water and citric acid-phosphate buffer with pH 3, 5 and 7. The samples were then transferred to 15 mL VWR centrifuge tubes and centrifuged at 2000 × g and 15 ℃ for 5 min in an Eppendorf Centrifuge 5804R. After centrifugation, an oil layer formed in the samples containing salmon meal. This was carefully removed with a pipette. The supernatant was then transferred into new test tubes.

Using the corresponding solvent, the protein extracts were diluted 1:25 and 1:100 for salmon meal and whey protein powder respectively. The solubility was determined by the Lowry method as described in section 2.2.2. The absorbance measurements were performed in triplicates.

2.2.4 The Effect of Different Temperatures on the Solubility

The effect of temperature on the solubility was determined by mixing herring protein powder and salmon meal with distilled water at six different temperatures. This was not performed on the whey protein powder, since the solubility was found (section 2.2.2) to be high (84 % in distilled water) at room temperature. 0.2 g HPP and salmon meal were dissolved in 10 mL distilled water at 30, 40, 50, 60, 70 and 100 ℃. The mixtures were transferred into 15 mL tubes and put in a water bath with corresponding temperatures for 15 min. The samples were centrifuged at 2000 × g and 20 ℃ for 5 min in an Eppendorf Centrifuge 5804R. Oil layers formed in the samples with salmon meal were removed with a pipette and the supernatant transferred into new test tubes. The protein extracts from HPP and salmon meal were diluted 1:5 and 1:25, respectively, with distilled water. The solubility was determined by the Lowry method as described in section 2.2.2. The absorbance measurements were performed in triplicates.

2.2.5 Amount of Acid Soluble Peptides

The amount of acid soluble peptides in the hydrolysates from herring protein powder was determined as described by Hoyle and Merritt (1994). 2.0 mL water soluble extract was mixed with 2.0 mL 20 % trichloroacetic acid (TCA, Sigma-Aldrich). The test tubes were left for 30 min before the samples were filtered through filter papers in funnels (Whatman quantitative filter papers 589/1, 12-25 μm). The content of acid soluble peptides was determined by the Lowry method as described in section 2.2.2. The absorbance measurements were performed in triplicates.

2.2.6 Swelling Capacity at 40 and 100 ºC

The swelling capacity was defined as amount of water absorbed by the powder in mL per gram powder. 5 g of herring protein powder, salmon meal and whey protein powder were weighed out and mixed with 20 g of distilled water with temperatures of 40 and 100 °C in a beaker. The solution was stirred well and left to stand for 15 min. The mixture was filtrated through a Buchner funnel with filter paper (Whatman quantitative filter papers 589/1, 12-25 μm). When no more water was dripping from the funnel, the amount of water in the Erlenmeyer flask was measured. The measurements were performed in triplicates. In order to find to find the amount of water in the wet filter, 20 g of distilled water was filtrated through a Buchner funnel with a pre-weighed filter paper. The amount of water absorbed by the filter was then calculated by subtracting the dry weight from the wet weight of the filter paper.

The amount of water absorbed by the powder was calculated by Equation 1:

$$W_P = W_T - W_E - W_F \quad (1)$$

Here, W_P is the water absorbed by the powder, W_T is the total amount of water, W_E is the amount of water in the Erlenmeyer flask and W_F is the amount of water absorbed by the filter.

2.2.7 Water Holding Capacity

The water holding capacity (WHC) of Atlantic cod (*Gadus morhua*) filets (Norway Sea Foods) were determined as described by Børresen (1980) and Eide et al. (1982), with some modifications. The fish filets, which were bought in a local food store (Rema 1000), were

thawed in a plastic bag and grinded with a food processor (Type Y92, Moulinex Illico). Approximately 2 g of the fish material was added into special sample holders that were weighed before and after the addition of fish. The measurements were performed in quadruplicates. The sample holders had a polyester membrane in the bottom that allowed water to pass during centrifugation, while the fish material was retained. The sample holders were placed into centrifuge tubes filled with glass beads as shown in Figure 6. After centrifugation at 210 × g and 4 °C for 5 min in a Sigma 202 centrifuge, the sample holders were weighed again.

In order to investigate whether the WHC could be improved by the addition of protein powders, the same procedure as described above was followed, but fish material was mixed with salmon meal or whey protein powder before the fish was added to the sample holders. The amount of powder added constituted 5, 10 and 20 % of the fish mass in the sample holder.

Dry matter content of minced fish and fish mixed with protein powder was determined by drying approximately 2 g in pre-weighed aluminium dishes in a heat cabinet (105 °C) for 24 h.

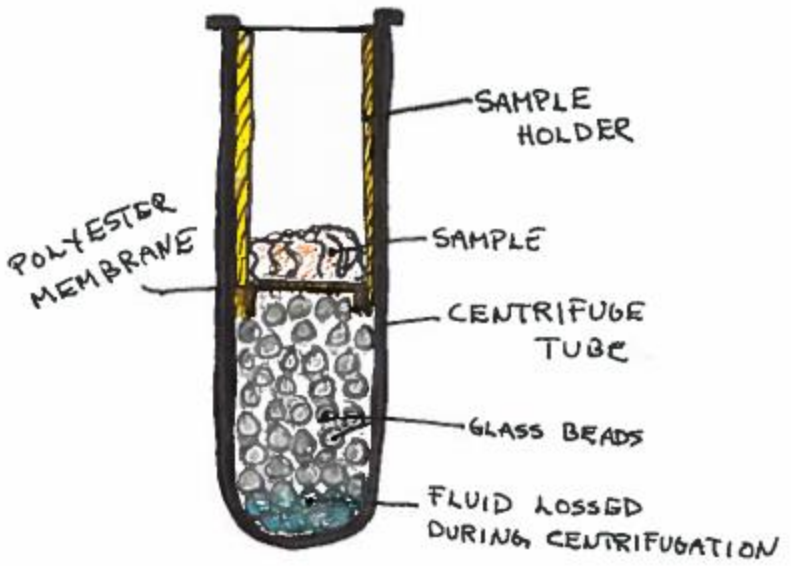

Figure 6. Centrifuge tube with sample holder. The sample holder have a polyester membrane in the bottom which allows liquid to pass during centrifugation. The centrifuge tubes were filled with glass beads to separate the fish sample and the liquid.

The WHC was calculated according to Equation 2:

$$WHC\ (\%) = \frac{V1-\Delta r}{V1} \cdot 100\ \% \quad (2)$$

Here, *V1* is the percentage of water before centrifugation and *Δr* is the percentage of weight loss due to centrifugation.

2.2.8 Emulsifying Properties

Emulsifying properties of salmon meal and whey protein powder was determined as described by Šližytė et al. (2009) with some modifications. The measurements were performed in quadruplicates. 5 mL of rapeseed oil was mixed with 5 mL of protein powder solution in a VWR 15 mL centrifuge tube. Three different concentrations of the protein powder were tested, namely 1, 2 and 5 %. The mixture of oil and protein powder solution was homogenized with an IKA T10 basic Ultra-Turrax for 90 s at level 6. The emulsion was centrifuged for 3 min at 2400 × g in an Eppendorf centrifuge 5804R. The volume of each fraction (oil, emulsion and water) was determined in mL by reading of the 15 mL centrifuge tube. The emulsifying capacity was expressed as mL of emulsified oil per 1 g of protein powder (Kinsella and Melachouris, 1976). The emulsions were left standing for 24 h in room temperature and the emulsion stability was expressed as percentage of initial emulsion remaining after 24 h and centrifugation at 2400 × g for 3 min (McClements, 2004).

2.2.9 Antioxidant Properties

The antioxidant property of whey protein powder was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay as described by Thiansilakul et al. (2007) with some modifications. The antioxidant property of salmon meal was investigated twice with this method, but it was found that the DPPH-method wasn`t suitable for this material.

0.15 mM DPPH (Sigma-Aldrich) in 96 % ethanol (VWR Chemicals) was prepared in a bottle covered with aluminum foil. The mixture was stirred at 4 ºC over night. Propyl gallate was used as a standard, and the same day as the experiment was conducted, 10 mM propyl gallate (Sigma-Aldrich) in 80 % methanol (VWR Chemicals) was prepared. A series of dilutions were prepared from the propyl gallate stock solution with volumes given in Appendix K.1.

Protein extracts were prepared by dissolving 0.05, 0.1 and 0.3 g of whey protein powder and salmon meal in 10 mL 80 % methanol. Then the samples were centrifuged at 2000 × g and 20 °C for 5 min in an Eppendorf Centrifuge 5804R. No oil layer was formed in the salmon meal samples when methanol was used as a solvent.

1.5 mL of protein extract or 1.5 mL standard solution was mixed with 1.5 mL of DPPH in 15 mL centrifuge tubes with lid and then vortexed. The samples were left in a dark place at room temperature for 30 min. The absorbance was read at 517 nm with a spectrophotometer (Ultrospec 2000, UV/Visible spectrophotometer, Pharmacia Biotech). 96 % ethanol was used as a reference. Each measurement was conducted in triplicates.

The scavenging effect of the protein powders was calculated according to Equation 3:

$$Scavenging\ effect\ (\%) = \frac{Blank\ absorbance - Sample\ absorbance}{Blank\ absorbance} \cdot 100\ \% \qquad (3)$$

In addition, the absorbance of salmon meal in pure 80 % methanol at different wavelengths (400, 450, 517, 570 and 734 nm) was measured with 96 % ethanol as reference.

2.2.10 Dry Matter Content

Approximately 0.6 g of herring protein powder, whey protein powder and salmon meal were transferred into pre-weighed aluminum weighing dishes and placed into a heating cabinet (105 °C) for 24 h. After cooling in a desiccator, the aluminum weighing dishes containing the dry matter were weighed again. The measurements were performed in duplicates.

2.2.11 Ash Content

Approximately 0.5 g of herring protein powder, whey protein powder and salmon meal were transferred into pre-weighed porcelain crucibles and placed into a muffle furnace (550 °C, Nabertherm) for 24 h. After cooling in a desiccator, the porcelain crucibles with ash were weighed again. The measurements were performed in duplicates.

2.2.12 Total Amount of Protein - C/N Analysis

All of the equipment used during this procedure was washed prior to using with acetone (VWR Chemicals) to remove any impurities. Between 0.5 and 1 mg of herring protein powder, whey protein powder and salmon meal were weighed in small tin capsules (5×9 mm, Santis Analytical) with a microbalance (MT5, Mettler Toledo). The tin capsules were closed, shaped into little round balls and placed in a 96-well plate. The measurements were performed as quintuplicates. The total amount of nitrogen in the powders was determined with a C/N Elemental analyser (Elemental Combustion System CHNS-O, ECS 4010, Costech) at SINTEF Fisheries and Aquaculture in Trondheim by engineer Marte Schei. By using a conversion factor of 6.25 (Coultate, 2016), the total amount of protein was calculated from the total amount of protein.

2.2.13 Total Lipid Content

Total lipid content of salmon meal was determined by the method described by Bligh and Dyer (1959) with some modifications The measurements were performed in duplicates. 5 g of sample was weighed out and 16 mL of distilled water, 40 mL of methanol (VWR Chemicals) and 20 mL of chloroform (VWR Chemicals) were added. The mixtures were homogenized with an IKA T 25 digital ULTRA-TURRAX® (6000 rpm) for 2 min before 20 mL of chloroform again was added and the mixtures were homogenized for 40 s. 20 mL of distilled water was added prior to homogenization for 40 s. During all homogenization, the centrifuge tubes where kept on ice in a ventilation hood. The centrifuge tubes were centrifuged at 4080 × g for 10-15 min at 4 ºC in a Sorvall Refridgerated RC5C Centrifuge. After centrifugation, the bottom phase (chloroform phase) was transferred into 50 mL centrifuge tubes. The total lipid content was determined by immediately transferring 2 mL of the chloroform phase of each parallel to two pre-weighed reagent tubes. The chloroform was evaporated on a heat block (60 °C) under a stream of nitrogen gas. After cooling in a desiccator for 1 h, the reagent tubes were weighed and percentage lipid content was calculated according to Equation 4:

$$Lipid\ content\ (\%) = \frac{a \cdot b}{c \cdot v} \cdot 100\ \% \qquad (4)$$

Here, *a* is the amount of evaporated fat (g), *b* is the volume of chloroform added (mL), *c* is the volume of evaporated chloroform (mL) and *v* is the weight of the sample (g).

2.2.14 Amino Acid Composition

The amino acid composition was determined with the method described by Blackburn (1978). Approximately 0.1 g of salmon meal, whey protein powder and herring protein powder were weighed into glass tubes with a flat bottom and a screw cap. The measurements were performed in triplicates. 2 mL of 6 M HCl (E. Merck) was added into each glass tube before they were put in a heat cabinet (105 ℃) for hydrolysis (22 h). The material from cooled samples was transferred into glass vials and NaOH (VWR Chemicals) was added until pH 7 was measured (Radiometer Copenhagen PHM210 Standard pH meter). The samples were then filtrated through a Whatman glass microfiber filter GF/C with suction. After filtration, the volumes were made up to 10 mL with de-ionized water. The herring protein powder, salmon meal and whey protein powder samples were then diluted 1:500, 1:500 and 1:750 with de-ionized water prior to being filtrated (0.2µm). At last, 0.205mL of the sample was added to HPLC (high pressure liquid chromatography) tubes. From each parallel two HPLC samples were made. The samples were then delivered to Siri Stavrum who performed reverse phase-HPLC analysis. An UltiMate® 3000 HPLC (Thermo Scientific) with Dionex RF 2000 fluorescence detector was used for this experiment. Cromelion software was used to process the data.

2.2.15 Free Amino Acids

This method was performed as described by Osnes and Mohr (1985). Protein extracts of salmon meal and whey protein powder with distilled water and citric acid-phosphate buffer with pH 3, pH 5 and pH 7 as solvent, were made in the same way as described in section 2.2.3. The amount of free amino acids in the herring protein powder hydrolysates were also determined. The measurements were performed in triplicates for the whey protein powder and salmon meal, and in quadruplicate for the herring protein powder hydrolysates.

1 mL of extract was transferred to Eppendorf tubes and 0.25 mL 10 % 5- sulfosalicylic acid dehydrate (SSA) (Merck KGaA) was added and then the tubes were mixed thoroughly. The samples were put in a refrigerator (4 ℃) for 30 min before they were centrifuged at 93 000 × g and 4 ℃ for 10 min in an Eppendorf Centrifuge 5415R. To check that the protein was fully precipitated, 0.5 mL from the supernatant of one of the parallels for the different extracts were transferred to new Eppendorf tubes and added 0.125 mL 10 % SSA and mixed. The samples were again put in the refrigerator for 30 min and centrifuged as described above. For samples with precipitation present after the second round, dilutions with de-ionized water

were made. Otherwise, dilutions were made from the samples with only one round of precipitation. Table 6 gives an overview of the number of precipitations and the dilution for the different samples.

Table 6. Overview of the number of precipitations and dilutions used for the different extracts. The type of solvent used for the different powders is also given.

Powder	Solvent	Number of precipitations	Dilution
Salmon meal	Distilled water and citric acid-phosphate buffer, pH 3, 5, 7	2	1:25
Whey protein powder	Distilled water and citric acid-phosphate buffer, pH 3	1	1:20
	Citric acid-phosphate buffer, pH 5 and 7	2	1:20
Herring protein powder hydrolysates	Water	1	1:10

Suitably diluted samples were filtrated (0.2 µm) and 0.205 mL was transferred into HPLC tubes. Reverse phase HPLC was conducted by Siri Stavrum in the same way as described in section 2.2.14.

2.2.16 Molecular Weight Distribution

The molecular weight distribution of salmon meal, whey protein powder and herring protein powder hydrolysates was analysed with gel filtration. Äkta FPLC (fast protein liquid chromatography, Amersham Biosciences) with column Superdex Peptide 10/300 GL was used. The detection wavelength was 280 nm and 0.05 M sodium-acetate buffer with pH 5 was used as an eluent. The flow speed was 0.5 mL/min.

0.05 M sodium-acetate buffer was prepared by mixing 357 mL 0.05 M acetic acid (VWR Chemicals) with 643 mL 0.05 M sodium acetate (Merck KGaA). The solution was adjusted to pH 5 with Radiometer Copenhagen PHM210 Standard pH meter. The buffer solution was then filtrated through a filter (0.2µm, Nalgene Rapid flow Bottle top filter, Thermo Scientific).

Samples were prepared by either mixing 0.1 g of salmon meal or 0.05 g of whey protein powder with 1 mL of 0.05 M sodium-acetate buffer in Eppendorf tubes that were centrifuged at 15 700 × g and 4 ℃ for 15 min in an Eppendorf Centrifuge 5415R. The oil layer formed in the salmon meal sample was removed with a pipette. The herring protein powder hydrolysates were not diluted and centrifuged at the same conditions as above.

2.2.17 Digestibility of Protein Powders

The digestibility of the powders with pepsin was analysed according to the AOAC Official Method 971.09 (AOAC, 1990). Herring protein powder, salmon meal, whey protein powder with neutral and chocolate taste together with casein (Merck) were digested with pepsin from porcine gastric mucosa (1200-2400 U/mg, Sigma Aldrich) mixed with 37 % HCl (E. Merck). The pepsin solution was made by diluting 3.05 mL HCl with 500 mL distilled water. The solution was heated to 40 ºC and then 1.0 g of pepsin was added to give a pepsin concentration of 0.2 %. The solution was gently stirred until the pepsin was completely dissolved. 0.5 g of the powders were added separately into 50 mL centrifuge tubes together with 20 mL pepsin solution. The mixtures were placed in a heating cabinet at 40 ºC for 16 h with stirring (160 rpm). After cooling for 1 h the samples were centrifuged for 20 min at 2000 × g and 20 ºC with an Eppendorf Centrifuge 5804R. The liquid phase was removed using a pipette. 10 mL of distilled water was added into each sample and shaken prior to centrifugation for 20 min at 2000 × g and 20 ºC. The liquid phase was again removed using a pipette. The samples were dried in a heating cabinet at 55 ºC for approximately 24 h. After cooling, the samples were weighed and the digestibility was calculated according to Equation 5:

$$Digestibility\ (\%) = \frac{M - M_D}{M} \cdot 100\ \% \qquad (5)$$

Here, M is the weight of the sample (g) and M_D is the weight of the sample after digestion and drying (g).

2.2.18 Preparation of Products Enriched with Protein Powder

Preparations of Bread Rolls

Wheat flour (Møllerens), finely grinded wholemeal (Møllerens), iodized salt (Jozo), olive oil (Ybarra), fresh yeast (Idun), water and herring protein powder, whey protein powder and salmon meal were used for the production of bread rolls according to Table 7. Equal amount of wheat flour and wholemeal were replaced with 0, 5, 10, 15 or 20 % protein power. Due to some larger particles, the salmon meal was crushed with a pestle and mortar prior to use.

Table 7. Formulations used for the preparation of bread rolls with replacement of wheat and wholemeal flour by herring protein powder, whey protein powder or salmon meal at different concentrations.

	Protein powder concentration [%]				
Ingredient	**0**	**5**	**10**	**15**	**20**
Wheat flour [g]	38.0	34.9	31.7	28.6	25.4
Wholemeal [g]	88.0	84.9	81.7	78.6	75.4
Protein powder [g]	0.0	6.3	12.6	18.9	25.2
Salt [tsp]	0.13	0.13	0.13	0.13	0.13
Water [90 mL]	0.9	0.9	0.9	0.9	0.9
Oil [tbs]	0.5	0.5	0.5	0.5	0.5
Yeast [g]	25.0	25.0	25.0	25.0	25.0

The doughs were made by mixing wheat flour, wholemeal, salt and different concentrations of protein powder. When the yeast was fully dissolved in water, oil was added and the solution was poured into the flour mixture. The doughs were kneaded until desired consistency and were left to ferment for 30 min in room temperature (20 ºC). The doughs made with whey protein powder, needed some extra wheat flour in order to get a consistency that it was possible to work with. Each dough was cut into 50 g portions that were shaped into bread rolls by hand. The bread rolls were again left to ferment for 30 min at room temperature. They were baked at 230 ºC for 13 min in an electric oven (Haka) and cooled at room temperature. Five bread rolls of each formulation were produced from the amounts given in Table 7.

Preparations of Drinking Yoghurt

In order to test whether a drinking yoghurt could be a suitable model product, Biola with blueberry taste (Tine) was mixed in a plastic cup with three different concentrations of whey and herring protein powder. 20 g of Biola was mixed with 1, 3 and 5 % (w/w, weight of Biola) protein powder and stirred well. This was not tested for the salmon meal.

The same amount of herring protein powder used above, was also dissolved in 20 mL distilled water in a 50 mL centrifuge tube. The samples were put into boiling water for 15 min, and then cooled. The samples were centrifuged at 2000 × g and 20 ℃ for 5 min in an Eppendorf Centrifuge 5804R. The supernatant was removed and the protein powder (precipitate) in the bottom was mixed with 20 g of Biola.

2.2.19 Sensory Analysis of Model Products

Bread Rolls

The sensory analyses were performed on the same day as the bread rolls were made. Each bread roll was sliced into 4 pieces. The different protein powder concentrations were given different three digit/letter codes and the correct piece of bread roll were laid out on paper plates as shown in Figure 7.

Figure 7. Paper plates used to serve the bread rolls. The plates were divided into 5 pieces with a three letter/digit code. Each plate contained bread rolls with concentrations of 0, 5, 10, 15 and 20 % protein powder.

The sensory analysis of the bread rolls baked with herring and whey protein powder was performed on the same day. The sensory analysis of bread rolls containing salmon meal was performed a couple of weeks later, but they were also baked the same day as tasted.

The assessors evaluated first the plate with bread rolls mixed with whey protein powder, then the plate with herring protein powder. The first sample on each plate, was a piece with no protein powder added. Then the samples were tasted in increasing protein powder concentration. In total, the assessors tasted 10 samples the first day. The bread rolls baked with salmon meal was tasted in the same order as above, with a total of 5 samples. The sensory evaluation was based on a 9-point hedonic scale (9 - like it extremely, 5 - neither like nor dislike it, 1 - dislike it extremely) (Lawless and Heymann, 2010). The three questionnaires used in the evaluation of the bread rolls are given in Appendix M.1.

For both sensory evaluations, it was used an untrained panel mainly consisting of female students in the age range 23-26 years old. In total, 17 people participated in the analysis of bread rolls enriched with herring and whey protein powder, while 18 people tasted the ones baked with salmon meal. Many of the students participating in the second round had already participated in the first and had a better knowledge of the product and procedure than some of the other participants.

The sensory analysis was performed in a study room at the university. The participants were given a time interval where they could come and therefore the number of students being in the assessment room varied. They assessors were not separated in any way, so they were able to hear and see each other.

Drinking Yoghurt

The mixtures with Biola and protein powders were tasted with increasing protein powder concentration by the author of this master thesis. The samples with whey protein powder were tasted first, then the samples with herring protein powder and at last the samples with herring protein powder that had been cooked. The sensory quality of Biola mixed with herring protein powder was determined to be so low that a more scientific sensory analysis with several assessors was not performed.

2.3 Statistical Analysis

Standard deviation (SD) has been used as a measurement for the uncertainty of the result presented. To calculate the SD, the function *STDEVA* in Microsoft Excel has been used.

The Analysis ToolPak with the function *t-Test: Paired Two Sample for Means* was used to perform a paired two-sample Student`s t-Test in Microsoft Excel.

3 Results and Discussion

3.1 Protein, Fat, Moisture and Ash Content

The chemical composition of a protein powder or a fish meal is influenced by raw material, production method and processing conditions (Šližytė et al., 2016a, Jensen and Keller, 1990). Proximate composition of the herring protein powder produced by ethanol extraction, salmon meal produced by cooking, pressing, drying and grinding of the raw material and the whey protein powder are given in Table 8. The production method of the whey protein powder is unknown. Measured values used to calculate the amount of crude protein, fat, moisture and ash content for the different powders can be found in Appendix B.1, B.2, B.3 and B.4, respectively.

Table 8. Proximate composition of herring protein powder, salmon meal and whey protein powder. The measurements of crude protein were performed in quintuplicates, fat content in quadruplicates and the moisture and ash content in duplicates. The values are given as mean ± SD.

Component [%]	Herring protein powder	Salmon meal	Whey protein powder
Crude protein*	81.5 ± 0.3	64.0 ± 5.3	74.7 ± 1.5
Fat	< 0.5**	17.3 ± 1.0	4.4**
Moisture	5.18 ± 0.04	5.3 ± 0.3	4.9 ± 0.1
Ash	1.47 ± 0.03	10.3 ± 0.3	3.2 ± 0.1
Sum	88.7	96.9	87.2

* Nitrogen·6.25. ** Obtained from the producer. Given in Appendix A.

Most proteins consist of approximately 16 % nitrogen (Coultate, 2016). The protein content of the powders was determined by multiplying the measured amount of total nitrogen with a specific conversion factor of 6.25. As seen from Table 8, all of the protein powders had a crude protein content above 60 %. According to the producers, the herring protein powder and the salmon meal should contain minimum 80.0 and 66.0 % protein, respectively, while the whey protein powder should contain 82.7 % protein. The protein content of the herring protein powder was 81.5 ± 0.3 %, while the whey protein powder and salmon meal contained 74.7 ± 1.5 and 64.0 ± 5.3 % protein, respectively. The protein content of the salmon meal is thus slightly below the given quality requirements. The measured protein content of the whey

protein powder was 8 % lower than the value given by the producer. The protein content of the herring protein powder is in agreement with the protein content measured for immature herring egg proteins (82.6 g/100 g) by Havenaar et al. (2016). It is approximately 8 % higher than the protein content measured in a protein powder from herring body (73.4 g/ 100 g) (Sathivel et al., 2004). The protein content of herring roe, which was the raw material for the HPP varies through the season (Larssen et al., 2014) and this may affect the protein content in the HPP. The protein content of a fish meal is usually lower than a protein powder meant for human consumption. The protein content of the salmon meal was similar to the protein content measured in a fish meal from Alaska white fish (67.2 g/100 g) (Sathivel et al., 2005a).

The difference in moisture content did not vary significantly between the powders. WPP had the lowest moisture content of 4.9 ± 0.1 % and salmon meal had the highest with a moisture content of 5.3 ± 0.3 %. None of the protein powders were stored in a desiccator. The herring and whey protein powder were stored in plastic boxes while the salmon meal was stored in a plastic bag. This may have affected the moisture content.

The largest variations between the powders were found in the fat and ash content. The herring and whey protein powder had a low ash content, which was 1.47 ± 0.03 and 3.2 ± 0.1 %, respectively, while salmon meal contained 10.3 ± 0.3 % ash. The ash content of the herring protein powder was much lower compared to the ash content in a protein powder made from herring body (17.7 g/100 g) (Sathivel et al., 2004). The ash content of the salmon meal was almost identical to the ash content of a fish meal produced from herring (10.4 g/100 g) (Tibbetts et al., 2006). Due to the higher content of bones, the ash content in a fish meal made from fish processing byproducts is generally higher compared to fish meals made from the whole fish (Sathivel et al., 2005a).

As illustrated in Figure 2, the herring protein powder was produced with ethanol extraction. This is a production method which effectively removes lipids from the proteins and hence results in a product with a low fat content (Kristinsson and Rasco, 2000b). Since the fat content of the herring protein powder was less than 0.5 %, the requirement of max 3.0 % fat, specified by the producer, was achieved. The salmon meal had the highest fat content (17.3 ± 1.0 %), which exceeds the 14 % quality level set by the producer. The production method shown in Figure 4 does not separate the lipids from the proteins as effectively as the ethanol extraction method, and results in a powder with a higher lipid content. The fat content of the commercial

whey protein powder was specified to be 4.4 g/100 g by the producer, which is similar to the fat content in a WPC (4.9 % dry basis) produced by Sinha et al. (2007). A high fat content is not desirable in a protein powder meant for human consumption. Oxidation of the lipids can have a negative influence on the sensory properties of the protein powder (Kristinsson and Rasco, 2000b). The fat content of a fish meal is influenced by the raw material. Fish meals made from fatty fishes such as salmon or herring will usually have a higher fat content compared to fish meals made from white fish (WINDSOR, 2001a).

The total sum of the protein, fat, moisture and ash content is also given in Table 8. When these components are added together, the total sum was 88.7, 96.9 and 87.2 % for herring protein powder, salmon meal and whey protein powder, respectively. It is therefore 11.3, 3.1 and 12.8 % of the powders, which is not accounted for, respectively. Havenaar et al. (2016) analysed the composition of herring roe proteins derived from whole eggs and found that they contained 3.2 g/100 g carbohydrates. It is therefore possible that approximately 3 % of the unaccounted material in the herring protein powder is carbohydrates. According to the producer, the whey protein powder contains approximately 4.9 g of carbohydrates. When the approximate amount of carbohydrates is included, the unaccounted material in the herring and whey protein powder is decreased to approximately 8.1 and 7.9 %. The fat content of these powders was not measured, so it is possible that these values were slightly higher than specified by the producers.

3.2 Amino Acid Composition

The nutritional value of proteins can be described by the amino acid composition. Different raw materials have different amino acid compositions (Opheim et al., 2015). Proteins from animal sources have generally a higher nutritional value compared to plant proteins, as plant proteins are deficient in some amino acids (Ustunol, 2014a). In order to find the amino acid composition, the protein powders were hydrolysed in 6 M HCl and analysed with reverse phase HPLC. In the calculations of the amino acids, the molecular weights without water were used. The total amino acid composition of herring protein powder, whey protein powder and salmon meal, together with the amino acid composition provided by the producers of the herring and whey protein powder, are given in Table 9. Calculated values for the amino acid composition for the powders can be found in Appendix C.

Table 9. Amino acid composition of herring protein powder (HPP), whey protein powder (WPP) and salmon meal compared with the amino acid composition provided by the producer for herring and whey protein powder. The number of HPLC measurements for the salmon meal were six. The number of HPLC measurements for WPP and HPP were four, because some of the samples were lost during the HPLC measurements and some were excluded since they deviated a lot from the other parallels. Values are given as mean ± SD.
[g/100 g protein powder].

Amino acid	**HPP**	**HPP** (Producer)	**WPP**	**WPP** (Producer)	**Salmon meal**
Aspartate	6.55 ± 0.37	6.68	7.55 ± 0.21	9.26	4.04 ± 0.15
Glutamate	**	10.3	13.47 ± 0.46	15.23	**
Asparagine	0.04 ± 0.05	nd	0.00 ± 0.00	nd	0.00 ± 0.00
Histidine	1.83 ± 0.04	1.82	1.12 ± 0.06	1.56	0.90 ± 0.07
Serine	3.68 ± 0.10	4.31	3.24 ± 0.07	4.20	2.09 ± 0.11
Glutamine	0.00 ± 0.00	nd	0.00 ± 0.00	nd	0.23 ± 0.04
Glycine/Arginine	3.88 ± 0.22	7.24	1.36 ± 0.03	3.31	3.34 ± 0.43
Threonine	4.83 ± 0.19	4.94	5.06 ± 0.10	5.79	1.95 ± 0.09
Alanine	7.06 ± 0.38	7.66	3.24 ± 0.08	4.23	2.48 ± 0.21
Tyrosine	3.82 ± 0.50	3.39	1.93 ± 0.04	2.31	1.44 ± 0.09
Aba	0.12 ± 0.04	nd	0.16 ± 0.01	nd	0.13 ± 0.01
Methionine	2.04 ± 0.26	2.30	1.47 ± 0.03	1.83	1.26 ± 0.06
Valine	5.87 ± 0.10	6.24	3.73 ± 0.08	4.93	1.98 ± 0.09
Phenylalanine	4.43 ± 1.18	3.31	2.11 ± 0.07	2.54	1.74 ± 0.08
Isoleucine	5.42 ± 0.05	5.34	4.38 ± 0.10	3.23	1.66 ± 0.07
Leucine	10.83 ± 1.75	9.25	7.13 ± 0.18	8.97	3.05 ± 0.13
Lysine	8.45 ± 2.11	6.30	6.87 ± 0.18	8.04	3.21 ± 0.13
Cysteine*	nd	0.909	nd	2.03	nd
Proline*	nd	4.76	nd	4.90	nd
Tryptophan*	nd	1.25	nd	1.27	nd
Total	68.84 ± 5.21	86.00/79.08*	62.82 ± 1.60	83.63/75.43*	29.63 ± 1.06

*The amino acids cysteine, proline and tryptophan were not measured with the acid hydrolysis. To make the comparison easier, the total amount of amino acids calculated from the values given by the producers, are given as "with/without cysteine, proline and tryptophan".
**Not included because of unreliable measurements. nd: not determined

As seen from Table 9, the measured amount of amino acids in the herring protein powder correlated well with the amino acid distribution provided by the producer. Glycine/arginine, leucine and lysine were the measured values that deviated the most. There were some problems with the glutamate standards used for the HPLC measurements. The glutamate values measured for the herring protein powder were above 1000 g/100 g protein powder, which is not possible. The problem with the glutamate values affected the salmon meal samples as well, and resulted in huge variations between the different parallels. The glutamate values for the HPP and the salmon meal were therefore not included in Table 9. According to the producer, the amount of glutamate should be approximately 10.3 g/100 g. If this value is added to the total sum in the second column in Table 9, the total sum becomes 79.14 g/100 g. This sum correlates well with the total amount of amino acids, without cysteine, proline and tryptophan given in the third column in the same Table (79.08 g/100 g). The amino acid distribution of the herring protein powder is slightly different from the amino acid distribution of a protein powder made from herring body (HBP), especially in the glycine/arginine content as shown in Table 2 (section 1.5.3).

As seen from Table 9, not all the amino acids were measured after the acid hydrolysis. Tryptophan is destroyed in acid hydrolysis and was therefore not detected. Asparagine (Asn) and glutamine (Gln) are unstable in acid and were converted to aspartic acid (Asp) and glutamic acid (Glu), respectively. The amount quantified with HPLC as Asp was hence the sum of Asn and Asp. Glu was quantified as the sum of Glu and Gln (Christensen, 2015). The amount of cysteine cannot be determined directly from the acid hydrolysed samples either (Fountoulakis and Lahm, 1998). The total amount of protein in the herring protein powder measured with the C/N analysis was 81.5 ± 0.3 g/100 g. If all of the amino acids had been measured after the acid hydrolysis, it may have been slightly above the protein content measured with the C/N analysis.

Except for isoleucine, the measured values for the amino acids in the whey protein powder were all lower compared to the values obtained from the producer. Some amino acids correlated better than others, but ideally the values should have been more similar. The measured total amount of amino acids was 62.8 ± 1.6 g/100 g. This is 12.6 g lower than specified by the producer when cysteine, proline and tryptophan are excluded. The molecular mass used to calculate the amount of amino acids (with or without water) would influence the results, but

would not give such a large difference as observed in Table 9. The total amount of amino acids from the acid hydrolysis is 11.88 g lower than the total protein content measured with the C/N analysis (74.7 ± 1.5 g/100 g). The dominating amino acids in the whey protein powder were aspartate, glutamate, leucine and lysine with 7.55 ± 0.21, 13.47 ± 0.46, 7.13 ± 0.18 and 6.87 ± 0.18 g/100 g, respectively. These were also the dominating amino acids in a WPC investigated by Sinha et al. (2007), as shown in Table 2. Whey proteins have a high content of the amino acid cysteine. As seen from Table 9, the WPP contained more than twice as much cysteine as the HPP. This sulphur containing amino acid can be used to produce glutathione, which protects the body against free radical damage (Farkye and Shah, 2014).

The amount of the different amino acids in the salmon meal was generally much lower compared to the HPP and WPP. As mentioned before, the amount of glutamate was not included in Table 9 due to the problems with the glutamate standards. The total amount of amino acids was measured to be 29.6 ± 1.1 g/100 g. The amount of glutamate was measured to be 11.31 g/100 g in a hydrolysate from salmon by-products (viscera, head and frames) (Opheim et al., 2015). By assuming the salmon meal contains glutamate in similar amounts as the salmon hydrolysate, the total amount of amino acids would be approximately 40.9 g/100 g. Even though a complete amino acid composition cannot be obtained after an acid hydrolysis, this value does not correlate with the amount of protein measured with the C/N analysis (64.0 ± 5.3 g/100 g). With the assumption of the amount of glutamate, the value from the acid hydrolysis is approximately 23 g lower than the value from the C/N analysis. An explanation for this difference may be loss of protein residues during the preparation of the HPLC samples. Another possible explanation could be an error with the HPLC standards, which may have resulted in an underestimation of the amino acids.

The protein content calculated from the total amino acid composition was generally lower compared to the protein content measured with the C/N analysis. As mentioned earlier, some of the amino acids are unstable in acids and cannot be measured after an acid hydrolysis. This will reduce the total amount of protein measured. Another explanation for the difference could be the conversion factor of value 6.25. This factor is based on the assumption that the average nitrogen content in a protein is 16 %, and by dividing 100 by 16 a conversion factor of 6.25 is obtained (Coultate, 2016). Nitrogen from other sources, such as free amino acids, amines, nucleotides and urea will also contribute to the protein content when the amount of protein is calculated based on the nitrogen content. Since the protein content varies between

different protein sources, this factor may lead to an underestimation or overestimation of the protein content. A conversion factor between 5.43 and 5.82 have been suggested for fish (Rustad, 2009). The conversion of nitrogen to protein have been thoroughly reviewed by Mariotti et al. (2008), and they proposed a set of specific conversion factors to be used for different foodstuff, with 5.6 as the default.

Of the 20 most common amino acids, humans are not able to synthesize half of them and these essential amino acids need to be provided through the diet. The essential amino acids are isoleucine, leucine, lysine, methionine, phenylalanine, threonine tryptophan and valine. The synthesis of the amino acids arginine and histidine can be insufficient during growth and are therefore some of the conditionally essential amino acids (Store norske leksikon, 2017).

The essential amino acid composition of herring protein powder, whey protein powder and salmon meal, together with suggested essential amino acid profiles by FAO/WHO for infants and adult humans are provided in Table 10. Values received from the producers of the herring and whey protein powders are also included.

Table 10. Essential amino acid composition of herring protein powder (HPP), whey protein powder (WPP) and salmon meal together with values specified by the producers of herring and whey protein powder. Suggested essential amino acid profiles by FAO/WHO are also included for infants and adult humans[b]. The values are given as averages. (n = 4 for HPP and WPP, n = 6 for salmon meal). [g/100 g protein powder].

Amino acid	**HPP**	**HPP** (Producer)	**WPP**	**WPP** (Producer)	**Salmon meal**	**FAO**[b] (infant)	**FAO**[b] (adult)
Histidine'	1.83	1.82	1.12	1.56	0.90	2.6	1.6
Isoleucine*	5.42	5.34	4.38	3.23	1.66	4.6	1.3
Leucine*	10.83	9.25	7.13	8.97	3.05	9.3	1.9
Lysine*	8.45	6.30	6.87	8.04	3.21	6.6	1.6
Methionine* + Cysteine	2.04[a]	3.21	1.47[a]	3.86	1.26[a]	4.2	1.7
Phenylalanine* + Tyrosine	8.25	6.70	4.04	4.85	3.18	7.2	1.9
Threonine*	4.83	4.94	5.06	5.79	1.95	4.3	0.9
Tryptophan*	nd	1.25	nd	1.27	nd	1.7	0.5
Valine*	5.87	6.24	3.73	4.93	1.98	5.5	1.3
TEAA	47.52	45.05	33.80	42.50	17.19	46.00	12.70
TEAA/TAA (%)	69.0	-	53.8	-	58.0	-	-

* Essential amino acids. ' Essential for infants and children in growth. TEAA: total essential amino acids. TAA: total amino acids. nd: Not determined. [a]Only methionine, since cysteine was not measured after the acid hydrolysis. [b](FAO/WHO, 1991).

