Product development using protein isolate produced by pH-shift process from Baltic herring (*Clupea harengus membras*)

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Ella Aitta

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AITTA, ELLA:

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Supervisors: MSc Tanja Kakko, PhD Annelie Damerau & Prof. Baoru Yang

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The aim of the study was to produce fish protein isolate using alkaline pH-shift processing and to use the protein isolate in products such as surimi and fish balls. Different pH-values and additives were used in order to find the processing method for the best colour and yield. Protein, lipid and moisture contents of the raw material, produced isolate and the products were determined. Furthermore, the surimi and fish balls were analysed with texture profile analyser and their volatile compounds were determined with head-space solid-phase microextraction combined with gas chromatography and mass spectrometry. Additionally, the colour of surimi from different pH-shift processes was determined by colorimetry. The results were compared to commercial surimi and fish products.

The alkaline pH-shift process using pH-values 11.2 and 5.4 as solubilisation and precipitation values, respectively, resulted in isolate yield of 84% of the raw material and a protein yield of 54%. The isolate contained 7.2-9.6% of protein, 3.7% of lipids and a moisture content of 85.3%. The lipid content of the isolate was relatively high, 25.2% of the dry-weight, making the isolate prone to oxidation. Next, titanium dioxide improved the colour of surimi when added to the paste before cooking However, the pH-shift processes using 11.5 as the solubilisation pH-value, or 6.5 as the precipitation value did not affect the colour of the isolate. In fact, the latter process led to a significantly softer surimi due to increased water content. Additionally, bentonite or activated carbon did not improve the colour of the isolate. More research ought to be done to improve the colour, which is an important aspect concerning the consumer acceptance.

The isolate showed good gelling properties, and the surimi made with it had similar texture profile to a commercial surimi scampi. Moreover, the isolate was successfully used in fish balls in concentrations as high as 50%. The addition of the protein isolate in the fish balls improved their texture without affecting the colour or taste adversely.

The raw material of this study was beheaded and gutted Baltic herring. The use of whole fish or production side-streams would make the process more environmentally friendly and economical. Thus, future studies should focus on the utilisation of whole fish. Additionally, more studies need to be conducted to improve the colour, reduce the lipid content and to increase the protein yield.

Keywords: Baltic herring, pH-shift process, fish protein isolate, texture profile analysis

Contents

Abbreviations

ALA α-linolenic acid

DALY disability adjusted life year

DHA docosahexaenoic acid

DL-PCB dioxin-like polychlorinated biphenyls

EPA eicosapentaenoic acid

EWP egg white powder

FA fatty acid

FPI fish protein isolate

FW fresh weight

HS-SPME-GC-MS headspace-solid phase microextraction-gas chromatography-mass spectrometry

OTC organotin compound

PBDE polybrominated diphenyl ether

PCA principal component analysis

PCB polychlorinated biphenyl

PCDD/F polychlorinated dioxin/furan

PFC perfluorinated compound

PFOA perfluorooctanoic acid

PFOS perfluorooctane sulfonate

TEQ toxic equivalence quantity

TG transglutaminase

TPA texture profile analysis

1. Introduction

1.1. Baltic herring

Baltic herring (*Clupea harengus membras*) is a subspecies of the Atlantic herring (*Clupea harengus*) living in the Baltic sea. Baltic herring is known for its high fat content and a distinguishable odour, which some consumers find unappealing after a few days of storage. However, it is the most important commercial catch in Finland both in value and quantity (Luonnonvarakeskus, 2019). In 2018, 126 million kilograms of Baltic herring was caught by Finnish fishing vessels, which comprised approx. 85% of the total fish catch. Furthermore, the value of Baltic herring out of all caught fish was approx. 70%. (Luonnonvarakeskus, 2019) Still, only 3% of the catch ends up on consumers plates whereas majority of the Baltic herring is used as animal feed. One of the factors that have decreased the use of Baltic fish over the years are environmental toxins, such as dioxins and polychlorinated biphenyl (PCB) compounds. However, the levels of these toxins have decreased during the 21st century and the amounts do not pose a threat to the general population any longer. For example, polychlorinated dioxins and furans (PCDD/Fs) and PCBs have decreased by approximately 80%, from approx. 20 to 5 pg/g fresh weight (FW) (expressed as toxic equivalence quantity (TEQ)), during the 31-year period between 1978–2009 (Airaksinen et al., 2014). The maximum level for these contaminants in fishery products is 6.5 pg/g FW set by the Commission Regulation (EC) No. 1881/2006. Another factor limiting the use of Baltic herring is its small size and abundance of bones which is both problematic for the automated filleting machinery and unappealing for consumers. Approx. 10% of the catch is too small for the automated filleting processing, and is used for fish meal and animal feed production instead (Pro Kala, 2017). Thirdly, younger generations consume less Baltic herring than the elderly, which is due to changes in eating habits and partly because of reduced availability of Baltic herring products in shops. Therefore, Baltic herring may be more consumer-friendly and profitable as a processed product, such as preserved fillets, fish patties or fish fingers.

Baltic herring, as other herrings and small pelagic fish can also be called dark muscle fish due to a higher proportion of dark muscle tissue. The darker muscle contains higher concentrations of pigments, fat and vitamins, which are beneficial for health but the higher contents of fat, myoglobin and hemoglobin often lead to increased lipid oxidation, causing rancid flavours and a decreased quality. (Murray and Burt, 1983; Park, 2005) If the dark muscle tissue is not removed from the fish, it gives the product a grey colour,

which consumers often reject. The issue with a darker colour can be overcome with coating (e.g. fish fingers) or trying to change the consumer acceptance by consistently linking the product with good quality. (Ministry of Agriculture, Fisheries and Food, 2001)

Baltic herring was the most important commercial fish in Finland until the 1980s, but the consumption of the fish as food has dropped from over 30 million kilograms down to 3.5–4 million in the past 30 years. Even though the consumption of Baltic herring has dropped ten-fold, the consumption of fish has nearly doubled in Finland since the 1980s. The decrease in the consumption of domestic fish has been replaced by an increase in imported fish, especially Norwegian salmon of which import has increased by 40%. Another big change in the Finnish fish market is the decreasing amount of wild fish which is being replaced with farmed fish, mainly salmon. (Natural Resources Institute Finland, 2019)

According to the European Commission (2017) 59% of Finns rank the origin of the product as the most important aspect when buying fishery and aquaculture products. Moreover, 62% of respondents would prefer buying domestic products over products from other countries. Additionally, over a third (34%) of the Finnish respondents think that “easy” and “quick to prepare” are important aspects to consider when buying fish. However, price seems to be one of the most important aspects considering the purchasing of fishery products. According to the study, 69% of Finns claim that they would buy more fish if it was cheaper. High price is not the only reason for the decrease in domestic fish consumption in Finland – the availability has also decreased, driving people to choose foreign fishery products.

High fish price, however, does not explain the decrease in the Baltic herring consumption. Filleted Baltic herring is one of the cheapest fishes in a supermarket, being over three times cheaper than filleted rainbow trout (Foodie.fi, 2019). When looking at the demographics, age seems to play the biggest role in the Baltic herring consumption: elderly people consume the largest amount. Further, as expected, people living on the coastal areas consume more Baltic herring than people from the inland regions. Interestingly, a larger group of consumers, who consume an average of over 10 grams of Baltic herring per day, come from all areas of Finland with no clear distinction between coastal and inland areas. (Elintarviketurvallisuusvirasto Evira, 2015)

In Finland, the amount of processed fish increased by 2.5-fold from 1993 to 2009, reaching 75 million kilograms by 2009. The four most important fish species for

processed fish products were trout, salmon, Baltic herring and herring, which altogether comprised over 90% of the fish raw material. Most of the processed fish is Baltic herring, which is most often frozen and exported abroad, mainly to Baltic countries for raw material to the canning industry. The dominant Baltic herring products on the Finnish market are fillets and preserved fillets in marinade (Figure 1). In 2009, approx. 1.8 million kilos of Baltic herring were frozen as fillets, 5.7 million kilos as fresh fillets and 215 tonnes were used as a raw material for convenience food. The amount of leftovers from beheading, gutting and filleting processes of Baltic herring comprise over four million kilos. The leftovers are mainly further frozen to feed fur animals. (Vielma et al., 2013)

Figure 1. *Most common Baltic herring products on the Finnish market include preserved fillets, fried fillets and fried breaded fillets, and smoked whole fish. Image: Pro Kala Ry.*

1.1.1. Composition and nutritional value

The recommended daily intake of dietary proteins for 18–64-year-old adults is 1.1–1.3 g/kg bw and for over 64-years-olds 1.2–1.4 g/kg bw. Therefore, an adult weighing 70 kg requires a daily amount of 77–91 grams of protein. However, all protein sources are not considered equal because the protein digestibility can vary vastly between different foods. For example, only 70% of the corn cereal protein is digested whereas the digestibility of egg protein is as much as 97%. (Joint Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition et al., 2007) Secondly, some protein sources, such as legumes, may lack certain essential amino acids (Evans and Bandemer, 1967). However, fish is regarded as a good source of protein due to its high protein and balanced amino

acid contents. Baltic herring contains approx. 16% protein, varying between the autumn and spring seasons (16.0–16.6%, respectively) (Elintarviketurvallisuusvirasto Evira, 2014a). Furthermore, it has been assessed that herring contains all essential amino acids in amounts exceeding the WHO/FAO/UNU amino acid requirements for adults expressed as mg/g protein, which are assessed by nitrogen balance (Joint Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition et al., 2007; Marmon, 2012).

Omega-3 (also called n-3) fatty acids (FAs) are also important to human health. These FAs are found in many vegetable sources but especially in fatty fish. Omega-3 FAs can be divided into α-linolenic acid (ALA; 18:3n-6) and long chain n-3 polyunsaturated FAs, main molecules being eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). ALA is an essential FA which is required for the synthesis of important FAs, including EPA and DHA, as precursors of eicosanoids. The intake of EPA and DHA is linked to several important functions: firstly, DHA is vital to the development of the foetal brain and retina and has also shown to benefit the problem solving skills of infants (Judge et al., 2007; Ramakrishnan et al., 2010) and secondly, these FAs have shown anti-inflammatory effects, reduction in the risk of cardiovascular disease and they may also play a role in the prevention of Alzheimer's disease (Bloomer et al., 2009; European Food Safety Authority, 2005; Tully et al., 2003). The total fat content of Baltic herring is approx. 5.6 g/100 g of the fresh weight. The total amount of n-3 FAs is approx. 1 g/100 g FW, of which 0.3 g/100 g is EPA and 0.4 g/100 g DHA. (Elintarviketurvallisuusvirasto Evira, 2015). The National Nutrition Council recommends that at least 1% of the total energy consumption for adults and over 2-year-old children should come from n-3 FAs, which equals to approx. 2 grams in a diet consisting of 2000 calories. The recommended daily consumption for DHA is 200 mg, whereas EPA does not have a specific recommendation. (Valtion ravitsemusneuvottelukunta, 2014) Therefore, Baltic herring is an excellent source of n-3 FAs, and the recommended daily amount of DHA is fulfilled with a 50-gram portion of fresh fish.

Fish is one of the most important sources of vitamin D in the human diet. Vitamin D is a fat-soluble vitamin, which can be found in Baltic herring in concentrations up to 17 µg/100g FW (Elintarviketurvallisuusvirasto Evira, 2014a). The current National recommendation for daily vitamin D is 10 µg for children and adults, and 20 µg for over 75-year-olds (Valtion ravitsemusneuvottelukunta, 2014). Therefore, for an adult a 60-gram portion of Baltic herring is enough for the recommended daily intake of vitamin D.

Volatile compounds create the aroma of foods and they are also among the essential indicators of freshness. The most important volatile compounds in fresh Baltic herring are heptadecane and 1-heptadecene. Additionally, branched chain aldehydes 2-methylbutanal and 3-methylbutanal can be found from baked fresh Baltic herring (1-day storage) in large quantities, comprising approx. 34% of the total amount. These compounds are created via Strecker degradation from the reaction of leucine and isoleucine with α-dicarbonyl compounds. (Aro et al., 2003, 2002) Another abundant compound in the baked fresh Baltic herring is hexanal, which has a green, plant-like aroma (Josephson et al., 1983). The composition of volatiles changes after storage, especially the proportions of 4-heptenal, 2-heptanone and octatriene which all increase significantly. Furthermore, after 3-6 days of storage at 6°C, the proportion of short chain acids, such as acetic, propanoic, 2-methylpropanoic and 3-methylbutanoic acid increases. During an extended storage, the long chain unsaturated FAs in cooked fish undergo oxidation, which leads to the increase of compounds such as hexanal, heptanal, 1-penten-3-ol and octadienes. Lastly, cresol and 3-methyl-1-butanol are compounds related to microbial spoilage which can also be found in fish after an extended storage. (Aro et al., 2003, 2002)

1.1.2. Environmental toxins

The National Nutrition Council of Finland recommends to consume fish twice a week varying between different fish species (Valtion ravitsemusneuvottelukunta, 2014). However, some fish species accumulate environmental toxins, such as heavy metals and dioxins, which is why the Finnish Food Authority has given an exception to this recommendation for large, over 17 cm long Baltic herring, trout and Baltic salmon. For example, children, adolescent, pregnant and fertile women should consume large Baltic herring maximum twice per month in 100-gram portions. (Ruokavirasto, 2019a) According to Elintarviketurvallisuusvirasto Evira (2015) the health benefits from Baltic herring consumption are greater than the potential health risks, especially for over 50 year-olds. The estimated decrease in heart diseases and heart-related deaths from Baltic herring consumption is -688 Disability Adjusted Life Year (DALY)/year. One DALY describes a lost year of "healthy" life and it is calculated as a sum of the years of life lost due to premature mortality and the years lost due to disability (World Health Organisation, 2019).

The maximum limit for dioxins in fishery products is 3.5 pg/g (TEQ) FW and for the combined dioxins and PCBs the value is 6.5 pg/g (TEQ) FW. However, Finland and Sweden have been granted a special permission to place Baltic fish exceeding the EU maximum limits of PCDD/Fs and/or DL-PCBs on the market, stated in Article 7 of the Commission Regulation (EC) No. 1881/2006. According to a research published in 2018, the combined amount of dioxins and PCBs exceeded the EU maximum levels only in larger, over 19 cm long Baltic herring caught from the Bothnian sea and the Bay of Bothnia while the mean value was less than 4 pg/g (TEQ) FW (Airaksinen et al., 2018). The dioxin and PCB-levels show a reducing trend according to previous reports from 2011 and 2004 (Hallikainen et al., 2011, 2004)

Elintarviketurvallisuusvirasto Evira (2015) has estimated the health risks of dioxins, especially by looking at tooth damage in children as is the most common and sensitive developmental disorder from dioxin exposure. One of the most important routes children are exposed to dioxins is though the mother to the foetus, and through breast milk. Tooth damage from dioxin exposure is estimated to cause annually approx. 11 disability adjusted life year (DALY/year). The health risk is only directed to the new-born children, which is about 1% of the Finnish population. Additionally, the cancer risk caused by dioxins for 0–19-year-olds is 1–2 DALY. However, not only does the mother's fish consumption contribute to the release of dioxins to breastmilk, but also the body's fat reserves with accumulated dioxins are broken down during breastfeeding. The research concludes that Finns eat so little Baltic herring that the current recommendations and limitations for fish consumption are satisfactory to protect the general population from dioxins and dioxin-like compounds *via* consumption of Baltic herring.