The essential amino acids in the herring protein powder exceed the requirements suggested for an adult by FAO/WHO. This applies for both the values measured after the acid hydrolysis and the values obtained from the producer. The essential amino acid requirements are much higher for infants and children in growth, compared to the requirements for an adult. As seen from Table 10, the values for the herring protein powder are slightly below the requirements for infants for some of the amino acids. The essential amino acid composition of the whey protein powder is also sufficient for an adult human. Except for threonine, all of the values are below the requirements for an infant. Although the amount of essential amino acids was significantly lower in the salmon meal, it exceeds the requirements for an adult human for all of the amino acids except histidine and methionine + cysteine. The essential amino composition of the salmon meal did not exceed the requirements for an infant.

The total amount of essential amino acids in the herring protein powder (producer value) is slightly above the value for the whey protein powder (producer values). It is much higher than

the total amount of essential amino acids required for an adult and almost as high as the total amount required by an infant. Based on the amino acid composition obtained from the acid hydrolysis, herring protein powder has the highest nutritional value, followed by the whey protein power and salmon meal in decreasing order. The ratio of the total essential amino acids to the total amino acids (TEAA/TAA) was highest in the herring protein powder (69 %). Salmon meal had a higher TEAA/TAA ratio (58.0 %) compared to the whey protein powder (53.8 %). Even though the amount of amino acids in the salmon meal was low compared to the other two, the percentage of essential amino acids was high.

3.3 Free Amino Acids

3.3.1 Salmon Meal and Whey Protein Powder

Not all amino acids are incorporated into proteins. The amount of free amino acids (FAA) were investigated for the salmon meal and whey protein powder dissolved in distilled water and citric acid-phosphate buffer with pH 3, 5 and 7. The total amounts of FAA in salmon meal and WPP are given in Figure 8 and 9, respectively. In the calculations of FAA, the molecular weights without water were used. Calculated values for the FAA in salmon meal and whey protein powder can be found in Appendix D.1 and D.2, respectively.

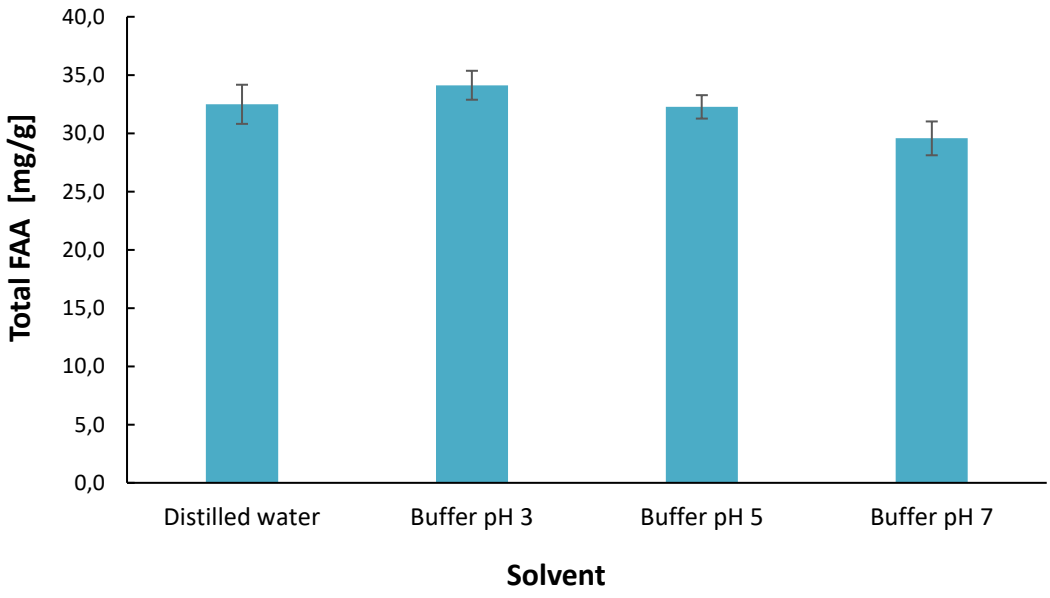

Figure 8. Total amount of free amino acids (FAA) (mg/g) in salmon meal dissolved in water and citric acid-phosphate buffer with pH 3, 5 and 7. The measurements were performed in triplicates. Values are given as mean ± SD.

The total amount of FAA did not vary significantly between the different solvents. It was highest in the citric acid-phosphate buffer with pH 3 (34.1 ± 1.2 mg/g) and decreased slightly with increasing pH. In citric acid-phosphate buffer with pH 5 and 7, the total amount of FAA was 32.3 ± 1.0 mg/g and 29.6 ± 1.5 mg/g, respectively. The total amount of FAA in distilled water was 32.5 ± 1.7 mg/g and thus almost the same as in the buffer with pH 5. The total amount of FAA in salmon meal dissolved in all the different solvents are slightly below the amount of FAA found in non-hydrolysed fish solubles from Atlantic salmon heads and backbones (41.52 mg/g protein) (Aspevik et al., 2016). Compared to the initial amount of FAA in hydrolysates made from salmon viscera, head and frames (endogenous enzymes, 212 mg/g hydrolysate) or only viscera (heat inactivated endogenous enzymes, 136 mg/g hydrolysate), the amount of FAA in the salmon meal was low (Šližytė et al., 2016a).

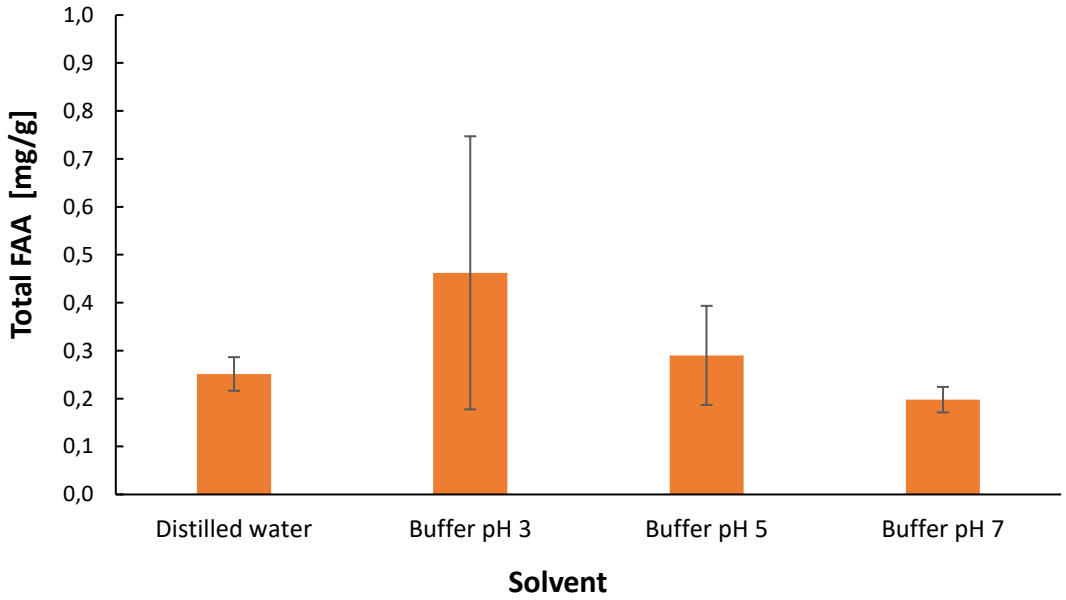

Figure 9. Total amount of free amino acids (FAA) (mg/g) in whey protein powder dissolved in dissolved water and citric acid-phosphate buffer with pH 3, 5 and 7. The measurements were performed in triplicates. Values are given as mean ± SD.

As with the salmon meal, the amount of FAA was highest in citric acid-phosphate buffer with pH 3 (0.46 ± 0.29 mg/g). The amount of FAA decreased with increasing pH and the total amount of FAA decreased to 0.198 ± 0.03 mg/g at pH 7. In distilled water the FAA content was 0.25 ± 0.04 mg/g, which was below the FAA content in the buffer with pH 5 (0.29 ± 0.10 mg/g). The measurements were performed in triplicates and there were major variations between the parallels for the FAA content for the whey protein powder dissolved in citric acid-phosphate buffer with pH 3. As seen from Figure 9, the standard deviation is therefore quite

high. The reason for the major variations between the parallels may be due to inaccurate pipetting, which would have resulted in varying amounts of SSA added to the extract. The amount of FAA in the whey protein powder was considerably lower than in the salmon meal. The salmon meal was made from trimmings and guts and it is possible that endogenous enzymes found in the guts have slightly hydrolysed the raw material and hence released some free amino acids before the enzymes were inactivated in the cooking stage.

The distributions of the amino acids in the different solvents are given in Figure 10 and 11 for salmon meal and whey protein powder, respectively.

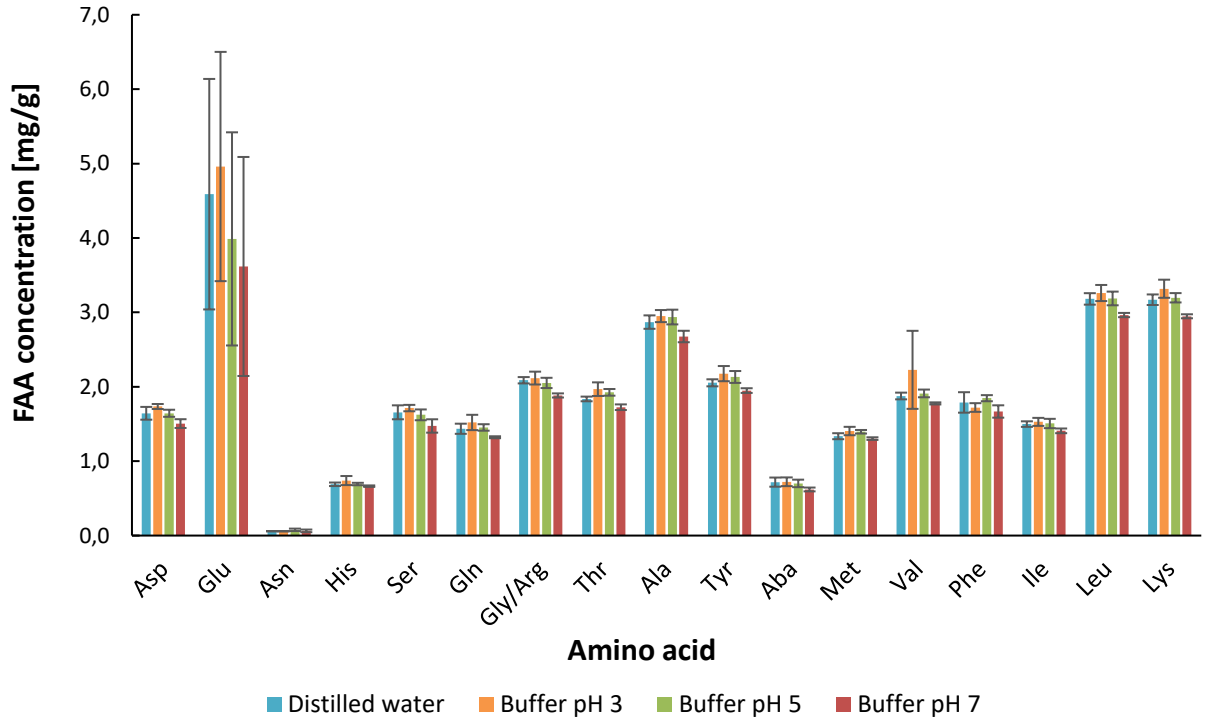

Figure 10. Distribution of free amino acids (FAA) (mg/g) in salmon meal dissolved in distilled water and citric acid-phosphate buffer with pH 3, 5 and 7. The measurements were performed in triplicates. Values are given as mean ± SD.

Free amino acids influence the taste of food significantly and major free amino acids of seafood muscle are taurine, proline, glycine, glutamine, alanine and arginine (Undeland et al., 2009). As seen from Table 10, the major FAA in the salmon meal were glutamate, alanine, leucine and lysine. These amino acids were also among the dominating amino acids in a hydrolysate made from rest raw material (viscera, head and frames) from Atlantic salmon (Opheim et al., 2015). The content of asparagine was very low, only 0.059 ± 0.001 mg/g in

distilled water. The other amino acids were quite evenly distributed. The amount of the different amino acids did not vary significantly between the different solvents. The highest amount of the different amino acids was generally obtained when the protein powder was dissolved in citric acid-phosphate buffer with pH 3. The standard deviations for the glutamate values were high. During the data processing of the HPLC samples, there were some problems with the glutamate standards, which may explain the high standard deviation calculated for this amino acid.

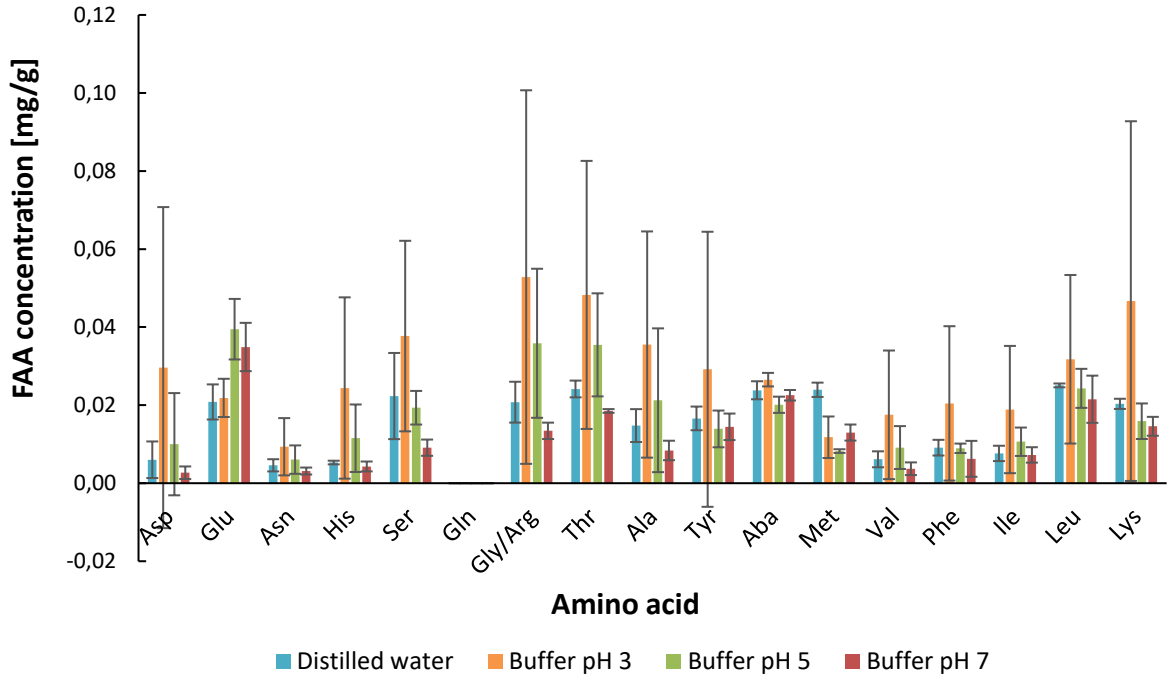

Figure 11. Distribution of free amino acids (FAA) (mg/g) in whey protein powder dissolved in distilled water and citric acid-phosphate buffer with pH 3, 5 and 7. The measurements were performed in triplicates. Values are given as mean ± SD.

The content of FAA in the whey protein powder was evenly distributed between the different amino acids. Glutamine was not detected as a free amino acid. The amount of FAA was highest when the whey protein powder was dissolved in citric acid-phosphate buffer with pH 3. The standard deviations, especially for values corresponding to whey protein powder dissolved in buffer with pH 3, were very high. The amount of FAA in the whey protein powder was generally very low, so it is possible that the variations displayed in Figure 11 were caused by errors with the detection due to the small amount of FAA. Due to the high SD of the values given in Figure 9 and 11 for whey protein powder dissolved in buffer at pH 3, the presented

values should only be considered as an indication of the amount of FAA, not the absolute volumes.

3.3.2 Herring Protein Powder Hydrolysate

As mentioned earlier, the solubility of the herring protein powder was investigated in the specialisation project, where the amount of soluble proteins was only 1.3 % in distilled water. In the same study, the amount of FAA in the HPP dissolved in distilled water and citric acid-phosphate buffer with pH 3, 5 and 7 was measured. Due to the low solubility, trypsin was used to hydrolyse the herring protein powder, and the amount of FAA after 30, 60 and 125 min of hydrolysis was investigated. The solubility as a function of hydrolysis time is given in section 3.6. Trypsin is a serine protease and cuts the peptide bonds at the C-terminal side of Lys and Arg residues (Nelson et al., 2013). If a Lys or Arg residue is located at the terminal end of the peptides, free amino acids will be released by the breakage of peptide bonds. The total amount of FAA in the herring protein powder hydrolysate as a function of hydrolysis time is given in Figure 12. Calculated values for the free amino acids in herring protein powder hydrolysates can be found in Appendix D.3.

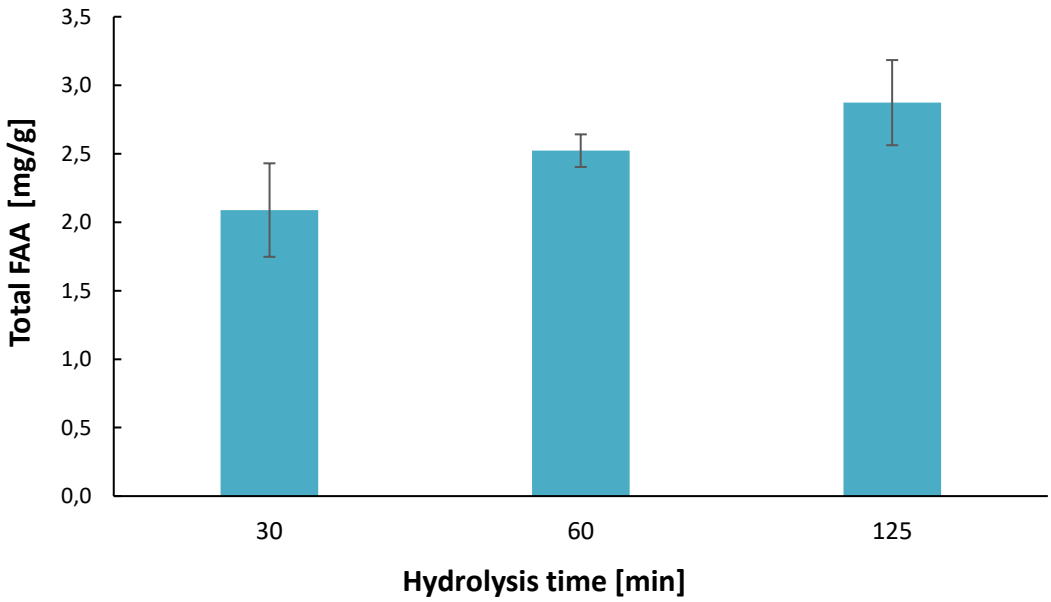

Figure 12. Total amount of free amino acids (FAA) in herring protein powder hydrolysate as a function of hydrolysis time (min). The measurements were performed in quadruplicates. Values are given as mean ± SD.

The total amount of FAA increased with increasing hydrolysis time. After 30 min of hydrolysis, the total amount of FAA was 2.1 ± 0.3 mg/g, and after another 95 min of hydrolysis it had increased to 2.9 ± 0.3 mg/g. These values are both higher than the values obtained for the unhydrolysed herring protein powder dissolved in citric acid-phosphate buffers. They ranged from 1.48 ± 0.03 to 1.68 ± 0.06 mg/g at pH 3 and 7, respectively. Measurements of the amount of FAA in unhydrolysed herring protein powder dissolved in distilled water should also have been performed. This was investigated in the specialization project, but due to problems with the instrument no results was achieved from these measurements. It was unfortunately not enough time to repeat these measurements.

Wu et al. studied the change in the FAA composition of protein hydrolysates from mackerel as a function of hydrolysis time. They found that the total amount of FAA increased with increasing time of hydrolysis (Wu et al., 2003). An increase in the FAA content as a function of the time of hydrolysis was also expected for the herring protein powder hydrolysates. Although the amount of FAA increased with increasing hydrolysis time, the change was very small. This indicates that the enzymatic hydrolysis was not very efficient, which is further discussed in section 3.6.1. The degree of hydrolysis (DH) was not determined for the herring protein powder hydrolysates, but is expected to be low. In hydrolysates, there is a positive correlation between the DH and a high amount of FAA (Šližytė et al., 2016a). A high DH usually results in a high amount of FAA. In addition to the DH, the type of proteolytic enzymes used will affect the amount of FAA. This was illustrated by Šližytė et al. who hydrolysed salmon heads, frames and viscera with different enzymes (Protamex, Papain and Bromelain) for 120 min (Šližytė et al., 2016a).

The amount of FAA in freeze-dried herring roe protein hydrolysates was investigated by Hansen (2011), who reported values in the range of 140 to 190 mg/g when different enzymes were used. These values are significantly higher compared to the values obtained for the herring protein powder hydrolysates. The higher amount of FAA in these hydrolysates can probably be explained by a higher degree of hydrolysis. All the herring roe hydrolysates studied by Hansen had a degree of hydrolysis above 27 %.

The content of different free amino acids were also investigated and the distribution of the free amino acids in herring protein powder hydrolysates after 30, 60 and 125 minutes of hydrolysis is given in Figure 13.

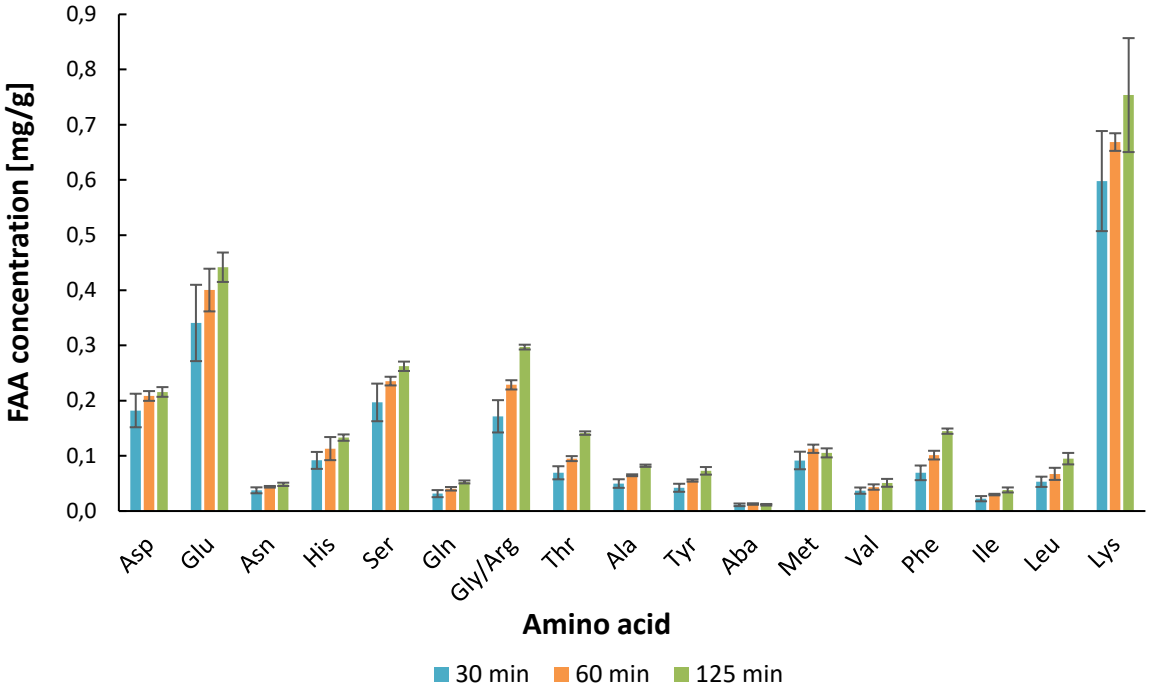

Figure 13. Distribution of free amino acids (FAA) (mg/g) in herring protein powder hydrolysates after 30, 60 and 125 min of hydrolysis. The measurements were performed in quadruplicates. Values are given as mean ± SD.

The amount of the different amino acids increased with increasing hydrolysis time. The dominating free amino acids were equal, independent of the time of hydrolysis. As seen from Figure 13, lysine was the major FAA in the hydrolysates made from herring protein powder. The amount of lysine increased from 0.60 ± 0.09 to 0.75 ± 0.10 mg/g from 30 to 125 minutes of hydrolysis, respectively. Aspartate, glutamate, serine and glycine/arginine were other dominating amino acids. Especially for proteins of plant origin, lysine is the limiting factor for the biological value of the protein (Belitz et al., 2009). Since fish proteins is a rich source of lysine, addition of fish proteins in food can increase the nutritional value of the product.

The dominating free amino acids in the herring protein powder hydrolysate were similar to the dominating FAA in the herring roe protein hydrolysates studied by Hansen (2011). However, the amounts of the dominating amino acids, lysine, glycine/arginine, serine and glutamate, were significantly higher in the herring roe protein hydrolysates.

3.4 Molecular Weight Distribution

The molecular weight distribution of salmon meal, whey protein powder and herring protein powder hydrolysates after 30, 60 and 125 min of hydrolysis were analysed with gel filtration in a FPLC system. The molecules were separated based on their molecular weights (size exclusion chromatography) in a column that separates molecules within a molecular range of 100-7000 Da. Smaller molecules will be retained in the column for a longer time than the larger molecules as they can move in and out of the particles in the column. The largest particles will hence be detected first and shown early in the chromatogram. Standards, together with the standard curve used to estimate the molecular weights of the peptide fractions in the protein powders are given in Appendix E.1. Retention time and estimated molecular weights of the major peptide fractions in salmon meal and whey protein powder are given in Table 11 and 12, respectively. Chromatograms can be found in Appendix E.2 and measured values used in the calculation of the molecular weights in Appendix E.3.

Table 11. Retention time (R_T) and estimated molecular weight (MW) of peptide fractions in salmon meal. The major factions are marked with a star (*).

R_T [min]	MW [Da]
33.70	900*
35.85	550*
39.32	260*
42.26	140*
52.88	13
56.50	6

The salmon meal consisted of four major fractions with molecular weights of approximately 900, 550, 260 and 140 Da. In addition, the chromatogram contained many peaks with low molecular weights, such as 13 and 6 Da. Several amino acids have molecular weights in the range of 130 to 150 Da and the peak with the molecular weight at around 140 Da may correspond to several free amino acids. The average molecular weight of an amino acid is 110 Da and glycine is the smallest amino acid with a molecular weight of 75 Da (Aylward and Findlay, 2008). Fractions with molecular weights smaller than glycine may thus not correspond to amino acids. Different columns can be used to separate structures with different molecular sizes. The peptide fractions were separated with a column that separates peptides with

molecular weights of 100-7000 Da and the accuracy of the molecular weights outside this range cannot be given. The retention time could be affected by the affinity the molecules have for the column material. As a result, larger molecules may use a longer time in the column than expected based on their molecular weights.

The molecular weight distribution of proteins in rest raw material from Atlantic salmon (head, skin, frame, viscera and belly flap) have been reported to lie in the range of 25-250 kDa. The proteins from viscera had a molecular weight around 10 kDa and is hence below this range (He et al., 2011). These molecular weights are much higher than the molecular weights found for the salmon meal in this study. However, it is important to mention that the solubility of salmon meal in distilled water was only 19.6 ± 0.8 % (section 3.6). It is possible that the higher molecular weight proteins were not solubilized in the buffer, and was thus not detected with the gel filtration.

Table 12. Retention time (R_T) and estimated molecular weight (MW) of peptide fractions in whey protein powder. The major factions are marked with a star (*).

R_T [min]	**MW [Da]**
15.42	49 000
21.32	13 000*
26.12	4700*

The whey protein powder was dominated by two peptide sizes, 13 000 and 4700 Da. It also contained a small fraction with a high molecular weight of 49 000 Da. These values are similar to the results obtained for the whey protein powder with chocolate taste during the specialization project. The chocolate whey protein powder was dominated by two peptide fractions of 14 000 and 4500 Da. The main proteins in whey are α- and β-lactoglobulin, but whey also consists of other proteins, such as bovine serum albumin (BSA) and Immunoglobulines. The molecular weight of α- lactoglobulin, β-lactoglobulin and BSA is 14 146, 18 300 and 66 433 Da respectively (Farkye and Shah, 2014). Some of the proteins in the whey protein powder were hydrolysed whey proteins (Appendix A.2) and the fractions of 13 000 and 4700 Da could be hydrolysed α- and β-lactoglobulin, while the smaller fraction of 49 000 could be hydrolysed BSA. The few peaks in the chromatogram indicate that the protein powder only consists of a few different peptide sizes.

The salmon meal consisted of peptide fractions with low molecular weights, especially compared to the whey protein powder. The fraction with a retention time of 42.26 min and a molecular weight around 140 Da could be free amino acids. Peptide fractions with molecular weights that low, were not detected for the whey protein powder. This correlates well with the fact that the content of free amino acids was significantly higher in the salmon meal compared to the whey protein powder.

Functional and bioactive properties, such as solubility, emulsification and antioxidative properties, are influenced by the molecular weights of the proteins, which will be further discussed in section 3.6, 3.7 and 3.10. (Wilding et al., 1984, Turgeon et al., 1991, Shahidi and Li, 2014). The molecular weight is also important for the sensory properties of a protein (Aspevik et al., 2016).

Retention time and estimated molecular weight of peptide fractions in the herring protein powder hydrolysate after 30, 60 and 125 min of hydrolysis are given in Table 13. Chromatograms can be found in Appendix E.2, and the measured values used in the calculation of the molecular weights in Appendix E.3. The molecular weight distribution of non-hydrolysed herring protein powder was investigated in the specialization project. For comparison, this result is also included in Table 13.

Table 13. Retention time (R_T) and estimated molecular weight (MW) of peptide fractions in non-hydrolysed herring protein powder and hydrolysates after 0, 30, 60 and 125 min of hydrolysis.

Hydrolysis time [min]	R_T [min]	MW [Da]
0	14.46	61 000
	17.70	30 000
	33.92	850
	35.50	600
30	34.19	800
	36.37	500
60	24.42	6800
	35.53	600
	37.66	400
125	34.15	800
	36.23	500

The chromatogram of the unhydrolysed herring protein powder consisted of four major peaks, and the detected peptide fractions had molecular weights of 61 000, 30 000, 850 and 600 Da. Only peptide fractions solubilized in the buffer could be measured with the gel filtration. As mentioned earlier, the solubility of the herring protein powder was very low. Hence, the insoluble parts of the powder probably consists of peptide fractions with high molecular weights, which were not detected.

The chromatogram for the herring protein powder after 30 min of hydrolysis was slightly different than for the unhydrolysed powder. It consisted of two major peaks with estimated molecular weights of 800 and 500 Da. As mentioned above, the unhydrolysed powder consisted of peptides with molecular weights of 850 and 600 Da. It is possible that the 800 and 500 Da fraction correspond to hydrolysed peptide fractions originally having molecular weights of 850 and 600 Da. The area under the curve was much larger for the two peaks in the chromatogram after 30 min of hydrolysis than compared to the unhydrolysed sample.

After 60 min of hydrolysis, the chromatogram consisted of three major peaks with molecular weights of 6800, 600 and 400 Da. The peptide fraction with a molecular weight of 6800 Da could be peptide fractions that were insoluble after 30 min of hydrolysis, but after further hydrolysis, became soluble. Except for the peak with a molecular weight of 6800 Da, peaks with lower molecular weights were measured after 60 min of hydrolysis compared to 30 min.

After 125 min of hydrolysis the chromatogram was again dominated by two peaks with molecular weights of 800 and 500 Da. The chromatogram after 125 min of hydrolysis was almost identical to the chromatogram obtained after 30 min of hydrolysis. The main difference was that the area under the curves was larger for the peaks after 125 min of hydrolysis compared to the peaks after 30 min. The minor change in molecular weights between 30 and 125 min of hydrolysis correlates with the small change in FAA content (section 3.3), amount of acid soluble peptides (section 3.5) and solubility (section 3.6) between 30 and 125 min of hydrolysis. The major change in the FAA content, amount of acid soluble peptides and solubility took place from 0 to 30 min of hydrolysis. A high reaction rate in the beginning of an enzymatic hydrolysis is common and a decrease could be a result of enzyme denaturation, which continuously decreases the enzyme concentration, or inhibition of the enzyme by the newly formed products (Belitz et al., 2009). If there had been major changes in the molecular weight distribution between 30 and 125 min of hydrolysis, a higher increase in the solubility would have been expected. It is possible that something happened during the gel filtration of the 60 min sample and that the peptide fractions were detected somewhat later than they should. This would have resulted in detection of peptide fractions with lower molecular weights such as the 600 and 400 Da peaks.

The molecular weight distribution of hydrolysed and unhydrolysed herring was studied by Liceaga-Gesualdo and Li-Chan (1999). The soluble fraction of unhydrolysed herring contained peptides in the range of 14.2 to 45 kDa, while the molecular weight of the herring protein hydrolysate was below 6.5 kDa. These values are much higher compared to the values presented in Table 13 for the herring protein powder hydrolysate. Only the unhydrolysed protein powder had some peptide fractions within that range of molecular weight.

3.5 Acid Soluble Peptides

The amount of acid soluble peptides in the herring protein powder hydrolysates after 30, 60 and 125 minutes of hydrolysis was determined by precipitating the proteins with trichloroacetic acid (TCA). The results are given in Figure 14 as a function of hydrolysis time. Absorbance measurements and standard curve of BSA can be found in Appendix F.1, while absorbance measurements of the herring protein powder hydrolysates are given in Appendix F.2.

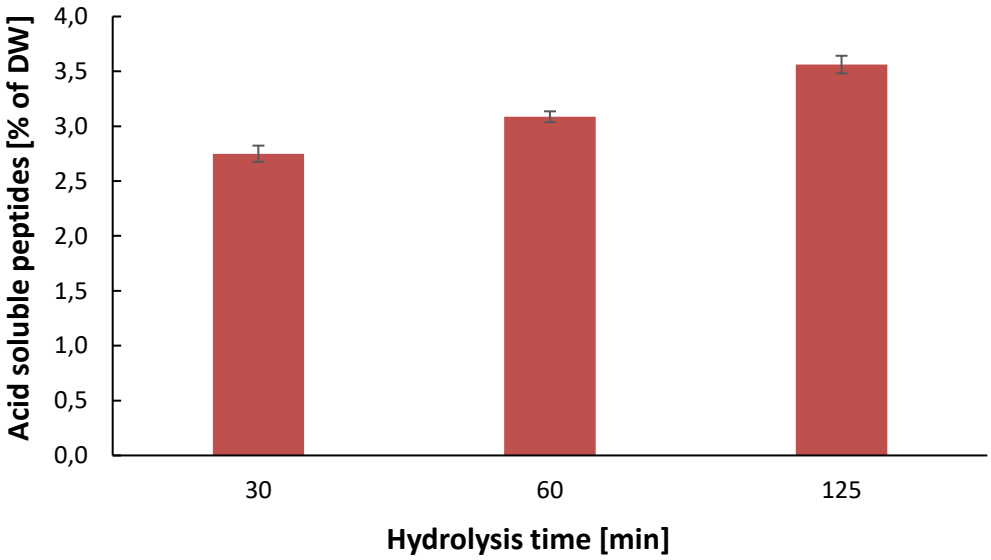

Figure 14. Amount of acid soluble peptides (soluble in 10 % TCA) in herring protein powder hydrolysates as a function of hydrolysis time (min) given as percentage of dry weight (DW). The absorbance measurements were performed in triplicates. Values are given as mean ± SD.

Most of the intact protein is precipitated by TCA, so this method determines the TCA soluble peptides and amino acids. The measurement of acid soluble peptides can be used to estimate the DH, but since it does not determine the number of broken peptide bonds, the DH is not directly measured (Rutherfurd, 2010).

As seen from Figure 14, the amount of acid soluble peptides increased with increasing hydrolysis time. After 30 min of hydrolysis, the amount of acid soluble peptides was 2.75 ± 0.07 % and increased to 3.56 ± 0.08 % after 125 minutes. The size of the peptides soluble in TCA varies and have been reported to lie between 3-4 amino acids in 10 % TCA (Greenberg and Shipe, 1979) and up to 20 amino acids in 12 % TCA (Rohm et al., 1996, Yvon et al., 1989). The low amount of acid soluble peptides indicates that the herring protein powder hydrolysates mainly consist of intact proteins, and that the hydrolysis with trypsin did not produce a

large amount of acid soluble peptides or free amino acids. This correlates well with the low amount of free amino acids presented in Figure 12 and the minor change observed in the molecular weight distribution in the hydrolysates as a function of hydrolysis time (section 3.4). The amount of acid soluble peptides in herring protein power before hydrolysis was not measured. It can be assumed to be below 2.75 ± 0.07 %, which was the measured value after 30 min of hydrolysis.

The amount of acid soluble peptides in hydrolysates from salmon by-products hydrolysed with Papain or/and Bromelain has been investigated by Skjellegrind (2013). Compared to the herring protein powder hydrolysates, these values were more than ten times as high (34-47 %). In addition, the salmon hydrolysates consisted of shorter peptides and had a higher amount of free amino acids (16-51 %) (Skjellegrind, 2013). These hydrolysates were produced with a different production method than the herring protein powder hydrolysates, which is an important reason for the difference.

The Biuret reaction is important for the colour formation in the Lowry method. This reaction requires at least three amino acids for detection and may therefore lead to an underestimation of the results (Johnsons, 2008). It is possible that the actual amount of acid soluble peptides is somewhat higher than the values presented in Figure 14. FAA is measured by the Lowry method, but they do not contribute as much to the colour formation in the Folin reaction as peptides above three residues (Peterson, 1979).

3.6 Solubility

Solubility is one of the most important functional properties of a protein, especially in a liquid product. In the specialization project, the solubility of herring protein powder was investigated both in water and in citric acid-phosphate buffer with pH 3, 5 and 7. As mentioned in section 3.3.2, the solubility in distilled water was less than 2 %. The herring protein powder was produced with ethanol extraction (Figure 2), which gives a powder with a high nutritional value and often low functional properties (Kristinsson and Rasco, 2000b). A protein powder produced with this method usually consists of denatured proteins. Denaturation leads to a conformational change in the structure of the proteins, which results in exposure of the hydrophobic groups that originally were located within the molecule (Lin et al., 2012). The exposure of the hydrophobic groups significantly lowers the solubility of the protein, which may explain the low solubility of the herring protein powder.

3.6.1 The Effect of Enzymatic Hydrolysis

Enzymatic hydrolysis is often a way to make proteins more soluble (Chobert et al., 1988) and denatured proteins are more susceptible to enzymatic hydrolysis compared to native proteins (Lin et al., 2012). As mentioned earlier, trypsin was used to hydrolyse the herring protein powder. The measured solubility of HPP in water before and after hydrolysis for 30, 60 and 125 min are given in Figure 15. Absorbance measurements and standard curve of BSA together with absorbance measurements of the herring protein powder hydrolysates can be found in Appendix G.1.