The amounts of perfluorinated compounds (PFCs), such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) have increased slightly after 2009 because of their increased use in textiles and industry as water and dirt repellents. The heavy use and slow degradation of these compounds may lead to increasing concentration in the Baltic sea in the future even though their use is now restricted. (Natural Resources Institute Finland, 2018) PFCs are a varied group of compounds, which have different half-lives and volatilities. Some of the compounds are hepatotoxic and disrupt the endocrine and immune systems. They may also be carcinogenic and genotoxic. (Mehtonen et al., 2016) The sales and usage of PFOS have been restricted under the EU Regulation (EC) No. 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of

Chemicals (REACH) starting from 2008. In 2009, PFOS were restricted through the Stockholm Convention on persistent organic pollutants and a year later, in 2010, these compounds were added to Annex I of Persistent Organic Pollutant (POP) protocol 850/2004. Furthermore, an Annex XVII to the EU Regulation (EC) No. 1907/2006 (REACH) restricts the use of PFOA and its derivatives from July 2020 onwards. The current restrictions have decreased the emissions of PFOS significantly (Chemical Watch, 2012). The concentration of PFOS in Baltic herring was measured in 2009 and it varied between 1–5 ng/g FW (Hallikainen et al., 2011). The tolerable daily intake of PFOS has been assessed by the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) as 150 ng/kg bw (European Food Safety Authority, 2012) and therefore, the amount in Baltic herring does not cause a health concern.

The concentration of mercury in Baltic herring is 0.015–0.037 mg/kg, which is below the maximum limit of 0.5 mg/kg wet weight for fishery products set by the Commission Regulation (EC) No. 1881/2006. The lowest amounts were measured in the Sea of Bothnia, which is the most important commercial fishing place for Baltic herring. (Ruokavirasto, 2019b) However, mercury, as well as PCDD/Fs and DL-PCBs accumulate in fatty fish, which is why fertile women have maximum recommendations for Baltic herring and salmon. A study found that 90% of fish liver samples, 95% of fish muscle samples and almost all mussel samples taken from the Baltic sea have mercury contents exceeding the value of 0.02 mg/kg, which is an environmental target value set by the EU. The EU Water Framework Directive allows the addition of background concentrations (OSPAR BAC) to the target value, but even then, 75% of fish samples and 91% of mussel samples exceeded the value. Even though the amount of mercury seems safe for human consumption, there is a risk of bioaccumulation and harmful effects in the predators. (Baltic Marine Environment Protection Commission (Helsinki Commission), 2010) The amount of arsenic, on the other hand, has increased since 2002, reaching levels of 0.46–1.4 mg/kg. The arsenic is mainly in organic form, which is considered harmless to humans. (Ruokavirasto, 2019b)

Organotin compounds (OTCs) *aka* stannases are compounds in which tin is linked to a hydrocarbon. Tributyltins are a group of OTCs which are used as industrial biocides and have been used in paints as antifungal agents. Previously, tributyltins were used in marine anti-biofouling agents of ocean-going ships but the concern in their toxicity resulted in bans of these compounds. There have been restrictions in the use of OTC in ship paints

since 1991, but EU set a total ban in 2008 in all ships that sail under its flag, which means that the existing paints need to be either removed or painted over. Unfortunately, large amounts of OTCs are found in bottom sediments of seas. According to Evira (2008), the mean concentration of OTCs in all Baltic fish is under 20 µg/kg FW in areas without organotin source, whereas in some areas, concentrations as high as 150–500 µg/kg FW were found. The mean concentration of OTCs in Baltic herring is 15 µg/kg FW. OTCs can cause developmental and behavioural problems in marine species. In human, the acute toxic syndromes include skin and respiratory tract irritation and neurotoxic effects, however, the long-term effects of exposure are not known. The tolerable daily intake (TDI) has been set by the European Food Safety Authority as 0.25 µg/kg bw/day for a group of OTC. (European Food Safety Authority, 2004) The amount is reached e.g. by consuming 100 grams of fish with an OTC concentration of 150 µg/kg FW.

Since many environmental toxins, such as PCDD/Fs are lipophilic, they are concentrated in the fat tissue. Furthermore, these toxins accumulate by time, and can be found from old, bigger Baltic herring in high concentrations. However, the bigger fish would be better for industrial purposes since the smallest fish are too small for the automated processes. Some of the lipids, and therefore lipophilic environmental toxins are removed during the normal fish processing which removes fatty tissue, such as skinning and filleting. For example, the production of Maatjes fillets and fillets for marinated herring pieces intended for salads removes only the upper skin, leaving the subcutaneous fat layer on the fillet. The process has shown a reduction of 23% of the total fat content, although the share of the removed skin is only 10–12% (Karl and Ruoff, 2007). Fish processing is further discussed in the next section.

1.2. Fish processing

Baltic herring is typically processed into fillets and marinated fillets or sold as whole fresh or smoked fish. Fish, especially small species, are usually not processed to minced fish, although minced roach is a recent addition on the Finnish market (Särkifood Oy, 2019). Surimi is one example of an untypical processed fish product on the Northern European market, although it is highly popular in Asia. It is one example of a product utilizing the gelation properties of fish protein. Surimi is typically made from Alaska pollock (*Theragra chalcogramma*), and it is processed into gelled products of different shapes.

Alaska pollock can be captured in large volumes and it is suitable for surimi production due to its white flesh. The traditional surimi paste production consists of mincing the fish, rinsing the meat multiple times with large amounts of water, refining, removing excess water with a screw press and the addition of cryoprotectants before freezing. (Park, 2005) Imitation crab meat is one example of a product made with surimi paste and additives, such as starch, oil, sugar, salt, egg white and crab aroma.

As alternative processing methods, pH-shift processing and enzymatic hydrolysis can be used to isolate proteins from whole fish or side stream products, such as fish frames, skins or heads. The industrial side-streams and the discards of marine products is estimated to be around 8% of the total catch. Based on this rate, the yearly average discard is approx. 7.3 million tons worldwide. (Kelleher, 2005) The pH-shift process enables the isolation of proteins and lipids while separating bones and scales. The process also leads to isolates with better gelling properties than the traditional surimi paste (Pérez-Mateos et al., 2004). Secondly, enzymatic hydrolysis utilises enzymes such as pepsin, neutrase or alcalase to recover proteins and lipids from fish (Liaset et al., 2000). Furthermore, fish frames and skins can be used as a raw material for fish collagen production, which has attracted interest in the global collagen markets (Kołodziejska et al., 2008). The following chapter describes the basics of the pH-shift process utilized in this study to isolate proteins from Baltic herring.

1.2.1. pH-shift process

The pH-shift processing has proven to be a promising way to solubilise and isolate proteins from fish. The pH-shift process allows the use of the smallest fish that the industry is currently not fully utilising for human consumption, allowing a more environmentally friendly and cost-effective production line. Furthermore, the process removes some of the pigments and lipids, which are prone to oxidation and may contain environmental toxins. The pH-shift process is based on the solubilisation of muscle proteins using either low or high pH (pH $\leq$3 or $\geq$10.8), after which the pH is shifted near the isoelectric point to precipitate the protein (Marmon and Undeland, 2010). The biochemical basis of the process is presented in Figure 2. Under alkaline conditions, proteins become negatively charged which induces protein–water interactions and makes proteins water soluble. Acidic conditions, on the other hand, induce positive charges on

proteins allowing weak hydrogen bonding with water molecules. Both processes lead to the solubilisation of proteins in the water phase, after which they can be precipitated by shifting the pH to the isoelectric point of the protein, where protein–protein interactions are favoured.

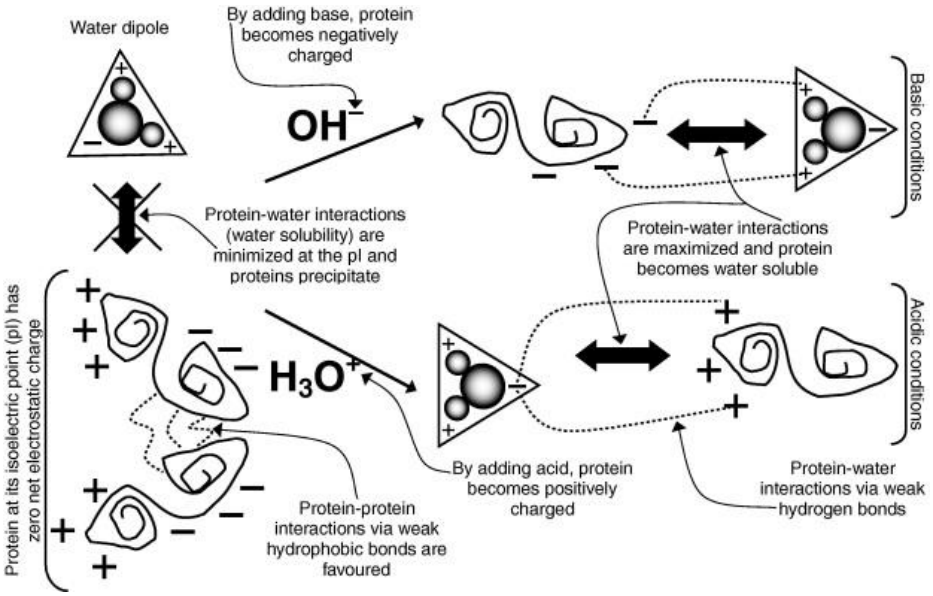

Figure 2. *The biochemical basis for protein solubilisation and precipitation by the pH-shift method. The figure is obtained from Gehring et al. (2011).*

The pH-shift process for the extraction of soluble proteins can be conducted in two ways: acid or alkali treatment. However, pelagic fish contain hemoglobin, which is probably the prime catalyst of lipid oxidation in fish. Acidic pH leads to a rapid oxidation of hemoglobin into monomers, dimers and free heme, and therefore, the acid treatment leads to oxidation reactions in the fish mass. (Park, 2005) Hemoglobin is more stable in alkaline pH, which is why the alkaline process is better for Baltic herring. The flow chart of the alkaline pH-shift process is presented in Figure 3.

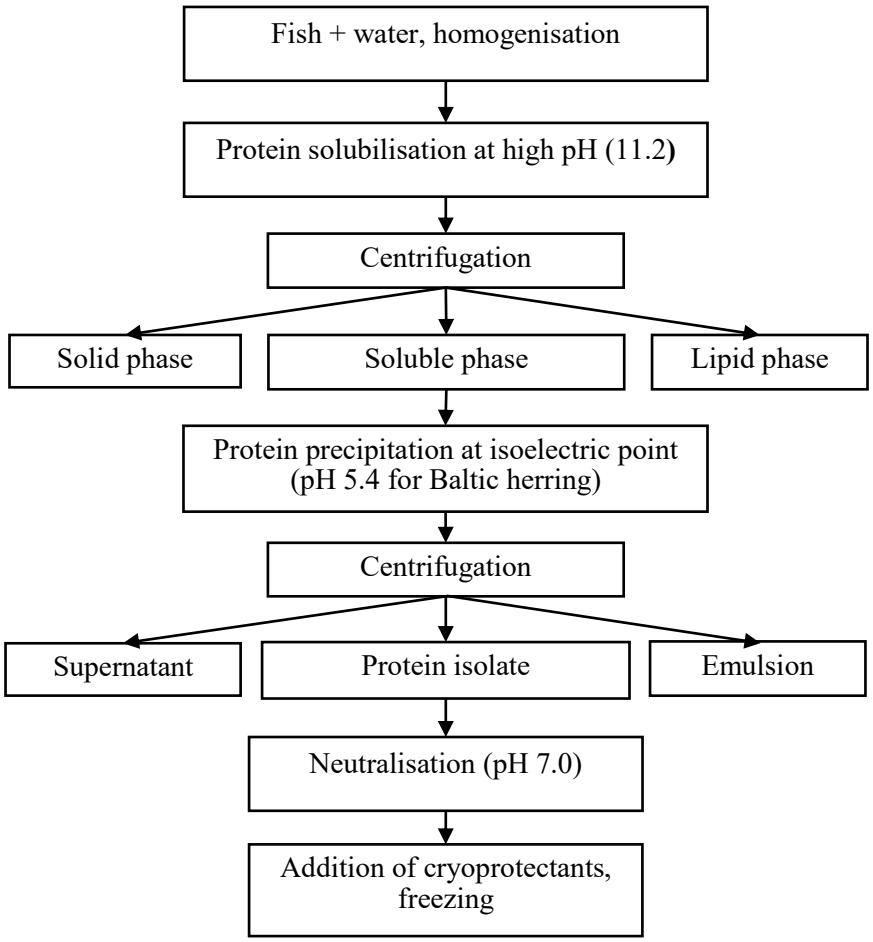

Figure 3. *A flowchart of the alkaline pH-shift process.*

The pH-shift process leads to protein concentrates with approx. 57–59% protein yield and a significant reduction in the ash and lipid contents (Marmon, 2012). The protein recovery and gel properties are affected by the different processing methods. For example, both acid and alkaline pH-shift processes give higher protein recoveries than the conventional surimi processing. Furthermore, a protein band sized 150 kD is visible after the pH-shift processes, which likely represents a partially hydrolysed myosin. The alkaline process has shown to disrupt the protein network more than the acid processing, forming a loosely structured network with no myofibrillar structure observed by confocal microscope. Myosin proteins myosin and actin are usually found in globular shapes, which seem to be disrupted during the pH-shift processing leading to a loose and homogenous network which consists of protein aggregates as well as thin and denser strands. However, the amino acid composition is not affected by the pH-shift process. (Marmon, 2012)

The pH-shift process allows an increased removal of lipids compared to the conventional surimi processing (Kristinsson et al., 2005). The removal of lipids also allows the reduction of fat-soluble environmental toxins. Both the acid and alkali processes have been shown to remove heavy metals, such as lipid-soluble methyl mercury and biphenyls, but the alkali process shows improved reductions compared to the acidic one (Park, 2005). A study reported that the amount of dioxin, DL-PCB and I-PCB were 70–80% lower in the protein isolates compared to the starting material (gutted herring). The levels of combined dioxin and DL-PCBs were above the EU limits in the gutted herring whereas the levels of the produced isolate was within the limits. (Marmon et al., 2009)

1.2.2. Fish protein isolate and its applications

Fish protein powders can be classified into two groups: concentrates and isolates. Fish protein concentrates contain approx. 65% of protein and 3% of lipids whereas the isolate should contain at least 80% of proteins and less than 0.75% of fat (Windsor, 1969). Here, the two types are not compared, and the term fish protein isolate (FPI) is used to describe both types. FPIs can be used for a wide range of products in addition to surimi. The isolate can be dried into fish powder, which can be easily incorporated into bread, biscuits and coating or other products, such as fish fingers. For example, the fortification of wheat bread with tilapia fish protein flour increased the protein content but did not affect the taste, aroma, crust or crumb colour or overall acceptability (Adeleke and Odedeji, 2010). However, most FPIs have a noticeable fish flavour and odour, which limits the use to products where such aromas are accepted. For example, fish protein powder decreased the sensory quality and consumer acceptance when it was incorporated into ice cream (Shaviklo et al., 2011).

The methods for drying the isolate into powder form include e.g. freeze drying, oven drying and spray drying. Different drying and processing methods as well as additives can affect the functional properties greatly, leading to completely different dehydrated products. For example, spray drying increases the water holding capacity significantly compared to freeze drying while the addition of 5% sucrose and 0.2% phosphate leads to an increase in the protein solubility in water (Shaviklo, 2015; Shaviklo et al., 2012).

1.2.3. Gelation

Fish proteins consists of myofibrillar, stroma and sarcoplasmic proteins. The myofibrillar proteins, mainly myosin and actin, are largely responsible for the gelation and water-binding properties of the muscle tissue. In fact, two thirds of the proteins in minced fish meat consist of myofibrillar proteins. Stroma, *aka* connective tissue proteins consist of primarily collagen and are almost totally insoluble in water and saline, and thus, they remain in the surimi paste together with the myofibrillar proteins during the surimi manufacturing process. Due to their low concentration in fish muscle, stroma proteins do not affect the gelling ability of surimi. Sarcoplasmic proteins include a variety of enzymes, such as transglutaminase (TG) and heat-stable proteinases as well as heme proteins, such as myoglobin and hemoglobin. The effect of sarcoplasmic proteins on the gelling properties depends on the composition of different proteins, which in turn varies within fish species. Heat stable proteinases worsen the gelling properties because they cleave and weaken the protein structure whereas TG promotes crosslinking, inducing gel formation. Sarcoplasmic proteins are water soluble and they are largely separated during the washing steps of the conventional surimi processing, which in turn concentrates the myofibrillar proteins with better gelling properties. In contrast, the pH-shift process recovers most of the sarcoplasmic proteins but there has been no indication of their interference in the gel formation. In fact, the pH-shift method produces gels with superior gelling properties compared to the conventionally washed surimi. The increased protein recovery also improves the overall protein yield of the pH-shift processing compared to the conventional method. (Park, 2005)

The protein isolates produced by the acid and alkaline processes show different gelling properties. For example, the alkaline processing produces gels with a higher gel strength than the acid process, both of which are better than the conventional surimi process. It is suggested that the pH-shift process induces protein–protein interactions and accessibility to endogenous microbial TG. (Pérez-Mateos et al., 2004) Freezing and addition of cryoprotectants have also a role in the gelling properties. The freeze/thaw process or the absence of cryoprotectants cause significant protein denaturation when the samples are kept at the pI. However, the addition of cryoprotectants, commonly sugars such as sorbitol and fructose, and adjusting the pH to 7.0 decrease the degree of protein denaturation. Furthermore, frozen isolates have shown higher strain and stress values compared to fresh isolates. (Thawornchinsombut and Park, 2006)

Small pelagic fish, such as sardine and mackerel, have shown poor gel characteristic because of the higher content of dark muscle, more specifically the higher amounts of lipids and myoglobin, and a higher ratio of sarcoplasmic to myofibrillar proteins. Another reason for the inferior gelation characteristics compared to white-fleshed fish is a lower pH due to acid content, which in turn causes a more rapid denaturation of proteins. (Park, 2005) The poorer gelation properties can be overcome by adding a substance with high water holding capacity such as egg albumen or a gelation agent such as TG. Egg albumen has been shown to increase connectivity of the surimi gel and contribute to a more compact network compared to a gel formed without albumen. Texture profile analysis (TPA) has shown increased hardness, cohesiveness, gumminess and chewiness with the addition of egg albumen in surimi made of sardine. Furthermore, scanning electron microscopy revealed a more compact network with more connectivity and smaller voids compared to surimi without added albumen (Benjakul, 2017). Additionally, TG induces the crosslinking reaction resulting in high molecular weight polymers, which ameliorates the gelling properties of surimi (Zaghbib and Arafa, 2016). The method and parameters for measuring gelling properties are described in the next section.

1.3. Quality analysis

The quality and sensory analyses are an essential part of the product development. Fish is easily oxidised, leading to rancid smell, unpleasant colour and harmful compounds. The volatile compounds, which create the aroma of food and can also reveal oxidation, can be studied with gas chromatography (GC) accompanied with a fibre absorbing the compounds (e.g. head-space solid-phase microextraction (HS-SPME)), which is a simple, cost-effective, solvent-free and easily automated method. Moreover, sensory analysis with either untrained or trained panellists is a valuable method to determine consumer acceptance.

The lipid content of food can be determined by solvent extraction methods, nonsolvent wet extraction methods or instrumental methods that rely on the physical and chemical properties of lipids, such as density or X-ray absorption. The choice of methods depends on the nature of the sample (dry versus moist), required precision and available instrument. (Carpenter, 2010) The fat content of fish is often determined by a method based on the Folch method, which is a common and simple method based on the solubility

of lipids on polar organic solvent (Folch et al., 1957). The sample is first homogenised with chloroform-methanol (2:1) mixture and filtered. Salt, most commonly KCl is added into the filtrate, after which the mixture is allowed to separate into two phases. The upper phase is removed (by siphoning, vacuum filtration etc.) and the remaining solution is washed with KCl-methanol (1:1) solution. The solvent is evaporated with a rotary evaporator and transferred to an autosampler bottle with the help of chloroform, which is finally allowed to evaporate to dryness under nitrogen flow. The lipid content can then be determined gravimetrically.

The protein content of food can be measured by a classical Kjeldahl method which is the official method for food protein determination by the AOAC International (Latimer, 2016). The method is based on the measurement of total nitrogen, which is converted to protein content using a conversion factor different for each food. The nitrogen in the sample is released into a strong acid, which is then neutralised and titrated. (Kjeldahl, 1883) The general conversion factor for food proteins is 6.25 which is based on the assumption that the general nitrogen content in food proteins is 16%. However, the measured nitrogen amount includes also free nitrogen and a wide range of other compounds, such as nitrate, ammonia, urea and alkaloids, leading to overestimation of the total protein. For example, a study on the protein content of different fish species in Brazil shows that the overall mean conversion factor for fish is 5.71. Therefore, the general conversion factor overestimates the protein content in fish by approx. 9%. (Diniz et al., 2013) The protein content can also be analysed by hydrolysing proteins to amino acids by hydrolysis and determining the amino acid residues chromatographically. However, the hydrolysis method (e.g. using 6 M HCl at 110 °C for 24 h) as described by Moore and Stein can lead to the destruction of amino acids, leading to underestimation of the protein content (Moore and Stein, 1963; Pickering and Newton, 1990).

The moisture content of foods can be easily determined gravimetrically. The sample is weighed before drying in an oven until the weight does not change between two consecutive measurements. The weight of the moisture lost during drying is divided by the initial weight of the sample to determine the moisture content. Protein, lipid and moisture content analyses form a basis for the fish composition analysis. The determination of carbohydrates is not necessary due to the very low amount of carbohydrates in fish in general (Sidwell et al., 1974). The rest of the weight is ash, which contains minerals, such as calcium, potassium, zinc, iron and magnesium (Lilly et al.,

2017). The composition of fish varies largely between different species and can vary within the species according to seasonal changes. For example, Baltic herring has the highest lipid content (11% of wet weight) in autumn and winter, and the lowest (4%) during spring and summer. The changes are due to periods of spawning and active feeding. (Aro et al., 2000)

Nutrients not only contribute to the aroma and health effects of the food, but also the texture. Protein, moisture and fat contents are all important factors in the hardness, rigidity and overall mouthfeel of foods. Texture analysis can be conducted with a sensory panel but also instrumentally with a texture profile analyser which measures the forces required to deform the sample, resembling the biting motion of food. Texture profile analyser can determine e.g. hardness, cohesiveness, gumminess, chewiness, springiness and adhesive force. The textural properties can be determined from a force-time curve (Figure 4). Hardness is the force required for a pre-determined deformation, for example 50% of the sample height, and it is measured as the peak force from the cycle. Fracturability is the force at the first significant break in the curve while cohesiveness estimates the strength of internal bonds in the sample and it is the ratio between the areas from the two cycles (A2 and A1). Adhesiveness is measured as the negative area from first compression cycle and it describes the work required to overcome the sticky forces between the probe and the sample. Gumminess, on the other hand, is the energy needed to break down a semisolid food until it is ready for swallowing, and it is measured by multiplying hardness by cohesiveness. Chewiness is a parameter used for solids foods, and it is calculated by multiplying hardness by cohesiveness and springiness. Springiness can also be called elasticity, and it describes the rate at which a deformed sample returns to its original size and shape. (Trinh, 2012)

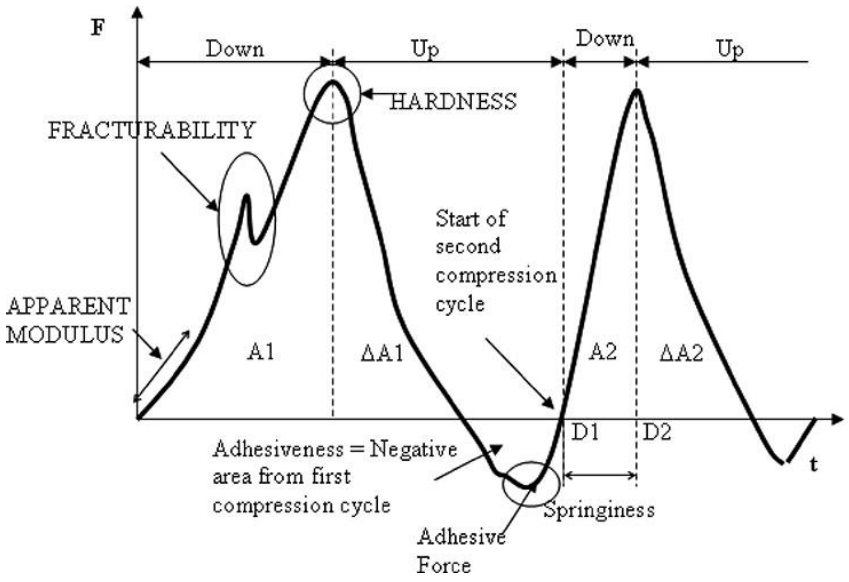

Figure 4. *Force-time curves from a two-bite test. The figure is adapted from Szczesniak (2002).*

Colour is an important sensory cue which affects the consumer's expectations on the taste, freshness and quality of food (Spence, 2015). Discolouration of food, such as fish, can be an indicator of oxidation reactions and worsened quality. Lipid oxidation correlates positively with myoglobin oxidation and is related to the darkening and discoloration of fish flesh (Chaijan and Panpipat, 2011). As previously mentioned, Baltic herring belongs to dark-muscled fish which are abundant in myoglobin and thus, prone to oxidation. The colour of foods can be determined with a colorimeter according to the CIE L*a*b* scale, where L* (lightness) describes the black to white axis, a* describes the red-green axis and b* describes the yellow-blue axis.

1.4. Aim of the study

The focus of the thesis was the production of protein isolate using Baltic herring followed by product development using the produced isolate. Different processing parameters were studied to achieve protein isolate with best colour and yield. The product development included the development of recipes and cooking methods for a surimi-like product and fish balls. Texture, chemical composition, volatile compounds and colour of the products were analysed and compared with literature and commercial samples.

2. Materials and methods

2.1. Materials

The raw material was gutted and beheaded Baltic herring from Martin Kala Oy (Turku, Finland). The fish was stored at -80 °C and thawed at 4 °C overnight before each experiment. Titanium dioxide (TiO_2) was purchased from Oy R. Österlund Ab (Helsinki, Finland) and the activated carbon was purchased from Tampereen Penli Oy (Tampere, Finland). The cheesecloth used for filtration during the pH-shift process was 100% cotton cheese cloth purchased from Decola (Jyväskylä, Finland). Hydrochloric acid and sodium hydroxide used in the pH-shift processing were purchased from Sigma-Aldrich Co. (Darmstadt, Germany). Sorbitol and fructose (Vinoferm® by Brouwland, Belgium) used as cryoprotectants were purchased from Lappo Oy (Piikkiö, Finland). Transglutaminase was received from BDF Natural Ingredients S.L. (Girona, Spain). The egg white powder used in the product development was received from Munax Oy (Laitila, Finland).

The commercial products (Table I) used as reference samples for texture profile analysis (TPA), colorimetry and head-space solid-phase microextraction (HS-SPME-GC-MS) were purchased from a local supermarket (Turku, Finland) in February 2019.

Table I. *Commercial products used as reference samples for product development.*

Product name	Product type	Producer/importer	Serial number
Pirkka Surimipuikko	Imitation crab stick	Made in Lithuania / Kesko Oyj, Finland	VO12 ASTI 864 2-3
Pirkka Surimisuikale	Imitation crab stick, sliced	Made in Lithuania / Kesko Oyj, Finland	U361 ASHRO84 2-2
Ravunmakuinen kalapuikko	Imitation crab stick	UAB„Plunges kooperatine prekyba'', Lithuania / Äyriäistukku Frisch Haus Finland Oy, Finland	U131 ASTI608
Surimi rapupuikko	Imitation crab stick	UAB„Plunges kooperatine prekyba'', Lithuania / Arvo Kokkonen Oy, Finland	T337 ASTI132 4-6
Surimi scampi	Imitation crab meat	Made in China / Äyriäistukku Frisch Haus Finland Oy, Finland	Produced 09.04.2018
Särkisen kalapyörykät	Fish ball	Apetit Ruoka Oy, Finland	Best before: 11.11.2019
Järvikalapihvi	Fish patty	Apetit Ruoka Oy, Finland	Best before: 08.11.2019
Pirkka Kalapuikko	Fish finger	Made in Estonia / Kesko Oyj, Finland	L271218

2.2. pH-shift processing

The basic steps of the pH-shift and gel formation processes were adopted from Grönroos (2018) and Marmon (2012). Five different methods presented on Table II were applied and compared to achieve the best yield and colour. The best method was then used for a bigger batch of fish to produce fish protein isolate (FPI) for product development.

Table II. *Different pH-shift methods to optimise yield and colour.* ***pH 1****: solubilisation pH and* ***pH 2****: precipitation pH.*

Sample	pH 1	pH 2	Water ratio	Filtration	Bleaching agent
1	11.2	5.4	1:9	cheese cloth	-
2	11.2	5.4	1:9	cheese cloth	titanium dioxide
3	11.2	5.4	1:9	cheese cloth	bentonite
4	11.2	6.5	1:9	cheese cloth	-
5	11.5	5.4	1:9	cheese cloth	-
6	11.2	5.4	1:9	cheese cloth	activated carbon

Defrosted Baltic herring was rinsed with water and then homogenised with water 1:9 (w/w, 1 part fish + 8 parts water) using Raw Mix 2 professional mixer (Rawmix Oy, Helsinki, Finland). The homogenate was poured in a reactor, which had a cooling set to 4 °C and mixing rate of 800 rpm/min. Bleaching agent (bentonite or activated carbon) was added to the homogenate of samples 3 and 6, respectively, before the stirring was started. Sample 2 is included on Table II, however, TiO_2 was added in the isolate before the gelation process and not during the pH-shift processing. 1 M NaOH was added with a precision pump Alaris Asena GH MK III (Becton, Dickinson and Company, Franklin Lakes, USA) at a constant speed of 30–40 mL/h until the alkaline target pH was reached. The homogenate was centrifuged at 4,000*g* for 20 minutes. Three layers were formed during the centrifugation: lipid layer, supernatant and precipitant. The supernatant was separated by decantering and filtering through 4-fold cheesecloth and a metal kitchen sieve and then poured back into the reactor. The lipid layer remained on the cheesecloth, and the precipitant at the bottom of the bottle. 1 M HCl was added at a constant speed until the pH value reached 5.4, after which the stirring was continued for further 5–10 minutes. The mixture was centrifuged again at 4,000*g* for 20 minutes, and three layers were formed. A waxy top layer was collected with a spoon, and the supernatant poured away. The bottom phase with precipitated proteins was collected with a spoon. Cryoprotectants (2 or 4 w-% fructose and sorbitol, respectively) were added to the protein

isolate according to the intended use. For the surimi, total amount of 8% of cryoprotectants was used whereas for the fish balls a lower amount of 4% was used. The pH of the isolate was adjusted to 7.0 with 4 M NaOH, and the isolate was frozen at -80°C.

2.3. Protein content

The protein contents of beheaded and gutted Baltic herring and FPIs with 4% and 8% of cryoprotectants, respectively, were analysed by VTT Technical Research Centre of Finland (Espoo, Finland) based on the classical Kjeldahl method which is the official method for food protein determination by the AOAC International (Latimer, 2016). Each sample was measured as duplicate and the protein contents were determined using the nitrogen conversion factor of 6.25.