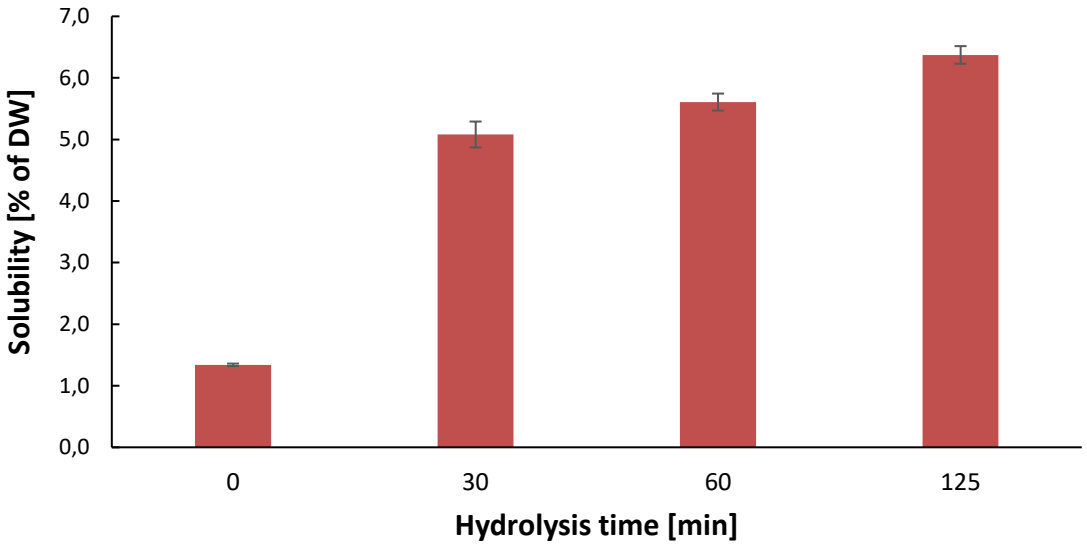

Figure 15. Solubility of herring protein powder (HPP) given as percentage of dry weight (DW). HPP was dissolved in distilled water and hydrolysed with trypsin in a water bath in two parallels. Aliquots were taken out for analysis after 30, 60 and 125 minutes. The values for the solubility with no hydrolysis were obtained during the specialization project (Autumn 2016). The absorbance measurements were performed in triplicates. Values are given as mean ± SD.

The solubility increased from 1.34 ± 0.02 % to 5.1 ± 0.2 % after hydrolysis for 30 min. Further hydrolysis did not increase the solubility significantly, so after 125 min the solubility of the herring protein powder hydrolysate had only increased to 6.37 ± 0.14 %. These numbers demonstrate that the hydrolysis with trypsin was most effective in the first 30 min of hydrolysis, but after this, the rate of hydrolysis decreased. The increase in solubility after hydrolysis can be explained by the newly exposed ionisable carboxyl and amino groups of the amino acids, which gives a higher hydrophilicity and hence a higher solubility (Kristinsson and Rasco, 2000b). The low solubility of the HPP may give an unattractive mouthfeel if the protein powder is added in a food product (Petersen, 1981). This will be discussed in section 3.13 and 3.14.

Since trypsin is an enzyme with a high specificity, and only cuts the peptide bonds at the C-terminal side of a Lys or Arg residue, the number of lysine and arginine residues in the protein will affect the degree of hydrolysis. As seen from the amino acid composition of the herring protein powder in Table 9, the concentrations of the two amino acids are not particularly low for either of them. A low amount of either lysine or arginine is probably not the reason for the minor increase in the solubility of the protein. The pH optimum for trypsin lies be-

tween 7 and 10 (Sipos and Merkel, 1970). Since the pH of the protein solution was not adjusted, it was most likely below pH 7 during the hydrolysis. This may have lowered the catalytic activity of the enzyme.

The solubility of whole herring and herring by-product hydrolysates was studied by Sathivel et al. (2003). Hydrolysates made from whole herring, herring body and head all had solubilities above 78 %, while the hydrolysate from herring gonad had approximately 56 % solubility. The solubility of the herring gonad hydrolysate is more than eight times higher than the solubility of the HPP hydrolysate after 125 min of hydrolysis. The hydrolysates produced by Sathivel et al. would have a higher potential as a food additive, especially in liquid products, due to the higher solubility.

Previous studies have demonstrated that enzymatic hydrolysis can be used to modify the properties of insoluble FPC (Cheftel et al., 1971, Archer et al., 1973, Hevia et al., 1976). Different enzymes have been tested, including pepsin, Papain, Bromelain and ficin. The increased solubility obtained after enzymatic hydrolysis in these studies was much higher compared to the increased solubility obtained after hydrolysis with trypsin. Due to the low solubility obtained with the ethanol extraction method, alternative production methods could be considered to make a protein powder from herring with a higher solubility. A production method patented by Lennart and Walton (1975), claims to produce a fish protein isolate with a high protein content (90-98 %), less than 0.5 % lipids and a bland flavour. The authors also claim that the proteins will be of high nutritional value and have a high solubility at all pH values.

3.6.2 The Effect of pH

The nature and the distribution of a protein`s net charge is affected by the pH of the solution (Pelegrine and Gasparetto, 2005). The net charge is zero when the pH reaches the isoelectric point (pI) of the protein. At this pH, the solubility of the protein is at it`s minimum. The solubility is usually higher both below and above the isoelectric point. Surface charges of the same sign produce repulsive forces between the molecules which enhance the solubility of the protein (Ustunol, 2014b). The solubility of salmon meal and whey protein powder dissolved in distilled water and citric acid-phosphate buffer with pH 3, 5 and 7 are presented in Figure 16 and 17, respectively. Absorbance measurements and standard curves of BSA together with absorbance measurements of salmon meal and whey protein powder can be found in Appendix G.2.

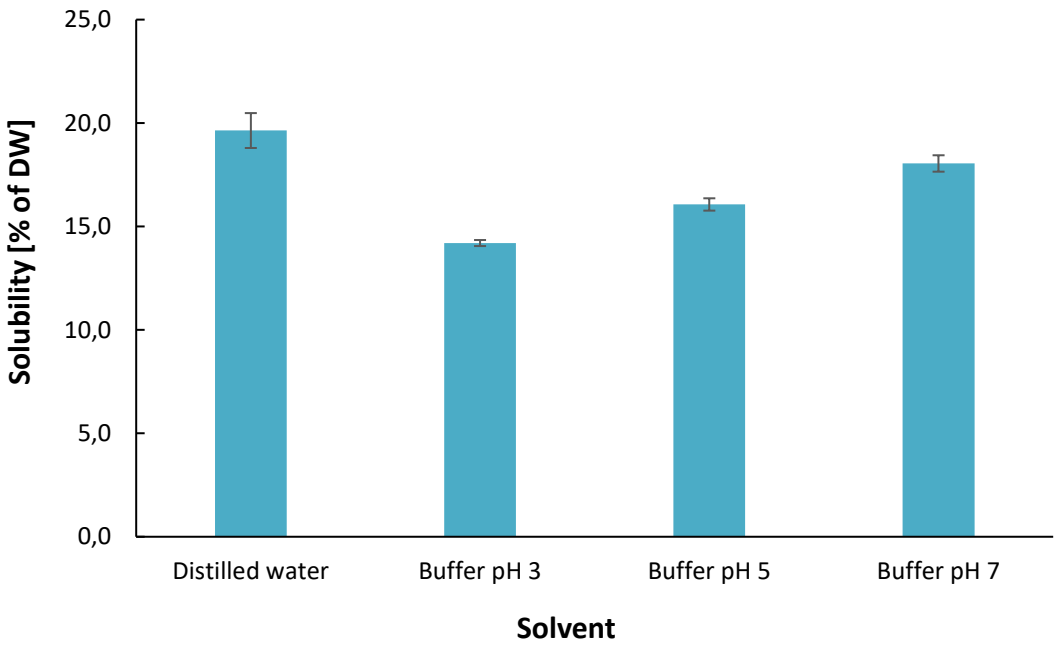

Figure 16. Solubility of salmon meal given as percentage of dry weight (DW). Salmon meal was dissolved in distilled water and citric acid-phosphate buffer with pH 3, pH 5 and pH 7. The measurements were performed in triplicates. Values are given as mean ± SD.

The solubility of the salmon meal was highest in distilled water (19.6 ± 0.8 %) and lowest in citric acid-phosphate buffer with pH 3 (14.2 ± 0.2 %). It increased with increasing pH and in buffer with pH 5 and 7, the solubility was 16.1 ± 0.3 and 18.1 ± 0.4 %, respectively. The solubility of heat stable, water soluble fish proteins from Atlantic salmon heads and backbones have been reported by Aspevik et al. (2016) to be 19.1 %. This value is similar to the solubility of salmon meal in distilled water. The amount of available information regarding functional properties of fish meal is much less compared to information about fish proteins and hydrolysates. However, Sathivel et al. (2005a) have investigated the functional properties of Alaska white fish meals and they found that the nitrogen solubility ranged from 27.5 to 42.2 %. The solubility of Alaska white fish meals was therefore higher than the solubility of the salmon meal.

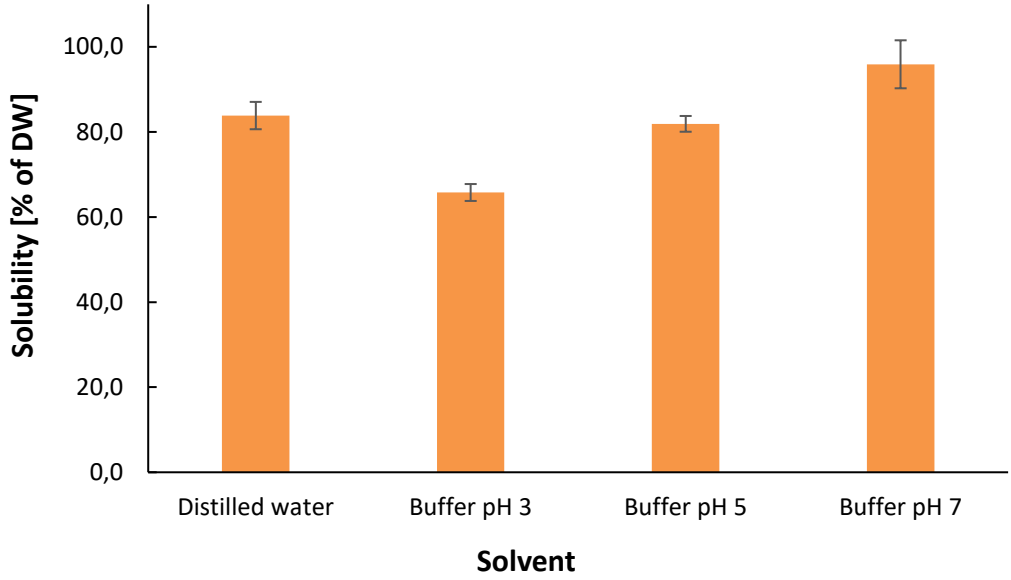

Figure 17. Solubility of whey protein powder (WPP) given as percentage of dry weight (DW). WPP was dissolved in distilled water and citric acid-phosphate buffer with pH 3, pH 5 and pH 7. The measurements were performed in triplicates. Values are given as mean ± SD.

The solubility of the whey protein powder dissolved in citric acid-phosphate buffer increased with increasing pH. It increased from 65.7 ± 3.2 % at pH 3 to 95.90 ± 5.6 % at pH 7. The solubility in citric acid-phosphate buffer with pH 5 (81.9 ± 1.9 %) was similar to the solubility of WPP dissolved in distilled water (83.9 ± 3.2 %). The amino acid composition of the whey protein powder was given in Table 9. Calculated from the values specified by the producer, 38 % of the total amount of amino acids were hydrophobic amino acids, while only 19 % were hydrophilic. A high amount of hydrophobic amino acids may have a negative impact on the solubility (Damodaran, 1996), but it seems not to have influenced the WPP negatively.

The high solubility of the whey protein powder is in agreement with the literature. Several studies have reported a high solubility of whey protein isolates or concentrates (Smith et al., 2016, Luck et al., 2013). As seen from Figure 17, the whey protein powder had a high solubility at even a low pH. A high solubility at low pH is a unique property and whey proteins can thus be used in acidic food and beverages (Pelegrine and Gasparetto, 2005, Kumar et al., 2010). Pelegrine and Gasparetto have demonstrated that the solubility of whey proteins is greatly affected by both pH and temperature. The isoelectric point of whey proteins is 4.5 (Pelegrine and Gasparetto, 2005) and the solubility is usually lowest at this pH. It was therefore expected that the solubility would be lower at pH 5 compared to pH 3, since this is closer to the isoelectric point. As seen from Figure 17, this was not the case.

3.6.3 The Effect of Temperature

Temperature is another factor that influences the solubility of a protein. Due to the low solubility of both herring protein powder (1.34 ± 0.02 %) and salmon meal (19.6 ± 0.8 %) in distilled water at room temperature, it was investigated whether an increase in temperature could make the proteins more soluble. The solubility as a function of temperature for the herring protein powder and salmon meal is given in Figure 18 and 19, respectively. Absorbance measurements and standard curves of BSA together with absorbance measurements of herring protein powder and salmon meal can be found in Appendix G.3. As already shown in Figure 17, the solubility of the whey protein powder was high in distilled water at room temperature. The solubility as a function of temperature was therefore not investigated for the whey protein powder.

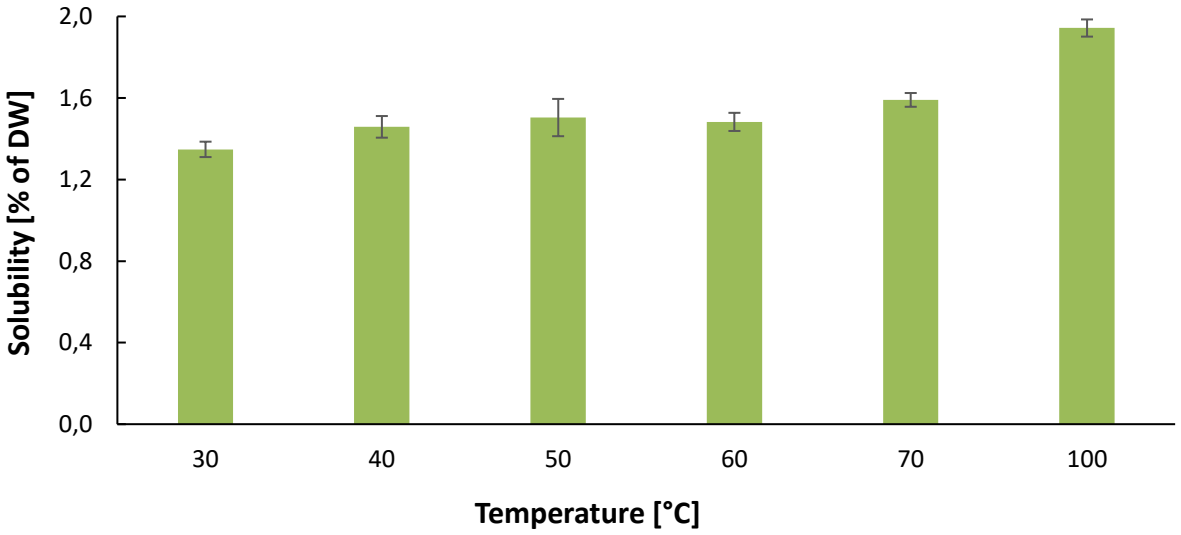

Figure 18. Solubility of herring protein powder (HPP) as a function of temperature given as percentage of dry weight (DW). HPP was dissolved in water at six different temperatures. The measurements were performed in triplicates. Values are given as mean ± SD.

The solubility increased from 1.35 ± 0.04 % at 30 °C to 1.94 ± 0.04 % at 100 °C. The increase in solubility was less than 1 % and the effect of increasing the temperature from 30 to 100 °C was thus minimal. The minor change in solubility as a function of temperature correlates well with the fact that the sensory properties of the herring protein powder was not improved by cooking the powder at 100 °C for 15 min. This will be discussed further in section 3.14.

An increase or decrease in temperature primarily affects the stability of the noncovalent interactions. The hydrogen and electrostatic interactions are weakened when the temperature is increased and high temperatures may cause the proteins to denature (Ustunol, 2014b). Since the herring protein powder most likely was denatured already, a change in the temperature would probably have less effect on the protein structure compared to the structure of the native protein. This might explain why the effect of increasing the temperature was so low.

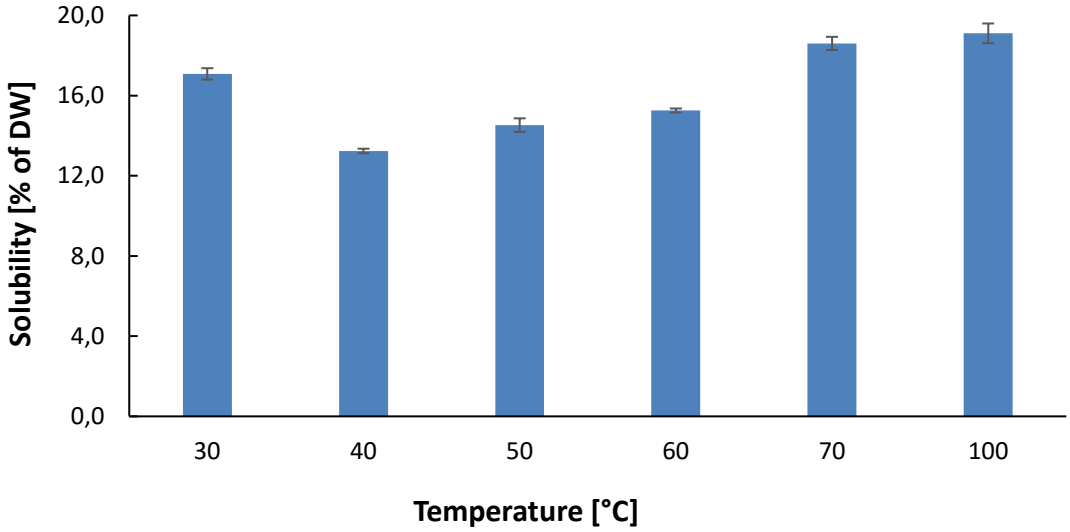

Figure 19. Solubility of salmon meal as a function of temperature given as percentage of dry weight (DW). Salmon meal was dissolved in water at six different temperatures. The measurements were performed in triplicates. Values are given as mean ± SD.

At room temperature, the solubility of the salmon meal was 19.6 ± 0.8 % in distilled water. When the salmon meal was mixed with water at 30 °C the solubility decreased slightly to 17.1 ± 0.3 %. Increasing the temperature to 40 °C decreased the solubility further to 13.24 ± 0.11 %, which was the lowest solubility measured for the salmon meal. When the temperature reached 50 °C, the solubility started to increase and it continued to increase until the temperature reached 100 °C, which was the last temperature investigated. At that point, the solubility was 19.1 ± 0.5 %, which was lower than the solubility measured at room temperature. Based in the values presented in Figure 16 and 19, it seems the salmon meal has the highest solubility around room temperature and at high temperatures (above 70 ºC).

When the pH and ionic strength are constant, the solubility of most proteins will increase with increasing temperature between 0 and 40 ºC (Damodaran, 1996). Although the solubility of

the salmon meal was not investigated below room temperature, an increase in solubility between this temperature range was not observed. As seen from Figure 19, the solubility of the salmon meal decreased from approximately 25 ºC to 40 ºC. When the temperature is increased above a certain level, the increasing thermal kinetic energy usually results in protein unfolding and exposure of nonpolar groups, which leads to a decrease in solubility (Damodaran, 1996). Thus, it is interesting that the solubility decreased until 40 ºC and then increased with higher temperatures.

The amino acid composition is important for the thermal stability of a protein. Hydrophobic amino acid such as Val, Ile, Leu and Phe, make the protein more resistant to heat compared to hydrophilic amino acids. It is also reported that proteins containing lower levels of Asn and Gln can withstand higher temperatures (Ustunol, 2014b). The herring protein powder has a higher content of the amino acids Val, Ile, Leu and Phe compared to the salmon meal. Although it is difficult to predict the stability of the proteins based on the results presented in Figure 18 and 19, the amino acid composition indicate that that the herring protein powder might be more resistant to heat compared to the salmon meal.

3.7 Emulsifying Properties

Since proteins have both hydrophobic and hydrophilic moieties, they can be used as stabilizers in multiple phase foods such as emulsions (Kristo and Corredig, 2014). The emulsion properties of salmon meal and whey protein powder were investigated and the amount of emulsion formed, emulsifying capacity and emulsion stability of the powders are given in Table 14. Measured and calculated values can be found in Appendix H.1 and H.2 for salmon meal and whey protein powder, respectively.

Table 14. Emulsion properties of salmon meal and whey protein powder (WPP). *Emulsion capacity* was defined as mL of emulsified oil per 1 g of protein powder. *Emulsion stability* was defined as percentage of emulsion remaining after one day at room temperature and centrifugation. The measurements were conducted in quadruplicates. The values are given as mean ± SD.

Protein powder concentration [%]	Emulsion formed [mL]		Emulsion capacity [mL/g]		Emulsion stability [%]	
	Salmon meal	WPP	Salmon meal	WPP	Salmon meal	WPP
1	1.9 ± 0.2	5.03 ± 0.05	36 ± 4	89 ± 2	22 ± 2	90 ± 14
2	2.8 ± 0.3	5.40 ± 0.14	27 ± 3	48 ± 1	16 ± 3	87 ± 10
5	4.8 ± 0.2	6.08 ± 0.15	17 ± 1	20 ± 0	11 ± 1	95 ± 4

As seen from Table 14, the amount of emulsion formed varied between the two protein powders. With a protein concentration of 1 %, the amount of emulsion formed was 1.9 ± 0.2 and 5.03 ± 0.05 mL for the salmon meal and whey protein powder, respectively. The amount of emulsion formed increased with increasing protein concentration. The highest protein powder concentration tested was 5 %, and with this concentration the emulsion formed had increased to 4.8 ± 0.2 and 6.08 ± 0.15 mL for the salmon meal and whey protein powder, respectively.

The emulsion capacity of the whey protein powder was superior to the salmon meal. With a protein concentration of 1 %, the emulsion capacity of whey protein powder and salmon meal was 89 ± 2 and 36 ± 4 mL oil/g powder, respectively. The emulsion capacity of acid WPC usually ranges from 38-52 mL oil per gram of protein (Farkye and Shah, 2014). The emulsion capacity of the salmon meal was slightly below this range, but the emulsion capacity of the whey protein powder was almost twice as high with a protein concentration of 1 %. The higher emulsion capacity of the whey protein powder indicates that the whey proteins can more rapidly adsorb and unfold to the oil-water interface and stabilize the oil droplets compared to the salmon meal. The hydrophilic parts of the protein bind water, while the hydrophobic parts bind the oil. This lowers the interfacial area and enables the formation of an emulsion. In addition, the whey protein powder had a higher solubility than the salmon meal. Before amphiphilic polymers, such as proteins, can exhibit their emulsifying properties, they must usually be fully dispersed and dissolved in an aqueous solution (McClements, 2004). In

their native form, proteins usually have their hydrophilic groups facing the aqueous environment, and the hydrophobic groups buried within the molecule. In order to work as an emulsifier, the hydrophobic groups of the protein must be able to unfold and interact with the lipid molecules at the interface (Hettiarachchy and Ziegler, 1994). The lower solubility of the salmon meal might thus be a reason for the lower emulsion capacity compared to the whey protein powder. The correlation between solubility and emulsion properties is especially evident up to 25 % protein solubility (Kristinsson and Rasco, 2000b).

The stability of the emulsions formed with salmon meal was very low, and the stability decreased with both increasing salmon meal concentration and emulsion formed. With a salmon meal concentration of 1 %, the emulsion stability was 22 ± 2 % and decreased to 11 ± 1 % when the salmon meal concentration increased to 5 %. The emulsion stability of a fish meal made from Alaska white fish was reported by Sathivel et al. (2005a) to be higher than the values obtained for the salmon meal and ranged from 62.1 to 67.1 %. The results presented in Table 14 show that even if the salmon meal was able to form an emulsion it was not able to stabilize it, in other words, it was not able to prevent gradual coalescence of the oil droplets. The lack of ability to stabilize the emulsion became especially evident when the volume of the emulsion increased. Emulsion properties are influenced by both the content and the classes of lipids, especially phospholipids and monoglycerides (Damodaran, 2005, Vaghela and Kilara, 1996). Vaghela and Kilara (1996) investigated different WPC and reported that the WPC with the highest lipid content had the lowest emulsion stability. The same effect on the emulsion stability has been reported by Patel and Kilara (1990). It is possible that the high fat content of the salmon meal (17.3 %) destabilized the emulsion formed with the proteins in the salmon meal and therefore resulted in the low emulsion stability.

The emulsion stability with the whey protein powder was also superior to the salmon meal. With a whey protein powder concentration of 1 % the emulsion stability was 90 ± 14 %, and increased to 95 ± 4 % when the whey protein concentration increased to 5 %. The high emulsion stability correlates well with the high emulsion stability of caprine and bovine whey protein concentrates reported by Sanmartín et al. (2013). Emulsion stability is correlated to protein solubility (Patel and Kilara, 1990) and as presented in Figure 17, the solubility of the whey protein powder was high. The size of the peptides is also important for good emulsion properties. Lee et al. suggested that the peptides should have a minimum length of 20 residues (Lee et al., 1987). The whey protein powder consisted of peptides of higher molecular weights

than the salmon meal (section 3.4). The molecular weight influences the conformative stability of the protein and this may also be a reason for the higher emulsion stability obtained with the whey protein powder compared to the salmon meal.

Salad dressings, mayonnaise, sausages and minced meat are products that would be immiscible without an emulsifier (Petersen, 1981). Based on the emulsifying properties demonstrated by the whey protein powder and salmon meal, the whey protein powder would be the preferred emulsifier of those two in such products. The whey protein powder would most likely be the preferred protein with respect to the taste as well. The taste of the salmon meal will be further discussed in section 3.13.

One of the parallels in the samples with 1 and 2 % of whey protein powder had a lower amount of emulsion compared to the other three. This is the reason for the high SD for the emulsion stability measured with 1 and 2 % of whey protein powder. Since the amount of the different phases, water, emulsion and oil are read manually, small changes are difficult to measure. The uncertainty of these measurements may therefore be higher than the calculated SDs shown in Table 14.

3.8 Water Holding Capacity

The water holding capacity (WHC) is defined as the ability of a protein matrix such as fish filet to absorb and retain water against applied gravitational force (Kristinsson and Rasco, 2000b). This is an important property in food applications. The ability of proteins to entrap water is associated with desirable textural properties of bakery products and with juiciness and tenderness of comminuted meat products (Damodaran, 1996). The WHC of Atlantic cod was measured with and without addition of either salmon meal or whey protein powder. Additions of 5, 10 and 20 % of protein powder were tested. Figure 20 presents minced cod filets with addition of different concentrations of protein powders.

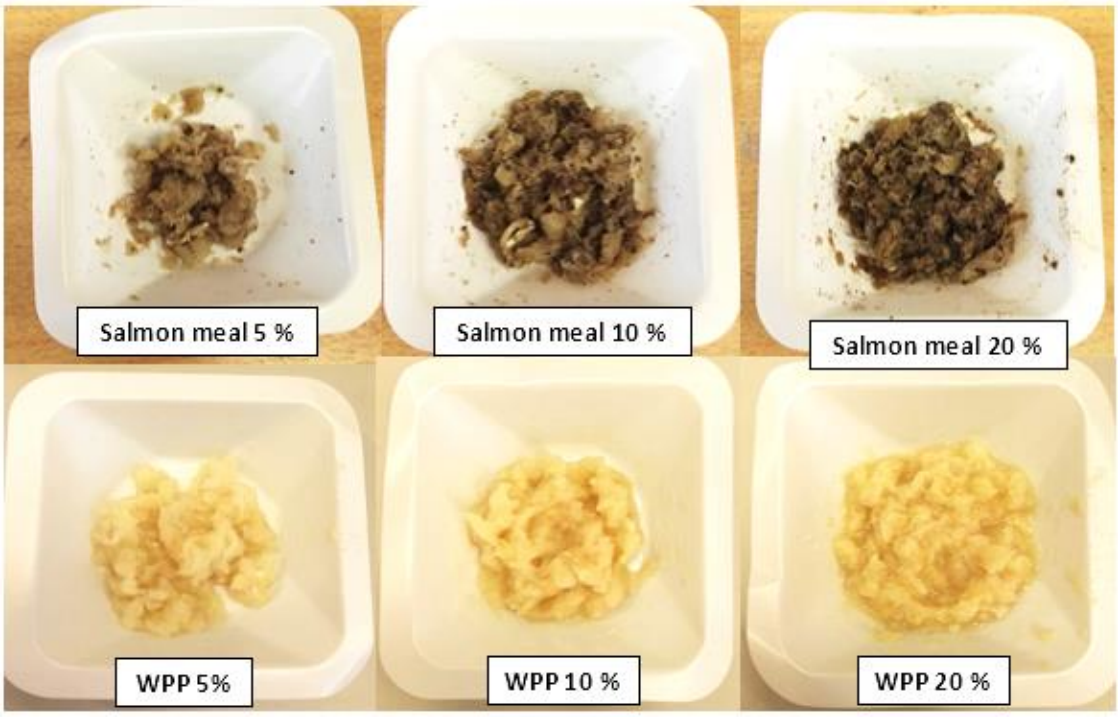

Figure 20. Grinded cod filets mixed with different concentrations (5, 10 and 20 %) of salmon meal and whey protein powder (WPP).

Addition of salmon meal or whey protein powder into minced cod filets changed the appearance of the mixture. Although it is not very clear from Figure 20, the consistency of the cod became slightly drier and firmer when mixed with salmon meal as the protein powder concentration increased. When the cod filet was mixed with the whey protein powder, the opposite happened. As the protein concentration increased, the mixture became significantly looser and more aqueous.

The observations described above correlates well with the results shown in Figure 21, where WHC is plotted as a function of protein powder concentration. Measured and calculated values can be found in Appendix I.1 and I.2 for salmon meal and whey protein powder, respectively.

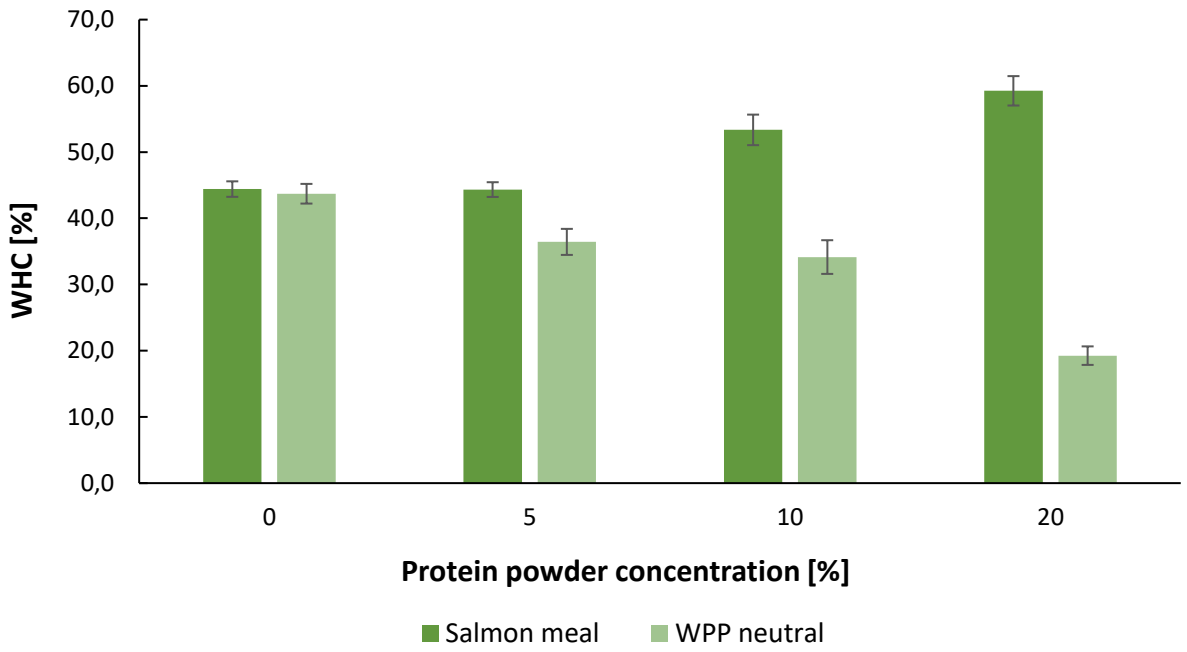

Figure 21. Water holding capacity (WHC) of Atlantic cod as a function of protein powder concentration (%). Three different protein concentrations were tested; 5, 10 and 20 %. No addition of protein powder is marked as 0. The WHC was measured on cod filets that had been thawed, frozen and then thawed again. The measurements were conducted in quadruplicates. Values are given as mean ± SD.

The WHC of cod without any addition of protein was 44 ± 1 %. It is important to mention that the cod filet had been thawed, frozen and then thawed again, and the initial WHC was therefore low. Both grinding and freezing disturbs the protein network and results in a reduced WHC compared to a WHC measured in material that has been treated more mildly.

Addition of 5 % salmon meal did not increase the WHC and it remained at 44 ± 1 %. Increasing the protein concentration to 10 and 20 % increased the WHC to 53 ± 2 and 59 ± 2 %, respectively. Šližytė et al. (2009) added fish protein hydrolysate from fresh cod backbones (2 % of minced muscle mass) to cod mince and found that this increased the WHC with 16 %. Addition of 20 % salmon meal only increased the WHC of cod with 15 %, so the water holding properties of the fish protein hydrolysate investigated by Šližytė et al. was by far superior to the salmon meal.

As seen from Figure 21, the WHC of cod mixed with whey protein powder decreased with increasing protein concentration. An addition of 5 % whey protein powder reduced the WHC capacity of cod filets to 36 ± 2 %. The addition of 10 and 20 % whey protein powder reduced

the WHC capacity further to 34 ± 3 and 19 ± 1 %, respectively. The decrease in WHC with the addition of protein powder was not expected, since it was assumed that an increase in the protein concentration would make more protein available to interact with and bind water, and hence increase the WHC. One possible explanation for the decreasing WHC, could be that the addition of whey proteins in some way disturbed the existing protein network in the fish mince and as a consequence, the water was drawn out of the protein network. In the specialization project, the WHC of a similar whey protein powder as the one used in this thesis was investigated. The main difference was that the whey protein powder used in the specialization project had a chocolate taste. The same trend with a reduction of WHC with increasing protein powder concentration was observed. It was investigated whether a change in the pH of the cod mixed with an increasing amount of protein powder could explain the decrease in WHC. As it was found that the change in pH was minor with the addition of protein powder, this is probably not the reason for the decrease in WHC.

The WHC is related to the amino acid composition of the protein (Damodaran, 1996). From a study on hydrolysates made from cod by-products, Šližytė et al. (2005) reported a linear relationship between the WHC in the FPH and certain amino acids. Decreasing amounts of alanine, glycine/arginine, hydroxyproline and the sum of hydrophobic amino acids increased the WHC of the fish mince. As seen from the values obtained after the acid hydrolysis in Table 9, the alanine content and the sum of nonpolar amino acids were lowest in the salmon meal which had the highest WHC. However, the glycine/arginine content was lowest in the whey protein powder. The relationship between the WHC and certain amino acids is therefore ambiguous based on the results obtained for the salmon meal and whey protein powder and do not entirely correlate with the relationship described by Šližytė et al. It is also important to mention that the amino acid composition presented in Table 9 is not complete and proline, which is a hydrophobic amino acid, is not included.

3.9 Swelling Capacity

Swelling is another property involving water. Wheat flour doughs and sausages are examples of foods where the absorption of water is important for the acceptance of the food (Pomeranz, 1985). The swelling capacity was defined as the amount of water taken up by the powder in mL per gram powder. In the literature, terms like water hydration and water absorption are used to describe the same quantity. The swelling capacity was determined for the herring protein powder and salmon meal at two different temperatures and the results are given in Figure 22. Measured and calculated values can be found in Appendix J. This was also investigated for the whey protein powder, but due to the high solubility most of the powder was solubilized and it was not possible to measure any swelling with this method. In addition, the water would not flow through the filter which was probably due to small molecules in the powder clogging the filter.

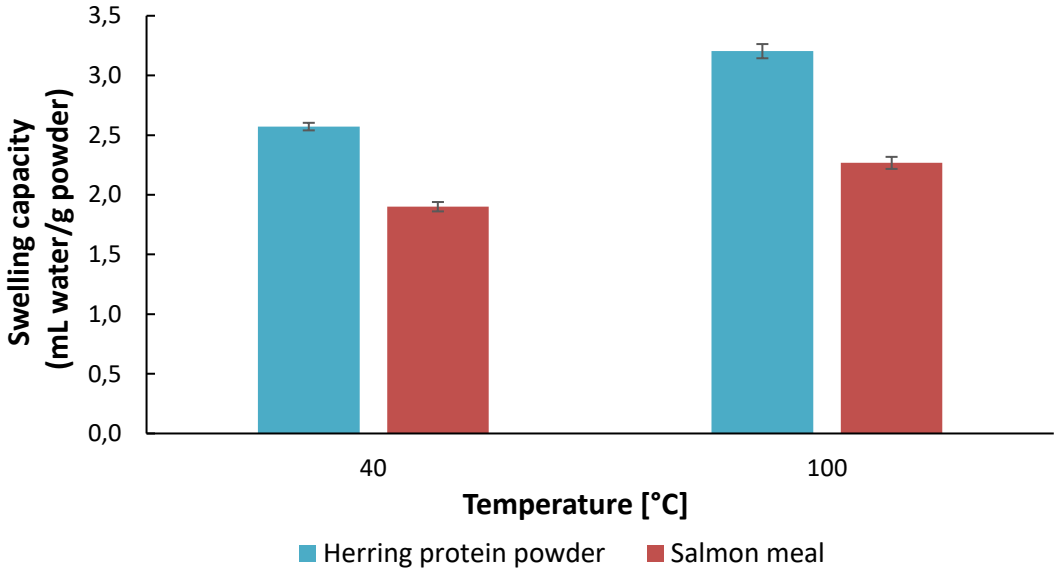

Figure 22. Swelling capacity (mL water/g powder) as a function of temperature (ºC) for herring protein powder and salmon meal. The measurements were performed in triplicates. Values are given as mean ± SD.

When the powders were mixed with water at 40 ºC the swelling capacity was 2.57 ± 0.03 and 1.90 ± 0.04 mL water/g powder for herring protein and salmon meal respectively. The swelling capacity increased with temperature for both of the powders. At 100 º C the swelling capacity of herring protein powder and salmon meal had increased to 3.20 ± 0.06 and 2.70 ± 0.05 mL water/g powder, respectively. The WHC of herring protein powder was measured in the specialization project. When 5, 10 and 20 % of herring protein powder was mixed with

cod filets, the WHC was 48 ± 1, 53 ± 1 and 66 ± 1 % respectively. The WHC of salmon meal for the same protein concentrations was 44 ± 1, 53 ± 2 and 59 ± 2 % (section 3.8). Both the swelling capacity and the WHC were somewhat higher for the herring protein powder compared to the salmon meal. Based on the result from these two powders, it seems like there is a positive correlation between the swelling capacity and the WHC. This is confirmed by the literature, as several studies have shown a positive correlation between WHC and the hydration capacity of proteins (Damodaran, 1996).

Water molecules can bind to several groups in a protein, and these include charged groups, uncharged polar residues and nonpolar groups. The various groups bind different amounts of water, and amino acid residues with charged groups bind the most. The amount of water taken up by the powder will thus be related to the amino acid composition of the protein (Damodaran, 1996). The herring protein powder had a higher content of charged amino acids compared to the salmon meal and this might explain the higher swelling capacity of the herring protein powder.

Sathivel et al. measured the water absorption for different fish meal samples and they ranged from 2.5 to 2.8 mL water/g protein (Sathivel et al., 2005a). These values are slightly above the swelling capacity measured for the salmon meal at 40 ºC, but correlates well with the value measured at 100 ºC. The swelling capacities measured for the herring protein power correlate with these values as well, and at 100 ºC the swelling capacity was higher compared to the values reported by Sathivel et al. The degree of denaturation of the proteins influences the swelling or water absorption capacities. A denatured protein has an increase in surface area to mass ratio compared to the native protein, and this leads to a higher water binding capacity (Damodaran, 1996). The degree of denaturation was not measured, but based on the solubility of the powders it can be assumed that the herring protein powder was most denatured. The herring protein powder also had a higher protein content which results in more proteins available to interact with water and hence a higher swelling capacity. A higher degree of denaturation at 100 ºC, compared to 40 ºC, could explain why the swelling capacity was highest at that temperature as well.