2.4. Lipid content

The lipid content was analysed by using a modified Folch extraction method (Folch et al., 1957). Approx. 3 grams of sample was weighed precisely in triplicate and homogenised with 20 mL of methanol using IKA® T25 Digital Ultra-Turrax® (IKA®-Werke GmbH & Co., Staufen, Germany), followed by the addition of 40 mL of chloroform and further 2 minutes of mixing with Ultra-Turrax. The sample was filtered through Whatman® filter paper Grade 0858 (Whatman®, Maidstone, U.K.) using vacuum filtration and the filtrate was poured to an extraction funnel. The solids were scraped from the filter paper back into the decanter glass followed by the addition of 20 mL of methanol and 40 mL of chloroform. The sample was mixed with Ultra-Turrax for 3 minutes and filtered as before. 10 mL of methanol and 20 mL of chloroform were added to rinse the beaker and Ultra-Turrax blade, after which it was filtered as before. The filtrate was poured into the separatory funnel. The Büchner flask was rinsed with 10 mL of methanol and 20 mL of chloroform which were poured into the separatory funnel. 37.5 mL of 0.88% KCl was added to the bottle and shaken to mix the phases. The sample was allowed to set for approx. 15–30 minutes, after which the upper phase was removed by pipetting. 75 mL of 1:1 (v/v) methanol:KCl was added into the solution and mixed again. The separatory funnel was transferred to 4 °C until the phases separated well. The bottom phase was then released into a boiling flask and the solvent was evaporated in a rotary evaporator.

Meanwhile, autosampler bottles were weighed. The evaporated lipid sample was pipetted into an autosampler bottle with the help of 1+1+½+½+½ mL of chloroform, which was then evaporated from the bottle under nitrogen flow. The sample was weighed to determine the fat content of the sample. In the end, 2 mL of chloroform was added to protect the sample which was frozen at -80 °C for further analysis.

2.5. Moisture content

The moisture contents of homogenised Baltic herring, fish protein isolate (FPI) (2% sorbitol and 2% fructose) and fish balls with 0, 15 and 50% of FPI were determined as duplicate by drying the samples to a constant weight at 105 °C in an oven (Memmert Loading Modell 100-800, Germany) as described by AOAC (Association of Official Analytical Chemists, 2005). The drying time was extended to approx. 20 hours. The samples were cooled down in a desiccator before weighing to avoid moisture absorbing into the sample during cooling down.

2.6. Product development

The FPI was used in surimi and fish balls. Different ingredients and cooking times were tested to achieve a product resembling the imitation crab meat products on the market. FPIs from different processes (from Table II) were also studied in order to determine changes in texture and colour. Moreover, the effect of FPI on texture, chemical composition and volatile compounds of fish balls were studied.

The protein isolate was defrosted overnight before each test, and different batches were combined to obtain enough isolate to produce surimi or fish balls. The isolate with 8% of cryoprotectants was used for the surimi tests whereas the isolate with 4% of cryoprotectants was used for the fish balls.

2.6.1. Cooking conditions

To study the effects of cooking time on the texture of surimi, four different methods were studied (Table III, samples A1–4). FPI was combined with 6% potato starch, 3% egg white powder (EWP), 1.5% NaCl and 0.1% TiO_2 and mixed thoroughly with Kenwood

Chef XL titanium (type KVL80, Kenwood Limited, Havant, U.K.) for 4*x*10 s at full speed. Sample 1 was preheated for 60 min at 45 °C, and then cooked for 30 min at 90 °C. Samples 2, 3 and 4 were cooked for 30, 35 and 40 min at 90 °C, respectively. The samples were cooled down at 4 °C and their textures were analysed together with two commercial samples (imitation crab stick from Kesko Oyj, Kesko, Finland and surimi scampi from Äyriäistukku Frisch Haus Finland Oy, Helsinki, Finland).

2.6.2. Transglutaminase and titanium dioxide

The effects of transglutaminase (TG) and TiO_2 additions were also studied (samples A5–9 on Table III). Two different concentrations of TiO_2 were studied: 0.1 w-% and 0.5 w-%. Furthermore, the effect of 0.2% and 0.5% of TG was studied with varying amounts of other additives. First, only 0.5% of TG was added to the isolate together with 1.5% NaCl. Due to excess water leaking the amount was reduced to 0.2%, amount of which was tested with and without EWP. The ingredients were mixed as described in the previous chapter and cooked according to the conditions presented on Table III.

2.6.3. The effect of different pH-shift processes

Isolates from different pH-shift processes (Table II) were studied regarding the quality of the surimi product. The batches from normal processing conditions (process 1 from Table II) were combined and mixed with a spoon. 6% potato starch, 3% EWP and 1.5% NaCl was added to each sample and mixed for 3*x*10 seconds at full speed with Bamix® Mono (ESGE Ltd, Mettlen, Switzerland). Additionally, 0.5% of TiO_2 was added to sample B2. The mixtures were transferred to 50 mL Falcon tubes, which had been coated with canola oil to prevent sticking prior to the addition. The samples were cooked in a water bath for 40 min at 90 °C. After cooking, the samples were cooled down at 4 °C and analysed with texture analyser.

Table III. *Surimi product development plan including variable ingredients and cooking conditions. Experiments A1–A9 were conducted to determine the right cooking conditions and amounts of additives, such as transglutaminase and TiO_2. Experiments B1–B6 had constant cooking conditions but were prepared from protein isolates from different pH-shift processes to determine the effects of different processes on colour and texture.*

Sample	TG (%)	TiO_2 (%)	NaCl (%)	EWP (%)	Potato starch (%)	Cooking tempe-rature (°C)	Cooking time (min)
A							
1	-	0.1	1.5	3	6	45/90	60/30*
2	-	0.1	1.5	3	6	90	30
3	-	0.1	1.5	3	6	90	35
4	-	0.1	1.5	3	6	90	40
5	0.5	-	1.5	-	-	50/80	30/5*
6	-	0.1	1.5	3	6	90	30
7	-	0.5	1.5	3	6	90	30
8	0.2	0.5	1.5	3	6	50/80	30/5*
9	0.2	0.5	1.5	-	6	50/80	30/5*
B							
1	-	-	1.5	3	6	90	40
2	-	0.5	1.5	3	6	90	40
3	-	-	1.5	3	6	90	40
4	-	-	1.5	3	6	90	40
5	-	-	1.5	3	6	90	40
6	-	-	1.5	3	6	90	40

**Samples A1, A5, A8 and A9 were cooked at two different temperatures for different times. For example, A1 was cooked for 60 minutes at 45 °C, followed by 30 minutes at 90 °C.*

2.6.4. Fish balls

The FPI with 4% of cryoprotectants was tested as an ingredient for fish balls and combined with a flavour and oat mixture by Kalaneuvos Oy (Sastamala, Finland), canola oil (Bunge Finland Oy, Raisio, Finland) and minced Baltic herring fillets (Martin Kala Oy, Turku, Finland). The fish ball recipe, including the composition of the dry ingredient mixture are not presented here due to the ongoing product development at Kalaneuvos Oy. The reference fish ball included oat mixture, canola oil, minced Baltic herring and water. Different amounts of FPI were used: 5%, 15%, 30% and 50%. The amount of minced fish, oil and dry ingredients were kept at constant weight for samples including 0, 5, 15 and 30% isolate, while the amount of added water was reduced, but their amounts were reduced to compensate the amount of isolate in the sample containing 50% FPI.

Filleted Baltic herring was minced with Kenwood Chef XL Titanium food mincer (CHEF/kMix, Kenwood Limited, Havant U.K.). All ingredients were combined and

mixed with the Kenwood Chef XL Titanium food processor. The mixtures were refrigerated for 1–2 hours before cooking in an oven at 200 °C for 8–10 minutes – until the inside temperature was 73–77 °C. The fish balls were cooled down at room temperature before transferring to a refrigerator overnight. TPA was conducted the following day.

2.7. Texture Profile Analysis

TPA was conducted using a QTS25 texture analyser (CNS Farnell Company, Borehamwood, U.K.). The measurements were carried out at room temperature. A cylindrical probe with 3.8 cm diameter was used to penetrate the sample at the rate of 60 mm/min to a total deformation of 50% of the sample length. Oil was used as a lubricant on the probe and the sample plate to prevent sticking. Samples were cut to pieces of 1.5*x*1.5*x*1.5 cm except the commercial fish patty, which was cut to 1.5*x*1.5*x*1 cm pieces due to the product size. The compression cycle was repeated twice for each sample to resemble two bites. Each sample was measured as duplicate or triplicate, according to the availability of the sample. The measured attributes were hardness, adhesiveness, cohesiveness, springiness and chewiness.

2.8. HS-HPME-GC-MS

The volatile compounds of the Baltic herring surimi, fish balls and commercial products from Table I were analysed with HS-HPME-GC-MS. The products were cut to even-sized cubes (1*x*1*x*1 cm). The instruments were Trace 1310 Gas Chromatograph (Thermo Scientific™, Waltham, U.S.) with SPB®-624 Fused Silica Capillary Column 60 m x 0.25 mm x 1.4 µm (Merck KGaA, Darmstadt, Germany) combined with ISQ 7000 Single Quadrupole Mass Spectrometer (Thermo Scientific™, Waltham, U.S.) together with TriPLUS RSH autosampler (Thermo Scientific™, Waltham, U.S.). The volatile compounds were absorbed with a DVB/CAR/PDMS Stableflex fibre (50/30 um film thickness, Supelco Inc., Bellefonte U.S.).

The incubation and extraction times of the samples were 20 min and 30 min, respectively. The samples were agitated at 40 °C. The fiber was cleaned at 250 °C for 2 minutes prior the extraction and 5 minutes after the extraction. The fibre was desorbed for 5 min at 240

°C in the injection port of the GC, which was operated in a splitless mode. Helium was used as a carrier gas in the gas chromatography at a flow rate of 1.4 mL/min. The oven temperature was 40 °C for 6 min which was then increased at the rate of 5 °C/min to 200 °C and held at 200 °C for 10 min. The ionisation energy of the MS was 70 eV and the scan range was from 40 to 300 amu. The results were given as peak areas. Identification of the compounds was performed by matching their mass spectra with the database NIST MS Search 2.3.

2.9. Colour

The whiteness of commercial surimi products and the surimi samples made with the FPI were determined by an Eoptis CLM-194 (EOPTIS SRL, Trento, Italy) colorimeter. The colorimeter reports values L*, a* and b*, which describe the black to white, red to green and yellow to blue axes, respectively. These values can be used to determine whiteness (W) values by using the following formula (Park, 2005):

$$W = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

The colour measurements were conducted from B-samples (Table III) as well as four commercial products: surimi sticks from Kesko Oyj (Kesko, Finland), Arvo Kokkonen Oy (Vantaa, Finland) and Äyriäistukku Frisch Haus Finland Oy (Helsinki, Finland), and surimi scampi from Äyriäistukku Frisch Haus Finland Oy (Helsinki, Finland). Each sample was measured as triplicate.

2.10. Statistical methods

The statistical significance of TPA and colorimetry results were determined with two-way t-test (p<0.05) assuming heteroscedasticity if the number of replicates was not equal. Paired, two-way t-test was used if the number or replicates was equal. Principal component analysis was conducted with The Unscrambler® X (version 10.4.1).

3. Results and discussion

3.1. Protein content

The protein contents of beheaded and gutted Baltic herring and FPIs with 4% (2% fructose and 2% sorbitol) and 8% (4% fructose and 4% sorbitol) of cryoprotectants are presented on Table IV. The protein content of the raw material was 14.7%, which is more than the 12.4% determined for gutted herring (Marmon, 2012) but less than the content of 16.3% for unprocessed Baltic herring (Elintarviketurvallisuusvirasto Evira, 2014b). The protein content on a dry-weight basis was calculated based on the moisture content presented on Table IV, which resulted in 74.4%, a higher content than 56.4% determined for gutted herring (Marmon, 2012).

The protein contents of the FPIs including 4% and 8% of cryoprotectants were 9.2% and 6.6%, respectively. The corresponding protein contents calculated without the cryoprotectants were 9.6% and 7.2%. In comparison, the alkali-made isolate from herring had a protein content of 17.8%, scaled to a 78% moisture content (Marmon, 2012), which however is different to the moisture content of 85.3% presented here. To a better comparison, the protein contents were calculated on the dry-weight basis. The protein content of the Baltic herring FPI without cryoprotectants was 64.9% of the dry weight compared to 80.9% for the herring isolate, indicating a significant difference in the protein contents.

The mean FPI yield was 82.5% of the amount of fish used. Additionally, the protein yield was 53.6%, which is slightly less than the yield of 57% achieved during the alkaline pH-shift processing of gutted herring (Marmon, 2012). The acid pH-shift processing has been linked to a higher protein yield (Nolsøe and Undeland, 2009) but it also induces lipid oxidation used with fish with high hemoglobin and myoglobin contents (Park, 2005). The protein yield also depends on the solubilisation pH used in the processing (Kim et al., 2003) fish species (Batista et al., 2003) and storage conditions (Davenport et al., 2005). The ash content was not determined in this study, however, based on literature, the ash contents of gutted herring and alkali-made isolate are 2.0% and 0.2%, respectively (Marmon, 2012).

Table IV. *Protein contents of homogenised, gutted and beheaded Baltic herring and fish protein isolates with 4% and 8% of cryoprotectants (cp), respectively; lipid contents of Baltic herring, FPI with 4% of cp and fish balls with 0, 15 and 50% of FPI and moisture contents of Baltic herring, FPI with 4% of cp, fish balls with 0, 15 and 50% of FPI and surimi samples 1 and 2. Both surimi samples contained 6% potato starch, 3% egg white powder and 1.5% NaCl. Surimi sample 2 contained additional 0.5% TiO_2.*

Sample	Protein (%)	Lipid (%)	Moisture (%)
Beheaded & gutted Baltic herring	14.73	6.10 ± 0.12	80.21 ± 0.00
FPI (4% cp)	9.18	3.72 ± 0.01	85.27 ± 0.01
FPI (8% cp)	6.63	N/A	N/A
Fish ball with 0% FPI	N/A	14.47 ± 0.62	59.72 ± 0.00
Fish ball with 15% FPI	N/A	13.98 ± 1.24	58.85 ± 0.03
Fish ball with 50% FPI	N/A	13.11 ± 0.62	64.44 ± 0.00
Surimi (sample 1)	N/A	N/A	75.40 ± 0.00
Surimi (sample 2)	N/A	N/A	74.27 ± 0.00

3.2. Lipid content

The lipid contents measured from homogenised, gutted and beheaded Baltic herring, FPI with 4% cryoprotectants (2% fructose and 2% sorbitol) and fish balls with 0, 15 and 50% of FPI are presented on Table IV. The lipid content of Baltic herring was 6.1% which is the same amount as reported by the Finnish Food Safety Authority (Elintarviketurvallisuusvirasto Evira, 2014b). The fish used in this study was caught in March 2019. A previous study showed that the lipid content of Baltic herring fillets is 6.3% during spring (Mar, Apr, May), 5.2% during summer (Jun, Jul, Aug), 10.4% during autumn (Sep, Oct, Nov) and 9.4% during winter (Dec, Jan, Feb) (Aro et al., 2000). Thus, the lipid content presented here is similar but seasonal variations, sample size, fish size (and age) and other factors can affect the lipid content. Furthermore, whole Baltic herring was used in this study instead of fillets. Based on the moisture content presented on Table IV, the lipid content of the raw material on a dry-weight basis is 30.8%.

The lipid content of the FPI was 3.7% which is similar result to an alkali-made isolate of herring containing 3.9% of lipids (Marmon, 2012). However, the lipid content presented in the study is expressed on 78% water content to be comparable to the raw material and therefore, the content would be smaller based on the moisture content of 85.3% analysed for the Baltic herring FPI. To compare the lipid reduction, the contents of the raw material

and FPI were calculated on dry basis. The lipid content of the FPI was 25.3% of the dry-weight, thus, the lipid reduction during the pH-shift process was 18%. However, based on wet weight, the reduction was 61%, which however is remarkably lower than the reductions achieved with acid and alkaline pH-shift processes on catfish muscle, 85% and 89%, respectively (Kristinsson et al., 2005). The fat content of the produced FPI is too high based on the recommended lipid contents of fish protein concentrates. The class A defined by FAO should contain less than 0.75% of fat whereas the class B should contain less than 3% (Windsor, 1969). During the pH-shift processing, low or high pH separates solubilized proteins from storage lipids and phospholipids, and the centrifugation step separates the compounds based on differences in density and solubility. One way to increase the removal of lipids is by increasing the centrifugal forces used for separation. Centrifugation at high centrifugal force (10,000*g*) has shown a significant increase in the removal of phospholipids compared to 4,000*g* (Liang and Kristinsson, 2006). However, high centrifugal forces may not be applicable in the food industry, where decanters and tricanters are often used for the separation (GN Separation, 2019).

High lipid content leads to oxidation, causing rancid smells, colour defects and harmful oxidation compounds. In comparison to the results presented here, the lipid content of freeze-dried saithe (*Pollachius virens*) FPI from the pH-shift process was 0.5–2%, which already showed increased oxidation compared to fish mince powder. The study showed that the oxidation started during the pH-shift process and was increased by freeze-drying. (Shaviklo et al., 2012) Therefore, methods for further removal of the lipids during the pH-shift process are needed, especially if the intended product is dried FPI. It has been shown that the addition of 5% ethanol during the pH-shift process leads to a lower lipid and contaminant contents in the protein isolate (Marmon, 2012). Furthermore, the addition of antioxidants such as propyl gallate or butylated hydroxyanisole during the pH-shift process can be used to reduce lipid oxidation (Raghavan and Hultin, 2009).

The lipid contents of the fish balls were significantly higher than that of Baltic herring or FPI due to the added oil. The fish ball with 0% FPI had a lipid content of 14.5%, whereas the fish balls with 15% and 50% FPI contained 14.0% and 13.1% of lipids, respectively. The addition of FPI reduces the lipid content even though the amount of added oil remained constant due to a decreased amount of dry-ingredients and minced Baltic herring, both of which contained more fat than the FPI. The variation was greater for the fish balls compared to other samples which can be explained by the non-homogenous

composition consisting of larger pieces of minced fish and oats. Furthermore, the fat might not be distributed evenly in the fish ball, leading to slightly variable contents in the samples. In comparison, the commercial fish ball used in this study contained 12 g/100 g of fat, of which 2.6 g/100 g were saturated, mainly from the cream used in the recipe. The commercial fish patty also contained 12 g/100 g of fat, of which 5.1 g/100 g was saturated fat due the cream and butter used in the recipe. Both commercial products contained added canola oil as well. (Apetit Group, 2019a, 2019b)

3.3. Moisture content

The moisture contents of the beheaded and gutted Baltic herring, FPI, fish balls with 0, 15 and 50% FPI and surimi samples B1 and B2 are also presented on Table IV. The moisture content of Baltic herring was 80.2% which is higher than the value of 60–75% reported in the literature. However, the moisture content varies between seasons, from 63–39% in autumn to 69–75% in spring (Timberg et al., 2011). The fish used in this study was caught in March, which explains the high moisture content. Furthermore, the moisture content of gutted herring has also been reported higher, at 78% (Marmon, 2012), which is similar to the value reported here.

The moisture content of the FPI with 4% of cryoprotectants (2% fructose and 2 % sorbitol) was 85.3% which is close to the value of 85.5% reported in a study using alkaline processing on the Common Carp (Tian et al., 2017). Moreover, the water contents of the surimi samples B1 and B2 were 75.4% and 74.3%, respectively. In comparison, the moisture content of conventional surimi varies between 72 and 77% (Park, 2005). The lower water content compared to the FPI was due to the dry ingredients potato starch and EWP added in the mixture. The fish ball with 0% FPI had a water content of 59.7%, which increased to 64.4% with the addition of 50% FPI. The moisture content of fish balls with 0% and 15% FPI did not differ much from each other.

3.3.1. TPA – cooking conditions

TPA results from surimi cooked at different conditions and two commercial samples (imitation crab stick from Kesko Oyj, Kesko, Finland and surimi scampi from Äyriäistukku Frisch Haus Finland Oy, Helsinki, Finland) are presented on Table V. Additionally, the TPA results on hardness and chewiness based on the different cooking conditions are presented as a graph in Figure 5. The results presented in this chapter determined the cooking conditions used for further surimi tests.

The commercial surimi stick was significantly softer than other samples, which was probably due to the processing method of commercial surimi. Most imitation crab sticks are moulded and cooked as sheets, which are then rolled to round shapes, and cut to a certain length. Therefore, the final product is porous and flexible. The commercial surimi scampi, on the other hand, had a solid form, which also lead to a much higher hardness value. Sample A1, which was precooked at 45 °C for an hour, had a higher hardness value than sample A2, though both were cooked for 30 minutes at 90 °C, however, the difference was not statistically significant ($p>0.05$). Samples A2 and A3, which were cooked for 35 and 40 minutes, respectively, were significantly ($p<0.05$) harder than A1.

The chewiness values increased by the increasing cooking time. Further, the precooked sample A1 also showed an increased, but not statistically significant ($p>0.05$) increase compared to A2. Only the commercial imitation crab stick had a significantly smaller chewiness value compared to other samples. Chewiness is calculated based on cohesiveness, hardness and springiness, and therefore changes in these aspects contribute to the chewiness value. Montejano et al. (1985) reported a positive correlation ($r = 0.74$) between hardness and sensory texture profile evaluation for food gels, including surimi gels. Therefore, the longest cooking time of 40 min, which resulted in the highest hardness and chewiness values, was applied to the following surimi tests. An earlier study has shown that the cooking time of 30 min is suitable for surimi gels, however, stainless steel tubes were used in the study which may have led to a quicker gel formation due to better heat transfer compared to the plastic tubes used in this study (Kelleher and Hultin, 2001).

Table V. *Texture profile analysis results of surimi samples with different cooking times and two commercial surimi products: (imitation crab stick from Kesko Oyj, Kesko, Finland and surimi scampi from Äyriäistukku Frisch Haus Finland Oy, Helsinki, Finland). The cooking conditions were;* ***A1****: 60 min at 45 °C followed by 30 min at 90 °C,* ***A2****: 30 min at 90 °C,* ***A3****: 35 min at 90 °C and* ***A4****: 40 min at 90 °C.*

Samples	Adhesiveness (gs)	Chewiness (gmm)	Cohesiveness
Commercial crab stick	-17.2 ± 0.20	7530 ± 370	0.786 ± 0.011
Commercial surimi scampi	-21.6 ± 3.50	13700 ± 2550	0.779 ± 0.054
A1	-41.8 ± 13.3	11800 ± 1690	0.717 ± 0.048
A2	-23.7 ± 12.5	11300 ± 823	0.763 ± 0.018
A3	-50.4 ± 16.4	13600 ± 1170	0.760 ± 0.018
A4	-38.3 ± 11.6	14400 ± 2660	0.741 ± 0.003
	Hardness (g)	**Springiness (mm)**	
Commercial crab stick	1620 ± 94.0	5.90 ± 0.028	
Commercial surimi scampi	3110 ± 422	5.66 ± 0.032	
A1	2520 ± 316	6.52 ± 0.193	
A2	2270 ± 203	6.53 ± 0.084	
A3	2720 ± 151	6.56 ± 0.241	
A4	2990 ± 470	6.50 ± 0.144	

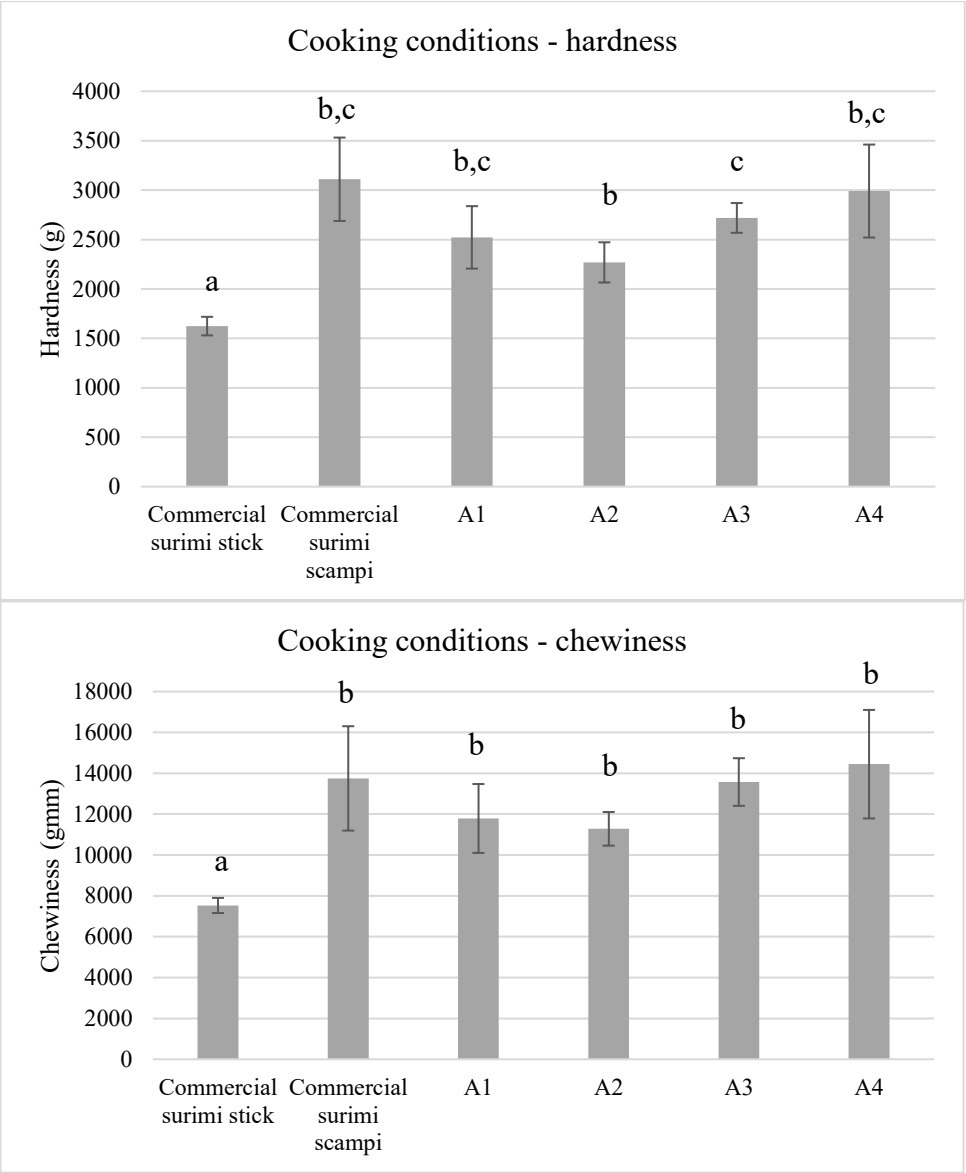

Figure 5*. Hardness and chewiness results from texture profile analysis of two commercial samples (imitation crab stick from Kesko Oyj, Kesko, Finland and surimi scampi from Äyriäistukku Frisch Haus Finland Oy, Helsinki, Finland) and surimi samples with different cooking times.* ***A1****: 60 min at 45 °C followed by 30 min at 90 °C;* ***A2****: 30 min at 90 °C;* ***A3****: 35 min at 90 °C and* ***A4****: 40 min at 90 °C. Statistically significant differences (p<0.05) are marked with different letters.*

3.3.2. TPA – transglutaminase and titanium dioxide

Table VI and Figure 6 present the TPA results from the TiO_2 and TG experiments. TG catalyses the formation of an isopeptide bond between glutamine and lysine, inducing cross-linking between separate molecules or within the same molecule (Stangierski and Baranowska, 2015). Egg white is commonly used in surimi to modify texture, inhibit gel-softening and to increase whiteness. The major protein components in egg white are

ovalbumin (54%), conalbumin (12%) and ovomucoid (11%). (Park, 2005). TG is able to use ovalbumin as a substrate after heat-treatment, inducing crosslinking and thus, gel strength (Giosafatto et al., 2012). However, it has not been evaluated whether the addition of EWP improves surimi gels treated with TG.

The samples containing TG were A5, A8 and A9. Samples A8 and A9 had less TG than sample A5 (0.2% vs. 0.5%) but included potato starch, which sample A5 did not. Additionally, sample A8 contained EWP in order to determine whether the egg proteins affect the TG function. Sample A9 had higher hardness and chewiness values than sample A8, but the differences were not statistically significant (p>0.05). However, sample A5 had significantly higher hardness and chewiness values compared to both A8 and A9. The results are likely to be caused by the bigger amount of enzyme in sample A5 leading to more crosslinking and a tighter protein network. Additionally, the samples were prepared in different days and from different batches of protein isolate, which might also affect the results.

The surimi gels with TG had visibly significant water leakage during the cooking, and therefore, TG was not used in the following FPI gels (B-samples, Table III). Water leakage was noticed with both 0.2% and 0.5% of TG, and despite the addition of EWP. TG increases water-holding capacity and forms a stable gel if an appropriate amount is used. Too high enzyme concentration leads to excess inter- and intrachain peptide cross-links and rather leads to the decrease in the water-holding capacity, as water is pushed out of the gel network (Gaspar and de Góes-Favoni, 2015). The water-leaking may therefore have been caused by too high amount of TG, and further study ought to be made to optimise the TG with and without additives, such as starch and EWP.

Sample A7, which had more TiO_2 than sample A6 (0.5% vs 0.1%), showed slightly increased hardness and chewiness values, although the difference was not statistically significant (p>0.05). A study on the effect of TiO_2 on carp protein gel has shown that the addition does not affect textural parameters (Taskaya et al., 2010). Since the addition of 0.5% showed visible whitening of the FPI gel, it was used in the following FPI gels (B-samples, Table III).