Both absorption of water and swelling change the appearance of a solution and lead to thickening and increased viscosity (Pomeranz, 1985). Figure 23 shows the filter cakes after filtration of herring protein powder and salmon meal at 40 and 100 °C.

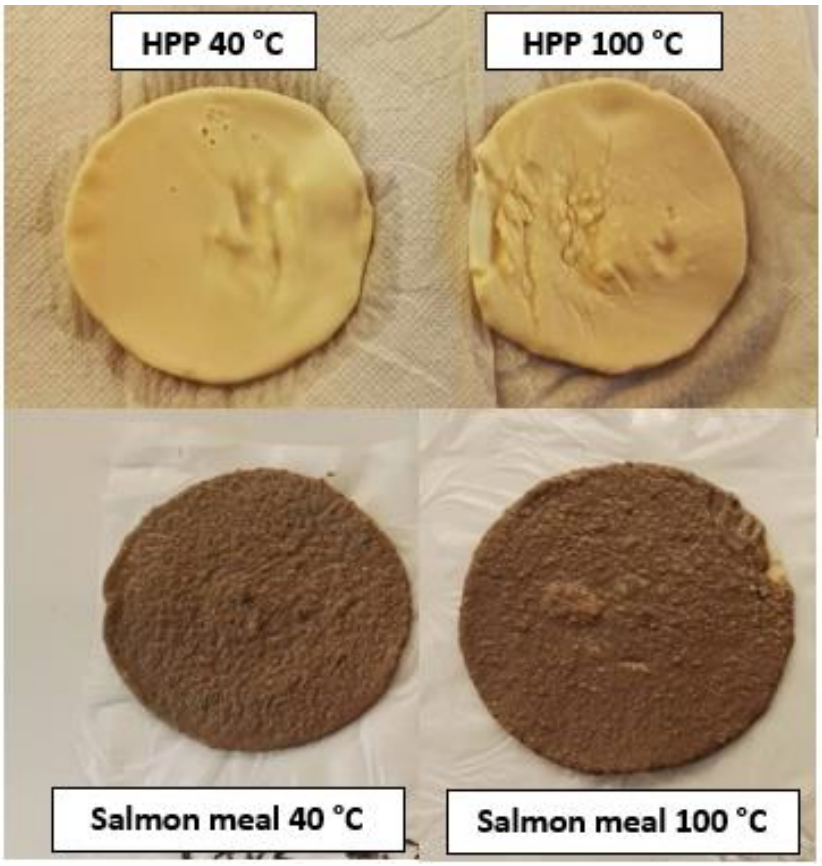

Figure 23. Filter cakes after filtration through a filter paper of herring protein powder (HPP) and salmon meal mixed with water at 40 and 100 °C.

The herring protein powder consisted of finer particles than the salmon meal, and this was reflected by the appearance of the filter cakes. The appearance of the filter cakes with herring protein powder was more homogenous compared to the salmon meal. The surface of the herring protein powder cake was also smoother. No clear difference in the appearance of the filter cakes was observed with respect to different temperatures, although the temperature affected the swelling capacity values as shown in Figure 22.

3.10 Antioxidant Properties

Antioxidants play an important role in the food industry by preventing degradation of food and extending their shelf life by inhibiting lipid oxidation. The antioxidant properties of whey protein powder and salmon meal were measured spectrophotometrically with the DPPH scavenging method. DPPH is a stable free radical with an absorbance maximum at 517 nm in methanol, and when DPPH encounter a proton donating substance, such as a protein, the radical is scavenged and the absorbance is reduced (Wu et al., 2003, Galla et al., 2012). The scavenging effect as a function of protein concentration for the whey protein powder is given in Figure 24. Absorbance measurements of the whey protein powder and the calculated scavenging effect are given in Appendix K.2. Propyl gallate was used as a standard and absorbance measurements, standard curve and the scavenging effect of propyl gallate can be found in Appendix K.2. The propyl gallate measurements were not directly used in the calculation of the scavenging effect of the protein, but used to check that the absorbance measurements of the protein powder were within the standard curve.

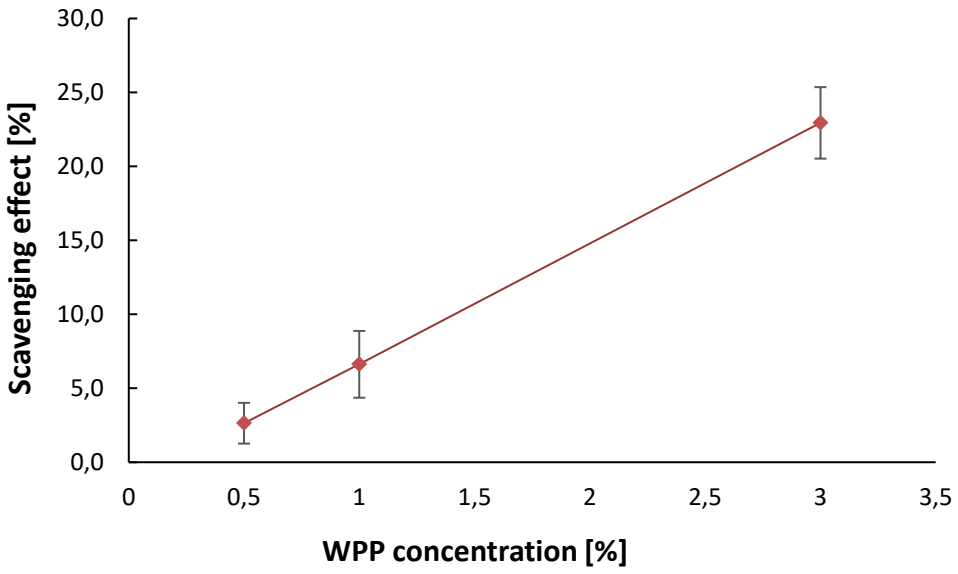

Figure 24. Scavenging of DPPH (2,2-diphenyl-1-picrylhydrazyl) (%) as a function of whey protein powder concentration (WPP) (%). The absorbance measurements were conducted in triplicates. Values are given as mean ± SD.

As seen from Figure 24, the scavenging of DPPH radicals increased linearly with increasing whey protein powder concentration. With a protein concentration of 0.5, 1 and 3 %, the scavenging effect from the whey protein powder was 3 ± 1, 7 ± 2 and 23 ± 2 %, respectively. The ability to scavenge DPPH radicals may indicate that the proteins have an antioxidant effect,

but other compounds in the powder may contribute as well. In the specialization project, the antioxidant properties of a whey protein powder with chocolate taste with a chemical composition of the protein similar to the neutral whey protein powder was investigated. With the same protein concentrations as used in Figure 24, this powder scavenged 35 ± 2, 60 ± 1 and 74 ± 1 % of the DPPH radicals. The chocolate whey protein powder scavenged more DPPH radicals at 0.5 % than the neutral whey protein powder did at 3 %. Cocoa have been reported to have antioxidant properties in many studies (Bubonja-Sonje et al., 2011, Schinella et al., 2010). It is therefore possible that is was the phenolic compounds from the cocoa powder in the chocolate whey protein powder that possessed the good antioxidant properties instead of the proteins.

Hydrolysates from cod backbones (60 min of hydrolysis) studied by Šližytė et al. scavenged more than 50 % of the DPPH radicals when a hydrolysate concentration of 0.25 % was tested (Šližytė et al., 2009). The proteins from this study was therefore much more effective in scavenging DPPH free radicals compared to the whey proteins. The DPPH method is one of the most popular methods for measuring the antioxidant properties of different compounds. Dawidowicz et al. studied different factors affecting the estimation of the antioxidant effect. This study showed that the type of solvent, together with water, hydrogen and metal ion content influence the DPPH measuremnts (Dawidowicz et al., 2012). Since the DPPH method is not a standardised method, different versions of the method are used. This makes it difficult to compare the existing litterature values.

The antioxidant properties of salmon meal were investigated twice with the DPPH scavenging method. The absorbance measurements of propyl gallate are given in Appendix K.3, together with the absorbance measurements for the salmon meal. As seen from Table 70 and 71 in Appendix K.3, the absorbance values increased with increasing protein concentration. If the salmon meal did not have any antioxidant properties the values should have been approximately the same at all concentrations, at least not increase. Since the absorbance measurements increased with increasing protein powder concentration it was not possible to calculate any scavenging effect of the DPPH free radicals for the salmon meal. The salmon meal may have antioxidant properties, but this could not be measured by the DPPH method. Other methods, such as the ABTS assay, could have been tested.

When DPPH free radicals are scavenged the colour changes gradually from deep violet to pale yellow. As seen from Figure 25, the extracts made from salmon meal dissolved in 80 % methanol (0.5 and 1 % salmon meal) have a slightly yellow colour. With a salmon meal concentration of 3 %, the extract has a distinct yellow colour. It is possible that the yellow colour of the extracts have influenced the absorbance measurements. The absorbance of the extracts at different wavelengths with 96 % ethanol as a reference can be found in Appendix K.4. These values show that the absorbance of the extract increased with increasing protein concentration and at 517 nm the absorbance measurements were higher than the measured values of the highest concentrations of propyl gallate.

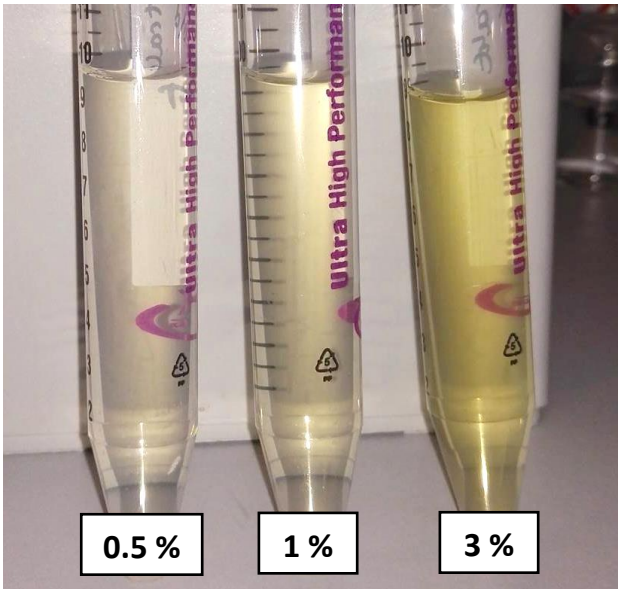

Figure 25. Extracts made from salmon dissolved in 80 % methanol. The extracts with 0.5 and 1 % salmon meal have a slightly yellow colour, which becomes clearly yellow when the salmon meal concentration increased to 3 %.

An overview of the production process of the salmon meal was given in Figure 4. As seen from this Figure, the salmon meal was added an antioxidant after the drying stage, which would also influence the DPPH scavenging. In order to measure the antioxidant properties of the salmon meal, a powder without any addition of antioxidant would be necessary. However, this is probably not the reason for the increasing absorbance values since an antioxidant would decrease the values.

3.11 Digestibility of the Protein Powders

Together with the amino acid composition, the digestibility is the main factor determining the quality of a protein source and this can be measured in several ways (World Health Organization, 2007). In this study, a pepsin/HCl solution was used to simulate the digestion of proteins in the stomach. Pepsin is a aspartic endopeptidase found in the stomach and cleaves the peptide bonds on the amino side of the amino acids Leu, Phe, Trp and Tyr (Nelson et al., 2013, Belitz et al., 2009). The digestibility of herring protein powder, salmon meal, whey protein powder with both a chocolate and a neutral taste are given in Table 15. Casein was used as a reference and is also included in Table 15. Measured values and calculated digestibility can be found in Appendix L.

Table 15. Digestibility of herring protein powder, salmon meal, whey protein powder (WPP) with chocolate and neutral taste and casein with a pepsin/HCl solution. The measurements were performed in triplicates. Values are given as mean ± SD.

Protein powder	Digestibility [%]
Herring protein powder	92.5 ± 0.4
Salmon meal	71.8 ± 0.8
WPP chocolate	93.6 ± 1.1
WPP neutral	99.3 ± 0.1
Casein	91.9 ± 0.6

Except for the salmon meal, all the powders had a digestibility above 90 %. The digestibility of the whey protein powder with chocolate taste and the herring protein powder was 93.6 ± 1.1 and 92.5 ± 0.4 %, respectively. The difference in the digestibility between these two powders was thus small, and they were similar to the digestibility of the reference protein, casein, which had a digestibility of 91.9 ± 0.6 %. As seen from Table 15, the neutral whey protein powder had the highest digestibility of 99.3 ± 0.1 %, which indicates that this protein powder consists of highly digestible proteins, actually more digestible than the reference protein. Havenaar et al. determined the digestion of immature herring roe proteins under human conditions in a gastrointestinal model (tiny-TIM). They found that the digestibility of immature herring egg proteins ranged from 71 to 92 % (Havenaar et al., 2016). The digestibility of the herring protein powder presented in Table 15 lies in the upper range of the digestibility values presented by Havenaar et.al. Digestibility of proteins in the stomach is not a simple, one-step procedure, such as the pepsin/HCl method. Digestibility values measured with the

tiny-TIM method probably give more realistic values compared to the pepsin/HCl method, as it is more similar to the true *in vivo* digestion.

As seen from Figure 15, the salmon meal had the lowest digestibility (71.8 ± 0.8 %). In order to meet the quality requirements set by the producer, the salmon meal should have a digestibility above 85 % (measured in mink). The digestibility measured with the pepsin/HCl method was more than 10 % below this value. As seen from Table 8 (section 3.1), the salmon meal had a much higher fat and ash content compared to the neutral whey protein powder and the herring protein powder. A lower digestibility of the salmon meal compared to the other two protein powders was therefore expected as pepsin would not digest neither fat nor inorganic substances found in the ash. It is also possible that these substances have made the substrate (proteins) less available to the active site of the enzyme. Preferably, the salmon meal should have been defatted before the digestion with pepsin/HCl. The high fat content of the salmon meal was discovered after the digestion of the salmon meal had been performed and it was not enough time repeat the measurements with a defatted salmon meal.

The production of a fish meal usually includes cooking, pressing, drying and grinding (WINDSOR, 2001a), and the processing conditions can influence the digestibility of a protein (Opstvedt et al., 2003, Jensen and Keller, 1990). The drying stage is crucial for maintaining the protein digestibility and it is important that it is done as gently as possible. Opstvedt et al. studied the effect on protein digestibility of different processing conditions in the production of fish meal. They found that fish meal (from Norwegian spring spawning herring) produced at high temperatures (>100 ºC) had a lower digestibility (905 g/1000 g digested) compared to the digestibility of a fish meal produced at low temperatures (< 70-80 ºC, 929 g/1000 g digested). The protein digestibility was determined in mink (Opstvedt et al., 2003). The drying conditions in the production of salmon meal are not known, but may be the reason for the lower digestibility of the proteins, as it is an important processing factor.

The digestibility of the different amino acids in a protein varies (Havenaar et al., 2016). Although this was not measured after the digestion with pepsin/HCl, the digestibility of both the essential amino acids and the protein should be high in order to be a high quality protein.

3.12 Appearance of Bread Rolls Enriched with Protein

Addition of proteins to familiar and frequently consumed products such as bread or bread rolls could be a way to increase the amount of protein in the food for people suffering from insufficient protein intake or malnutrition. Addition of high quality proteins would increase both the protein content of the food and the nutritional quality, due to a higher content of essential amino acids. Bread rolls were made from mixtures of wheat flour, wholemeal and various amounts of protein powder. The appearance of the bread rolls with different amounts of protein powder (0, 5, 10, 15 and 20 %) is given in Figure 26.

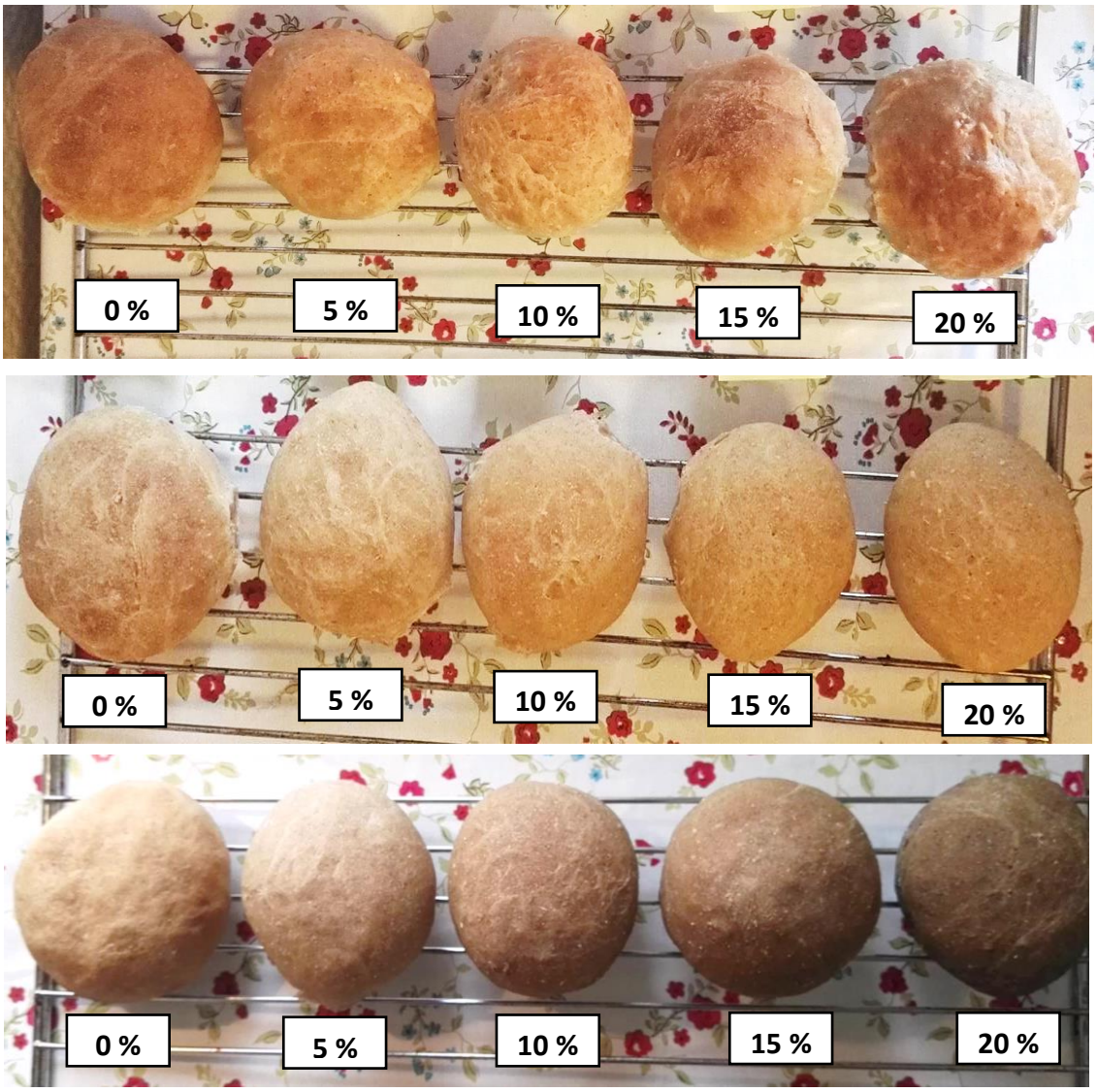

Figure 26. The appearance of bread rolls made from mixtures of wheat flour, whole meal and various amounts of protein powder (0, 5, 10, 15 and 20 %). Bread rolls made with whey protein are shown at the top, bread rolls made with herring protein powder in the middle, while the bread rolls made with salmon meal are at the bottom.

The appearance of the bread rolls varied both with the type of protein added and the protein concentration. As seen from Figure 26, the surfaces of the bread rolls made with whey protein powder (5 to 20 %) were somewhat uneven. The doughs made with this type of protein powder were very sticky compared to the dough without protein powder, which made it difficult to form the doughs into bread rolls. As the protein concentration increased, the dough became more and more sticky and difficult to work with. Some extra wheat flour was added to all of the doughs with whey protein powder in order to achieve a consistency that was possible to make bread rolls from. In section 3.8, Figure 21 shows the WHC of cod as a function of whey protein powder, where the WHC decreased with increasing protein concentration. This correlates well with the fact that the dough became more sticky and less firm with increasing protein concentration. Although no rheology measurements were performed, it was observed that the consistency of the dough changed with addition of whey protein powder.

The doughs made with herring protein powder was very easy to work with. A seen from Figure 26, the bread rolls made with herring protein powder were almost identical to the reference, which had no addition of protein powder. As with the whey protein powder, the consistency of the dough changed with increasing protein powder concentration. With increasing protein powder concentration, the dough became firmer and less sticky. As with the WHC of cod mixed with herring protein powder measured in the specialisation project, an increasing firmness was observed with increasing protein powder concentration. The consistency of the dough changed with the addition of salmon meal as well, but not as much as with the herring protein powder. This correlates well with the fact that the WHC of the salmon meal was not as high as the WHC of the herring protein powder.

As seen from Figure 26, the colour of the bread rolls varied from light to dark brown. The bread rolls baked with whey protein powder had a more golden appearance compared to the bread rolls with herring protein powder. The colour of the bread rolls with herring protein powder did not change significantly and was almost identical for all protein concentrations. The bread rolls with whey proteins became slightly darker/more golden with increasing protein concentration. The whey protein powder contained sugar (4.9 g/100 g) and the colour change could be due to the Maillard reaction. Brown compounds are produced when amino acids react with reducing sugars at high temperatures. The amino groups reacting with the sugars may belong to a free amino acid or the side-chains of amino acids in proteins (Coultate, 2016). The most obvious colour change was observed for the bread rolls baked

with salmon meal. Since the salmon meal was brown, the bread rolls became darker with increasing salmon meal concentration. As seen from Figure 26, the appearance of the bread roll baked with 20 % salmon meal was significantly darker than the reference.

The bread rolls were sliced in halves to get a better visualization of the texture. The texture of the bread rolls mixed with and without the different protein powders are given in Figure 27.

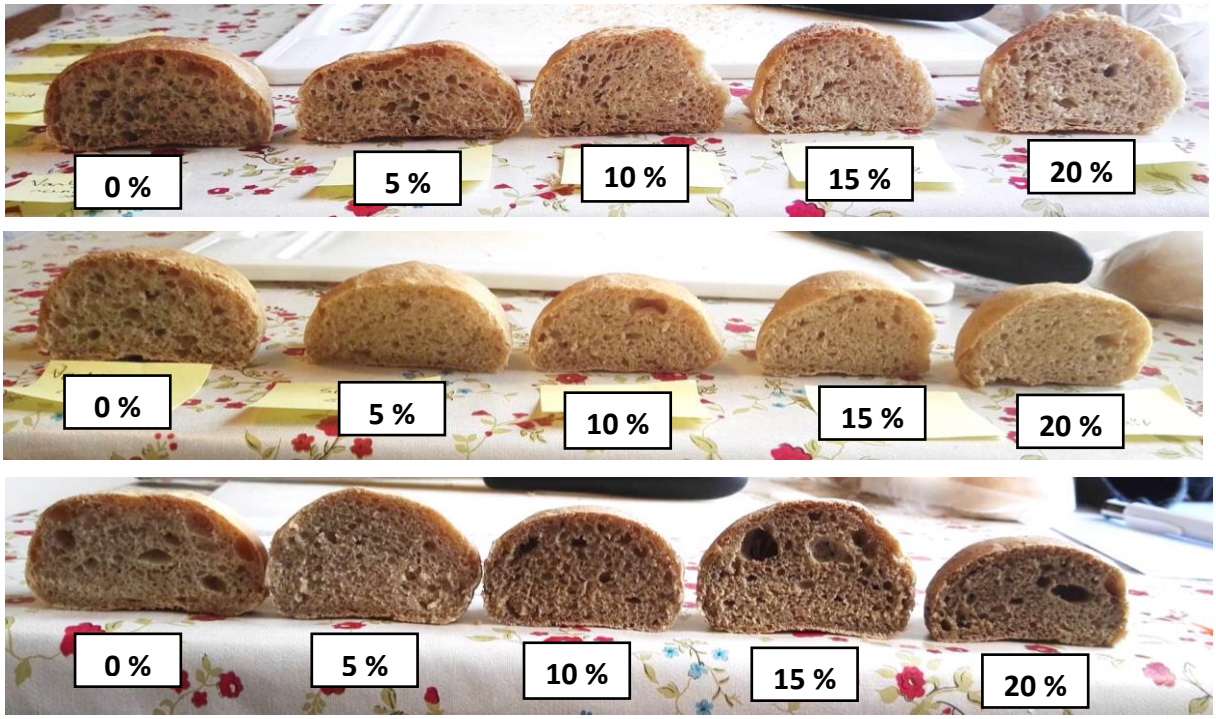

Figure 27. The texture of bread rolls made from mixtures of wheat flour, whole meal and various amounts of protein powder (0, 5, 10, 15 and 20 %). Bread rolls made with whey protein are shown at the top, bread rolls with herring protein powder in the middle and bread rolls with salmon meal are shown at the bottom.

It was not observed a major difference in the texture of the bread rolls with respect to protein concentration or the type of protein powder added. Compared to the reference with no protein added, the volumes of the bread rolls were similar for all protein concentrations. Sidwell et al. studied the physical and sensory characteristics of doughs and bread containing various concentrations of FPC from red hake. They found that the addition of FPC decreased the volumes of the bread, and in addition the crumb became darker, coarser and more compact (Sidwell and Hammerle, 1970). Since a bread is much larger than a bread roll, it is possible that any changes in the volumes would have been more evident if breads had been made instead. Although the bread rolls were quite similar, they became slightly more compact (smaller air bubbles) with increasing protein concentration. This is perhaps easiest to observe when the bread

rolls with 20 % whey or herring protein powder are compared to the reference. The bread rolls with the highest concentrations (15 and 20 %) of salmon meal contain lager bubbles than the others, but this is probably due to the kneading of the dough, not the protein powder.

3.13 Sensory Analysis of Bread Rolls Enriched with Protein

In order to test the acceptance of the bread rolls, a sensory evaluation was performed by an untrained panel, mainly consisting of female students in the age range 23-26 years old. The sensory evaluation was based on a 9-point hedonic scale for the sensory attributes (appearance, smell, texture and taste) and a 5-point hedonic scale for the purchase intent. The results from the sensory analysis of bread rolls mixed with whey protein powder, herring protein powder and salmon meal are given in Figure 28, 29 and 30, respectively. The data from the sensory evaluations are given in Appendix M.2.

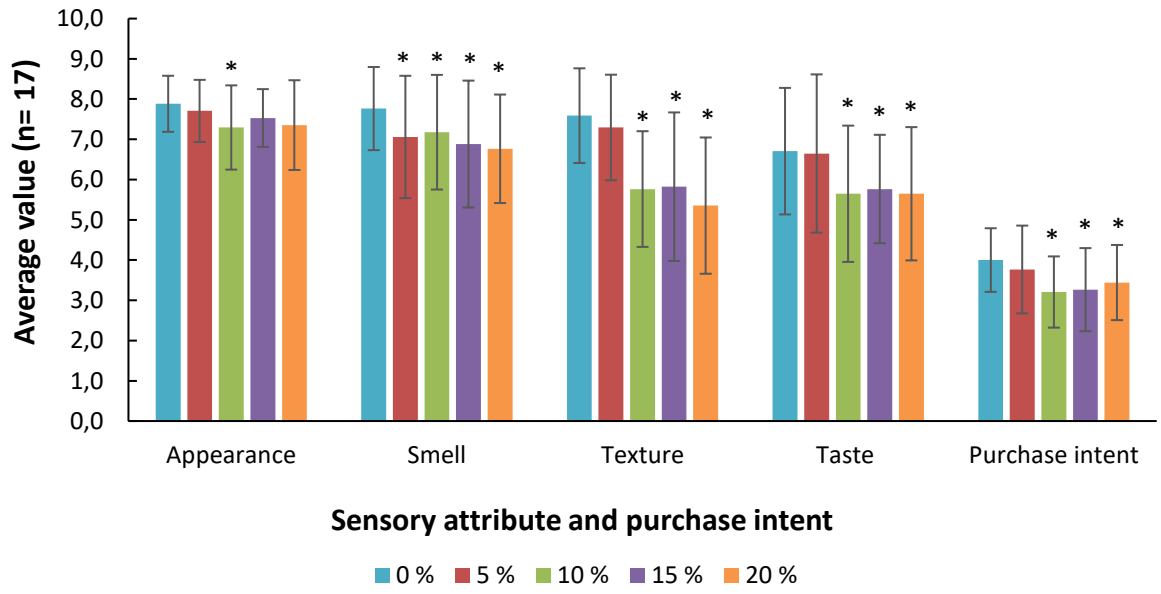

Figure 28. Average values of appearance, smell, texture, taste and purchase intent for bread rolls baked with various amount of whey protein powder (0, 5, 10, 15 and 20 %). The sensory evaluation was based on a 9-point hedonic scale (9 - like it extremely, 5 - neither like nor dislike it, 1 - dislike it extremely), except for the purchase intent which was based on a 5-point hedonic scale (5 - certainly would buy it, 3 - might buy it/might not buy it, 1 - certainly would not buy it). The total number of assessors was 17. The values are given as mean ± SD. The star (*) indicates a value significantly different ($P < 0.05$) from the reference value calculated from a one sided t-test.

As seen from Figure 28, the assessors generally liked the appearance of the bread rolls baked with whey protein powder. The bread rolls with 5 % WPP received similar acceptance as the reference and the smell was the only attribute statistically different from the reference. The bread rolls with 10, 15 and 20 % whey protein powder were not as well accepted as the one with only 5 %. It was especially the texture and the taste that received a lower score. As seen from Figure 28, there was not a major difference in the sensory attributes between the bread rolls added 10-20 % protein powder. The purchase intent of the reference bread roll was 4 ± 0.8, and with the addition of 5 % WPP it decreased to 3.8 ± 1.1. The purchase intent for bread rolls added 10 to 20 % was slightly lower than the bread rolls with
5 % and ranged from 3.2 ± 0.9 to 3.4 ± 0.9.

Some of the assessors reported that some of the bread rolls with the highest concentrations were slightly dry and hard. As mentioned earlier, the whey protein powder had a low WHC, which may have caused a higher water loss during the baking in the oven and hence resulted in drier bread rolls.

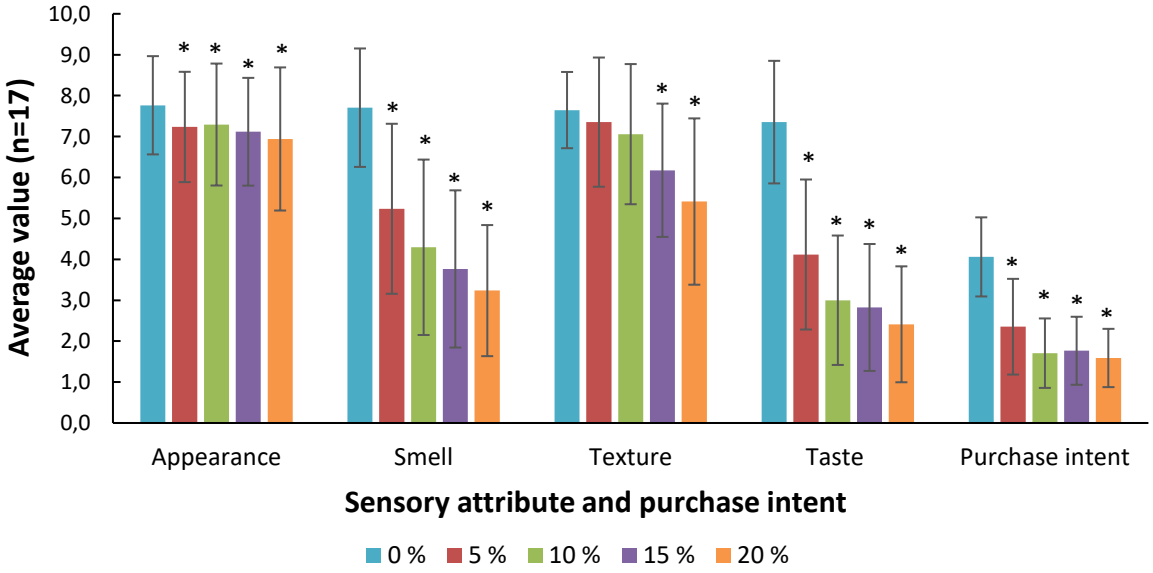

Figure 29. Average values of appearance, smell, texture, taste and purchase intent for bread rolls baked with various amounts of herring protein powder (0, 5, 10, 15 and 20 %). The sensory evaluation was based on a 9-point hedonic scale (9 - like it extremely, 5 - neither like nor dislike it, 1 - dislike it extremely), except for the purchase intent which was based on a 5-point hedonic scale (5 - certainly would buy it, 3 - might buy it/might not buy it, 1 - certainly would not buy it). The total number of assessors was 17. The values are given as mean ± SD. The star (*) indicates a value significantly different ($P < 0.05$) from the reference value calculated from a one sided t-test.

Compared to the bread rolls baked with whey protein powder, the acceptance of the bread rolls baked with herring protein powder was much lower. The appearance was the attribute that changed the least. Bread rolls with HPP obtained a slightly lower score for the appearance compared to both the reference and the bread rolls baked with whey protein powder. As seen from Figure 29, the attributes that received the lowest score were the smell and the taste. The difference in acceptance was obvious at only 5 % of herring protein powder. Generally, the value of all of the attributes decreased with increasing protein concentration. The texture of the bread rolls also decreased with increasing protein concentration, but not as much as the smell and taste. The reasons for the lower acceptance of the herring protein powder compared to the whey protein powder were mainly due to the fishy odour and taste. This was reported by many of the assessors and was most prominent at in the bread rolls with 15 and 20 % protein powder.

Several of the assessors described a powdery and grainy mouthfeel of the bread rolls with the highest concentrations of the herring protein powder. This was especially a problem with the bread rolls added 20 % protein powder, which also lead to a dry feeling in the mouth. One major problem with the herring protein powder was the very low solubility in water (section 3.6). Petersen reported that a low solubility can lead to an unattractive appearance and sandy mouthfeel when used in a product (Petersen, 1981). The low solubility may therefore be the reason for the bad mouthfeel.

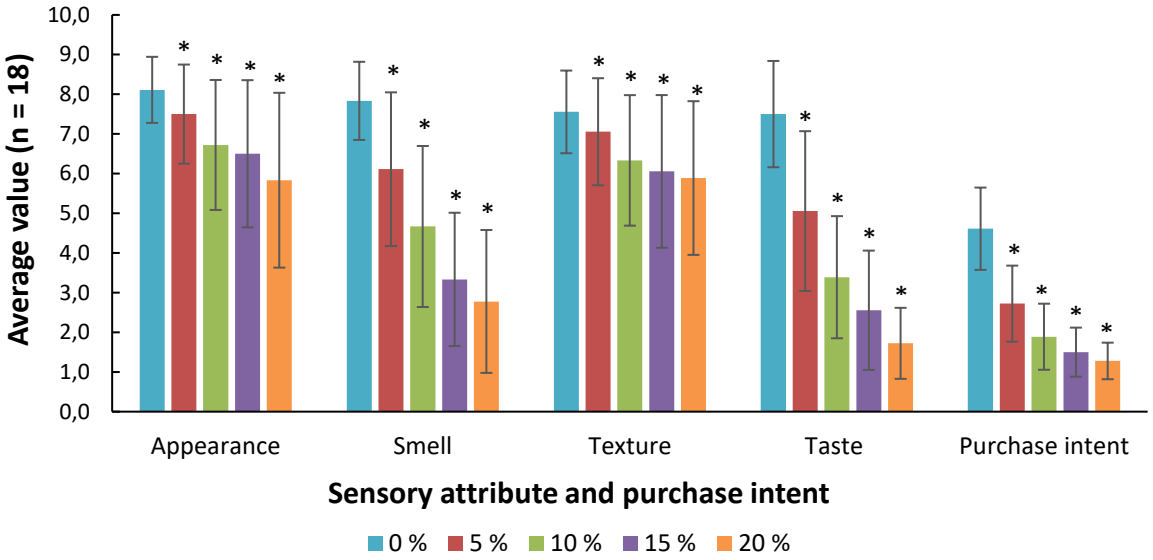

Figure 30. Average values of appearance, smell, texture, taste and purchase intent for bread rolls baked with various amounts of salmon meal (0, 5, 10, 15 and 20 %). The sensory evaluation was based on a 9-point hedonic scale (9 - like it extremely, 5 - neither like nor dislike it, 1 - dislike it extremely), except for the purchase intent which was based on a 5-point hedonic scale (5 - certainly would buy it, 3 - might buy it/might not buy it, 1 - certainly would not buy it). The total number of assessors was 18. The values are given as mean ± SD. The star (*) indicates a value significantly different ($P < 0.05$) from the reference value calculated from a one sided t-test.

As seen from Figure 30, the bread rolls made with salmon meal received the lowest acceptance for their appearance. High concentrations of salmon meal led to a dark appearance which was less accepted compared to the bread rolls with less protein and hence a brighter colour. The bread rolls with 5 and 10 % salmon meal received a better acceptance for their taste and smell compared to the herring protein powder, but slightly lower values for their appearance and texture. With addition of 15 and 20 % salmon meal, the bread rolls received the lowest acceptance compared to both the whey and herring protein powder. The purchase intent of the 5 and 10 % bread rolls was 2.7 ± 1.0 and 1.9 ± 0.8, respectively. This is higher than the purchase intent for the herring protein powder with the same concentrations (2.4 ± 1.2 and 1.7 ± 0.8, respectively). At higher concentrations the bread rolls with herring protein powder received slightly higher purchase intent values compared to the ones with salmon meal.

Several of the assessors reported that the fishy odour or the smell of cod liver oil was higher in the bread rolls made with salmon meal compared to the ones with herring protein powder. The fat content was significantly higher in the salmon meal compared to the herring protein

powder (section 3.1). Oxidation of unsaturated fatty acids found in fish products can result in an unpleasant fishy odour (Zeng and Huang, 2012). The higher fat content of the salmon meal can therefore explain the more distinct fishy odour compared to the bread rolls with herring protein powder. Reduction of the fat content will limit the lipid oxidation and hence the fishy odour.

The total score, where all the sensory attributes and the purchase intent have been summarized for each protein concentration, is given in Figure 31.