Table VI. *Texture profile analysis results of surimi made of Baltic herring protein isolate with varying amounts of transglutaminase and TiO_2.* ***A5****: 1.5% NaCl and 0.5% transglutaminase, cooked for 30 min at 50 °C and for 5 min at 80 °C;* ***A6****: 6% potato starch, 3% egg white powder, 1.5% NaCl and 0.1% TiO_2, cooked for 30 min at 90 °C;* ***A7****: 6% potato starch, 3% egg white powder, 1.5% NaCl and 0.5% TiO_2, cooked for 30 min at 90 °C;* ***A8****: 6% potato starch, 3% egg white powder, 1.5% NaCl and 0.2% TiO_2, cooked for 30 min at 50 °C and for 5 min at 80 °C and* ***A9****: 6% potato starch, 1.5% NaCl and 0.2% TiO_2, cooked for 30 min at 50 °C and for 5 min at 80 °C.*

Samples	**Adhesiveness (gs)**	**Chewiness (gmm)**	**Cohesiveness**
A5	-3.89 ± 3.30	5010 ± 268	0.640 ± 0.0294
A6	-10.7 ± 6.48	5230 ± 777	0.730 ± 0.0163
A7	-16.3 ± 4.69	6120 ± 729	0.717 ± 0.0125
A8	-12.6 ± 8.77	1920 ± 513	0.543 ± 0.0655
A9	-5.44 ± 2.12	2640 ± 262	0.610 ± 0.0616
	Hardness (g)	**Springiness (mm)**	
A5	1090 ± 14.4	7.23 ± 0.399	
A6	1110 ± 176	6.43 ± 0.088	
A7	1270 ± 209	6.77 ± 0.526	
A8	505 ± 49.1	6.80 ± 0.511	
A9	611 ± 56.5	7.16 ± 0.507	

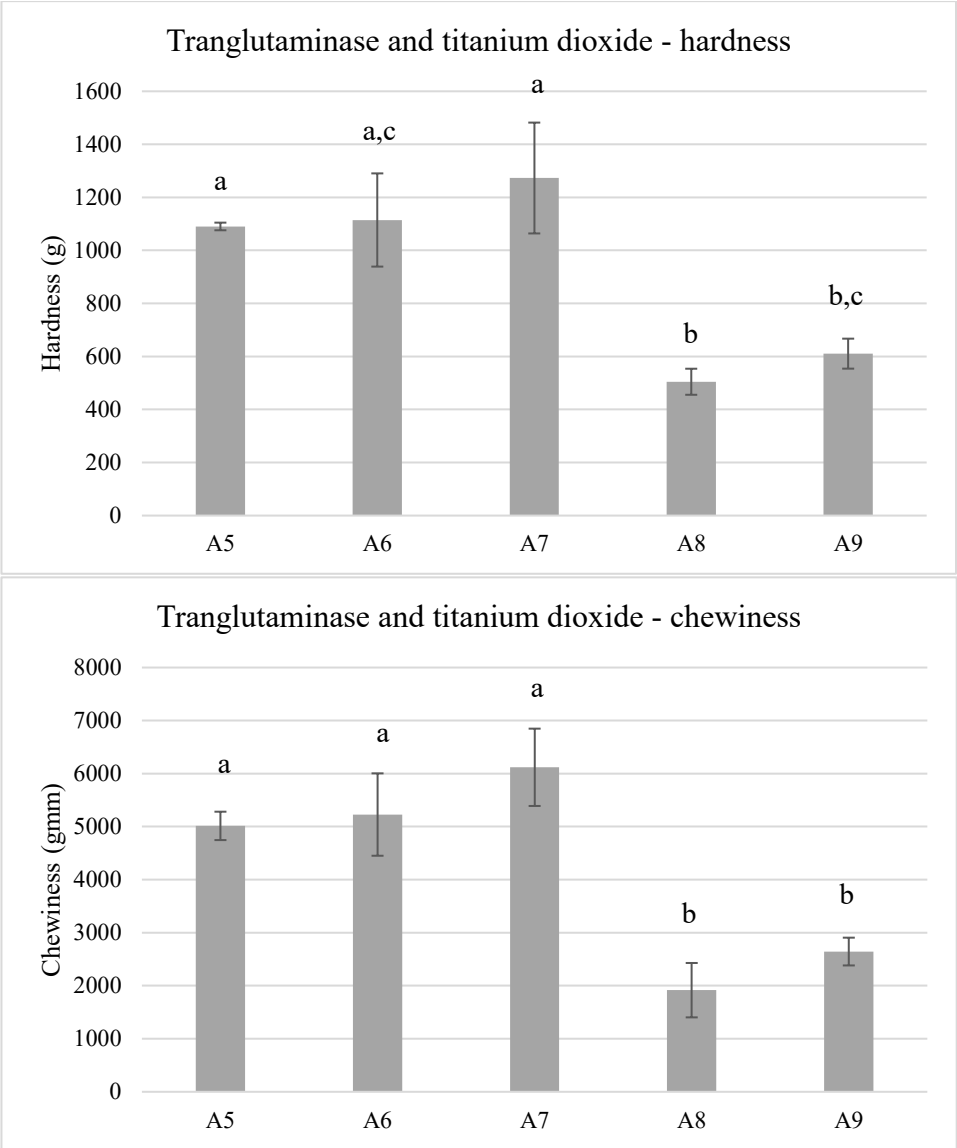

***Figure 6**. Hardness and chewiness results from texture profile analysis of surimi samples with different amounts of TiO_2 and transglutaminase. **A5**: 1.5% NaCl and 0.5% transglutaminase, cooked for 30 min at 50 °C and for 5 min at 80 °C; **A6**: 6% potato starch, 3% egg white powder, 1.5% NaCl and 0.1% TiO_2, cooked for 30 min at 90 °C; **A7**: 6% potato starch, 3% egg white powder, 1.5% NaCl and 0.5% TiO_2, cooked for 30 min at 90 °C $_2$; **A8**: 6% potato starch, 3% egg white powder, 1.5% NaCl and 0.2% TiO_2, cooked for 30 min at 50 °C and for 5 min at 80 °C and **A9**: 6% potato starch, 1.5% NaCl and 0.2% TiO_2, cooked for 30 min at 50 °C and for 5 min at 80 °C. Statistically significant differences (p<0.05) are marked with different letters.*

3.3.3. TPA – different pH-shift processes

TPA results of samples B1–5 and commercial surimi scampi (Äyriäistukku Frisch Haus Finland Oy, Helsinki, Finland) are shown on Table VII while graphs on hardness, cohesiveness and chewiness are shown in Figure 7. All samples contain 6% potato starch, 3% EWP and 1.5% NaCl, and they were cooked for 40 min at 90 °C. The differences

come from additives (0.5% of TiO_2 in B2) or differences in the pH-shift processing (B3–6).

According to the results, samples B1 and B2 both resemble the commercial surimi scampi in hardness, chewiness and cohesiveness. The mean hardness and chewiness values are slightly smaller, yet the differences are not statistically significant ($p>0.05$). B1 and B2 contained FPI produced by the alkaline pH-shift processing while B2 contained additional 0.5% of TiO_2. B2 had significantly increased ($p<0.05$) cohesiveness and chewiness values compared to B1, but the hardness values did not differ significantly. As mentioned in the previous chapter, the addition of TiO_2 should not affect the texture of surimi (Taskaya et al., 2010). The results reported here show increased cohesiveness and chewiness with the addition of TiO_2, resulting from increased internal bonds in the surimi resisting breakage. Bigger sample pool and more measurements ought to be done to confirm the result since the difference is more likely to come from slight differences in the compositions, cooking and storing conditions, or from measurement errors.

Sample B3 was made of FPI from a pH-shift process including bentonite as a possible whitening agent. Bentonite is used in animal feed as a binder, anti-caking agent and coagulant and in the oil industry as a decolourizing agent. The use of bentonite as a food additive has been authorised under Directive (EC) 95/2, with a specification established by Directive (EC) 2008/84. In their study, Cerbulis (1978) used bentonite to precipitate proteins from cheese whey. A concentration of 3% at pH 4.6 showed an approx. 100% protein recovery and also removed most riboflavin from whey, resulting in a colourless supernatant. However, it has not been assessed how bentonite reacts during the pH-shift processing, and whether it precipitates some of the target proteins. The addition of bentonite (sample B3) did not lead to differences ($p>0.05$) in hardness, chewiness or cohesiveness values compared to B1, meaning that the addition of bentonite during the pH-shift processing did not affect the FPI gelling properties.

Sample B6, which contained activated carbon as a whitening agent during the pH-shift process, did not differ from B1 or the commercial surimi scampi in hardness, chewiness or cohesiveness, which means that the addition of activated carbon did not affect the FPI in terms of texture. Activated carbon is used in the food industry as a deodorisation, decolourisation and detoxification agent. For example, it has been shown to reduce some

environmental toxins from cod liver oil (Maes et al., 2005). The results on the effect of activated carbon on the colour of the FPI are presented in the Colour-chapter.

Sample B4 was made of FPI with a different precipitation pH (6.5 instead of 5.4). The sample was significantly (p<0.05) softer, less chewy and had a decreased cohesiveness value compared to the commercial surimi scampi and sample B1. The FPI from the abovementioned pH-shift process contained significantly more water than the FPI from the normal pH-shift process since the weight of the isolate was approx. 3 times bigger compared to the normal process. Since the same amount of FPI was added to each sample, the added moisture content has likely led to a higher total water content and a decreased protein content. Since all surimi samples were cooked under the same conditions, the resulting surimi likely had a higher moisture content as well and thus, a softer texture. The results were different than presented by Marmon (2012), which showed superior properties for the protein isolate when the precipitation pH 6.5 was used. The study showed a more homogenous microstructure of proteins, less lipid oxidation and improved gelation properties, such as gel strength. Furthermore, the water content was only 0.4 percentage points higher than in the isolate from the process using a precipitation pH of 5.4. However, a much higher centrifugal forces, 8,000g were used in their study, which might have led to a better separation and removal of the excess water that remained in the isolate in this study.

Sample B5 was made of FPI from a pH-shift process where the solubilisation pH was increased from 11.2 to 11.5. The pH-value 11.2 used in the normal process was obtained from a previous study, which assessed the value based on the alkali consumption and solubility (Marmon, 2012). However, shifting the pH from 11.5 to 12.5 has shown the maximal protein yield for herring and also improved heme pigment removal, breaking force and whiteness, but at the same time requiring two-fold volume of alkali (Abdollahi and Undeland, 2019). The results show that B5 was softer than sample B1, yet the difference was not statistically significant (p>0.05). However, the cohesiveness and chewiness values of B5 were significantly lower. The lowered cohesiveness value means that the sample B5 did not retain its form between the 1st and 2nd compression cycle as well as B1, indicating worsened gelling properties.

In conclusion, the alkaline pH-shift process with a solubilisation pH-value at 11.2 and precipitation pH at 5.4 produced FPI with good gelling properties. The FPI can be used

to make surimi similar in texture to the commercial surimi scampi when cooked for 40 min at 90 °C. The processes with bentonite and activated carbon did not have significant effects on the texture but the processes with different solubilisation and precipitation pH-values decreased the surimi quality by causing softening and/or weakening of the gel.

Table VII. *Texture profile analysis results of commercial surimi scampi and surimi samples made with Baltic herring protein isolate obtained from different pH-shift processes.* ***Sample B1****: alkaline pH-shift process,* ***B2****: alkaline pH-shift process with 0.5% added TiO_2 before cooking,* ***B3****: isolate with bentonite used during the pH-shift process, B****4****: isolate with a higher precipitation pH-value (6.5 instead of 5.4),* ***B5****: isolate with a higher solubilisation pH (11.5 instead of 11.2) and* ***B6****: isolate with activated carbon used during the pH-shift process.*

Samples	Adhesiveness (gs)	Chewiness (gmm)	Cohesiveness
Commercial surimi scampi	-8.49 ± 10.8	4310 ± 516	0.71 ± 0.02
B1	-24.8 ± 3.67	3580 ± 126	0.67 ± 0.01
B2	-28.9 ± 1.02	4130 ± 214	0.73 ± 0.01
B3	-24.2 ± 0.73	3730 ± 389	0.68 ± 0.00
B4	-18.3 ± 5.04	938 ± 186	0.54 ± 0.08
B5	-24.4 ± 9.28	1440 ± 214	0.42 ± 0.04
B6	-45.2 ± 3.04	2790 ± 569	0.54 ± 0.09
	Hardness (g)	**Springiness (mm)**	
Commercial surimi scampi	1470 ± 284	4.18 ± 0.27	
B1	1360 ± 42.5	3.95 ± 0.06	
B2	1260 ± 118	4.50 ± 0.19	
B3	1280 ± 145	4.28 ± 0.04	
B4	428 ± 19.3	4.03 ± 0.08	
B5	1070 ± 4.50	3.23 ± 0.23	
B6	1440 ± 10.0	3.56 ± 0.11	

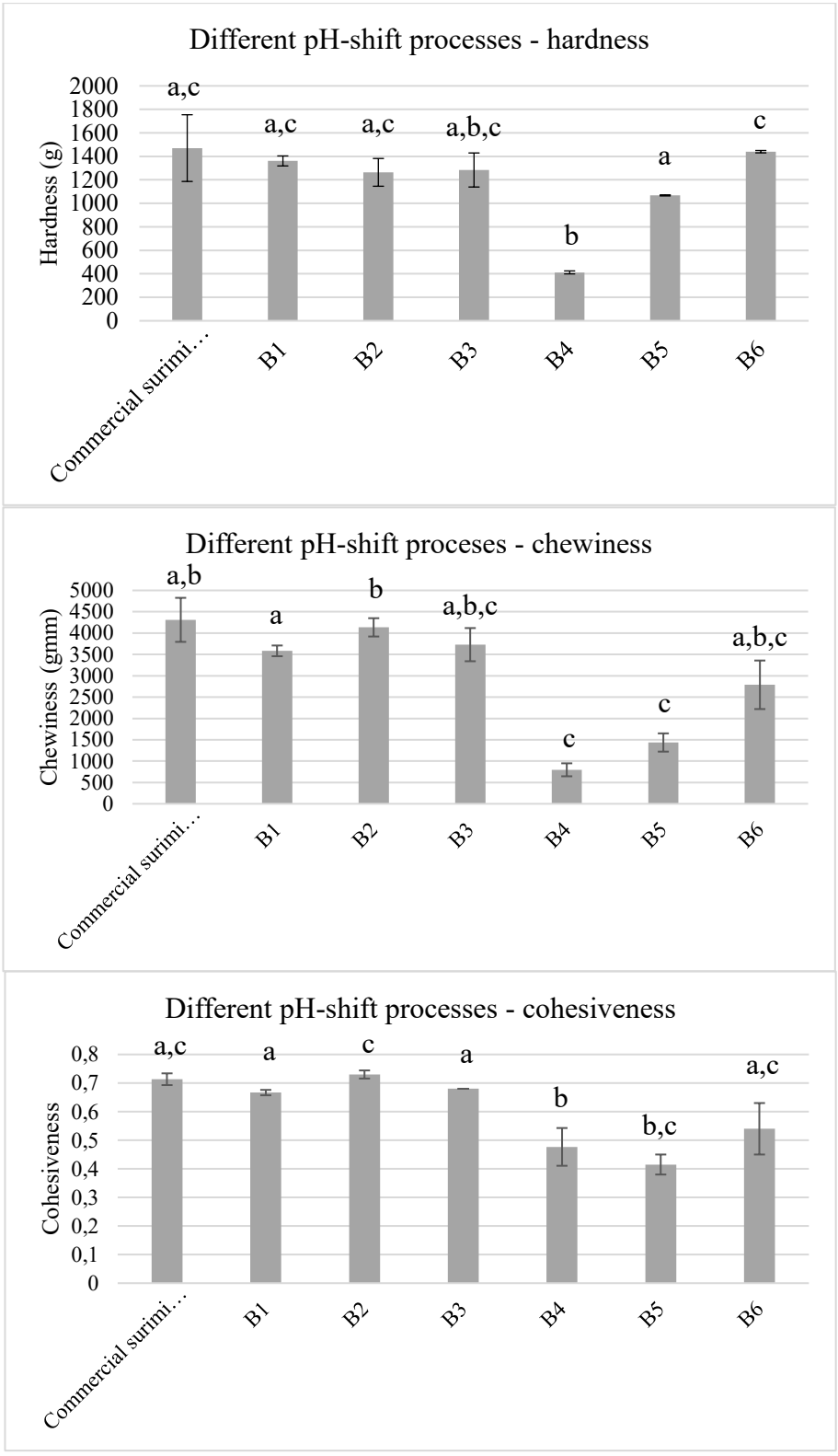

Figure 7. *Hardness, chewiness and cohesiveness results from texture profile analysis of surimi samples made with Baltic herring protein isolates from different pH-shift processes.* ***B1****: alkaline pH-shift process,* ***B2****: alkaline pH-shift process with 0.5% added TiO_2 before cooking,* ***B3****: isolate with bentonite used during the pH-shift process, B****4****: isolate with a higher precipitation pH-value (6.5 instead of 5.4),* ***B5****: isolate with a higher solubilisation pH (11.5 instead of 11.2) and* ***B6****: isolate with activated carbon used during the pH-shift process. Statistically significant differences (*$p<0.05$*) are marked with different letters.*

3.3.4. TPA – fish balls

Four different concentrations of Baltic herring protein isolate were added in the fish ball mixture: 5, 15, 30 and 50%. Fish ball without FPI was used as a reference sample. The textures of the cooked fish balls were analysed with texture profile analyser using a commercial fish ball and patty (Apetit Ruoka Oy, Säkylä, Finland) as additional reference samples. The results are shown on Table VIII and graphs on hardness, cohesiveness and chewiness are presented in Figure 8.

All lab-made samples were remarkably softer than the two commercial samples. Furthermore, the lab-made samples were significantly less chewy than the commercial fish ball, except the sample with 30% of FPI. However, a panel of five people considered the texture pleasant and resembling home-made fish balls. A larger sensory panel could be organised for the fish balls to determine which added amount gives the best mouthfeel. Additionally, all samples kept their shape throughout cooking and cooling down. The addition of FPI increased the hardness and therefore, the sample with 50% FPI was significantly harder than the reference sample with 0% FPI. Controversially, the hardness of the 5% FPI sample was higher than the ones with 15 and 30% FPI. The difference might be due the slight differences in incubation or cooking times, which could cause hardening of the surface. The cohesiveness, which estimates the strength of internal bonds in the sample, remains similar throughout the samples with no clear trend with the addition of FPI.

Table VIII. *Texture profile analysis results of fish balls with 0, 5, 15, 30 or 50% of Baltic herring protein isolate, and two commercial reference products; fish ball and fish patty from Apetit Ruoka Oy (Säkylä, Finland).*

Samples	Adhesiveness (gs)	Chewiness (gmm)	Cohesiveness
0%	-1.21 ± 1.51	348.18 ± 56.38	0.41 ± 0.04
5%	-1.40 ± 1.01	379.38 ± 30.19	0.34 ± 0.04
15%	-3.96 ± 1.83	273.30 ± 87.23	0.35 ± 0.01
30%	-2.67 ± 1.77	531.63 ± 25.24	0.41 ± 0.02
50%	-0.59 ± 0.56	392.16 ± 33.32	0.41 ± 0.04
Commercial fish ball	-4.42 ± 4.17	915.09 ± 187.94	0.42 ± 0.03
Commercial fish patty	-0.67 ± 0.32	594.26 ± 108.83	0.44 ± 0.00
	Hardness (g)	**Springiness (mm)**	
0%	282.33 ± 25.94	3.02 ± 0.38	
5%	354.33 ± 54.31	3.13 ± 0.16	
15%	314.67 ± 62.23	2.41 ± 0.36	
30%	323.00 ± 23.72	2.50 ± 0.20	
50%	420.00 ± 15.51	2.30 ± 0.04	
Commercial fish ball	758.67 ± 110.86	2.89 ± 0.29	
Commercial fish patty	870.00 ± 107.32	1.54 ± 0.11	

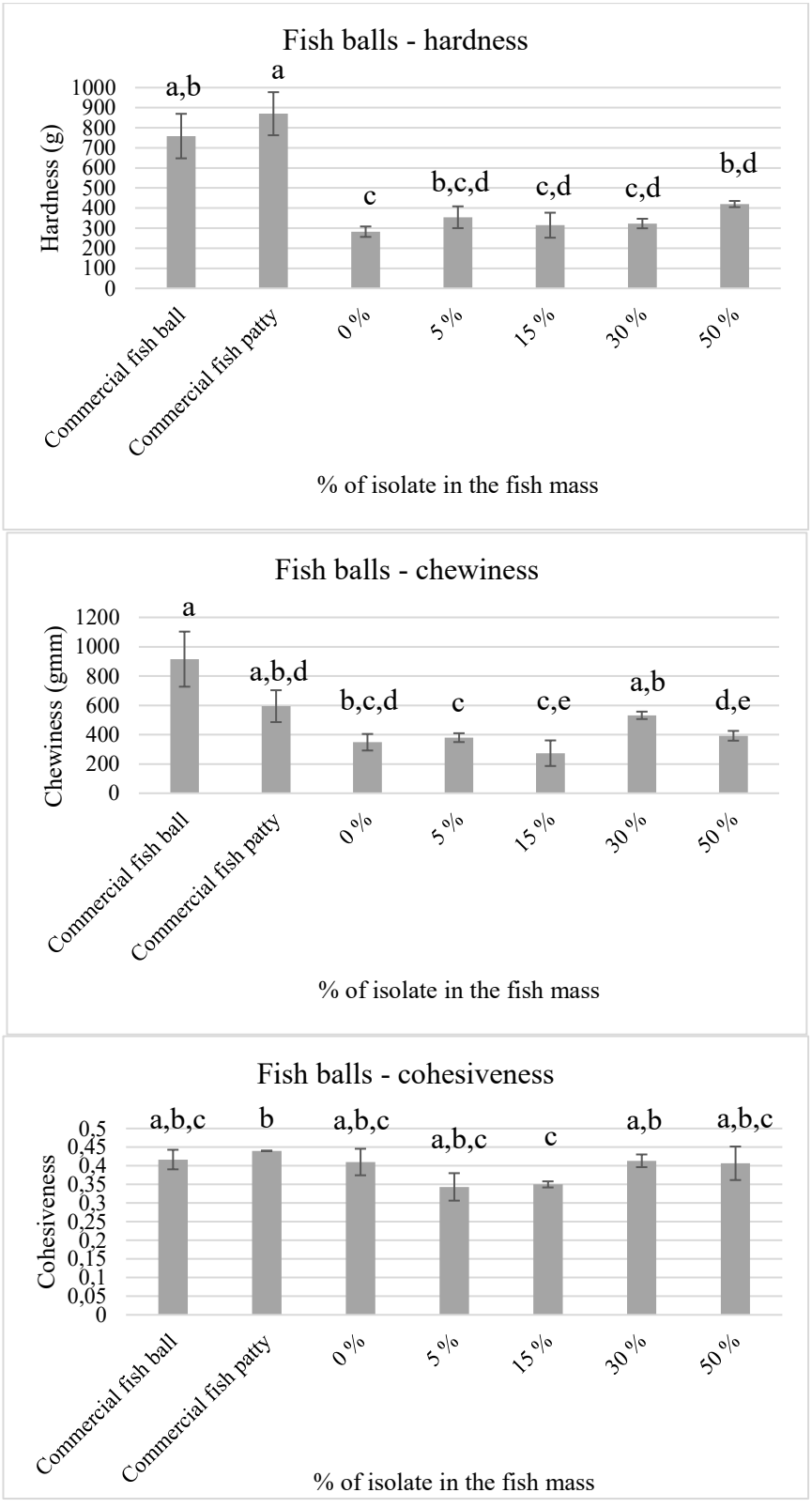

Figure 8. *Hardness, chewiness and cohesiveness values from texture profile analysis of fish balls with 0, 5, 15, 30 or 50% of Baltic herring protein isolate. Two commercial products were used as references. Statistically significant differences ($p<0.05$) are marked with different letters.*

3.4. HS-SPME-GC-MS

Headspace solid-phase microextraction (HS-SPME) was used in combination with gas chromatography-mass spectrometry (GC-MS) to determine volatile organic compounds from the lab-made surimi samples, fish balls and commercial samples. The method is semiquantitative and allows the comparison between the areas (amounts) of different volatile compounds between the samples. Quantitative analysis could be conducted by adding internal standards with known concentrations, however, which was not applied in this study. The identified peaks and their presence in commercial surimi products and surimi samples B1–6 are presented on Table IX whereas the compounds in commercial fish products and fish balls are presented on Table X. Figures 9, 10 and 11 show the compositional differences (PCA bi-plot) between the samples, commercial products and Baltic herring, as well as the volatile compounds identified from the GC-MS- analysis results.

Table IX. *Table of the volatile compounds found in commercial fish ball and fish patty (Apetit Ruoka Oy, Säkylä, Finland), and fish finger (Kesko Oyj, Kesko, Finland), as well as fish balls made with Baltic herring protein isolate (0, 15 and 50%).*

Peak	RT	Commercial fish patty	Commercial fish ball	Commercial fish finger	Fish ball with 0% FPI	Fish ball with 15% FPI	Fish ball with 50% FPI
methanethiol	**6.92**	x	x	x	x	x	x
ethanol	**9.07**				x	x	x
propanal	**9.98**				x	x	x
2-propanone	**10.25**				x	x	x
dimethyl sulfide	**10.33**	x		x			
2-propanol	**10.55**	x	x	x			
carbon disulfide/dithioxomethane	**10.74**				x	x	x
1-butanol	**14.49**	x	x	x			
2,3-butanedione	**14.58**	x	x	x			
2-butanone	**14.97**	x	x	x			
ethyl acetate	**15.04**	x	x	x			
3-methylbutanal	**17.60**	x	x	x	x	x	x
2-methylbutanal	**17.99**	x	x	x	x	x	x
2-ethylfuran	**18.78**	x	x	x	x	x	x
1-penten-3-ol	**19.36**	x	x	x	x	x	x
pentanal	**19.67**	x	x	x	x	x	x
1-methylsulfanyl-1-propene	**20.83**				x	x	x
dimethyl disulfide	**21.81**				x	x	x
3-methyl-1-butanol	**21.91**		x	x			

Peak	RT	Commercial fish patty	Commercial fish ball	Commercial fish finger	Fish ball with 0% FPI	Fish ball with 15% FPI	Fish ball with 50% FPI
2-methyl-2-butenal	**22.28**				x	x	x
hexane/1-chloranylnonane	**22.52**				x	x	x
hexanal	**24.43**				x	x	x
caryophyllene	**25.27**	x	x	x	x	x	x
2-methyl-1,4-diazine	**25.40**	x	x	x			
2-hexenal	**27.30**	x	x	x			
1-hexanol	**27.53**	x	x	x			
2-furanmethanol	**28.03**	x	x	x			
2-heptanone	**28.28**	x	x	x			
2-methyl-5-(1-methylethyl)-1,3-cyclohexadiene	**28.58**				x	x	x
heptanal	**28.64**	x	x	x			
3,4-dimethylthiophene	**28.7**				x	x	x
2-pinene	**28.99**	x	x	x	x	x	x
4(10)-thujene/á-phellandrene	**30.76**				x	x	x
-pinene/7-methyl-3-methylene-1,6-octa-diene	**30.89**				x	x	x
2(10)-pinene	**31.07**				x	x	x
2-pentylfuran	**31.16**	x	x	x			
1-octen-3-ol	**31.60**	x	x	x			
3-carene	**32.04**				x	x	x
benzaldehyde	**32.07**	x	x				
octanal	**32.50**	x	x	x			
D-limonene	**32.68**	x	x	x	x	x	x
P-cymene	**32.81**	x	x	x	x	x	x
2,4-heptadien-1-ol	**33.00**	x	x	x			
4(10)-thujene/á-phellandrene	**33.04**				x	x	x
5-ethyl-2,2,3-trimethylheptane	**33.10**	x	x				
eucalyptol	**33.27**				x	x	x
2,4-heptadienal	**33.76**	x	x	x			
2-mercapto-5,6,7,12-tetrahydropyrimido[5',6':6,7]cyclo-hepta[b]indole	**35.22**				x	x	x
L-linalool	**35.89**				x	x	x
nonanal	**36.04**	x	x	x	x	x	x
propyl disulfide	**36.28**				x	x	x
3,5-octadien-2-one	**36.46**	x	x	x			
terpinen-4-ol	**39.04**				x	x	x
dill ether (3R,3aR,7aS)-3,6-dimethyl-2,3,3a,4,5,7a-hexahydro-1-benzofuran	**39.26**				x	x	x
2,4-decadienal	**43.73**	x	x	x			
2,4-decadienal	**44.86**			x			

Table X. *Major volatile compounds of commercial surimi products, surimi made with fish protein isolate and homogenised Baltic herring (**Bh**). Commercial samples are **C1**: crabstick (Äyriäistukku Frisch Haus Finland Oy, Helsinki, Finland); **C2**: surimi scampi (Äyriäistukku Frisch Haus Finland Oy, Helsinki, Finland); **C3**: crabstick (Arvo Kokkonen Oy, Vantaa, Finland), **C4**: crabstick (Kesko Oyj, Kesko, Finland) and **C5**: sliced crabstick (Kesko Oyj, Kesko, Finland). The surimi samples are: **B1**:isolate from alkaline pH-shift process, **B2**: B1 with 0.5% of additional TiO_2, **B3**: isolate with bentonite added during the pH-shift process, B**4**: isolate with a higher precipitation pH (6.5 instead of 5.4), **B5**: isolate with a higher solubilisation pH (11.5 instead of 11.2) and **B6**: isolate with activated carbon added during the pH-shift process.*

Compound	RT	C1	C2	C3	C4	C5	B1	B2	B3	B4	B5	B6	Bh
methanethiol	**6.92**	x	x	x	x	x	x	x	x	x	x	x	
dimethyl sulfide	**10.33**	x	x	x	x	x							x
2-propanol	**10.55**	x	x	x	x	x	x	x	x	x			
1-butanol	**14.49**	x	x	x	x	x	x	x	x	x	x	x	x
2,3-butanedione	**14.58**	x	x	x	x	x				x	x		x
2-butanone	**14.97**	x	x	x	x	x	x	x	x	x	x	x	x
ethyl acetate	**15.04**	x	x	x	x		x	x					
3-methylbutanal	**17.60**	x	x	x	x	x	x	x	x	x	x	x	x
2-methylbutanal	**17.99**	x	x	x	x	x	x	x	x	x	x	x	x
2-ethylfuran	**18.78**	x	x	x	x	x	x	x	x	x	x	x	
1-penten-3-ol	**19.36**	x	x	x	x	x	x	x	x	x	x	x	x
pentanal	**19.67**	x	x	x	x	x	x	x	x	x	x	x	x
3-methyl-1-butanol	**21.91**	x	x	x	x	x							
3-methyl-1-butanol/2-methyl-1-butanol	**22.06**	x	x	x	x	x							x
hexanal	**24.37**	x	x	x	x	x	x	x	x	x	x	x	x
2-methyl-1,4-diazine	**25.40**	x	x	x	x	x	x		x	x	x		x
2-hexenal	**27.30**	x	x	x		x	x	x	x	x	x	x	x
1-hexanol	**27.53**	x	x	x	x	x	x				x		x
2-furanmethanol	**28.03**	x	x	x	x	x	x		x		x		x
2-heptanone	**28.28**	x	x	x	x	x	x	x	x	x	x	x	
heptanal	**28.64**	x	x	x	x	x	x	x	x	x	x	x	x
2-pinene	**28.99**	x	x	x	x	x	x	x	x	x	x	x	x
2-pentylfuran	**31.16**	x	x	x	x	x	x	x	x	x	x	x	x
1-octen-3-ol	**31.60**	x	x	x	x	x	x	x	x	x	x	x	x
benzaldehyde	**32.07**	x	x	x	x	x	x	x	x	x	x	x	
octanal	**32.50**	x	x	x	x	x	x	x	x	x	x	x	x
D-limonene	**32.68**	x	x	x	x	x	x	x	x	x	x	x	x
P-cymene	**32.81**	x	x	x	x	x	x	x	x	x	x	x	x
2,4-heptadien-1-ol	**33.00**	x	x	x	x	x	x	x	x	x	x	x	x
5-ethyl-2,2,3-trimethylheptane	**33.10**					x							
2,4-heptadienal	**33.76**	x	x		x	x	x	x	x	x	x	x	x
nonanal	**36.04**	x	x	x	x	x	x	x	x	x	x	x	x
3,5-octadien-2-one	**36.46**	x		x		x	x	x	x	x	x	x	x
2,4-decadienal	**43.73**						x					x	x
2,4-decadienal	**44.86**	x	x				x	x		x	x		x

Figure 9 presents a bi-plot of the PCA including commercial samples, homogenised Baltic herring and surimi samples B1–6. Principal components 1 and 2 explain 92% of the variation between the samples. The difference between 2-propanol and the rest of the volatile compounds explain the variation in the X-axis (PC1), which accounts for 79% of the compositional variation in the dataset. The samples separating within the X-axis are three commercial crabsticks, which separate from the surimi samples, Baltic herring and commercial fish ball and patty. According to Windsor (1969), 2-propanol is used in the dehydration step of the production of fish protein concentrates. The 2-propanol in the commercial surimi products may therefore originate from the processing steps as contamination. However, the quantification of the solvent was not possible with the results.