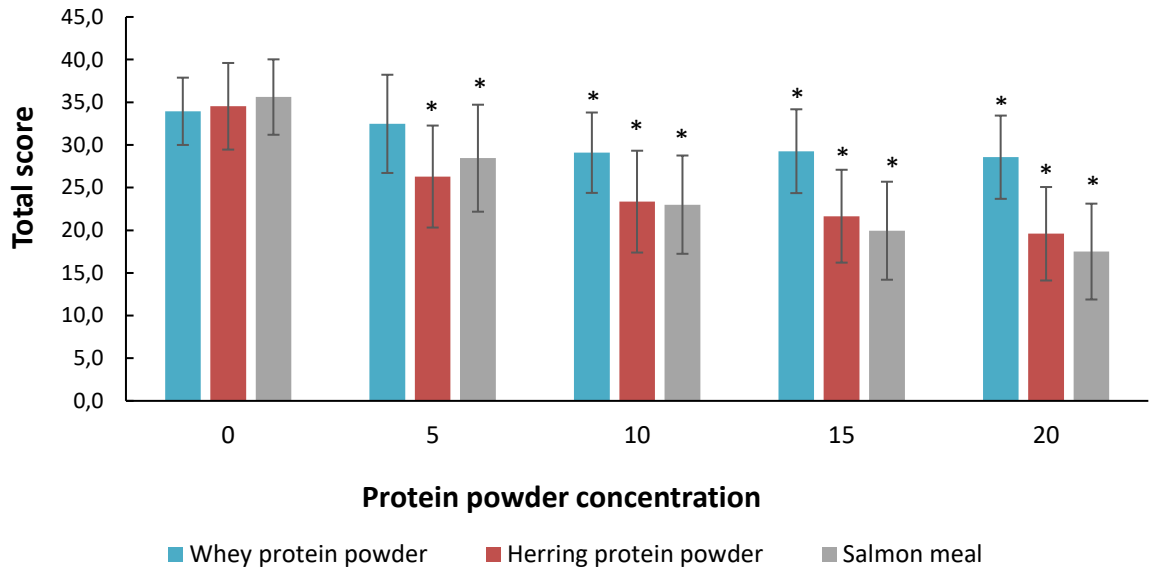

Figure 31. The total score (sum of all sensory attributes (appearance, smell, texture, taste) and purchase intent) for bread rolls baked with different concentrations of whey protein powder (WPP), herring protein powder (HPP) and salmon meal. The total number of assessors was 17 for WPP and HPP, while 18 people tasted the bread rolls with salmon meal. The values are given as mean ± SD. The star (*) indicates a value significantly different ($P < 0.05$) from the reference value for the different protein powders calculated from a one sided t-test.

As seen from Figure 31, the bread rolls with whey protein powder received the highest total score for all protein concentrations. The total score ranged from 32.5 ± 5.8 to 28.6 ± 4.9 for bread rolls with 5 and 20 % whey protein powder, respectively. The total score for bread rolls with 5 % salmon meal was higher than for bread rolls with 5 % herring protein powder, 28.4 ± 6.3 and 26.3 ± 6.0, respectively. The difference in the total score for the bread rolls with either 10 % herring protein powder or salmon meal was minor. At the highest protein concentrations (15 and 20 %), the bread rolls with herring protein powder received a higher acceptance than the bread rolls with the salmon meal. The total score of the reference bread rolls

(35.6 ± 4.4) was more than twice as high compared to the total score of the bread rolls with 20 % salmon meal (17.5 ± 5.6).

The assessors tasted bread rolls without extra protein three times. As seen from Figure 31, there was some variations in the total score for the reference bread rolls. The values ranges from 33.9 ± 3.9 to 35.6 ± 4.4, so the accuracy of the assessors was good.

The results from the sensory evaluation of bread rolls mixed with protein powder are in agreement with other studies. Sidwell et al. found that bread enriched with 5 and 10 % FPC were well accepted by the judges, but higher concentrations were not as acceptable (Sidwell and Hammerle, 1970). FPC supplemented into Arabic bread and Indian bread was also well accepted up to 10 % (Nikkila et al., 1976). The appearance, taste, texture and the colour of bread with different concentrations of fish meal from Red-tailed Brycon were evaluated by an untrained adult panel in a study by Bastos et al. (2014). When all the sensory attributes were evaluated, the highest acceptance was for the bread with 5 and 10 % fish flour. Based on these sensory evaluations it seems as if bread enriched with 5 or 10 % protein powder/fish meal have the highest potential. The same concentrations as mentioned above got the highest acceptance in the sensory evaluation with herring protein powder and salmon meal as well.

The major drawbacks with the addition of fish protein powder to the bread rolls were the effect on the smell and the taste. The bread rolls were tasted without any spread, such as butter, cheese, jam etc. If they had been tasted with something that could mask the taste, it might result in a higher acceptance when it comes to these attributes. Traditional taste masking techniques used in the drug industry include the use of sweeteners, amino acids, flavouring agents and microencapsulation (Deepak et al., 2012). Encapsulation of the herring protein powder or the salmon meal could be used to mask the unpleasant taste and smell. A casein hydrolysate has been successfully encapsulated with spray-drying. The encapsulation with gelatine and soy protein isolate decreased the bitter taste of the hydrolysate (Favaro-Trindade et al., 2010).

The preference for a product may vary with geographic location, age, sex, lifestyle, values and product usage. The number and type of assessors is thus of great importance in affective testing (Kemp et al., 2009). The assessors participating in the sensory analysis consisted of mainly female students in the age range 23-26 years old. This is a narrow part of the popula-

tion and it is possible that the results would have been different if a broader age range participated. Preferably, it should also have been more people participating in the sensory evaluation. There is a limited amount of bread rolls one person can manage to produce when the sensory evaluation is performed the same day as they are baked. For practical reasons, it was therefore not possible to have a higher number of assessors without any extra help. Since the sensory evaluations were performed in a study room with no separation of the assessors, they were able to both see and hear each other. This could of course have an influence on the results. In addition, some of the people participating in the evaluation had a better knowledge of the ingredients in the bread rolls than others, which also may have influenced their acceptance of the products.

Most of the assessors had never participated in a sensory analysis before and were not familiar with the 9-point hedonic test scale used to assess the sensory attributes of the bread rolls. Some of the judges found the 9-point hedonic scale difficult to use because of too many intervals. Hence, it might have been better to use a 7-point hedonic scale to make it easier for the assessors.

3.14 Drinking Yoghurt mixed with Biola

A drinking yoghurt, such as Biola, was thought of as a possible model product. Biola with blueberry taste was mixed with 1, 3 and 5 % (w/w) of whey protein powder and herring protein powder. In addition, the herring protein powder was cooked in boiling water for 15 min prior to mixing with Biola. The observations from the tasting can be found in Table 16.

Table 16. Subjective observations from the tasting of Biola with whey protein powder (WPP) and untreated and cooked herring protein powder (HPP). Three different protein powder concentrations (PPC) were tested. It was not any difference between the pretreated and the untreated mixture of Biola mixed with herring and the observations are therefore described together.

Protein powder	PPC [%]	Appearance	Smell	Taste
WPP	1	No change	No change	Almost identical to the one with no addition
	3	No change	No particular change	Neither good nor bad
	5	Somewhat brighter in the colour	Somewhat different smell, but not unpleasant	Does not taste as Biola anymore, but the taste is not particular unpleasant
HPP (untreated + cooked)	1	No change	Not able to smell any particular unpleasant smell	Neither good nor bad. Somewhat strange after taste
	3	No change	The protein powder smell is distinct and not very pleasant	Not eatable. Powdery and grainy mouthfeel. Left with a dry feeling in the mount
	5	No change	The protein powder smell is very distinct and worse compared to the 3 %	Similar to the one with 3 %, but even more powdery and grainy.

It was easy to mix whey protein powder with Biola and the powder dissolved without problems. The smell and taste changed some with increasing protein powder concentration, especially with 5 % WPP, but it was not unpleasant. Mixing the herring protein powder with Biola was more difficult due to the low solubility of the protein powder. The smell was not very appealing with 3 and 5 % HPP. The major problem was the taste. Even if the solution was stirred well, the mixtures with 3 and 5 % HPP felt very powdery and grainy in the mouth. The cooking of the powder prior to mixing with the Biola did not improve the problems with the grainy mouthfeel and the very unpleasant after taste. Although the observations listed in Table 16 are subjective and only the opinions from one person, it was concluded that Biola was not a good model product. Based on this, it was not performed a more comprehensive sensory analysis as with the bread rolls.

The herring protein powder used in this thesis has also been the raw material in another study. The effect from daily consumption of the herring protein powder compared to a whey protein powder has been investigated by Solheim (2016). In this study, the participants consumed 20 g of herring protein powder mixed with water daily. Nine of the twenty one people who participated in the study, resigned due to the taste, smell and consistency of water mixed with protein powder. Liquid model products, such as water or Biola, are not well suited for the herring protein powder due to the low solubility, which results in a grainy mouthfeel.

3.15 Protein and Essential Amino Acid Content of the Bread Rolls

3.15.1 Protein Content

The protein content in wheat is low (Rosell, 2011) and the quantity of protein could be increased by addition of a protein powder. Elderly people, especially hospitalized elderly people, is a group that could benefit from products enriched with proteins as they often have an insufficient protein intake (van Bokhorst–de van der Schueren et al., 2012, Leistra et al., 2011). The approximate protein content of the bread rolls baked with different concentrations of whey protein powder, herring protein powder and salmon meal are given in Table 17. Since the protein content of the protein powders measured with the C/N analysis was slightly lower than the protein content specified by the producers, calculations based on both values are included in Table 17. The formulations given in Table 7 (section 2.2.18) was used to calculate the protein content of the whole dough. The calculated values were then divided by five, since five bread rolls were made from each dough. The approximate composition of wheat flour and wholemeal used in these calculations can be found in Appendix N. As mentioned in section 3.12, some extra wheat flour was added to the dough mixed with whey protein powder. This has not been taken into account when the protein content of the bread rolls mixed with whey protein was calculated.

Table 17. Proximate protein content of one bread roll [g] for the different concentrations of the whey protein powder (WPP), herring protein powder (HPP) and salmon meal. It is given in parenthesis whether the protein content specified by the producer or the protein content from the C/N analysis was used in the calculations.

	Protein powder concentration [%]				
Protein powder	**0**	**5**	**10**	**15**	**20**
WPP (Producer)	3.0	3.9	4.7	5.6	6.5
WPP (C/N)	3.0	3.8	4.5	5.3	6.1
HPP (Producer)	3.0	3.9	4.9	5.8	6.8
HPP (C/N)	3.0	3.9	4.7	5.6	6.4
Salmon meal (C/N)	3.0	3.6	4.3	4.9	5.6

The addition of protein powder increased the amount of protein in the bread rolls. A bread roll without extra protein added contained approximately 3.0 g protein. The protein content of the protein powders were given in Table 8 (section 3.1). As seen from Table 8 and 17, the protein powder with the highest protein content resulted in the highest protein content of the bread rolls as well. The increase in protein content of the bread rolls was smallest with addition of salmon meal, due to it having the lowest protein content of the protein powder. Addition of 5 % protein powder increased the protein content of the bread rolls with less than 1 % for all of the powders. Although the protein content was higher in bread rolls with 5 % protein powder compared to those without any addition, the differences were so small that it probably does not have any effect. With the addition of 20 % protein powder, the protein content of the bread rolls increased to 6.1, 6.4 and 5.6 g for the WPP, HPP and salmon meal, respectively. If two bread rolls mixed with 20 % HPP (producer value) are consumed both for breakfast and for lunch, the protein intake would increase from 12.0 to 27.1 g. The protein intake from bread rolls is thus more than doubled by addition of 20 % HPP.

The nutritional effect from bread and drinking yoghurt enriched with whey protein concentrate in older adults in a rehabilitation center and in acute hospitalized older adults have been investigated in two different studies with promising results (van Til et al., 2015, Stelten et al., 2015). In the study at the rehabilitation center, the patients in the intervention group received bread with approximately 7 g protein per bread slice, while the bread for the control group contained approximately 4 g protein. Around 20 g of the daily protein intake came from bread

enriched with protein and 32 g from enriched drinking yoghurt. The effect from eating a reasonable amount of bread rolls per day with only 5 and 10 % protein addition may be insufficient. Especially if it is desired to reach a total amount of protein as high as the one the intervention group consumed on a daily basis. The bread rolls would most likely have to be enriched with at least 15 or 20 % protein powder in order to actually give a significant increase in the protein intake. The bread rolls with 15 and 20 % HPP or salmon meal were not well accepted in the sensory evaluation. Bread rolls baked with 15 and 20 % WPP were less accepted than the ones with lower concentrations, but the judges were generally positive to the bread rolls baked with this protein powder. Based on the sensory properties of the powders, WPP is probably the powder with the highest potential, especially if the protein addition must be above 10 %.

3.15.2 Essential Amino Acids

The low content of essential amino acids (EAA), such as lysine, is the reason for the low protein quality of wheat flour (Coultate, 2016). Animal proteins have a high content of lysine (Belitz et al., 2009), and by adding a fish protein powder to baked products, the nutritional quality could be increased. The mean values for essential amino acid requirements for a healthy adult are given in Table 18. Based on these requirements, the necessary intake of the different amino acids for an adult weighing approximately 70 kg are also included.

Table 18. Mean values for essential amino acid (EAA) requirements for a healthy adult (World Health Organization, 2007). Cysteine is a metabolic product from methionine catabolism, while tyrosine is a metabolic product of phenylalanine. Cysteine and tyrosine are therefore dependent on sufficient methionine and phenylalanine supply, respectively, and these values are for that reason given as "methionine + cysteine" and "phenylalanine + tyrosine".

Essential amino acid	mg/kg body weight per day	The amount an adult with a body weight of 70 kg need to consume per day [g]
Histidine	10	0.7
Isoleucine	20	1.4
Leucine	39	2.7
Lysine	30	2.1
Methionine + cysteine	15	1.1
Methionine	10.4	0.7
Cysteine	4.1	0.3
Phenylalanine + tyrosine	25	1.8
Threonine	15	1.1
Tryptophan	4	0.3
Valine	26	1.8
Total EAA	**184**	**12.9**

The results from the acid hydrolysis, given in Table 9 (section 3.2), were used together with the amino acid composition of wheat flour and wholemeal, given in Appendix N, to calculate the amount of essential amino acids in one bread roll with different concentrations of whey protein powder, herring protein powder and salmon meal. The total amount of essential amino acids in one bread roll with different protein powder concentrations is given in Table 19. Calculated values can be found in Appendix O.

Table 19. Total amount of essential amino acids [g] in one bread roll with different concentrations of whey protein powder, herring protein powder and salmon meal.

	Protein powder concentration [%]				
Protein powder	**0**	**5**	**10**	**15**	**20**
Whey protein powder	1.1	1.5	1.9	2.3	2.6
Herring protein powder	1.1	1.7	2.2	2.8	3.3
Salmon meal	1.1	1.3	1.5	1.6	1.8

Without any addition of protein, the amount of EAA in one bread roll was 1.1 g. As expected, the amount of EAA increased with increasing protein powder concentration. The total amount of EAA increased the most with the herring protein powder, followed by the whey protein powder and salmon meal, respectively. With addition of 5 % HPP the total amount of EAA was 1.7 g, and this increased to 3.3 g when the HPP concentration was 20 %. With addition of 20 % WPP and salmon meal, the amount of EAA increased to 2.6 and 1.8 g, respectively. The addition of 5 % HPP resulted in an EAA content similar to addition of 20 % salmon meal. The content of essential amino acids in salmon meal measured after the acid hydrolysis was approximately 30 g lower compared to the herring protein powder, and this was reflected in the lower content of EAA in the bread rolls.

The content of the different essential amino acids in bread rolls with different concentrations of whey protein powder is given in Figure 32. Calculated values used to produce Figure 32, can be found in Appendix O.

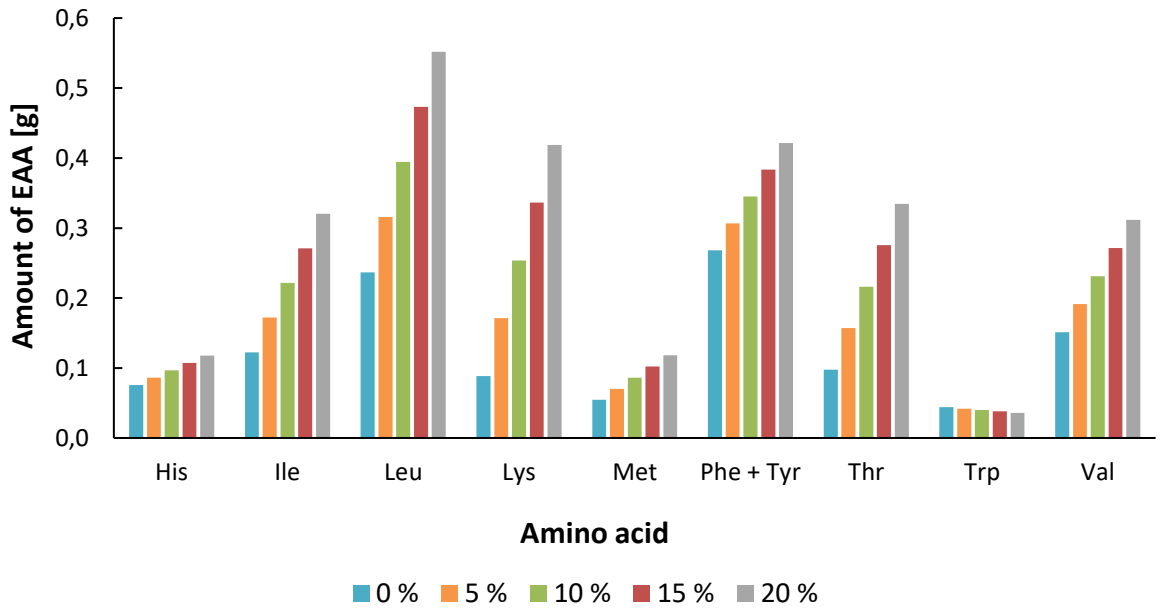

Figure 32. The amounts of the different essential amino acids (EAA) [g] in one bread roll with different concentrations (0, 5, 10, 15 and 20 %) of whey protein powder added.

As seen from Figure 32, the amount of the different essential amino acids increased with increasing whey protein powder concentration. The values from phenylalanine and tyrosine were presented together to make the comparison with the requirements for essential amino acids presented in Table 18 easier. Tryptophan could not be measured after the acid hydrolysis, so the tryptophan values presented in Figure 32, are only the amount found in the wheat flour and wholemeal flour. The amount of flour was replaced with protein powder, which explains the decrease in the tryptophan values. The bread rolls with whey protein powder had a high content of both leucine and lysine. Without any addition of protein powder the lysine content was 0.09 g in one bread roll, but with addition of 20 % whey protein powder, the lysine content increased to 0.42 g, which is almost five times higher. However, since the ε-amino group of lysine is very reactive, food processing such as cooking results in losses of this amino acid (Belitz et al., 2009), and the lysine content could thus be lower than shown in Figure 32.

The number of bread rolls with different concentrations of whey protein powder necessary to consume per day to meet the daily requirements of essential amino acids are given in Table 20. Since cysteine could not be measured after the acid hydrolysis, only the amount of methionine was used in the calculations, and 10.4 mg/kg daily was used as the methionine requirement.

Table 20. Approximate number of bread rolls with different concentrations of whey protein powder necessary to consume per day to meet the daily requirements of essential amino acids given in Table 18. These values are based on requirements for an adult weighing approximately 70 kg.

	Protein powder concentration [%]				
Essential amino acid	**0**	**5**	**10**	**15**	**20**
Histidine	9	8	7	7	6
Isoleucine	11	8	6	5	4
Leucine	12	9	7	6	5
Lysine	24	12	8	6	5
Methionine	13	10	8	7	6
Phenylalanine + tyrosine	7	6	5	5	4
Threonine	11	7	5	4	3
Tryptophan	6	7	7	7	8
Valine	12	10	8	7	6

The number of bread rolls necessary to consume per day was reduced with increasing protein concentration. The addition of 5 % WPP reduced the number of bread rolls necessary to consume in order to meet the lysine requirements from 24 to 12. This was further reduced to 5 bread rolls when the WPP concentration was increased to 20 %. As seen from Table 20, the number of bread rolls varied for the different amino acids. Lysine was the limiting amino acid, so it also had the highest number of bread rolls necessary to consume without any protein addition. The values in Table 20 represent the number of bread rolls necessary to consume if bread rolls were the only source of essential amino acids in the diet of an adult weighing approximately 70 kg. The number of bread rolls would be reduced for all protein concentrations if protein consumed from other sources had been included as well. Most importantly, Table 20 illustrates how addition of animal proteins can be used to increase the nutritional quality of a food product based on wheat flour.

The content of the different essential amino acids in bread rolls with different concentrations of herring protein powder is given in Figure 33. Calculated values used to produce Figure 33, can be found in Appendix O.

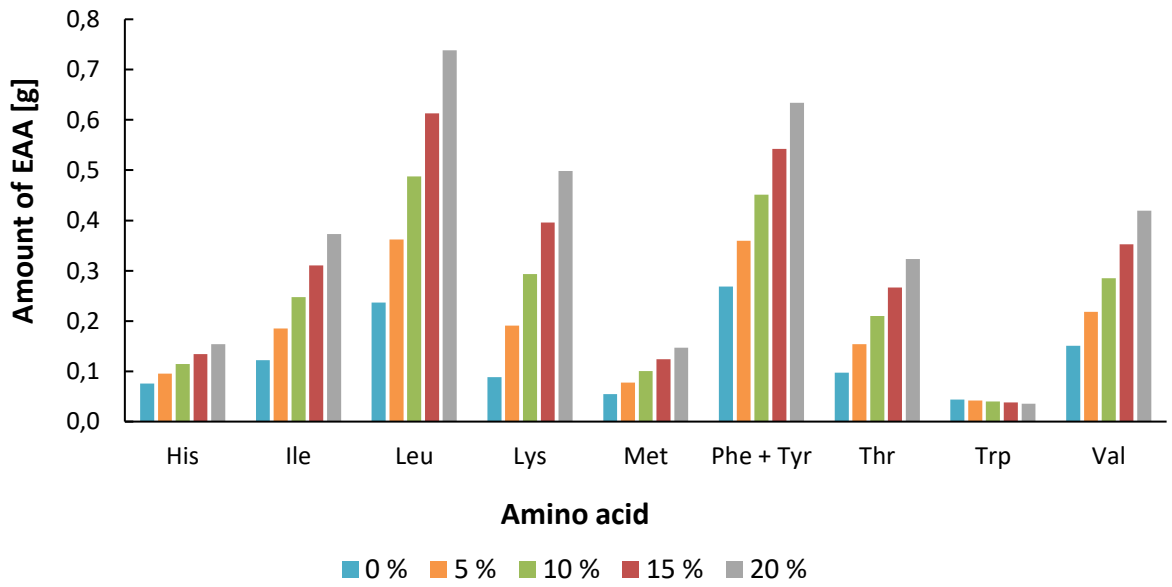

Figure 33. The amounts of the different essential amino acids (EAA) [g] in one bread roll with different concentrations (0, 5, 10, 15 and 20 %) of herring protein powder added.

As with the addition of WPP, the amount of the different amino acids increased with increasing herring protein powder concentration. The herring protein powder had a higher content of EAA compared to the WPP, and the amount of EAA in one bread roll was therefore highest for the bread rolls with herring protein powder. The increase in the leucine, lysine and phenylalanine + tyrosine content was high for bread rolls with HPP. Addition of 15 and 20 % HPP increased the amount of lysine from 0.09 g (reference bread roll) to 0.40 and 0.50 g, respectively. The amount of lysine was approximately 4.5 and 5.5 times higher as the reference bread roll with addition of 15 and 20 % HPP, respectively. Based on the higher amount of EAA, as shown in Figure 33, the bread rolls with HPP would have a higher nutritional quality compared to the bread rolls without.

The number of bread rolls with different concentrations of herring protein powder necessary to consume per day to meet the daily requirements of essential amino acids are given in Table 21.

Table 21. Approximate number of bread rolls with different concentrations of herring protein powder necessary to consume per day to meet the daily requirements of essential amino acids given in Table 18. These values are based on requirements for an adult weighing approximately 70 kg.

	Protein powder concentration [%]				
Essential amino acid	**0**	**5**	**10**	**15**	**20**
Histidine	9	7	6	5	5
Isoleucine	11	8	6	5	4
Leucine	12	8	6	4	4
Lysine	24	11	7	5	4
Methionine	13	9	7	6	5
Phenylalanine + tyrosine	7	5	4	3	3
Threonine	11	7	5	4	3
Tryptophan	6	7	7	7	8
Valine	12	8	6	5	4

Due to a higher content of EAA in HPP, the number of bread rolls necessary to consume on a daily basis was lower for the HPP compared to the WPP. As mentioned earlier, the amount of tryptophan could not be measured after the acid hydrolysis, so these values are only based on the amount of tryptophan found in wheat flour and wholemeal. This is also the reason for the increasing numbers for tryptophan as shown in Table 21, since the amount of flour decreased

with increasing protein powder concentrations. If the amount of tryptophan found in herring protein powder had been included, these values would have decreased with increasing protein concentration as well. When the tryptophan value is excluded the average amount of bread rolls with 20 % HPP necessary to consume on a daily basis for an adult weighing 70 kg is 4. This can easily be achieved by consuming two bread rolls for breakfast and two for lunch. The major problem is of course that the bread rolls with 20 % HPP were not well accepted by the assessors in the sensory evaluation.

The content of the different essential amino acids in bread rolls with different concentrations of salmon meal is given in Figure 34. Calculated values used to produce Figure 34 can be found in Appendix O.

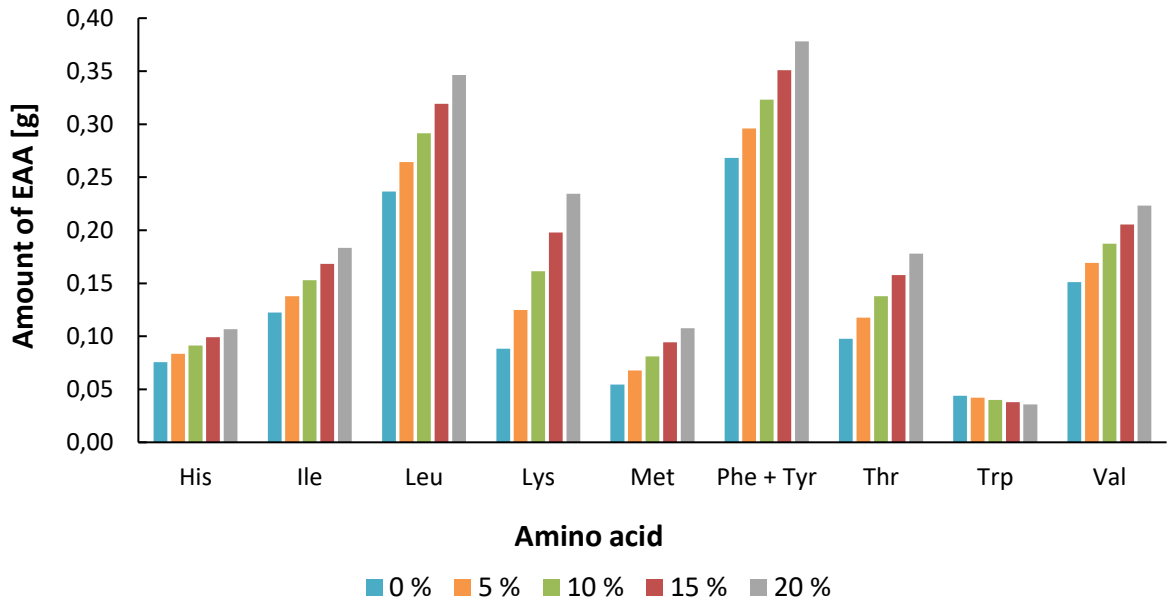

Figure 34. The amount of the different essential amino acids (EAA) [g] in one bread roll with different concentrations (0, 5, 10, 15 and 20 %) of salmon meal added.

The amount of EAA in bread rolls with salmon meal increased with increasing protein powder concentration, but the increase was not as high as for WPP or HPP. The lysine content in a bread roll with 20 % salmon meal was 0.23 g, which is less than half the amount of lysine in a bread roll with 20 % HPP (0.50 g). Due to a lower EAA content of salmon meal, this protein powder was not as efficient as HPP or WPP in increasing the nutritional value of the bread rolls.

The number of bread rolls with different concentrations of salmon meal necessary to consume each day to meet the daily requirements of essential amino acids are given in Table 22.

Table 22. Approximate number of bread rolls with different concentrations of salmon meal necessary to consume per day to meet the daily requirements of essential amino acids given in Table 18. These values are based on requirements for an adult weighing approximately 70 kg.

	Protein powder concentration [%]				
Essential amino acid	**0**	**5**	**10**	**15**	**20**
Histidine	9	8	8	7	7
Isoleucine	11	10	9	8	8
Leucine	12	10	9	9	8
Lysine	24	17	13	11	9
Methionine	13	11	9	8	7
Phenylalanine + tyrosine	7	6	5	5	5
Threonine	11	9	8	7	6
Tryptophan	6	7	7	7	8
Valine	12	11	10	9	8

The bread rolls with salmon meal need to be consumed at the highest quantity, except for the bread rolls without addition. The addition of salmon meal decreased the number of bread rolls necessary to consume per day to meet the daily requirements, but not at the same extent as HPP or WPP. When the tryptophan value is excluded, the average amount of bread rolls added 20 % salmon meal necessary to consume on a daily basis for an adult weighing 70 kg is 7. Compared to the average number of bread rolls necessary to consume per day calculated for HPP, it increased with 3 bread rolls for salmon meal. Considering the fact that the bread rolls with 15 and 20 % salmon meal were less accepted compared to the herring protein powder, it might be difficult to consume as much as 7 bread rolls with 20 % salmon meal every day.

4 Conclusion

This work has demonstrated the differences in nutritional, functional, bioactive and sensory properties of protein powders made from herring roe, salmon by-products and whey. The protein content of the different protein powders varied from 64.0 to 81.5 %, and this affected the content of essential amino acids. Both HPP and WPP had a high nutritional value due to high content of essential amino acids and high digestibility.

The solubility of the herring protein powder was low, and enzymatic hydrolysis with trypsin only improved the solubility with 5 %. The hydrolysis was therefore not very efficient in solubilizing the proteins. Increasing the temperature of the solvent did not significantly improve the solubility of the native HPP.

The WPP demonstrated better functional properties compared to the salmon meal. The solubility of the whey protein powder was high and the emulsion properties were good. The WPP can therefore be used in nutrition drinks or as an emulsifier in multiple phase foods. Due to a solubility below 20 % and a low emulsion stability, the salmon meal would not be suitable for the same application areas. Addition of salmon meal into minced cod filets increased the WHC, and the HPP had a slightly better swelling capacity than the salmon meal. Due to their ability both to bind and entrap water, the HPP and the salmon meal could be used in baked products or sausages to increase the juiciness. However, these application areas may be limited by their sensory properties.

The bread rolls with WPP received the best acceptance. The low solubility of the HPP resulted in a grainy mouthfeel, especially at high protein concentrations. A fishy odour and taste were reported for the bread rolls with salmon meal. In addition, the dark colour of this powder would be undesirable if it is added into light coloured products. The low sensory properties of the HPP and salmon meal make it difficult to add these powders in products made for human consumption. Due to a high nutritional value and good sensory properties, the WPP is probably the protein powder with the highest potential, especially if the addition of the powder has to be above 10 %. If the sensory properties of the HPP can be improved, this would make it more compatible with the WPP as a nutritional supplement.

Although some of the bread rolls were not well received, this work has demonstrated that the nutritional quality of wheat products can be improved by addition of proteins from an animal source. With addition of 20 % HPP or WPP, the number of bread rolls necessary to consume per day to meet the daily requirements of lysine was reduced from 24 to 4 and 5 bread rolls, respectively. The nutritional health of people suffering from insufficient protein or essential amino acid intake could therefore be improved by consuming protein enriched products.

5 Further Work

This work has shown that the nutritional quality of wheat products can be improved by addition of proteins with a high content of essential amino acids. Although the theoretical content of the essential amino acids was calculated for the different bread rolls, some amino acids, such as lysine, are lost during food processing. The proximate composition of the bread rolls after baking would therefore be interesting to analyse. This would have given a better estimate of the increase in nutritional value of the bread rolls by addition of protein.

The consistency of the dough changed with addition of the protein powders. Especially with the addition of WPP. Rheology measurements, such as a frequency sweep, could be used to say something about the stickiness or the elasticity of the dough. The texture and the colour of the bread rolls were only evaluated by observation. Measurements of the texture, such as a texture profile analysis, and the colour of the bread rolls can be performed in the future. The texture analysis would be useful in order to evaluate how the texture changed with increasing protein concentration.

The bread rolls were tasted without any spread, such as butter, jam or cheese. If they had been tasted with something that could mask the taste, this could have resulted in a higher acceptance. Whether a spread has a positive effect, is something that should be investigated.

The grainy mouthfeel of the HPP would most likely be reduced if the solubility of the powder increased. Due to the minor change with trypsin, enzymatic hydrolysis with other enzymes could be performed. Commercial enzymes, such as Alacase, Papain, Bromelain, Protamex or even a combination of some of these could be tested. If the fat content of the salmon meal is reduced, this would probably result in a higher acceptance for this powder as well.

Elderly adults, especially hospitalised elderly adults, is a group that could benefit from food with extra protein added. The acceptance of a product may vary with age, lifestyle or sex, so the results from the sensory analysis might have been different if a test panel with a higher average age had been used. A sensory evaluation where the target group was used as participants is therefore something that can be performed.

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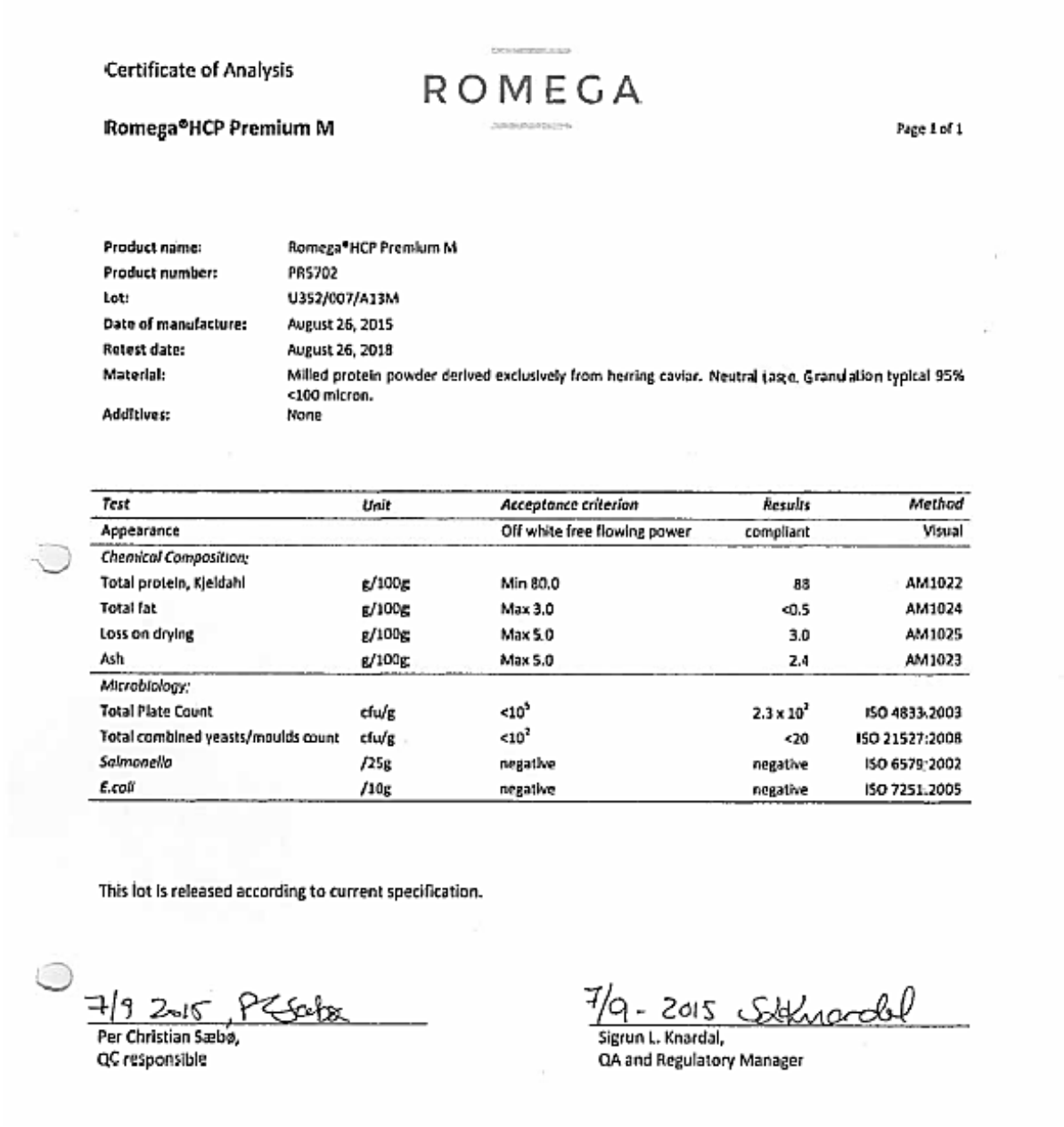
Appendix

A. Product Information about the Raw Materials

A.1 Herring Protein Powder

A certificate of analysis and the total amino acid composition for the herring protein powder, provided by the producer, are given in Figure 35 and 36, respectively.



Certificate of Analysis

ROMEGA

Romega®HCP Premium M

Page 1 of 1

Product name:	Romega®HCP Premium M
Product number:	PR5702
Lot:	U352/007/A13M
Date of manufacture:	August 26, 2015
Retest date:	August 26, 2018
Material:	Milled protein powder derived exclusively from herring caviar. Neutral taste. Granulation typical 95% <100 micron.
Additives:	None

Test	*Unit*	*Acceptance criterion*	*Results*	*Method*
Appearance		Off white free flowing power	compliant	Visual
Chemical Composition:				
Total protein, Kjeldahl	g/100g	Min 80.0	88	AM1022
Total fat	g/100g	Max 3.0	<0.5	AM1024
Loss on drying	g/100g	Max 5.0	3.0	AM1025
Ash	g/100g	Max 5.0	2.4	AM1023
Microbiology:				
Total Plate Count	cfu/g	$<10^5$	2.3×10^2	ISO 4833:2003
Total combined yeasts/moulds count	cfu/g	$<10^2$	<20	ISO 21527:2008
Salmonella	/25g	negative	negative	ISO 6579:2002
E.coli	/10g	negative	negative	ISO 7251:2005

This lot is released according to current specification.

7/9 2015, PCSæbø
Per Christian Sæbø,
QC responsible

7/9 - 2015 SLKnardal
Sigrun L. Knardal,
QA and Regulatory Manager

Figure 35. A certificate of analysis for the herring protein powder. The certificate gives information about chemical composition of the protein powder, microbiology measurements and the methods used to analyse the material.

AR-16-MO-004804-01
EUNOMO2-00040354

eurofins

Sample code:	440-2016-0503-050	Sampled on:	21.04.2016
Description:	Proteinpulver	Sampled by:	Oppdragsgiver
Client Sample:	Romega HCP Premium M, Lot U352/007/A13M-2	Analysis date:	03.05.2016

	Analysis		Result	Unit	LOQ:	MU	Method
a)	**Amino acids (acid hydrolysis)**						
a)	DI004	Aspartic acid	6.68	g/100 g	0.017	6%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Serine	4.31	g/100 g	0.016	7%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Glutamic acid	10.3	g/100 g	0.021	7%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Proline	4.76	g/100 g	0.02	8%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Glycine	2.87	g/100 g	0.019	7%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Alanine	7.66	g/100 g	0.015	6%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Valine	6.24	g/100 g	0.016	8%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Isoleucine	5.34	g/100 g	0.035	8%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Leucine	9.25	g/100 g	0.015	8%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Tyrosine	3.39	g/100 g	0.023		ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Phenylalanine	3.31	g/100 g	0.031	6%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Histidine	1.82	g/100 g	0.02	10%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Lysine	6.30	g/100 g	0.014	8%	ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Arginine	4.37	g/100 g	0.01	6%	ISO 13903:2005, EU 152/2009 (F)
a)	**Tryptophane**						
a)	DJ009	Tryptophan (Total)	1.25	g/100 g	0.01	8%	EU 152/2009
a)	**Cystine, methionine (oxidative)**						
a)	DJ011	Cystein +Cystine	0.909	g/100 g	0.006	10%	ISO 13903:2005, EU 152/2009 (F)
a)	DJ011	Methionine	2.30	g/100 g	0.024	10%	ISO 13903:2005, EU 152/2009 (F)
a)	**Amino acids (acid hydrolysis)**						
a)	DI004	Hydroxyproline	<0.05 (LOQ)	g/100 g	0.05		ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Ornithine	<0.05 (LOQ)	g/100 g	0.05		ISO 13903:2005, EU 152/2009 (F)
a)	DI004	Threonine	4.94	g/100 g	0.006		ISO 13903:2005, EU 152/2009 (F)

Figure 36. Amino acid composition analyzed by Eurofins Steins Laboratorium. Symbol description: LOQ: Limit of quantification, MU: Uncertainty of measurement

A.2 Whey Protein Powder

The ingredients, nutritional facts and the total amino acid composition of the whey protein powder with a neutral taste, provided by the producer, can be found in Table 23, 24 and 25, respectively.

Table 23. Ingredients in the whey protein powder.

Ingredients
Whey protein isolate
Whey protein concentrate
Hydrolysed whey protein (OptiPEP^{TM})

Table 24. Nutritional facts per 100 g for the whey protein powder.

Nutritional Facts	**[g/100 g]**
Energy	1620 kJ/ 382 kcal
Protein	82.7
Total Carbohydrates	4.9
- Sugars	4.9
- Lactose	2.7
Total fat	4.4
- Saturated fatty acids	2.4
Dietary fiber	0.0
Salt	540 mg

Table 25. Amino acid composition for the whey protein powder. The values are given as g amino acid per 100 g protein powder.

Amino acid	**[g/100 g]**
Alanine	4.23
Arginine	1.94
Aspartic acid	9.26
Cysteine	2.03
Glutamic acid	15.23
Glycine	1.37
Histidine	1.56
Isoleucine	3.23
Leucine	8.97
Lysine	8.04
Methionine	1.83
Phenylalanine	2.54
Proline	4.90
Serine	4.20
Threonine	5.79
Tryptophan	1.27
Tyrosine	2.31
Taurine	0.00
Valine	4.93

B. Protein, Fat, Moisture and Ash Content

B.1 Protein Content

The protein content of the different protein powders were given in Table 8 in section 3.1. The amount of powder weighed out, measured values from the C/N analysis, calculated protein content and the standard deviations for the different samples are given in Table 26.

Table 26. Results from the C/N analysis, weight of the samples and calculated protein content for whey protein powder (WPP), herring protein powder (HPP) and salmon meal. The average amount of protein together with the SD for each powder are also included.

Material	Parallel	Carbon [µg/capsule]	Nitrogen [µg/capsule]	Weight of the sample [mg]	Protein content [mg/g]	Protein content [g/100 g]	Average protein content [g/100 g]	SD
WPP	# 1	406.079	102.833	0.858	749.1	74.9	74.7	1.5
	# 2	480.287	124.079	1.020	760.3	76.0		
	# 3	335.786	84.654	0.733	721.8	72.2		
	# 4	377.884	96.551	0.803	751.5	75.1		
	# 5	358.803	91.231	0.756	754.2	75.4		
HPP	# 1	335.982	91.528	0.702	814.9	81.5	81.5	0.3
	# 2	359.679	98.075	0.753	814.0	81.4		
	# 3	312.442	84.999	0.651	816.0	81.6		
	# 4	332.886	90.773	0.692	819.8	82.0		
	# 5	323.549	88.078	0.679	810.7	81.1		
Salmon meal	# 1	417.682	98.147	0.899	682.3	68.2	64.0	5.3
	# 2	375.026	83.183	0.948	548.4	54.8		
	# 3	285.397	64.025	0.594	673.7	67.4		
	# 4	274.755	60.823	0.587	647.6	64.8		
	# 5	350.531	79.466	0.769	645.9	64.6		

B.2 Fat Content

The fat content of the salmon meal was determined and the result was given in Table 8. Measured values together with the calculated amount of fat and the SD are given in Table 27.

Table 27. Measured values used in the determination of the fat content in salmon meal. The average amount of fat together with the SD are also included.

Sample	Parallel	Weight of the sample [g]	Empty reagent tube [g]	Reagent tube with fat [g]	Amount of fat in reagent tube [g]	Lipid content [%]	Average amount of lipids [%]	SD
A	# 1	5.0232	13.6000	13.6450	0.0450	17.92		
	# 2	5.0232	21.2506	21.2919	0.0413	16.44		
							17.3	1.0
B	# 1	5.0006	19.7640	19.8086	0.0446	17.84		
	# 2	5.0006	19.2807	19.3212	0.0405	16.20		

2 mL of the chloroform phase was added into each reagent tube.

B.3 Moisture Content

The moisture content of the herring protein powder, whey protein powder and salmon meal were determined, and the results were given in Table 8. Measured values together with the calculated moisture content and the SD are given in Table 28.

Table 28. Measured values used in the determination of the moisture content of herring protein powder (HPP), whey protein powder (WPP) and salmon meal. The average moisture content, together with the SD for each powder are also included.

Material	Parallel	Weighing dish [g]	Weighing dish + powder [g]	Weighing dish + powder (after drying) [g]	Dry matter content [%]	Moisture content [%]	Average [%]	SD
HPP	#1	1.8446	2.5141	2.4796	94.85	5.15	5.18	0.04
	#2	1.8357	2.4493	2.4173	94.78	5.22		
WPP	#1	1.8380	2.4733	2.4417	95.03	4.97	4.9	0.1
	#2	1.8324	2.4536	2.4236	95.17	4.83		
Salmon meal	#1	1.8327	2.4825	2.4470	94.54	5.46	53	0.3
	#2	1.8144	2.4203	2.3896	94.93	5.07		

B.4 Ash Content

The ash content of the herring protein powder, whey protein powder and salmon meal were determined and the results were given in Table 8. Measured values together with the calculated ash content and the SD are given in Table 29.

Table 29. Measured values used in the determination of the ash content of herring protein powder (HPP), whey protein powder (WPP) and salmon meal. The average ash content together with the SD for each powder are also included.

Material	Parallel	Crucible [g]	Crucible + powder [g]	Crucible + ash [g]	Ash content [%]	Average [%]	SD
HPP	#1	19.8978	20.4000	19.9051	1.45	1.47	0.03
	#2	16.5155	17.0179	16.5230	1.49		
WPP	#1	20.1382	20.6564	20.1549	3.2	3.2	0.1
	#2	18.6167	19.1330	18.6329	3.1		
Salmon meal	#1	20.0265	20.5310	20.0797	10.5	10.3	0.3
	#2	20.2118	20.7119	20.2623	10.1		

C. Amino Acid Composition

The protein powders were hydrolysed with HCl and analysed with HPLC. The detected amount of the different amino acids was multiplied with the corresponding molecular weights and divided by 1000 to calculate the amino acid concentration (µg/mL). The content of the different amino acids was then calculated according to Equation 6:

$$Amino\ acid\ (\frac{mg}{g}) = \frac{C \cdot V \cdot D}{1000 \cdot M} \quad (6)$$

Here, *C* is the concentration of the amino acid (µg/mL), *V* is the total volume of the sample after neutralization (10 mL), *D* is the dilution, and *M* is the weight of the sample (g). The value was divided by 1000 to get the correct unit.

The calculated amount of the different amino acids in the herring protein powder, salmon meal and whey protein powder are Table 30, 31 and 32, respectively.

Table 30. Amino acid composition (mg/g) of herring protein powder. Six measurements were performed in total. Parallel number 3 and 4 were not included in the average, due to deviations from the other parallels. The glutamate values were above 1000 g/ 100 g, which is impossible, and were not included in the calculated total amount of amino acids or in Table 9.

Amino acid	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**Average [mg/g]**	**SD**
Aspartate	62.10	62.42	49.81	48.29	69.14	68.25	65.48	3.73
Glutamate	10824.92	11037.09	8933.55	8557.72	12301.76	12132.90	0.00	0.00
Asparagine	0.85	0.91	0.00	0.00	0.00	0.00	0.44	0.51
Histidine	18.64	17.79	12.46	12.06	18.66	18.08	18.29	0.43
Serine	36.10	35.67	26.40	26.21	37.66	37.58	36.75	1.02
Glutamine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycine /Arginine	40.74	40.80	0.00	25.27	37.03	36.81	38.84	2.22
Threonine	46.29	47.05	35.54	34.83	50.10	49.61	48.26	1.87
Alanine	67.52	67.18	51.95	50.82	74.18	73.47	70.59	3.75
Tyrosine	42.83	42.14	24.99	24.11	34.10	33.75	38.20	4.95
Aba	1.58	1.56	0.84	1.09	0.82	0.87	1.21	0.42
Methionine	22.49	22.67	14.27	14.08	18.56	17.68	20.35	2.60
Valine	59.44	59.44	41.50	41.06	57.46	58.30	58.66	0.96
Phenylalanine	54.22	54.85	24.04	24.24	33.60	34.50	44.29	11.83
Isoleucine	54.47	54.65	38.56	0.00	53.93	53.68	54.18	0.45
Leucine	123.02	124.04	66.72	65.48	92.89	93.39	108.34	17.55
Lysine	102.53	103.03	46.87	45.20	66.58	65.77	84.48	21.14
Total	732.81	734.18	433.93	412.74	644.70	641.73	688.36	52.14

Table 31. Amino acid composition (mg/g) of salmon meal. Six measurements were performed in total. Parallel 1 was lost during the HPLC measurements. Parallel 2 was not included in the average due to deviations from the other measurements. Problems with the glutamate standard resulted in substantial variations between the different parallels, with respect to glutamate. These values were hence not included in the calculated total amount of amino acids or in Table 9.

Amino acid	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**Average [mg/g]**	**SD**
Aspartate		129.00	41.60	38.19	40.41	41.35	40.39	1.5
Glutamate		0.96	1.62	97.18	0.00	103.25	0.00	0.0
Asparagine		0.00	0.00	0.00	0.06	0.00	0.02	0.0
Histidine		7.10	8.00	9.15	9.39	9.51	9.01	0.7
Serine		20.24	19.62	20.58	21.75	21.85	20.95	1.1
Glutamine		1.19	1.83	2.37	2.57	2.63	2.35	0.4
Glycine /Arginine		33.66	34.46	36.48	38.16	28.22	34.33	4.3
Threonine		16.77	18.53	19.05	20.04	20.46	19.52	0.9
Alanine		21.51	22.55	24.13	25.21	27.45	24.83	2.1
Tyrosine		14.39	13.26	14.20	14.97	15.20	14.41	0.9
Aba		1.14	1.16	1.28	1.36	1.51	1.33	0.1
Methionine		36.14	11.87	12.37	13.03	13.20	12.62	0.6
Valine		18.74	18.67	19.63	20.51	20.52	19.83	0.9
Phenylalanine		32.71	16.50	17.05	18.03	18.07	17.41	0.8
Isoleucine		30.69	15.70	16.50	17.19	17.20	16.65	0.7
Leucine		57.37	28.77	30.38	31.42	31.44	30.50	1.3
Lysine		60.78	30.28	31.96	33.07	33.17	32.12	1.3
Total		482.38	282.80	293.31	307.19	301.78	296.27	10.6

Table 32. Amino acid composition (mg/g) of whey protein powder. Six measurements were performed in total. Average value and SD are also included.

Amino acid	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**Average [mg/g]**	**SD**
Aspartate	74.16	75.99	79.00	76.46	73.96	73.33	75.48	2.11
Glutamate	131.24	134.77	142.86	136.76	131.92	130.63	134.70	4.63
Asparagine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Histidine	11.38	11.62	11.92	10.77	10.79	10.49	11.16	0.56
Serine	31.92	33.15	33.15	32.62	31.83	31.77	32.40	0.65
Glutamine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Glycine /Arginine	13.42	13.84	13.94	13.61	13.40	13.29	13.58	0.26
Threonine	50.20	51.49	52.02	50.75	49.75	49.46	50.61	1.00
Alanine	31.66	32.55	33.70	32.97	31.61	31.96	32.41	0.82
Tyrosine	19.26	19.65	19.94	19.36	18.88	18.97	19.34	0.40
Aba	1.65	1.72	1.60	1.65	1.45	1.67	1.62	0.09
Methionine	14.84	14.58	15.19	14.81	14.48	14.38	14.71	0.30
Valine	36.26	37.47	38.58	37.65	37.11	36.58	37.27	0.83
Phenylalanine	20.20	20.52	21.96	21.18	21.66	20.83	21.06	0.67
Isoleucine	42.81	43.64	45.57	44.43	43.21	43.10	43.79	1.04
Leucine	69.65	71.45	74.32	72.47	70.29	69.89	71.35	1.80
Lysine	66.88	68.72	71.73	69.67	66.98	67.95	68.66	1.84
Total	615.54	631.15	655.49	635.16	617.29	614.29	628.16	15.97

D. Free Amino Acids

The protein was precipitated with SSA and the amount of free amino acids in salmon meal, whey protein powder and herring protein powder hydrolysates were analysed with HPLC. The detected amount of the different amino acids was multiplied with the corresponding molecular weights and divided by 1000 to calculate the amino acid concentration (µg/mL). The content of the different amino acids was then calculated according to Equation 7:

$$Free\ amino\ acid\ (\frac{mg}{g}) = \frac{C \cdot V \cdot D \cdot S}{1000 \cdot M} \quad (7)$$

Here, *C* is the concentration of the amino acid (µg/mL), *V* is the volume of extract (mL), *D* is the dilution, *S* is the dilution factor due to the addition of SSA and *M* is the weight of the sample (g). The value was divided by 1000 to get the correct unit. The dilution factor was 1.25 or 1.25^2 and corresponds to one or two precipitations with SAA, respectively.

D.1 Salmon Meal

The calculated amount of free amino acids in salmon meal dissolved in distilled water and citric acid-phosphate buffer with pH 3 are given in Table 33.

Table 33. Amount of free amino acids in salmon meal dissolved in distilled water and citric acid-phosphate buffer with pH 3. The measurements were performed in triplicates. Average values and SD are also included.

	Distilled water					Buffer pH 3				
Amino acid	**#1**	**#2**	**#3**	**Average [mg/g]**	**SD**	**#1**	**#2**	**#3**	**Average [mg/g]**	**SD**
Asp	1.64	1.73	1.56	1.64	0.09	1.72	1.77	1.71	1.73	0.03
Glu	5.31	5.64	2.81	4.59	1.55	5.80	5.90	3.18	4.96	1.54
Asn	0.06	0.06	0.06	0.06	0.001	0.06	0.06	0.06	0.06	0.00
His	0.66	0.70	0.71	0.69	0.02	0.68	0.80	0.75	0.74	0.06
Ser	1.64	1.76	1.58	1.66	0.09	1.73	1.75	1.66	1.71	0.04
Gln	1.36	1.47	1.48	1.44	0.07	1.44	1.48	1.64	1.52	0.10
Gly/Arg	2.04	2.11	2.12	2.09	0.04	2.05	2.09	2.21	2.12	0.09
Thr	1.80	1.86	1.85	1.84	0.03	1.92	1.91	2.07	1.97	0.09
Ala	2.78	2.96	2.87	2.87	0.09	2.88	2.93	3.04	2.95	0.08
Tyr	2.00	2.09	2.07	2.05	0.05	2.11	2.13	2.29	2.18	0.10
Aba	0.65	0.74	0.76	0.72	0.06	0.67	0.71	0.79	0.72	0.06
Met	1.29	1.36	1.36	1.34	0.04	1.35	1.41	1.46	1.41	0.06
Val	1.82	1.91	1.89	1.88	0.05	1.87	2.83	1.98	2.23	0.52
Phe	1.63	1.86	1.88	1.79	0.14	1.67	1.70	1.79	1.72	0.06
Ile	1.46	1.52	1.52	1.50	0.04	1.49	1.51	1.59	1.53	0.05
Leu	3.09	3.21	3.24	3.18	0.08	3.17	3.24	3.38	3.26	0.11
Lys	3.12	3.25	3.14	3.17	0.07	3.22	3.28	3.45	3.32	0.12
Total	32.34	34.24	30.89	32.49	1.68	33.83	35.49	33.06	34.12	1.24

The calculated amount of free amino acids in salmon meal dissolved in citric acid-phosphate buffer with pH 5 and 7 are given in Table 34.

Table 34. Amount of free amino acids in salmon meal dissolved in citric acid-phosphate buffer with pH 5 and 7. The measurements were performed in triplicates. Average values and SD are also included.

	Buffer pH 5					Buffer pH 7				
Amino acid	**#1**	**#2**	**#3**	**Average [mg/g]**	**SD**	**#1**	**#2**	**#3**	**Average [mg/g]**	**SD**
Asp	1.69	1.65	1.59	1.64	0.05	1.57	1.49	1.46	1.51	0.06
Glu	5.64	3.08	3.24	3.99	1.43	5.32	2.79	2.74	3.62	1.47
Asn	0.09	0.08	0.06	0.08	0.02	0.05	0.08	0.05	0.06	0.02
His	0.68	0.71	0.69	0.69	0.02	0.65	0.67	0.67	0.66	0.01
Ser	1.71	1.59	1.57	1.62	0.07	1.58	1.41	1.43	1.47	0.09
Gln	1.41	1.46	1.49	1.45	0.04	1.31	1.32	1.33	1.32	0.01
Gly/Arg	1.98	2.12	2.05	2.05	0.07	1.85	1.91	1.89	1.88	0.03
Thr	1.88	1.97	1.93	1.93	0.04	1.69	1.77	1.71	1.73	0.04
Ala	2.85	3.05	2.92	2.94	0.10	2.62	2.76	2.64	2.68	0.08
Tyr	2.04	2.20	2.15	2.13	0.08	1.92	1.98	1.95	1.95	0.03
Aba	0.64	0.72	0.74	0.70	0.05	0.61	0.60	0.65	0.62	0.03
Met	1.37	1.41	1.41	1.40	0.02	1.29	1.30	1.32	1.30	0.02
Val	1.87	1.97	1.89	1.91	0.05	1.77	1.79	1.76	1.78	0.01
Phe	1.84	1.89	1.81	1.85	0.04	1.72	1.57	1.71	1.67	0.08
Ile	1.46	1.58	1.48	1.51	0.06	1.38	1.44	1.40	1.41	0.03
Leu	3.10	3.28	3.18	3.19	0.09	2.96	2.99	2.94	2.96	0.03
Lys	3.13	3.25	3.21	3.20	0.06	2.93	2.98	2.94	2.95	0.03
Total	33.38	32.01	31.42	32.27	1.00	31.24	28.87	28.60	29.57	1.45

D.2 Whey Protein Powder

The calculated amount of free amino acids in whey protein powder dissolved in distilled water and citric acid-phosphate buffer with pH 3 are given in Table 35.

Table 35. Amount of free amino acids in whey protein powder dissolved in distilled water and citric acid-phosphate buffer with pH 3. The measurements were performed in triplicates. Average values and SD are also included.

	Distilled water					Buffer pH 3				
Amino acid	**#1**	**#2**	**#3**	**Average [mg/g]**	**SD**	**#1**	**#2**	**#3**	**Average [mg/g]**	**SD**
Asp	0.011	0.003	0.004	0.006	0.005	0.010	0.077	0.002	0.030	0.041
Glu	0.025	0.022	0.016	0.021	0.004	0.020	0.027	0.018	0.022	0.005
Asn	0.006	0.004	0.004	0.005	0.002	0.007	0.017	0.003	0.009	0.007
His	0.006	0.006	0.005	0.005	0.000	0.017	0.050	0.005	0.024	0.023
Ser	0.035	0.017	0.015	0.022	0.011	0.062	0.038	0.013	0.038	0.024
Gln	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gly/Arg	0.027	0.017	0.018	0.021	0.005	0.034	0.107	0.017	0.053	0.048
Thr	0.026	0.022	0.024	0.024	0.002	0.033	0.088	0.025	0.048	0.034
Ala	0.020	0.011	0.013	0.015	0.004	0.027	0.068	0.011	0.036	0.029
Tyr	0.013	0.018	0.019	0.017	0.003	0.011	0.070	0.007	0.029	0.035
Aba	0.021	0.025	0.025	0.024	0.002	0.025	0.026	0.028	0.027	0.002
Met	0.025	0.024	0.022	0.024	0.002	0.010	0.018	0.008	0.012	0.005
Val	0.008	0.005	0.005	0.006	0.002	0.012	0.036	0.005	0.018	0.016
Phe	0.011	0.009	0.007	0.009	0.002	0.011	0.043	0.007	0.020	0.020
Ile	0.010	0.007	0.006	0.008	0.002	0.012	0.038	0.007	0.019	0.016
Leu	0.025	0.025	0.026	0.025	0.000	0.023	0.056	0.016	0.032	0.022
Lys	0.022	0.019	0.020	0.020	0.001	0.011	0.030	0.099	0.047	0.046
Total	0.292	0.235	0.227	0.251	0.035	0.325	0.790	0.272	0.462	0.285

The calculated amount of free amino acids in whey protein powder dissolved in citric acid-phosphate buffer with pH 5 and 7 are given in Table 36.

Table 36. Amount of free amino acids in whey protein powder dissolved in citric acid-phosphate buffer with pH 5 and 7. The measurements were performed in triplicates. Average values and SD are also included.

	Buffer pH 5					Buffer pH 7				
Amino acid	**#1**	**#2**	**#3**	**Average [mg/g]**	**SD**	**#1**	**#2**	**#3**	**Average [mg/g]**	**SD**
Asp	0.004	0.025	0.001	0.010	0.013	0.002	0.002	0.005	0.003	0.002
Glu	0.036	0.048	0.034	0.039	0.008	0.031	0.032	0.042	0.035	0.006
Asn	0.005	0.010	0.003	0.006	0.004	0.003	0.002	0.004	0.003	0.001
His	0.007	0.022	0.007	0.012	0.009	0.005	0.005	0.003	0.004	0.001
Ser	0.024	0.019	0.015	0.019	0.004	0.009	0.011	0.007	0.009	0.002
Gln	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gly/Arg	0.017	0.055	0.036	0.036	0.019	0.012	0.013	0.016	0.013	0.002
Thr	0.022	0.049	0.035	0.035	0.013	0.018	0.019	0.018	0.019	0.000
Ala	0.011	0.043	0.010	0.021	0.018	0.009	0.006	0.011	0.008	0.002
Tyr	0.013	0.019	0.010	0.014	0.005	0.014	0.011	0.018	0.014	0.003
Aba	0.018	0.021	0.022	0.020	0.002	0.022	0.021	0.024	0.023	0.001
Met	0.008	0.009	0.008	0.008	0.000	0.013	0.011	0.015	0.013	0.002
Val	0.007	0.015	0.005	0.009	0.005	0.003	0.003	0.006	0.004	0.002
Phe	0.010	0.009	0.008	0.009	0.001	0.007	0.001	0.010	0.006	0.005
Ile	0.010	0.015	0.008	0.011	0.004	0.006	0.006	0.010	0.007	0.002
Leu	0.022	0.030	0.021	0.024	0.005	0.018	0.018	0.028	0.022	0.006
Lys	0.014	0.021	0.013	0.016	0.005	0.016	0.016	0.012	0.015	0.002
Total	0.226	0.409	0.235	0.290	0.103	0.188	0.177	0.228	0.198	0.027

D.3 Herring Protein Powder Hydrolysates

The calculated amount of free amino acids in herring protein powder hydrolysates after 30 and 60 min of hydrolysis are given in Table 37.

Table 37. Amount of free amino acids in herring protein powder hydrolysates after 30 and 60 min of hydrolysis. The hydrolysis was performed in duplicates (H1 and H2) and two measurements were performed for both of them. The average value and SD, calculated from the measurements of both H1 and H2, are also included.

	30 min						60 min					
Amino acid	**H1#1**	**H1#2**	**H2#1**	**H2#2**	**Average [mg/g]**	**SD**	**H1#1**	**H1#2**	**H2#1**	**H2#2**	**Average [mg/g]**	**SD**
Asp	0.203	0.214	0.158	0.155	0.182	0.030	0.218	0.214	0.203	0.200	0.209	0.009
Glu	0.370	0.425	0.284	0.285	0.341	0.069	0.445	0.421	0.372	0.364	0.400	0.039
Asn	0.041	0.044	0.034	0.032	0.038	0.005	0.045	0.045	0.044	0.042	0.044	0.001
His	0.105	0.105	0.077	0.080	0.092	0.015	0.140	0.110	0.114	0.089	0.113	0.021
Ser	0.220	0.232	0.170	0.165	0.197	0.034	0.240	0.243	0.225	0.235	0.235	0.008
Gln	0.000	0.039	0.028	0.028	0.032	0.006	0044	0.042	0.037	0.038	0.040	0.003
Gly/Arg	0.191	0.203	0.146	0.147	0.172	0.029	0.240	0.230	0.223	0.221	0.229	0.008
Thr	0.077	0.081	0.062	0.056	0.069	0.012	0.101	0.097	0.092	0.091	0.095	0.005
Ala	0.056	0.057	0.045	0.041	0.050	0.008	0.067	0.066	0.063	0.064	0.065	0.002
Tyr	0.049	0.048	0.039	0.034	0.042	0.007	0.056	0.054	0.058	0.053	0.055	0.002
Aba	0.014	0.012	0.009	0.010	0.011	0.002	0.013	0.013	0.011	0.014	0.013	0.001
Met	0.113	0.093	0.085	0.076	0.092	0.016	0.104	0.114	0.122	0.111	0.113	0.008
Val	0.042	0.042	0.032	0.032	0.037	0.006	0.049	0.046	0.040	0.039	0.044	0.005
Phe	0.081	0.080	0.057	0.059	0.069	0.013	0.111	0.103	0.098	0.093	0.101	0.008
Ile	0.027	0.026	0.020	0.017	0.022	0.005	0.031	0.029	0.028	0.031	0.030	0.001
Leu	0.057	0.064	0.045	0.046	0.053	0.009	0.077	0.071	0.052	0.069	0.067	0.011
Lys	0.679	0.673	0.522	0.517	0.598	0.091	0.682	0.680	0.648	0.665	0.669	0.016
Total	2.325	2.437	1.810	1.781	2.088	0.341	2.664	2.577	2.430	2.418	2.522	0.119

The calculated amount of free amino acids in herring protein powder hydrolysates after 125 min of hydrolysis is given in Table 38.

Table 38. Amount of free amino acids in in herring protein powder hydrolysates after 125 min of hydrolysis. The hydrolysis was performed in duplicates (H1 and H2) and two measurements were performed for both of them. The average value and SD, calculated from the measurements of both H1 and H2, are also included.

	125 min					
Amino acid	**H1#1**	**H1#2**	**H2#1**	**H2#2**	**Average [mg/g]**	**SD**
Asp	0.224	0.222	0.208	0.209	0.216	0.009
Glu	0.464	0.466	0.419	0.418	0.442	0.027
Asn	0.050	0.052	0.046	0.046	0.048	0.003
His	0.138	0.130	0.126	0.138	0.133	0.006
Ser	0.269	0.270	0.256	0.254	0.262	0.008
Gln	0.055	0.055	0.051	0.050	0.053	0.003
Gly/Arg	0.301	0.297	0.293	0.000	0.297	0.004
Thr	0.145	0.143	0.139	0.138	0.141	0.003
Ala	0.085	0.082	0.081	0.080	0.082	0.002
Tyr	0.073	0.083	0.068	0.068	0.073	0.007
Aba	0.011	0.013	0.010	0.012	0.011	0.001
Met	0.109	0.115	0.098	0.100	0.105	0.008
Val	0.057	0.057	0.045	0.045	0.051	0.007
Phe	0.149	0.149	0.140	0.141	0.145	0.005
Ile	0.042	0.043	0.034	0.034	0.038	0.005
Leu	0.104	0.104	0.086	0,086	0.095	0.010
Lys	0.842	0.844	0.670	0.659	0.754	0.103
Total	3.117	3.127	2.768	2.479	2.873	0.311

E. Molecular Weight Distribution

E.1 Standards and Standard Curve

The molecular weight of peptide fractions in the protein powders was estimated by establishing a relationship between retention volume (V_R) and molecular weight (MW) of three different standards. The standards and their corresponding molecular weights are listed in Table 39. The available coefficient, K_{av} was calculated with Equation 8:

$$K_{av} = \frac{V_R - V_0}{V_T - V_0} \quad (8)$$

where V_0 = void volume = 8 mL, V_R = retention volume and V_T = total volume = 24 mL.

Table 39. Standards used to establish a relationship between molecular weight (MW), retention volume (V_R) and available coefficient (K_{av}). The retention volume was calculated by multiplying the retention time (min) with the flow rate (0.5 mL/min).

Standard	Retention time [min]	V_R [mL]	K_{av} [-]	MW [g/mol]
Aprotinin	23.7	11.85	0.24	6512
Vitamin B12	34.125	17.06	0.57	1355
Aspartate (Asp)	40.89	20.45	0.78	133,1

The logarithm of the molecular weight was plotted as a function of the available coefficient for the different standards. Figure 37 shows the linear relationship between the molecular weight and the availability coefficient.

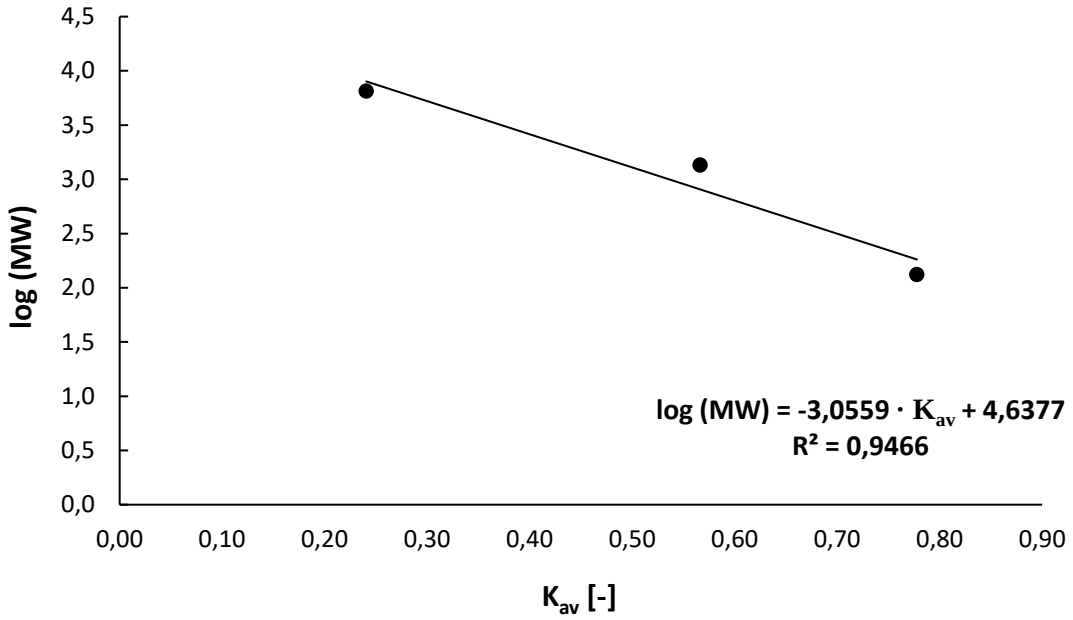


Figure 37. Standard curve used to estimate the molecular weight of peptide fractions in the protein powders. The logarithm of molecular weight, log (MW) as a function of available coefficient, K_{av}.

The linear relationship obtained from linear regression as shown in Figure 37, is given in Equation 9. This equation was used to estimate the molecular weights of peptide fractions in the protein powders.

$$\log(MW) = -3.0559 * K_{av} + 4.6377 \quad (9)$$

Where *MW*, is the molecular weight, K_{av} is the available coefficient and the numbers are the constants obtained from linear regression.

E.2 Chromatograms

The molecular weight distribution of the protein powders were investigated with gel filtration. The chromatograms from the FPLC measurements for salmon meal and whey protein powder are given in Figure 38 and 39, respectively.

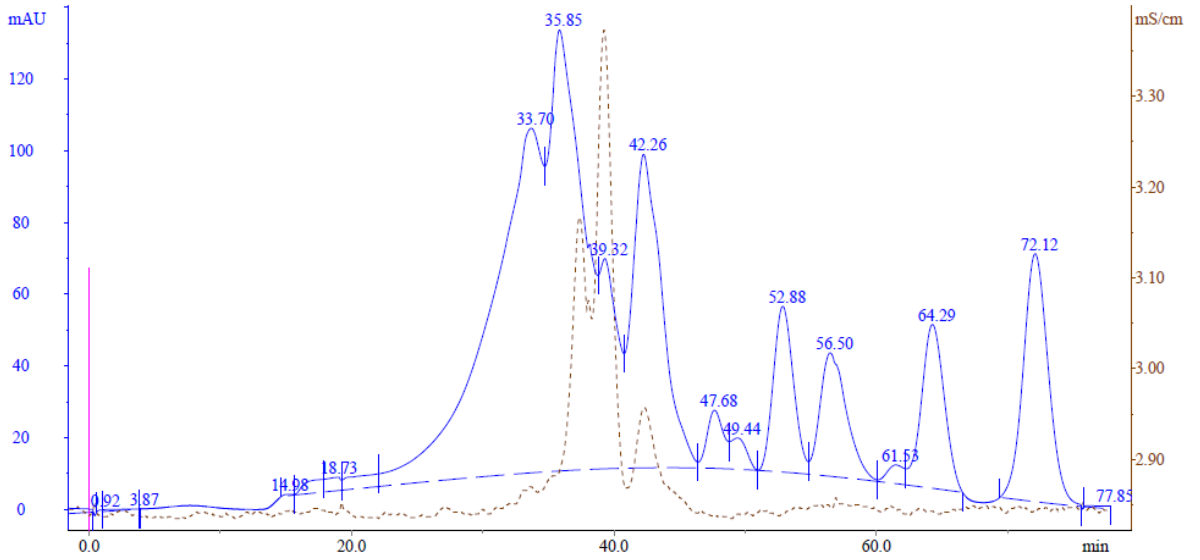


Figure 38. Molecular weight distribution of salmon meal. The blue line represents the UV measurement in milliabsorbance units (mAU) as a function of retention time (min). The number above the different peaks is the corresponding retention time.

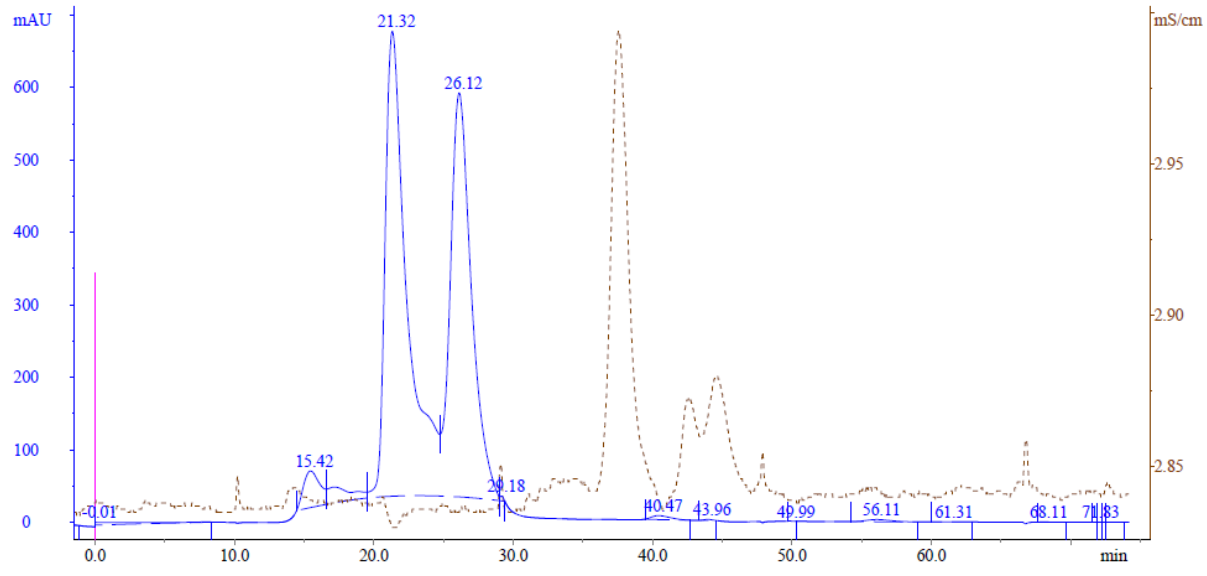


Figure 39. Molecular weight distribution of whey protein powder. The blue line represents the UV measurement in milliabsorbance units (mAU) as a function of retention time (min). The number above the different peaks is the corresponding retention time.

The chromatograms from the FPLC measurements for the herring protein hydrolysates after 30, 60 and 125 minutes of hydrolysis are given in Figure 40, 41 and 42, respectively.

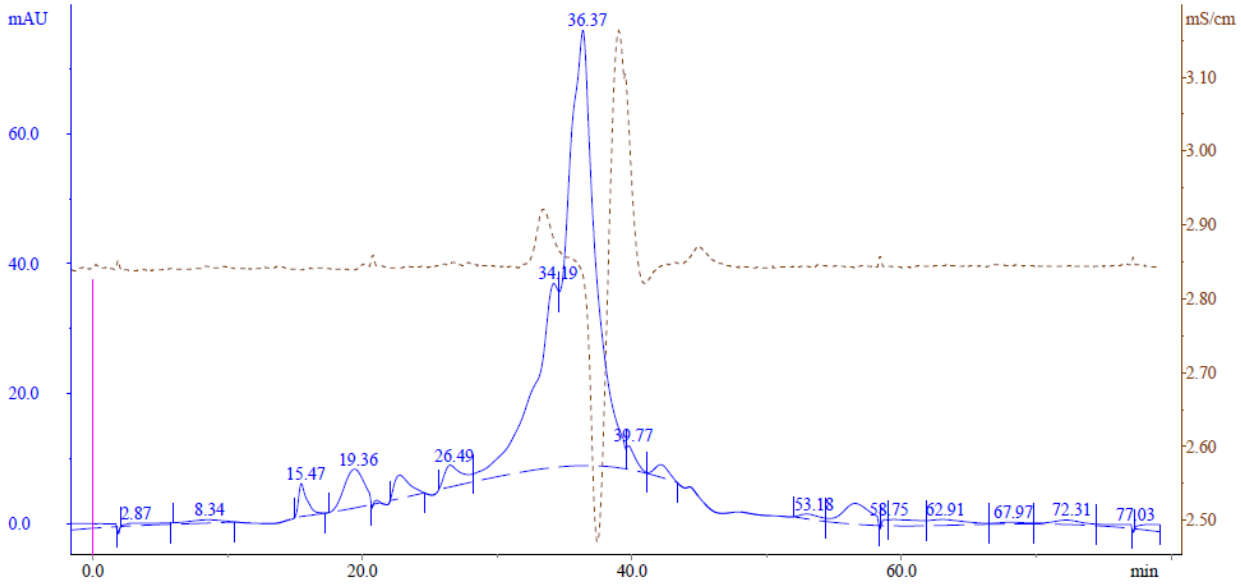


Figure 40. Molecular weight distribution of herring protein powder hydrolysate after 30 min. The blue line represents the UV measurement in milliabsorbance units (mAU) as a function of retention time (min). The number above the different peaks is the corresponding retention time.

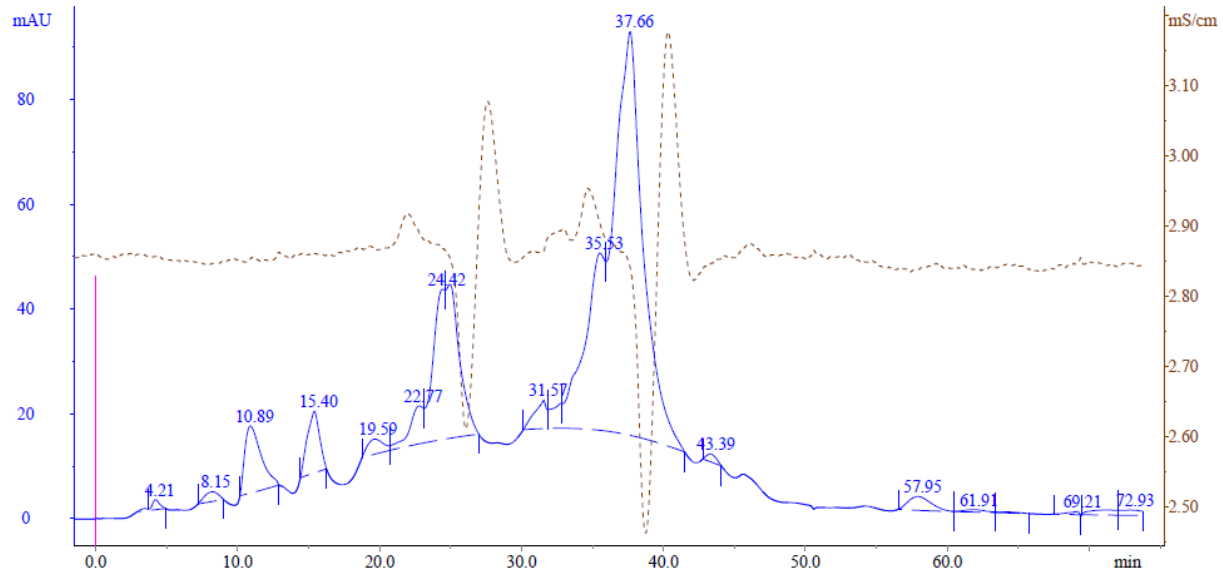


Figure 41. Molecular weight distribution of herring protein powder hydrolysate after 60 min. The blue line represents the UV measurement in milliabsorbance units (mAU) as a function of retention time (min). The number above the different peaks is the corresponding retention time.

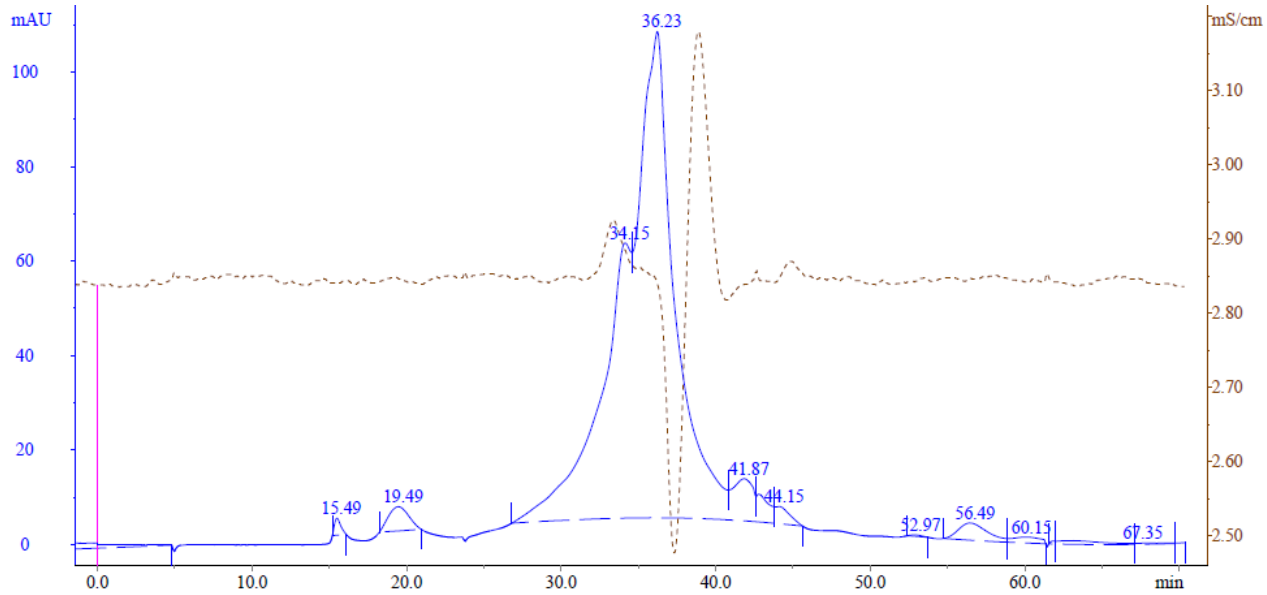


Figure 42. Molecular weight distribution of herring protein powder hydrolysate after 125 min. The blue line represents the UV measurement in milliabsorbance units (mAU) as a function of retention time (min). The number above the different peaks is the corresponding retention time.

E.3 Estimated Molecular Weight

Equation 8 was used to calculate the available coefficient, while Equation 9 was used to estimate the molecular weights of the detected peptide fractions. The retention time, retention volume, available coefficient and estimated molecular weight of detected peptide fractions in salmon meal and whey protein powder are given in Table 40 and 41, respectively

Table 40. The retention time (R_T), retention volume (V_R), available coefficient (K_{av}) and estimated molecular weight (MW) of detected peptide fractions in salmon meal. The retention volumes were calculated by multiplying the retention time with the flow rate (0.5 mL/min).

R_T [min]	V_R [mL]	K_{av} [-]	Log (MW) [-]	MW [Da]
33.70	16.85	0.55	2.95	886
35.85	17.93	0.62	2.74	552
39.32	19.66	0.73	2.41	257
42.26	21.13	0.82	2.13	135
52.88	26.44	1.15	1.12	13
56.5	28.25	1.27	0.77	6

Table 41. The retention time (R_T), retention volume (V_R), available coefficient (K_{av}) and estimated molecular weight (MW) of detected peptide fractions in whey protein powder. The retention volumes were calculated by multiplying the retention time with the flow rate (0.5 mL/min).

R_T [min]	V_R [mL]	K_{av} [-]	Log (MW) [-]	MW [Da]
15.42	7.71	-0.02	4.69	49322
21.32	10.66	0.17	4.13	13478
26.12	13.06	0.32	3.67	4691

The retention time, retention volume, available coefficient and estimated molecular weights of detected peptide fractions in herring protein powder hydrolysates after 0, 30, 60 and 125 minutes of hydrolysis are given in Table 42.

Table 42. The retention time (R_T), retention volume (V_R), available coefficient (K_{av}) and estimated molecular weight (MW) of detected peptide fractions in herring protein powder hydrolysates after 0, 30, 60 and 125 min of hydrolysis. The retention volumes were calculated by multiplying the retention time with the flow rate (0.5 mL/min).

Time of hydrolysis [min]	R_T [min]	V_R [mL]	K_{av} [-]	Log (MW) [-]	MW [Da]
0	14.46	7.23	-0.05	4.78	60915
	17.70	8.85	0.05	4.48	29875
	33.92	16.96	0.56	2.93	844
	35.50	17.75	0.61	2.78	596
30	34.19	17.10	0.57	2.90	795
	36.37	18.19	0.64	2.69	492
60	24.42	12.21	0.26	3.83	6817
	35.53	17.77	0.61	2.77	592
	3766	18.83	0.68	2.57	371
125	34.15	17.08	0.57	2.90	802
	36.23	18.12	0.63	2.71	508

F. Acid Soluble Peptides

F.1 Absorbance Measurements of BSA and Standard Curve

Absorbance measurements of the standard, bovine serum albumin (BSA), and standard curve used to determine the amount of acid soluble peptides in the herring protein powder hydrolysates are given in Table 43 and Figure 43, respectively.

Table 43. Measured absorbance (OD 750 nm) and calculated average for different concentrations of bovine serum albumin (BSA). The measurements were performed in triplicates.

Concentration [ug/ml]	**12.5**	**25**	**50**	**100**	**150**	**200**	**300**
	0.039	0.077	0.139	0.262	0.361	0.463	0.621
OD 750	0.044	0.076	0.137	0.261	0.383	0.444	0.619
	0.043	0.077	0.144	0.264	0.367	0.450	0.629
Average	0.042	0.077	0.140	0.262	0.370	0.452	0.623

The standard curve was obtained by plotting average absorbance at 750 nm as a function of BSA concentration.

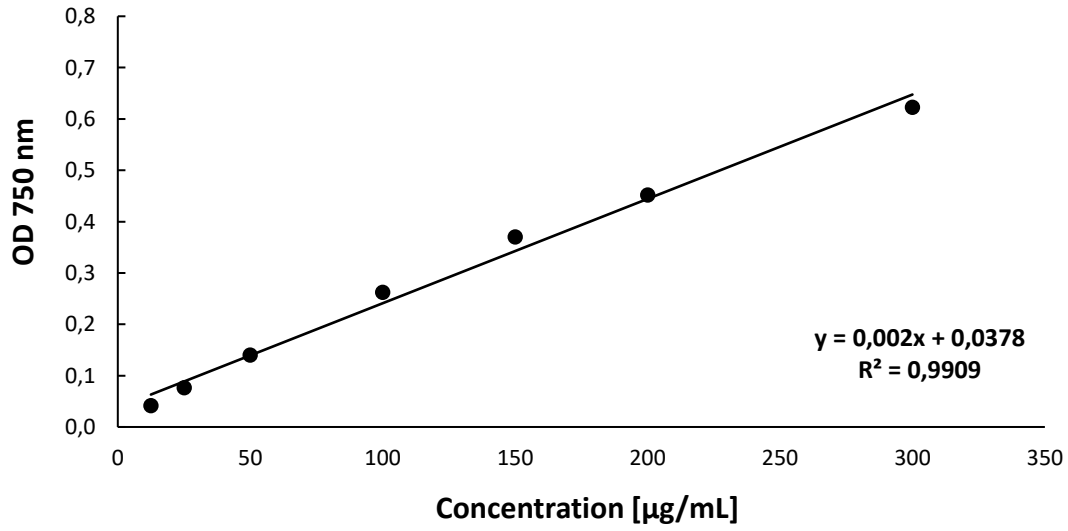


Figure 43. Standard curve used in determination of acid soluble peptides in herring protein powder hydrolysates. Measured absorbance (OD 750 nm) as a function of bovine serum albumin (BSA) concentration (µg/mL).

F.2 Measurements for the Herring Protein Powder Hydrolysates

Measured values, including absorbance measurements and calculated amount of acid soluble peptides in the herring protein powder hydrolysates, can be found in Table 44. The values presented in Figure 14 in section 3.5 are the averages from the values obtained for hydrolysate 1 and hydrolysate 2 after 30, 60 and 125 min of hydrolysis.

Table 44. Amount of powder weighed out, volume of extract, dilution, absorbance measurements and calculated amount of acid soluble peptides of herring protein powder hydrolysates after 30, 60 and 125 min of hydrolysis. Absorbance measurements were performed in triplicates.

		Hydrolysate 1			Hydrolysate 2		
Hydrolysis time [min]		**30**	**60**	**125**	**30**	**60**	**125**
Weight of powder [g]		10	10	10	10	10	10
Volume extract [mL]		101.3	101.3	101.3	99.9	99.9	99.9
Dilution		1:20	1:20	1:20	1:20	1:20	1:20
OD 750 nm	**#1**	0.314	0.352	0.392	0.311	0.348	0.410
	#2	0.323	0.350	0.383	0.310	0.345	0.408
	#3	0.322	0.353	0.392	0.313	0.349	0.401
Amount of acid soluble peptides [% of DW]	**#1**	2.75	3.13	3.53	2.69	3.05	3.66
	#2	2.84	3.11	3.44	2.68	3.02	3.64
	#3	2.83	3.14	3.53	2.71	3.06	3.57

G. Solubility

G.1 Herring Protein Powder Hydrolysates

Absorbance measurements of the standard, bovine serum albumin (BSA), and the standard curve used to determine the amount of soluble proteins of herring protein powder hydrolysates are given in Table 45 and Figure 44, respectively.

Table 45. Measured absorbance (OD 750 nm) and calculated average for different concentrations of bovine serum albumin (BSA). The measurements were performed in triplicates.

Concentration [µg/mL]	**12.5**	**25**	**50**	**100**	**150**	**200**	**300**
	0.040	0.064	0.138	0.258	0.360	0.452	0,626
OD 750 nm	0.044	0.064	0.140	0.251	0.360	0.452	0,624
	0.043	0.067	0142	0.254	0.361	0,455	0,621
Average	0.042	0.065	0.140	0.254	0.360	0.453	0.624

The standard curve was obtained by plotting average absorbance at 750 nm as a function of BSA concentration.

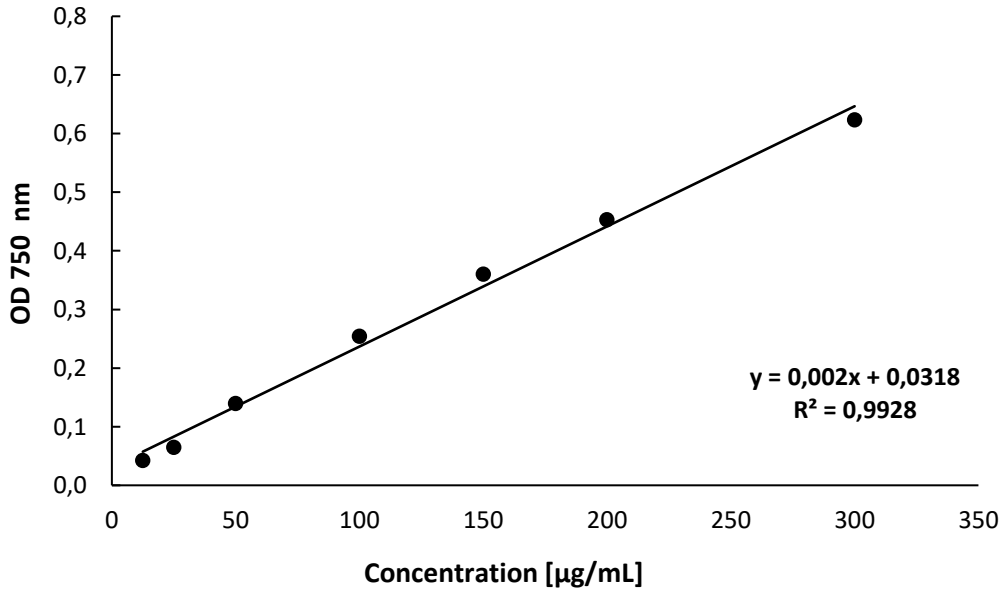


Figure 44. Standard curve used in determination of soluble proteins in herring protein powder hydrolysates. Measured absorbance (OD 750 nm) as a function of bovine serum albumin (BSA) concentration (µg/mL).

Measured values, including absorbance measurements and calculated amount of soluble proteins in herring protein powder hydrolysates, can be found in Table 46. The values presented in Figure 15 in section 3.6 are the averages from the values obtained for hydrolysate 1 and hydrolysate 2 after 30, 60 and 125 min of hydrolysis.

Table 46. Amount of powder weighed out, volume of extract, dilution, absorbance measurements and calculated amount of soluble peptides in herring protein powder hydrolysates after 30, 60 and 125 min of hydrolysis. Absorbance measurements were performed in triplicates.

		Hydrolysate 1			Hydrolysate 2		
Weight of powder [g]			10.0			10.0	
Volume extract [mL]			101.3			99.9	
Dilution			1:25			1:25	
Hydrolysis time [min]		**30**	**60**	**125**	**30**	**60**	**125**
OD 750 nm	**#1**	0.462	0.491	0.535	0.428	0.469	0.564
	#2	0.448	0.490	0.540	0.434	0.494	0.570
	#3	0.462	0.492	0.537	0.440	0.496	0.561
Protein solubility [% of DW]	**#1**	5.32	5.68	6.22	4.83	5.33	6.49
	#2	5.14	5.66	6.28	4.90	5.63	6.56
	#3	5.32	5.69	6.24	4.98	5.66	6.45

G.2 Dissolved in Distilled Water and Citric Acid-Phosphate Buffer

Salmon meal and whey protein powder were dissolved in distilled water and citric acid-phosphate buffer with pH 3, pH 5 and pH 7.

G.2.1 Salmon Meal

Absorbance measurements of the standard bovine serum albumin (BSA) and the standard curve used to determine the amount of soluble proteins in salmon meal dissolved in distilled water and citric acid-phosphate buffer with pH 3, pH 5 and pH 7 are given in Table 47 and Figure 45, respectively.

Table 47. Measured absorbance (OD 750 nm) and calculated average for different concentrations of bovine serum albumin (BSA). The measurements were performed in triplicates.

Concentration [µg/mL]	12.5	25	50	100	150	200	300
	0.044	0.085	0.151	0.277	0.391	0.484	0.665
OD 750 nm	0.045	0.086	0.156	0.278	0.397	0.484	0.648
	0.050	0.088	0.157	0.282	0.403	0.498	0.662
Average	0.046	0.086	0.155	0.279	0.397	0.489	0.658

The standard curve was obtained by plotting average absorbance at 750 nm as a function of BSA concentration.

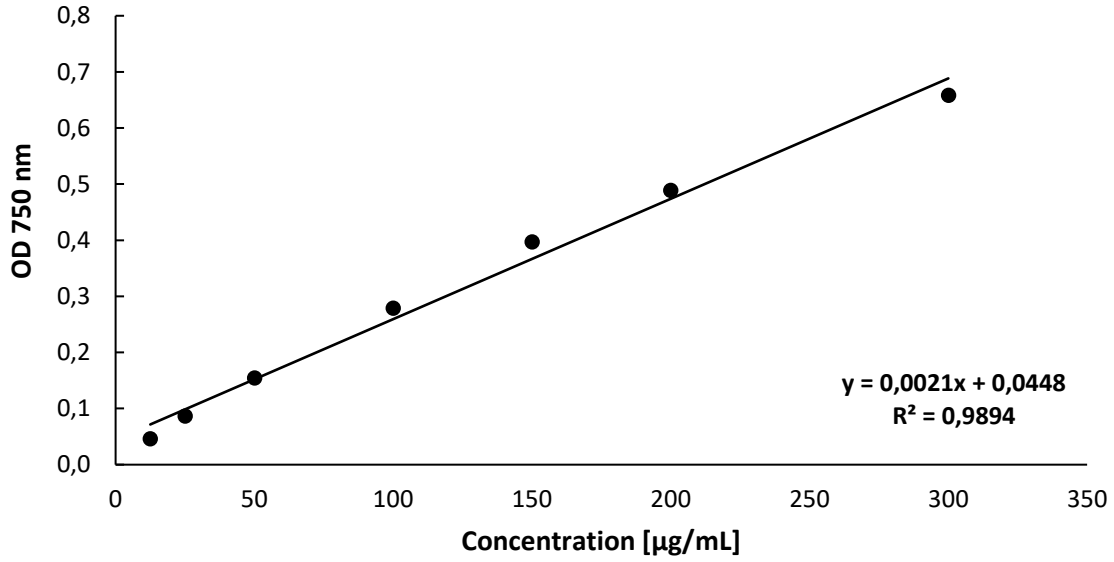


Figure 45. Standard curve used in determination of soluble proteins in salmon meal dissolved in distilled water and citric acid-phosphate buffer. Measured absorbance (OD 750 nm) as a function of bovine serum albumin (BSA) concentration (µg/mL).

Measured values, including absorbance measurements and calculated amount of soluble proteins in salmon meal dissolved in different solvents, can be found in Table 48.

Table 48. Amount of powder weighed out, volume of extract, dilution, absorbance measurements and calculated protein solubility of salmon meal in distilled water and citric acid-phosphate buffer with pH 3, 5 and 7. Absorbance measurements were performed in triplicates.

		Distilled water	Buffer pH 3	Buffer pH 5	Buffer pH 7
Weight of powder [g]		0.2007	0.2015	0.2006	0.2001
Volume extract [mL]		10	10	10	10
Dilution		1:25	1:25	1:25	1:25
OD 750 nm	**#1**	0.369	0.288	0.320	0.347
	#2	0.382	0.293	0.327	0.360
	#3	0.398	0.290	0.317	0.357
Protein solubility [% of DW]	**#1**	18.82	14.06	15.99	17.60
	#2	19.58	14.35	16.39	18.36
	#3	20.51	14.18	15.81	18,.18
	Average	19.64	14.20	16.06	18.05
	SD	0.84	0.15	0,30	0.40

G.2.2 Whey Protein Powder

Absorbance measurements of the standard, bovine serum albumin (BSA), and the standard curve used to determine the amount of soluble proteins in whey protein powder dissolved in distilled water and citric acid-phosphate buffer with pH 3, pH 5 and pH 7 are given in Table 49 and Figure 46, respectively.

Table 49. Measured absorbance (OD 750 nm) and calculated average for different concentrations of bovine serum albumin (BSA). The measurements were performed in triplicates.

Concentration [µg/mL]	**12.5**	**25**	**50**	**100**	**150**	**200**	**300**
OD 750 nm	0.038	0.075	0.140	0.251	0.360	0.444	0.599
	0.043	0.087	0142	0.304	0.353	0.449	0.589
	0.039	0.089	0.142	0.252	0.360	0.435	0.607
Average	0.040	0.084	0.141	0.269	0.358	0.443	0.598

The standard curve was obtained by plotting the average absorbance at 750 nm as a function of BSA concentration.

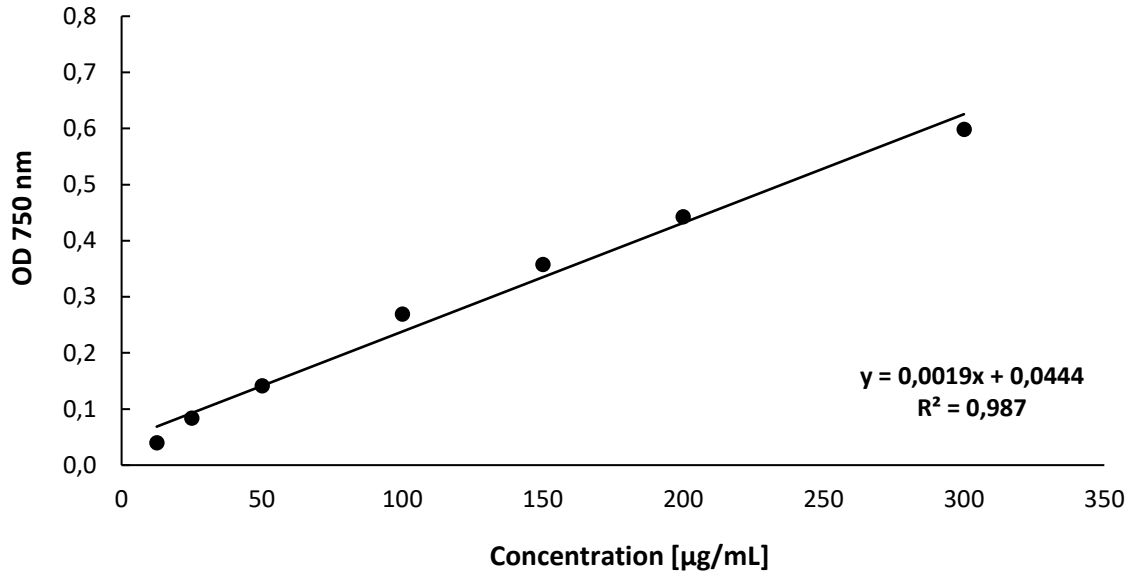


Figure 46. Standard curve used in determination of soluble proteins in whey protein powder dissolved in distilled water and citric acid phosphate buffer. Measured absorbance (OD 750 nm) as a function of bovine serum albumin (BSA) concentration (µg/mL).

Measured values, including absorbance measurements and calculated amount of soluble proteins in whey protein powder dissolved in different solvents can be found in Table 50.

Table 50. Amount of powder weighed out, volume of extract, dilution, absorbance measurements and calculated protein solubility of whey protein powder in distilled water and citric acid-phosphate buffer with pH 3, 5 and 7. Absorbance measurements were performed in triplicates.

		Distilled water	Buffer pH 3	Buffer pH 5	Buffer pH 7
Weight of powder [g]		0.1997	0.2020	0.2013	0.2008
Volume extract [mL]		10	10	10	10
Dilution		1:100	1:100	1:100	1:100
OD 750 nm	**#1**	0.383	0.293	0.360	0.430
	#2	0.361	0.304	0.359	0.392
	#3	0.362	0.308	0.372	0.430
Protein solubility [% of DW]	**#1**	87.55	63.55	80.96	99.16
	#2	81.86	66.36	80.70	89.39
	#3	82.12	67.38	84.03	99.16
	Average	83.85	65.77	81.90	95.90
	SD	3.21	1.99	1.86	5.64

G.3 Dissolved in Distilled Water with Different Temperatures

The herring protein powder and the salmon meal were dissolved in distilled water with different temperatures.

G.3.1 Herring Protein Powder

Absorbance measurements of the standard, bovine serum albumin (BSA), and the standard curve used to determine the amount of soluble proteins in herring protein powder dissolved in distilled water with different temperatures, are given in Table 51 and Figure 47, respectively.

Table 51. Measured absorbance (OD 750 nm) and calculated average for different concentrations of bovine serum albumin (BSA). The measurements were performed in triplicates.

Concentration [µg/mL]	12.5	25	50	100	150	200	300
	0.051	0.077	0.144	0.273	0.376	0.483	0.672
OD 750 nm	0.045	0.112	0.145	0.308	0.377	0.484	0.647
	0.044	0.084	0.144	0.294	0.373	0.482	0.663
Average	0.047	0.091	0.144	0.292	0.375	0.483	0.661

The standard curve was obtained by plotting average absorbance at 750 nm as a function of BSA concentration.

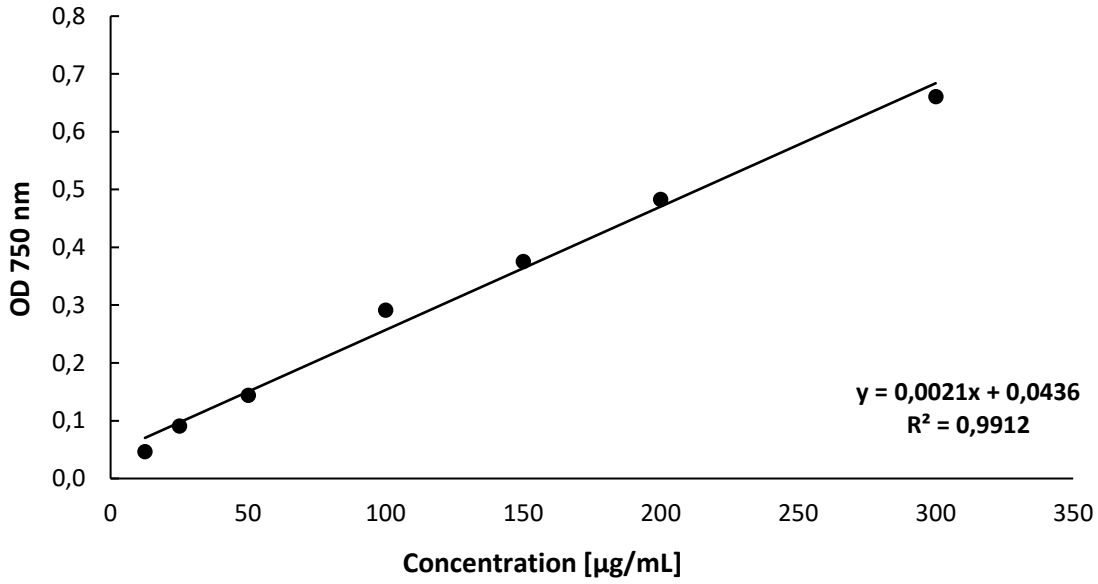


Figure 47. Standard curve used in determination of soluble proteins in herring protein powder dissolved in distilled water with different temperatures. Measured absorbance (OD 750 nm) as a function of bovine serum albumin (BSA) concentration (µg/mL).

Measured values, including absorbance measurements and calculated amount of soluble proteins in herring protein powder dissolved in distilled water with different temperatures can be found in Table 52.

Table 52. Amount of powder weighed out, volume of extract, dilution, absorbance measurements and calculated protein solubility of herring protein powder dissolved in distilled water with six different temperatures.*The standard curve in Figure 48 was used in the determination of protein solubility at 100 ºC.

		Temperature [°C]					
		30	**40**	**50**	**60**	**70**	**100***
Weight of powder [g]		0.1994	0.1993	0.2000	0.1992	0.2004	0.2014
Volume extract [mL]		10	10	10	10	10	10
Dilution		1:5	1:5	1:5	1:5	1:5	1:5
OD 750 nm	**#1**	0.156	0.163	0.163	0.167	0.178	0.176
	#2	0.157	0.168	0.177	0.168	0.178	0.182
	#3	0.162	0.172	0.176	0.174	0.183	0.177
Protein solubility [% of DW]	**#1**	1.32	1.40	1.40	1.45	1.57	1.91
	#2	1.33	1.46	1.56	1.46	1.57	1.99
	#3	1.39	1.51	1.55	1.53	1.63	1,93
	Average	1.35	1.46	1.50	1.48	1.59	1.94
	SD	0.04	0.05	0.09	0.04	0.03	0.04

G.3.2 Salmon Meal

Absorbance measurements of the standard, bovine serum albumin (BSA), and the standard curve used to determine the amount of soluble proteins in salmon meal dissolved in distilled water with different temperatures, are given in Table 53 and Figure 48, respectively.

Table 53. Measured absorbance (OD 750 nm) and calculated average for different concentrations of bovine serum albumin (BSA). The measurements were performed in triplicates.

Concentration [µg/mL]	**12.5**	**25**	**50**	**100**	**150**	**200**	**300**
OD 750 nm	0.033	0.066	0.126	0.239	0.336	0.421	0.571
	0.034	0.064	0.126	0.242	0.337	0.427	0.587
	0.035	0.071	0.129	0.237	0.342	0.415	0.577
Average	0.034	0.067	0.127	0.239	0.338	0.421	0.578

The standard curve was obtained by plotting average absorbance at 750 nm as a function of BSA concentration.

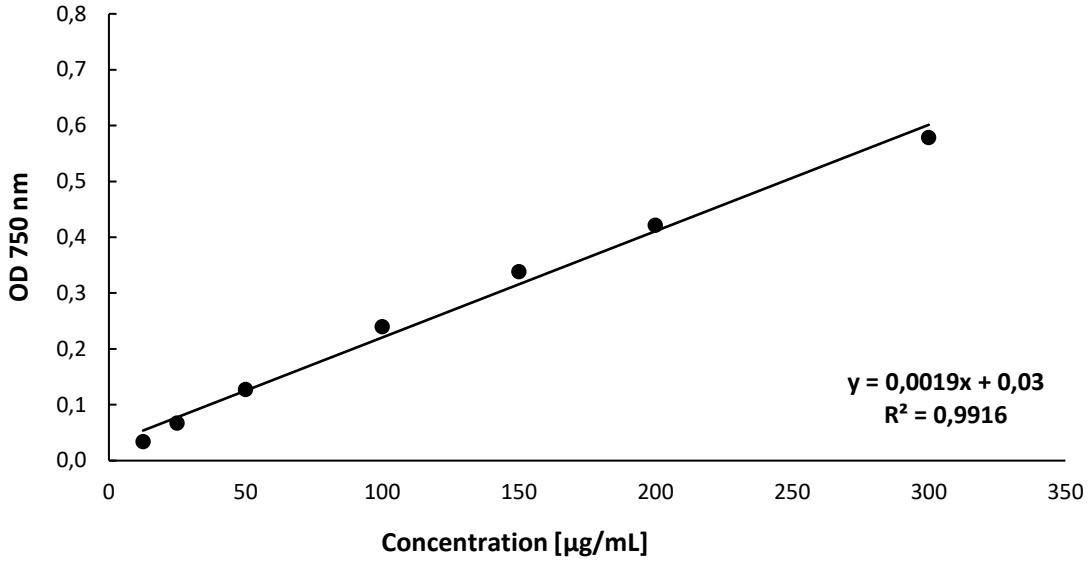


Figure 48. Standard curve used in the determination of soluble proteins in salmon meal dissolved in distilled water with different temperatures. Measured absorbance (OD 750 nm) as a function of bovine serum albumin (BSA) concentration (µg/mL).

Measured values, including absorbance measurements and calculated amount of soluble proteins in salmon meal dissolved in distilled water with different temperatures, can be found in Table 54.

Table 54. Amount of powder weighed out, volume of extract, dilution, absorbance measurements and calculated protein solubility of salmon meal dissolved in distilled water with six different temperatures.

		Temperature [°C]					
		30	**40**	**50**	**60**	**70**	**100**
Weight of powder [g]		0.2021	0.2013	0.2015	0.2019	0.2004	0.2027
Volume extract [mL]		10	10	10	10	10	10
Dilution		1:25	1:25	1:25	1:25	1:25	1:25
OD 750 nm	**#1**	0.288	0.231	0.247	0.263	0.309	0.314
	#2	0.295	0.234	0.256	0.265	0.314	0.322
	#3	0.296	0.234	0.256	0.266	0.319	0.329
Protein solubility [% of DW]	**#1**	16.76	13.11	14.14	15.15	18.28	18.60
	#2	17.21	13.30	14.72	15.28	18.60	19.13
	#3	17.28	13.30	14.72	15.34	18.93	19.59
	Average	17.08	13.24	14.53	15.26	18.60	19.11
	SD	0.28	0.11	0.34	0.10	0.33	0.49

H. Emulsion Properties

H.1 Salmon meal

The volumes of water, emulsion and oil were measured after centrifugation the same day as homogenization and after 24 h and centrifugation, for different concentrations of salmon meal (1, 2 and 5 %). The results are given in Table 55 and the calculated emulsion capacity and emulsion stability are given in Table 56.

Table 55. Volumes of water, emulsion and oil (mL) after centrifugation the same day as homogenization and after 24 h and centrifugation, for three different concentrations (1, 2 and 5 %) of salmon meal. The weight of the powder is also included. The measurements were performed in quadruplicates.

		After centrifugation			After 24 h and centrifugation		
Parallel	**Powder [g]**	**H_2O [mL]**	**Emulsion [mL]**	**Oil [mL]**	**H_2O [mL]**	**Emulsion [mL]**	**Oil [mL]**
# 1	0.04996	4.9	2.0	3.1	4.9	0.4	4.7
# 2	0.05036	5.0	2.0	3.0	5.0	0.4	4.6
# 3	0.05026	4.9	1.6	3.5	5.0	0.4	4.6
# 4	0.05029	5.0	1.8	3.2	5.0	0.4	4.6
# 1	0.10237	4.9	3.1	2.0	4.9	0.5	4.6
# 2	0.10017	4.9	3.0	21	5.0	0.4	4.6
# 3	0.10025	4.9	2.5	2.6	5.0	0.5	4.5
# 4	0.10011	5.0	2.5	2.5	4.9	0.4	4.6
# 1	0.25050	4.5	48	0.7	4.7	05	4.8
# 2	0.25150	4.5	4.5	1.0	4.8	0.5	4.7
# 3	0.25090	4.5	4.9	0.6	4.8	0.5	4.7
# 4	0.25180	4.5	4.9	0.6	4.9	0.6	4.5

Table 56. Volume of emulsion formed, together with the calculated amount of emulsified oil, emulsion capacity and emulsion stability with three different concentrations of salmon meal. *Emulsion capacity* was defined as mL of emulsified oil per 1 g of protein powder. *Emulsion stability* was defined as percentage of emulsion remaining after one day at room temperature and centrifugation. The measurements were performed in quadruplicates.

Protein concentration [%]	Parallel	Emulsion formed [mL]	Emulsified oil [mL]	Emulsion capacity [mL/g]	Emulsion stability [%]
1	#1	2.0	1.9	38.03	20.00
	#2	2.0	2.0	39.71	20.00
	#3	1.6	1.5	29.84	25.00
	#4	1.8	1.8	35.79	22.22
	Average	**1.9**	-	**36**	**22**
	SD	**0.2**	-	**4**	**2**
2	#1	3.1	3.0	29.31	16.13
	#2	3.0	2.9	28.95	13.33
	#3	2.5	2.4	23.94	20.00
	#4	2.5	2.5	24.97	16.00
	Average	**2.8**	-	**27**	**16**
	SD	**0.3**	-	**3**	**3**
5	#1	4.8	4.3	17.17	10.42
	#2	4.5	4.0	15.90	11.11
	#3	4.9	4.4	17.54	10.20
	#4	4.9	4.4	17.47	12.24
	Average	**4.8**	-	**17**	**11**
	SD	**0.2**	-	**1**	**1**

H.2 Whey Protein Powder

The volumes of water, emulsion and oil were measured after centrifugation the same day as homogenization and after 24 h and centrifugations, for different concentrations of whey protein powder (1, 2 and 5 %). The results are given in Table 57 and calculated emulsion capacity and emulsion stability with the different concentrations are given in Table 58.

Table 57. Volumes of water, emulsion and oil (mL) after centrifugation the same day as homogenization and after 24 h and centrifugation, with three different concentrations (1, 2 and 5 %) of whey protein powder. The weight of the powder is also included. The measurements were performed in quadruplicates.

		After centrifugation			After centrifugation and 24 h		
Parallel	**Powder [g]**	**H_2O [mL]**	**Emulsion [mL]**	**Oil [mL]**	**H_2O [mL]**	**Emulsion [mL]**	**Oil [mL]**
# 1	0.0505	4.5	5.0	0.5	4.5	4.9	0.6
# 2	0.0526	4.5	5.0	0.5	5.0	5.0	0.5
# 3	0.0507	4.5	5.1	0.4	4.5	3.5	2.0
# 4	0.0504	4.5	5.0	0.5	4.8	4.6	0.6
# 1	0.1018	4.5	5.3	0.2	4.5	5.0	0.5
# 2	0.1005	4.5	5.4	0.1	4.5	4.1	1.4
# 3	0.1013	4.5	5.3	0.2	4.4	5.1	0.5
# 4	0.1019	4.3	5.6	0.1	4.4	4.6	1.0
# 1	0.2502	3.7	6.3	0.0	4.0	5.6	0.2
# 2	0.2496	4.0	6.0	0.0	4.2	5.7	0.1
# 3	0.2509	4.0	6.0	0.0	4.0	5.9	0.1
# 4	0.2519	4.0	6,0	0.0	4.0	5.8	0.2

Table 58. Volume of emulsion formed, together with the calculated amount of emulsified oil, emulsion capacity and emulsion stability for three different concentrations (1, 2 and 5 %) of whey protein powder. *Emulsion capacity* was defined as mL of emulsified oil per 1 g of protein powder. *Emulsion stability* was defined as percentage of emulsion remaining after one day at room temperature and centrifugation. The measurements were performed in quadruplicate.

Protein concentration [%]	**Parallel**	**Emulsion formed [mL]**	**Emulsified oil [mL]**	**Emulsion capacity [mL/g]**	**Emulsion stability [%]**
1	#1	5.0	4.5	89.11	98.00
	#2	5.0	4.5	85.55	100.00
	#3	5.1	4.6	90.73	68.63
	#4	5.0	4.5	89.29	92.00
	Average	**5.03**	-	**89**	**90**
	SD	**0.05**	-	**2**	**14**
2	#1	5.3	4.8	47.15	94.34
	#2	5.4	4.9	48.76	75.93
	#3	5.3	4.8	47.38	96.23
	#4	5.6	4.9	48.09	82.14
	Average	**5.40**	-	**48**	**87**
	SD	**0.14**	-	**1**	**10**
5	#1	6.3	5.0	19.98	88.89
	#2	6.0	5.0	20.03	95.00
	#3	6.0	5.0	19.93	98.33
	#4	6.0	5.0	19.85	96.67
	Average	**6.08**	-	**19.9**	**95**
	SD	**0.15**	-	**0,1**	**4**

I. Water Holding Capacity

I.1 Salmon Meal

The weight of the sample before and after centrifugation, weight loss and water holding capacity of cod and cod mixed with 5, 10 and 20 % salmon meal are given in Table 59. The water content of the fish used in the calculation of the WHC are given in Table 60.

Table 59. Water holding capacity (WHC) of cod (F1-F4) and cod mixed with 5 % salmon meal (S1P5- S4P5), 10 % salmon meal (S1P10- S4P10) and 20 % salmon meal (S1P20-S4P20). The second and third column contain the weight of the fish before and after centrifugation, respectively. Percentage weight loss due to centrifugation, is given in the fourth column. These measurements were performed on cod filets that had been thawed, frozen, and then thawed again. The measurements were performed in quadruplicates.

Sample	Weight of the fish [g]	Weight of fish after centrifugation [g]	Weight loss [%]	WHC [%]	Average WHC [%]	SD
F1	2.0150	1.1008	45.5	44.9	44	1
F2	2.0154	1.0711	46.9	43.1		
F3	2.0027	1.0752	46.3	43.8		
F4	2.0159	1.1150	44.7	45.8		
S1P5	2.1050	1.1679	44.5	43.9	44	1
S2P5	2.1112	1.1791	44.2	44.4		
S3P5	2.1106	1.2040	43.0	45.9		
S4P5	2.1161	1.1632	45.0	43.2		
S1P10	2.2004	1.4132	35.8	52.3	53	2
S2P10	2.2044	1.4291	35.2	53.1		
S3P10	2.2086	1.4032	36.5	51.4		
S4P10	2.2024	1.4864	32.5	56.6		
S1P20	2.4059	1.6941	29.6	575.4	59	2
S2P20	2.4025	1.7299	28.0	59.7		
S3P20	2.4172	1.7830	26.2	62.2		
S4P20	2.4047	1.6986	29.4	57.744		

Table 60. Water content, V1 (%) of fish (F) and fish mixed with 5, 10 and 20 % salmon meal (SP5, SP10 and SP20). The second row gives the weight of the fish sample. The weight of the sample after 24 h in a heat cabinet (dry weight) is given in the third.

	F	**SP5**	**SP10**	**SP20**
Weight of the fish [g]	3.8292	3.6919	3.8691	4.6546
Dry weight of fish [g]	0.6741	0.7629	0.968	1.4228
Water content, V1[%]	82.4	79.3	75.0	69.4

I.2 Whey Protein Powder

The weight of the sample before and after centrifugation, weight loss and water holding capacity of cod and cod mixed with 5, 10 and 20 % whey protein powder are given in Table 61. The water content of the fish used in the calculation of the WHC are given in Table 62.

Table 61. Water holding capacity (WHC) of cod (F1-F4) and cod mixed with 5 % whey protein powder (W1P5- W4P5), 10 % whey protein powder (W1P10- W4P10) and 20 % salmon meal (W1P20- W4P20). The second and third column contain the weight of the fish before and after centrifugation, respectively. Percentage weight loss due to centrifugation is given in the fourth column. These measurements were performed on cod filets that had been thawed, frozen, and then thawed again. The measurements were performed in quadruplicate.

Sample	Weight of the fish [g]	Weight of fish after centrifugation [g]	Weight loss [%]	WHC [%]	Average WHC [%]	SD
F1	2.0070	1.0571	47.3	43.0	44	1
F2	1.9990	1.0516	47.4	43.0		
F3	2.2017	1.0621	47.5	42.9		
F4	2.0079	1.1058	44.9	45.9		
W1P5	2.1084	1.0889	48.4	39.1	36	2
W2P5	2.1258	1.0592	50.2	36.8		
W3P5	2.1043	1.0122	51.9	34.6		
W4P5	2.1002	1.0227	51.3	35.3		
W1P10	2.2096	1.1026	50.1	34.3	34	3
W2P10	2.1878	1.1326	48.2	36.7		
W3P10	2.2023	1.0381	52.9	30.7		
W4P10	2.2110	1.1135	49.6	34.9		
W1P20	2.4101	1.0579	56.1	21.3	19	1
W2P20	2.3003	0.9631	58.1	18.4		
W3P20	2.4166	1.0227	57.7	19.1		
W4P20	2.4147	1.0073	58.3	18.2		

Table 62. Water content, V1 (%) of fish (F) and fish mixed with 5, 10 and 20 % whey protein powder (WP5, WP10 and WP20). The second row gives the weight of the fish sample. The weight of the sample after 24 h in a heat cabinet (dry weight) is given in the third.

	F	WP5	WP10	WP20
Weight of the fish [g]	3.4014	3.6432	3.6077	3.7846
Dry weight of fish [g]	0.575	0.7528	0.8573	1.0872
Water content, V1[%]	83.1	79.3	76.2	71.3

J. Swelling Capacity

The swelling capacity was defined as the amount of water taken up by the powder in mL per gram powder. In order to calculate the swelling capacity, it was necessary to measure the amount of water absorbed by the filter paper. Measurements for the amount of water in the filter paper are given in Table 63.

Table 63. Amount of absorbed water in the filter paper.

	Dry filter	**Wet filter**	**Absorbed water in the filter paper**
Weight [g]	0.3249	1.0376	0.7127

Measured values and the calculated swelling capacity at 40 and 100 ºC for the herring protein powder and salmon meal are given in Table 64 and 65, respectively.

Table 64. Measured values and the calculated swelling capacity (mL water/g powder) at 40 and 100 ºC for herring protein powder. The average swelling capacity and the SD are also included.

	40 °C					100 °C				
	#1	**#2**	**#3**	**Average**	**SD**	**#1**	**#2**	**#3**	**Average**	**SD**
Powder [g]	5.0039	5.0097	5.0051			5.0033	5.0019	5.0007		
Filter [g]	0.3250	0.3248	0.3260			0.3235	0.3251	0.3248		
Erlenmeyer flask [g]	114.5379	124.1973	120.1390			124.1996	104.7872	138.8774		
Total amount of water [g]	20.0070	20.0212	20.0015			20.0744	20.0378	20.0356		
Erlenmeyer flask + water after filtration [g]	121.1394	130.5834	126.4233			127.1897	108.2618	142.3514		
Water in Erlenmeyer flask after filtration [g]	6.6015	6.3861	6.2843			2.9901	3.4746	3.474		
Water taken up by the powder [mL]	12.69	12.92	13.00	12.87	0.16	16.37	15.85	1585	16.02	0.30
Swelling capacity	2.54	2.58	260	2.57	0.03	3.27	3.17	3.17	3.20	0.06

Table 65. Measured values and the calculated swelling capacity (mL water/g powder) at 40 and 100 ºC for salmon meal. The average swelling capacity and the SD are also included.

	40 °C					100 °C				
	#1	**#2**	**#3**	**Average**	**SD**	**#1**	**#2**	**#3**	**Average**	**SD**
Powder [g]	5.0027	5.0046	5.0039			5.0046	5.0018	5.0114		
Filter [g]	0.3235	0.3253	0.3260			0.3257	0.3240	0.3261		
Erlenmeyer flask [g]	108.3509	109.5525	104.8087			124.1961	114.5388	133.2489		
Total amount of water [g]	20.0049	20.0249	20.014			20.0408	20.0019	20.0072		
Erlenmeyer flask + water after filtration [g]	118.0227	119.2432	114.8291			131.9457	122.4502	141.4501		
Water in Erlenmeyer flask after filtration [g]	9.6718	9.6907	10.0204			7.7496	7.9114	8.2012		
Water taken up by the powder [mL]	9.62	9.62	9.28	9.51	0.20	11.58	11.38	11.09	11.35	0.24
Swelling capacity	1.92	1.92	1.85	1.90	0.04	2.31	2.27	2.21	2.27	0.05

K. Antioxidant Properties

K.1 Dilution Series of Propyl Gallate

As mentioned in section 2.2.9, a series of dilution was prepared from the propyl gallate stock solution, and the volumes of propyl gallate and methanol are given in Table 66.

Table 66. Volumes of propyl gallate (stock solution) and 80 % methanol used in the preparation of the propyl gallate standard solution.

Concentration [µM]	**0**	**10**	**20**	**30**	**40**	**50**
Stock solution [µL]	0	10	20	30	40	50
80 % methanol [mL]	10	9.99	9.98	9.97	9.96	9.95

K.2 Whey Protein Powder

Absorbance measurements, standard curve and the scavenging effect of propyl gallate prepared for the determination of the antioxidant properties of whey protein powder, are given in Table 67 and Figure 49 and 50, respectively. The measurements of propyl gallate were used to check whether the absorbance measurements of the whey protein powder were within the standard curve.

Table 67. Measured absorbance (OD 517 nm) for different concentrations of propyl gallate (uM) used to produce Figure 49. The measurements were performed in triplicates.

Concentration [uM]	**0**	**10**	**20**	**30**	**40**	**50**
	0.751	0.543	0.325	0.146	0.039	0.035
OD 517 nm	0.739	0.530	0.306	0.133	0.038	0.037
	0.746	0.534	0.307	0.121	0.039	0.036
Average	0.745	0.536	0.313	0.133	0.039	0.036

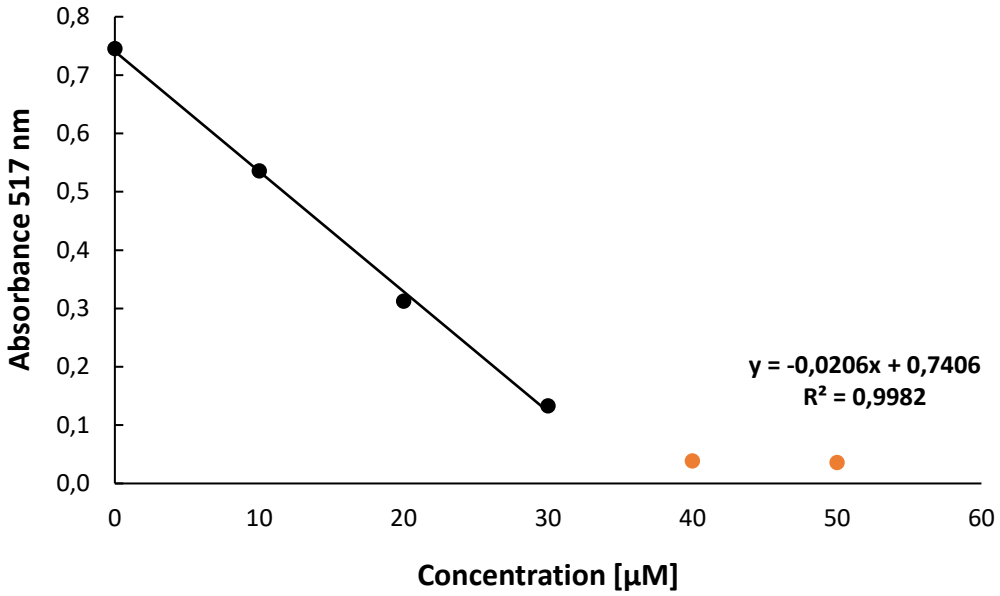


Figure 49. Standard curve for propyl gallate. Absorbance at 517 nm as a function of propyl gallate concentration (µM). The regression line was found for the first four absorbance measurement. The absorbance measurements were performed in triplicates.

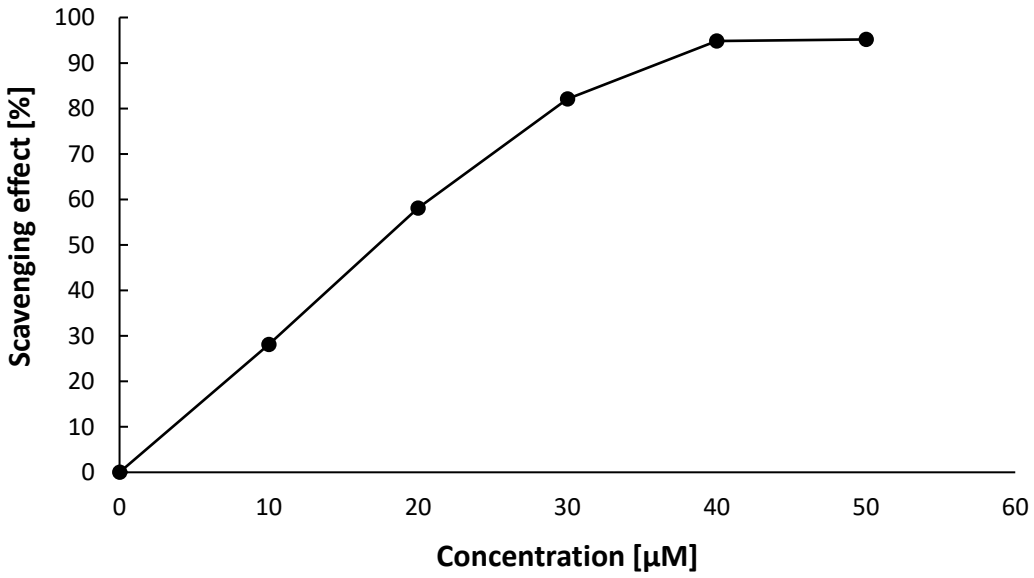


Figure 50. Scavenging of DPPH free radicals (%) as a function of propyl gallate concentration (µM). The absorbance measurements were performed in triplicates. The values are given as (absorbance of blank – absorbance of propyl gallate)/absorbance of blank ·100%.

The amount of whey protein powder dissolved in methanol, volume of extract, absorbance measurements and scavenging effect are given in Table 68.

Table 68. Amount of powder weighed out, volume of extract, absorbance measurements at 517 nm and scavenging effect against DPPH free radicals for whey protein powder in 80 % methanol. Absorbance measurements were performed in triplicates.

		Protein concentration [%]		
		0.5	**1**	**3**
Weight of powder [g]		0.0504	0.1038	0.2999
Volume extract [mL]		10	10	10
OD 517 nm	#1	0.737	0.715	0.595
	#2	0.723	0.683	0.562
	#3	0.717	0.690	0.566
	Average	0.726	0.696	0.574
Scavenging effect [%]	Average	2.6	6.6	22.9

K.3 Salmon Meal

The absorbance measurements of propyl gallate are given in Table 69. The measurements of propyl gallate were used to check whether the absorbance measurements of the whey protein powder were within the standard curve. The amount of salmon dissolved in methanol, volume of extract and absorbance measurements are given in Table 70 and 71. The antioxidant properties of salmon meal was investigated twice, but as seen from Table 70 and 71, the absorbance increased where it should have decreased. Hence, it was not possible to calculate the scavenging effect of the DPPH free radicals.

Table 69. Measured absorbance (OD 517 nm) for different concentrations of propyl gallate (uM) for the first (Nr. 1) and the second (Nr. 2) time the antioxidant properties of salmon meal was investigated. The measurements were performed in triplicates.

	Concentration [uM]	0	10	20	30	40	50
		0.883	0.600	0.413	0.180	0.068	0.062
Nr. 1	**OD 517 nm**	0.881	0.586	0.422	0.182	0.072	0.066
		0.858	0.585	0.413	0.168	0.066	0.062
	Average	0.874	0.590	0.416	0.177	0.069	0.063
		0.867	0.648	0.371	0.192	0.066	0.052
Nr. 2	**OD 517 nm**	0.863	0.638	0364	0.175	0.056	0.051
		0.852	0.623	0.359	0.162	0.056	0.054
	Average	0.861	0.636	0.365	0.176	0.059	0.052

Table 70. Amount of powder weighed out, volume of extract and absorbance measurements at 517 nm for salmon meal in 80 % methanol. Absorbance measurements were performed in triplicates. The values presented below are the measurements for the first investigation of the antioxidant properties of salmon meal.

		Protein concentration [%]		
		0.5	**1**	**3**
Weight of powder [g]		0.0508	0.1034	0.3012
Volume extract [mL]		10	10	10
	#1	0.778	0.913	1.643
OD 517 nm	#2	0.747	0.892	1.617
	#3	0.751	0.906	1.612

Table 71. Amount of powder weighed out, volume of extract and absorbance measurements at 517 nm for salmon meal in 80 % methanol. Absorbance measurements were performed in triplicates. The values presented below are the measurements for the second investigation of the antioxidant properties of salmon meal.

		Protein concentration [%]		
		0.5	**1**	**3**
Weight of powder [g]		0.0500	0.1005	0.2996
Volume extract [mL]		10	10	10
OD 517 nm	#1	0.774	0.865	1.434
	#2	0.749	0.623	1.418
	#3	0.744	0.619	1.411

K.4 Additional Absorbance Measurements of Salmon Meal Extracts

The absorbance of salmon meal in 80 % methanol at different wavelengths (400, 450, 517, 570 and 734 nm) were measured with 96 % ethanol as reference, and the results are given in Table 72.

Table 72. Absorbance measurements of different concentrations of salmon meal in 80 % methanol at different wavelengths with 96 % ethanol as reference.

	Protein concentration [%]		
Wavelength [nm]	**0.5**	**1**	**3**
400	0.092	0.156	0.322
450	0.051	0.096	0.203
517	0.029	0.050	0.082
570	0.021	0.039	0.054
734	0.018	0.019	0.037

L. Digestibility

The protein powders were digested with pepsin. Measurements and calculated digestibility of herring protein powder, salmon meal, whey protein powder with chocolate and neutral taste, together with casein are given in Table 73.

Table 73. Measured values and calculated digestibility of herring protein powder (HPP), salmon meal, whey protein powder (WPP) with chocolate and neutral taste, together with casein. Average digestibility and the SD are also given.

Material	Parallel	Empty centrifuge tube [g]	Centrifuge tube + powder [g]	Centrifuge tube + remaining powder after digestion and drying [g]	Digestibility [%]	Average digestibility [%]	SD
HPP	#1	11.5755	12.0776	11.6154	92.1		
	#2	11.5752	12.0761	11.6111	92.8	92.5	0.4
	#3	11.7074	12.2095	11.7452	92.5		
Salmon meal	#1	11.6260	12.1350	11.7657	72.6		
	#2	11.4829	11.9900	11.6300	71.0	71.8	0.8
	#3	11.6000	12.1080	11.7424	72.0		
WPP chocolate	#1	11.5648	12.0654	11.5980	93.4		
	#2	11.5245	12.0214	11.5502	94.8	93.6	1.1
	#3	11.6985	12.1989	11.7349	92.7		
WPP neutral	#1	11.5869	12.0989	11.5905	99.3		
	#2	11.6963	12.1994	11.6999	99.3	99.3	0.1
	#3	11.4816	11.9840	11.4847	99.4		

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Material	Parallel	Empty centrifuge tube [g]	Centrifuge tube + powder [g]	Centrifuge tube + remaining powder after digestion and drying [g]	Digestibility [%]	Average digestibility [%]	SD
Casein	#1	11.6002	12.1046	11.6395	92.2		
	#2	11.5328	12.0395	11.5717	92.3	91.9	0.6
	#3	11.6317	12.1324	11.6755	91.3		

M. Sensory Analysis

M.1 Questionnaires used in the Sensory Analysis

It was performed a sensory evaluation based on a 9-point hedonic scale for the sensory attributes (appearance, smell, texture and taste) and a 5-point hedonic scale for the purchase intent. The questionnaires used in the sensory analysis of the bread rolls fortified with whey protein powder, herring protein powder and salmon meal are given in Figure 51, 52 and 53, respectively.

Assessor: **Date:**

Sensory Analysis of Bread Rolls #1

You are provided a plate with five different bread rolls enriched with protein powder where each sample is labelled with a three-digit code. Please taste the samples in the following order. Use the water provided to cleanse your mouth before tasting each sample:

XIY VPP KXW PAS QRT

Indicate by giving each sample a number from 1 to 9 based on the scale below whether you liked or did not like each attribute. Indicate also on a scale from 1 to 5 what your attitude would be if you found the sample for sale. Please record your numbers in Table 1.

How much do you like each attribute of the sample?	
I like it extremely	9
I like it very much	8
I like it moderately	7
I like it slightly	6
I neither like nor dislike it	5
I dislike it slightly	4
I dislike it moderately	3
I dislike it very much	2
I dislike it extremely	1

Purchase intent	
I certainly would buy it	5
I might buy it	4
I might buy it/ I might not buy it	3
I might not buy it	2
I certainly would not buy it	1

Table 1: Please record your chosen value for each attribute here

Sample	Appearance (Utseende)	Smell (Lukt)	Texture (Konsistens)	Taste (Smak)	Purchase intent
XIY					
VPP					
KXW					
PAS					
QRT					

Comments:

Thank you for your participation!

Figure 51. Questionnaire used in the sensory analysis of bread rolls fortified with whey protein powder.

Assessor: **Date:**

Sensory Analysis of Bread Rolls #2

You are provided a plate with five different bread rolls enriched with protein powder where each sample is labelled with a three-digit code. Please taste the samples in the following order. Use the water provided to cleanse your mouth before tasting each sample:

XYZ MSL M6G KL9 0J4

Indicate by giving each sample a number from 1 to 9 based on the scale below whether you liked or did not like each attribute. Indicate also on a scale from 1 to 5 what your attitude would be if you found the sample for sale. Please record your numbers in Table 2.

How much do you like each attribute of the sample?	
I like it extremely	9
I like it very much	8
I like it moderately	7
I like it slightly	6
I neither like nor dislike it	5
I dislike it slightly	4
I dislike it moderately	3
I dislike it very much	2
I dislike it extremely	1

Purchase intent	
I certainly would buy it	5
I might buy it	4
I might buy it/ I might not buy it	3
I might not buy it	2
I certainly would not buy it	1

Table 2: Please record your chosen value for each attribute here

Sample	Appearance (Utseende)	Smell (Lukt)	Texture (Konsistens)	Taste (Smak)	Purchase intent
XYZ					
MSL					
M6G					
KL9					
0J4					

Comments:

Thank you for your participation!

Figure 52. Questionnaire used in the sensory analysis of bread rolls fortified with herring protein powder.

Assessor: **Date:**

Sensory Analysis of Bread Rolls #3

You are provided a plate with five different bread rolls enriched with protein powder where each sample is labelled with a three-digit code. Please taste the samples in the following order. Use the water provided to cleanse your mouth before tasting each sample:

KSL BL6 LS9 PQM TRT

Indicate by giving each sample a number from 1 to 9 based on the scale below whether you liked or did not like each attribute. Indicate also on a scale from 1 to 5 what your attitude would be if you found the sample for sale. Please record your numbers in Table 1.

How much do you like each attribute of the sample?	
I like it extremely	9
I like it very much	8
I like it moderately	7
I like it slightly	6
I neither like nor dislike it	5
I dislike it slightly	4
I dislike it moderately	3
I dislike it very much	2
I dislike it extremely	1

Purchase intent	
I certainly would buy it	5
I might buy it	4
I might buy it/ I might not buy it	3
I might not buy it	2
I certainly would not buy it	1

Table 1: Please record your chosen value for each attribute here

Sample	Appearance (Utseende)	Smell (Lukt)	Texture (Konsistens)	Taste (Smak)	Purchase intent
KSL					
BL6					
LS9					
PQM					
TRT					

Comments:

Thank you for your participation!

Figure 53. Questionnaire used in the sensory analysis of bread rolls fortified with salmon meal.

M.2 Results from the Sensory Analysis

Seventeen people participated in the sensory analysis of bread rolls mixed with whey and herring protein powder, while eighteen participated in the sensory analysis of bread rolls mixed with salmon meal. The results from the analysis of bread rolls mixed with different concentrations (0, 5, 10, 15 and 20 %) of whey protein powder are given in Table 74 and 75.

Table 74. The results from the sensory analysis of bread rolls mixed with different concentrations (0, 5, 10, 15 and 20 %) of whey protein powder with respect to appearance, smell and texture.

	Appearance					Smell					Texture				
Participant	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**
1	8	7	5	8	6	9	9	8	8	7	7	8	4	2	2
2	8	8	9	8	7	6	5	4	4	5	9	8	8	7	7
3	8	7	7	6	5	8	4	7	4	5	8	7	5	5	5
4	8	8	7	8	7	8	8	9	9	8	8	7	6	8	6
5	9	8	8	8	8	9	7	7	7	7	8	8	4	3	2
6	6	8	6	8	9	8	6	8	6	7	7	8	4	4	4
7	8	8	8	8	8	8	8	8	8	8	6	7	6	7	7
8	9	9	9	9	9	9	9	9	9	9	9	9	7	7	6
9	8	9	8	8	9	8	9	8	8	5	9	9	8	7	6
10	7	6	6	7	8	7	7	8	7	8	7	7	7	8	7
11	8	8	8	8	7	8	7	7	7	7	7	7	6	6	5
12	7	7	7	7	7	8	6	6	7	6	8	7	7	7	6
13	8	8	8	7	7	8	8	8	8	8	8	7	7	7	7
14	8	7	7	7	7	5	5	5	5	5	6	4	4	5	5
15	8	8	7	7	8	7	6	5	5	5	5	5	4	3	3
16	8	7	7	7	7	8	8	8	8	8	8	7	6	6	6
17	8	8	7	7	6	8	8	7	7	7	9	9	5	7	7
Average	7.9	7.7	7.3	7.5	7.4	7.8	7.1	7.2	6.9	6.8	7.6	7.3	5.8	5.8	5.4
SD	0.7	0.8	1.0	0.7	1.1	1.0	1.5	1.4	1.6	1.3	1.2	1.3	1.4	1.8	1.7

Table 75. The results from the sensory analysis of bread rolls mixed with different concentrations (0, 5, 10, 15 and 20 %) of whey protein powder with respect to taste, purchase intent and total score.

	Taste					Purchase intent					Total score				
Participant	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**
1	7	8	4	3	4	3	4	2	1	2	34	36	23	22	21
2	3	5	4	6	8	2	3	2	4	4	28	29	27	29	31
3	8	4	6	5	4	5	2	3	2	2	37	24	28	22	21
4	7	7	4	5	8	4	4	2	3	4	35	34	28	33	33
5	9	9	5	5	5	5	5	3.5	3.5	3.5	40	37	27.5	26.5	25.5
6	5	8	5	6	5	4	5	3	3	4	30	35	26	27	29
7	8	8	8	7	7	4	4	4	4	4	34	35	34	34	34
8	9	9	8	7	6	5	5	5	5	5	41	41	38	37	35
9	8	9	7	7	7	5	5	4	4	4	38	41	35	34	31
10	6	5	7	7	7	4	3	4	4	4	31	28	32	33	34
11	7	6	7	6	7	4	4	4	4	4	34	32	32	31	30
12	7	3	5	6	4	4	2	3	3	2	34	25	28	30	25
13	7	8	8	8	7	4	4	4	4	4	35	35	35	34	33
14	5	4	4	3	3	3	2	3	2	3	27	22	23	22	23
15	5	5	4	5	3	4	3	2	2	2	29	27	22	22	21
16	7	7	7	6	5	4	4	3	3	3	35	33	31	30	29
17	6	8	3	6	6	4	5	3	4	4	35	38	25	31	30
Average	6.7	6.6	5.6	5.8	5.6	4.0	3.8	3.2	3.3	3.4	33.9	32.5	29.1	29.3	28.6
SD	1.6	2.0	1.7	1.3	1.7	0.8	1.1	0.9	1.0	0.9	3.9	5.8	4.7	4.9	4.9

The results from the evaluation of bread rolls mixed with different concentrations (0, 5, 10, 15 and 20 %) of herring protein powder are given in Table 76 and 77.

Table 76. The results from the sensory analysis of bread rolls mixed with different concentrations (0, 5, 10, 15 and 20 %) of herring protein powder with respect to appearance, smell and texture.

	Appearance					Smell					Texture				
Participant	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**
1	4	3	3	3	2	9	2	2	2	1	7	3	2	2	1
2	9	8	8	8	8	9	4	2	1	1	9	9	8	8	8
3	8	7	5	6	6	8	2	2	2	2	8	7	7	7	7
4	8	7	7	6	4	9	8	6	4	4	7	8	8	7	2
5	9	7	7	7	7	9	7	4	4	3	8	9	9	7	7
6	8	7	7	7	7	6	6	6	3	4	8	8	6	6	5
7	8	8	8	8	8	8	8	8	7	6	8	8	8	8	4
8	9	9	9	9	9	9	4	4	4	4	9	9	9	4	4
9	9	9	9	8	9	9	4	3	3	4	9	9	9	8	8
10	7	7	7	7	6	8	3	3	2	2	8	7	7	6	6
11	8	8	8	8	8	8	6	3	3	2	7	7	7	6	7
12	7	7	7	7	7	6	6	5	5	3	7	7	7	7	7
13	8	8	8	8	8	8	8	8	8	7	8	8	8	7	7
14	7	7	7	7	7	4	4	3	2	2	6	6	6	6	6
15	7	7	7	7	7	6	5	3	5	3	6	5	5	5	4
16	8	8	8	7	8	7	4	3	3	3	7	7	7	4	4
17	8	6	9	8	7	8	8	8	6	4	8	8	7	7	5
Average	7.8	7.2	7.3	7.1	6.9	7.7	5.2	4.3	3.8	3.2	7.6	7.4	7.1	6.2	5.4
SD	1.2	1.3	1.5	1.3	1.7	1.4	2.1	2.1	1.9	1.6	0.9	1.6	1.7	1.6	2.0

Table 77. The results from the sensory analysis of bread rolls mixed with different concentrations (0, 5, 10, 15 and 20 %) of herring protein powder with respect to taste, purchase intent and total score.

	Taste					Purchase intent					Total score				
Participant	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**
1	8	2	1	1	1	4	1	1	1	1	32	11	9	9	6
2	9	3	2	1	1	5	1	1	1	1	41	25	21	19	19
3	7	2	1	1	1	4	1	1	1	1	35	19	16	17	17
4	7	2	2	3	1	4	1	1	2	1	35	26	24	22	12
5	9	4	3	3	3	5	2	2	2	2	40	29	25	23	22
6	7	6	5	3	2	4	4	3	2	2	33	31	27	21	20
7	9	8	4	4	3	5	4	3	3	2	38	36	31	30	23
8	9	2	1	1	2	5	1	1	1	1	41	25	24	19	20
9	9	5	4	5	5	5	3	2	3	3	41	30	27	27	29
10	7	3	2	2	2	4	2	1	1	1	34	22	20	18	17
11	8	6	4	2	1	4	3	2	1	1	35	30	24	20	19
12	6	4	3	2	2	4	2	1	1	1	30	26	23	22	20
13	8	7	7	6	6	4	4	3	3	2	36	35	34	32	30
14	4	3	2	2	2	1	2	1	1	1	22	22	19	18	18
15	5	5	3	3	3	4	3	2	2	2	28	25	20	22	19
16	6	4	3	5	3	3	2	1	2	2	31	25	22	21	20
17	7	4	4	4	3	4	4	3	3	3	35	30	31	28	22
Average	7.4	4.1	3.0	2.8	2.4	4.1	2.4	1.7	1.8	1.6	34.5	26.3	23.4	21.6	19.6
SD	1.5	1.8	1.6	1.6	1.4	1.0	1.2	0.8	0.8	0.7	5.1	6.0	6.0	5.4	5.5

The results from the sensory analysis of bread rolls mixed with different concentrations (0, 5, 10, 15 and 20 %) of salmon meal are given in Table 78 and 79.

Table 78. The results from the sensory analysis of bread rolls mixed with different concentrations (0, 5, 10, 15 and 20 %) of salmon meal with respect to appearance, smell and texture.

	Appearance					Smell					Texture				
Participant	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**
1	8	8	7	9	8	8	6	2	2	1	8	8	8	8	7
2	8	5	5	5	5	8	4	4	4	4	7	4	5	5	5
3	8	8	7	7	7	8	7	5	5	4	7	7	7	7	7
4	9	7	5	5	5	8	5	2	1	1	8	7	7	7	7
5	8	7	5	4	3	8	7	3	2	1	7	6	6	4	3
6	8	8	8	8	8	8	8	7	3	3	8	6	4	4	6
7	9	9	9	9	9	9	4	4	3	3	9	9	9	9	9
8	8	6	6	6	5	7	7	7	4	3	6	7	4	4	4
9	6	5	4	4	4	6	4	3	2	2	5	5	5	5	5
10	8	8	7	7	7	8	8	7	5	5	8	8	8	8	8
11	9	8	4	4	2	9	8	5	2	1	8	7	5	4	3
12	8	8	8	7	6	7	7	6	5	3	8	9	8	8	7
13	7	7	7	7	5	7	2	2	2	1	7	7	7	7	7
14	8	7	7	6	4	7	4	3	3	4	7	7	4	4	4
15	9	9	8	7	6	9	7	5	3	2	9	7	6	6	5
16	9	9	9	9	9	9	8	6	4	2	9	9	8	8	8
17	9	9	9	9	9	9	9	9	8	8	8	8	8	8	8
18	7	7	6	4	3	6	5	4	2	2	7	6	5	3	3
Average	8.1	7.5	6.7	6.5	5.8	7.8	6.1	4.7	3.3	2.8	7.6	7.1	6.3	6.1	5.9
SD	0.8	1.2	1.6	1.9	2.2	1.0	1.9	2.0	1,7	1,8	1.0	1.3	1.6	1.9	19

Table 79. The results from the sensory analysis of bread rolls mixed with different concentrations (0, 5, 10, 15 and 20 %) of salmon meal with respect to taste, purchase intent and total score.

	Taste					Purchase intent					Total score				
Participant	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**	**0**	**5**	**10**	**15**	**20**
1	8	3	2	1	1	5	2	1	1	1	37	27	20	21	18
2	7	3	2	2	2	4	2	2	2	2	34	18	18	18	18
3	8	4	2	1	1	8	3	2	1	1	39	29	23	21	20
4	8	4	3	2	1	5	2	2	1	1	38	25	19	16	15
5	8	4	2	4	3	5	2	1	2	1	36	26	17	16	11
6	8	6	4	2	3	4	3	2	1	2	36	31	25	18	22
7	9	4	3	3	2	5	2	2	2	1	41	28	27	26	24
8	6	3	2	2	2	3	2	1	1	1	30	25	20	17	15
9	6	4	3	2	1	4	2	1	1	1	27	20	16	14	13
10	8	8	5	4	2	4	4	2	1	1	36	36	29	25	23
11	8	7	4	2	1	5	4	1	1	1	39	34	19	13	8
12	8	6	3	2	1	4	3	2	2	1	35	33	27	24	18
13	6	2	2	1	1	4	1	1	1	1	31	19	19	18	15
14	5	5	4	4	2	4	3	2	2	1	31	26	20	19	15
15	9	8	4	3	2	5	4	3	2	2	41	35	26	21	17
16	9	8	5	3	1	5	4	3	2	2	41	38	31	26	22
17	9	8	8	7	4	5	4	4	3	2	40	38	38	35	31
18	5	4	3	1	1	4	2	2	1	1	29	24	20	11	10
Average	7.5	5.1	3.4	2.6	1.7	4.6	2.7	1.9	1.5	1.3	35.6	28.4	23.0	19.9	17.5
SD	1.3	2.0	1.5	1.5	0.9	1.0	1.0	0.8	0.6	0.5	4.4	6.3	5.8	5.7	5.6

N. Amino Acid Composition of Wheat Flour and Wheat Grain

The proximate composition of wheat flour and wheat grain used in the calculations of the protein content of the bread rolls, are given in Table 80. The amino acid compositions are also included. Although it was wholemeal which was used in the bread rolls, the amino acid composition was not easily available. The amino acid composition of wheat grain was therefore used instead.

Table 80. Proximate composition (%) of wheat grain and wheat flour (Rosell, 2011).

	Wheat grain	**Wheat flour**
Energy (kcal)	329	364
Total carbohydrate (g)	68	76.3
Dietary fibre (g)	12	2.7
Total fat (g)	1.9	1
Saturated fat (g)	0.3	0.2
Monounsaturated fat (g)	0.3	0.1
Polyunsaturated fat (g)	0.8	0.4
Protein (g)	15.4	10.3
Amino acids		
Tryptophan (mg)	195	127
Threonine (mg)	433	281
Isoleucine (mg)	541	357
Leucine (mg)	1038	710
Lysine (mg)	404	228
Methionine (mg)	230	183
Cysteine (mg)	404	219
Phenylalanine (mg)	724	520
Tyrosine (mg)	441	312
Valine (mg)	679	415
Arginine (mg)	702	417
Histidine (mg)	330	230
Alanine (mg)	555	332
Aspartic acid (mg)	808	435
Glutamic acid (mg)	4946	3479
Glycine (mg)	621	371
Proline (mg)	1680	1198
Serine (mg)	663	516

O. Essential Amino Acid Content of one Bread Roll

The essential amino acid composition of the protein powders (section 3.2) were used together with the amino acid composition of the wheat flour and wheat grain (Appendix N), to calculate the content of essential amino acids in the dough. The formulation used for the different doughs were given in Table 7 (section 2.2.18). The content of the different essential amino acids (EAA) in the doughs was calculated according to Equation 10:

$$EAA\ (dough) = EAA_{wheat\ flour} + EAA_{wholemeal} + EAA_{protein\ powder} \quad (10)$$

The content of the different amino acids in *one* bread roll was found by dividing the values calculated with Equation 10 by five, since five bread rolls were made from one dough.

The content of the different EAA in one bread roll baked with different concentrations of whey protein powder, herring protein powder and salmon meal are given in Table 81, 82 and 83.

Table 81. The content of the different essential amino acids (EAA) in one bread roll added varying amounts (0, 5, 10, 15 and 20 %) of whey protein powder.

	Protein powder concentration [%]				
Essential amino acid	**0**	**5**	**10**	**15**	**20**
His	0.08	0.09	0.10	0.11	0.12
Ile	0.12	0.17	0.22	0.27	0.32
Leu	0.24	0.32	0.39	0.47	0.55
Lys	0.09	0.17	0.25	0.34	0.42
Met	0.05	0.07	0.09	0.10	0.12
Phe + Tyr	0.27	0.31	0.34	0.38	0.42
Thr	0.10	0.16	0.22	0.28	0.33
Trp	0.04	0.04	0.04	0.04	0.04
Val	0.15	0.19	0.23	0.27	0.31
Total EAA	1.1	1.5	1.9	2.3	2.6

Table 82. The content of the different essential amino acids (EAA) in one bread roll added varying amounts (0, 5, 10, 15 and 20 %) of herring protein powder.

	Protein powder concentration [%]				
Essential amino acid	**0**	**5**	**10**	**15**	**20**
His	0.08	0.10	0.11	0.13	0.15
Ile	0.12	0.19	0.25	0.31	0.37
Leu	0.24	0.36	0.49	0.61	0.74
Lys	0.09	0.19	0.29	0.40	0.50
Met	0.05	0.08	0.10	0.12	0.15
Phe + Tyr	0.27	0.36	0.45	0.54	0.63
Thr	0.10	0.15	0.21	0.27	0.32
Trp	0.04	0.04	0.04	0.04	0.04
Val	0.15	0.22	0.29	0.35	0.42
Total EAA	1.1	1.7	2.2	2.8	3.3

Table 83. The content of the different essential amino acids (EAA) in one bread roll added various amounts (0, 5, 10, 15 and 20 %) of salmon meal.

	Protein powder concentration [%]				
Essential amino acid	**0**	**5**	**10**	**15**	**20**
His	0.08	0.08	0.09	0.10	0.11
Ile	0.12	0.14	0.15	0.17	0.18
Leu	0.24	0.26	0.29	0.32	0.35
Lys	0.09	0.12	0.16	0.20	0.23
Met	0.05	0.07	0.08	0.09	0.11
Phe + Tyr	0.27	0.30	0.32	0.35	0.38
Thr	0.10	0.12	0.14	0.16	0.18
Trp	0.04	0.04	0.04	0.04	0.04
Val	0.15	0.17	0.19	0.21	0.22
Total EAA	1.1	1.3	1.5	1.6	1.8