PC2 accounts for additional 13% of the compositional variation, which is visible in the differences within the Y-axis. Dimethyl sulfide correlates with the commercial surimi scampi, which is separated from the rest of the samples. Dimethyl sulfide is a volatile sulfur compound which has a role as a marine metabolite. The compound is known for its foul odour, which is formed e.g. by cooking cabbage and seafood (PubChem, 2019). Most volatile compounds are grouped in the middle of the axes in a proximity with the surimi samples, Baltic herring and the commercial fish ball and patty, which means that there is not much variation in the samples concerning these compounds. In conclusion, the surimi made of Baltic herring FPI largely resembles the aroma of the commercial products and Baltic herring, which indicates that the pH-shift process does not affect the volatile compounds by e.g. oxidation.

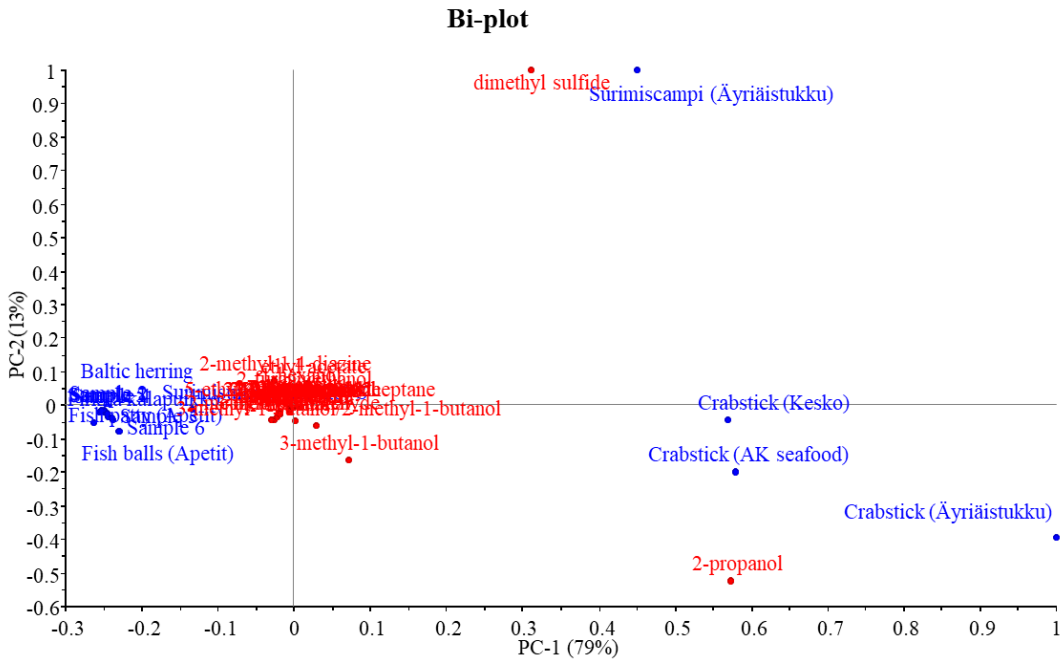

***Figure 9**. A bi-plot of all commercial samples, homogenised Baltic herring and surimi samples B1–6 based on the principal component analysis of the HS-SPME-GC-MS results on volatile compounds.**B1**:isolate from alkaline pH-shift process, **B2**: B1 with 0.5% of TiO_2 added before cooking, **B3**: isolate with bentonite added during the pH-shift process, B**4**: isolate with a higher precipitation pH (6.5 instead of 5.4), **B5**: isolate with a higher solubilisation pH (11.5 instead of 11.2) and **B6**: isolate with activated carbon added during the pH-shift process. Principal components 1 and 2 explain 92% of the variation between the samples. Surimi scampi correlates with dimethyl sulfide whereas three different crabsticks correlate with 2-propanol. Other samples and volatile compounds are grouped closely together.*

Figure 10 presents the same data as Figure 9 excluding the commercial crabsticks and surimi scampi in order to separate the rest of the samples better. The plot shows a larger variation between the samples than in Figure 9, separating the commercial products from surimi samples and Baltic herring, respectively. Principal components 1 and 2 explain 83% of the variation, PC1 accounting for 52% and PC2 for 31% of the compositional variation. All four commercial samples separate from both the Baltic herring and surimi samples, and they correlate with hexanal and D-limonene. Hexanal is a common flavour compound in fish but also a common indicator of oxidative reactions. It gives fish 'green' and general oxidised, paint-like flavours (Jónsdóttir et al., 2005; Karahadian and Lindsay, 1989). The correlation of hexanal with the commercial products indicates increased oxidation. The products were frozen but may have been prepared weeks or months before the measurement. Further, the packages did not include information on the fishing or preparation dates. D-limonene, on the other hand, is a common flavouring agent which

can be found in orange rind, dill oil, cumin and bergamot (Arn and Acree, 1998). Dill and lemon flavourings are commonly used in fish products.

2-methylbutanal and 3-methylbutanal correlate with the homogenised Baltic herring and are separated from the rest of the samples and aroma compounds. Both 2-methylbutanal and 3-methylbutanal are characterised with a malty, dark chocolate flavour (Lapsongphon et al., 2015) which may arise as a result of microbial spoilage. Other compounds indicating microbial spoilage odours are 1-propanol, 2-propanol and 1-butanol (Nicolay, 2006). 2-propanol can be found from all commercial samples and surimi samples B1–4 whereas 1-butanol is present in all commercial and surimi samples as well as the homogenised Baltic herring.

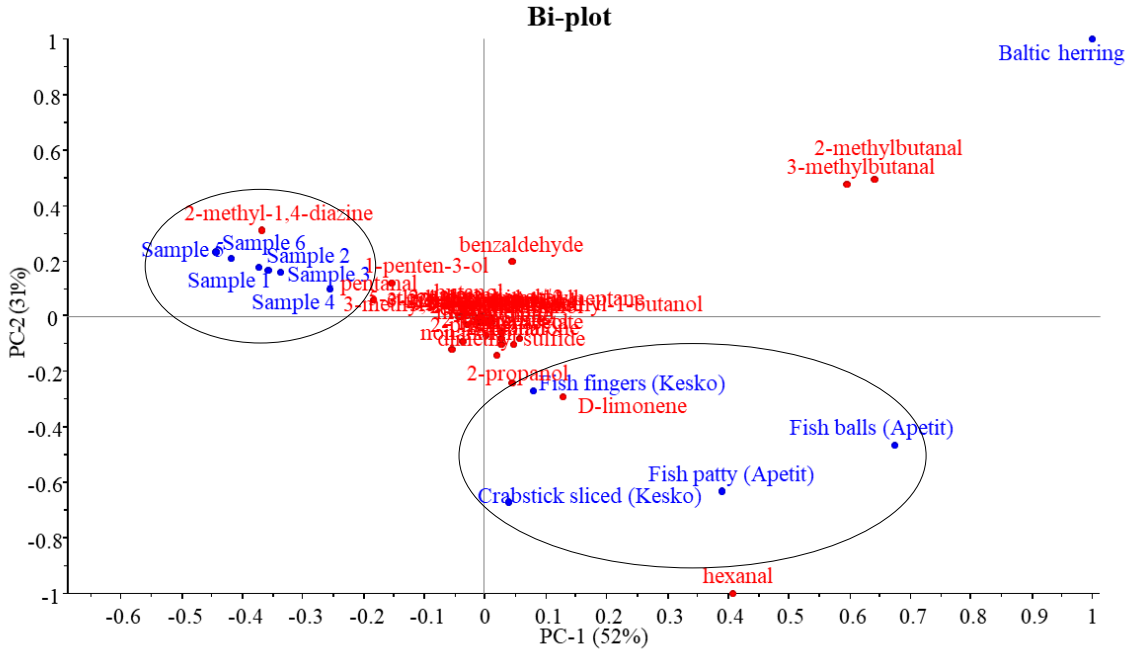

Figure 10. *Principal component analysis of the HS-SPME-GC-MS results on volatile compounds. A bi-plot of a principal component analysis including four commercial samples (outliers from figure A left out), homogenised Baltic herring and surimi samples B1–6.* ***B1****:isolate from alkaline pH-shift process,* ***B2****: B1 with 0.5% of* TiO_2 *added before cooking,* ***B3****: isolate with bentonite added during the pH-shift process, B****4****: isolate with a higher precipitation pH (6.5 instead of 5.4),* ***B5****: isolate with a higher solubilisation pH (11.5 instead of 11.2) and* ***B6****: isolate with activated carbon added during the pH-shift process. Principal components 1 and 2 explain 83% of the variation between samples. The homogenised Baltic herring is separated from other samples and correlates with 2-methylbutyraldehyde and 3-methylbutanal. The commercial samples are also separated from both the Baltic herring and the surimi samples, and correlate with hexanal, D-limonene and 2-propanol. The surimi samples group together and correlate most with 2-methyl-1,4-diazine, pentanal and 1-penten-3-ol.*

Figure 11 shows a bi-blot of the fish ball data together with the volatile compounds. Principal components 1 and 2 explain 99% of the compositional variation. The duplicate samples 1-1 and 1-2 (with 0% FPI) explain most of the variation within the X-axis even though the samples have the same composition and cooking time and thus, should not differ from each other. Flavour compounds, such as 1-pinene, 4(10)-thujene, 2(10)-pinene, 3-carene, D-limonene and cymene (Table III), which come from herbs and flavourings in the dry mixture are present in both fish balls 1-1 and 1-2, however, in significantly larger quantities in the latter sample, shown as an increased area in the chromatogram (not presented here). Some reasons for this difference might be the nonhomogeneous composition of the dry mixture as well as differences on the surface areas of the samples analysed by HS-SPME-GC-MS. Furthermore, two volatile compounds: 3-carene and D-limonene, explain the compositional variation within the Y-axis, which somewhat separates the fish balls 2 and 4 (15 and 50% FPI, respectively) from the fish ball with no FPI. Both 3-carene and D-limonene are citrus aromas (Miyazawa et al., 2013), which were used as flavourings in the dry mixture used in the fish ball recipe. The result indicates that D-limonene correlates more with the fish ball with 0% FPI whereas 3-carene correlates with the fish balls with 15 and 50% of FPI, respectively. Each fish ball was made with the same dry mixture, the most likely source of these flavourings, and therefore, the differences comes most likely from the nonhomogeneous composition of the dry mixture or differences in the sample surface areas.

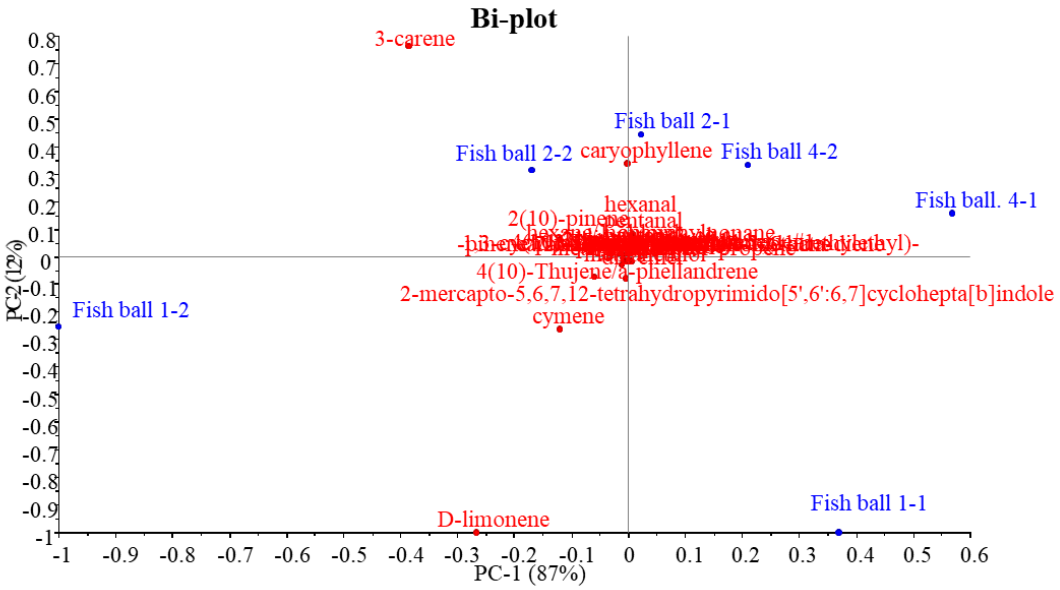

Figure 11. *Principal component analysis of the HS-SPME-GC-MS results on volatile compounds. Duplicate samples of fish balls;* ***fish ball 1****: 0% Baltic herring protein isolate,* ***fish ball 2****: 15% protein isolate and* ***fish ball 4****: 50% protein isolate. Principal components 1 and 2 explain 99% of the compositional variation.*

3.5. Colour

The colour of the surimi samples B1–6 and commercial surimi sticks from Kesko Oyj (Kesko, Finland), Arvo Kokkonen Oy (Vantaa, Finland) and Äyriäistukku Frisch Haus Finland Oy (Helsinki, Finland), and surimi scampi from Äyriäistukku Frisch Haus Finland Oy (Helsinki, Finland) and the colorimetry results are shown in Figures 12 and 13, respectively. The four commercial surimi products are whiter than the Baltic herring surimi, which is expected due to the use of white-fleshed fish in the surimi industry. A previous study reported a whiteness value of approx. 85 for a protein gel made with rainbow trout (Tahergorabi et al., 2012). The commercial crab sticks used in this study have a similar whiteness value, whereas the surimi scampi and sample B2, which contains TiO_2, have whiteness values of approx. 75. The difference in whiteness is significant between the first, third and fourth commercial sample and all the Baltic herring samples. The commercial surimi scampi is significantly whiter than samples B5 and B6, but the difference is not significant with samples B1–4. Samples B1 and B2 differ only in the added TiO_2 content – the addition of 0.5% of TiO_2 causes a statistically significant increase in whiteness.

Different solubilisation or precipitation pH does not cause a significant difference in the whiteness compared to the normal process. Furthermore, the addition of bentonite during the process does not affect the colour, but the addition of activated carbon causes a significant decrease in whiteness. It is likely that the fine activated carbon powder does not separate enough during the process and some stays in the isolate causing the darkening. Most of the activated carbon was removed with the lipid layer after first centrifugation, shown as a black lipid phase. Since the pH-shift process led to a 61% reduction in the lipids per wet weight, a method for better lipid reduction might also reduce the amount of activated carbon left in the supernatant. Since activated carbon has shown to reduce lipophilic environmental toxins (Maes et al., 2005), it could be utilised in the pH-shift processing for the removal of toxins, as long as it can be removed fully during the process.

According to these results, the only effective whitening method was the addition of TiO_2. The 0.5% addition increased the whiteness to the level of commercial surimi scampi, but not to the level of surimi sticks. According to literature, pressure treatment has shown increased whiteness values compared to heat-induced surimi gels. The rearrangement of water molecules achieved with the pressure treatment seemed to modify the gel network, producing a more transparent gel. Furthermore, the pressure treatment with 400 MPa led to increased gel strength for Alaska pollock surimi but not for Pacific whiting surimi compared to the heat-induced gels. (Tabilo-Munizaga and Barbosa-Cánovas, 2004) Pressure treatment might therefore improve both colour and gel strength, without the need for food additives.

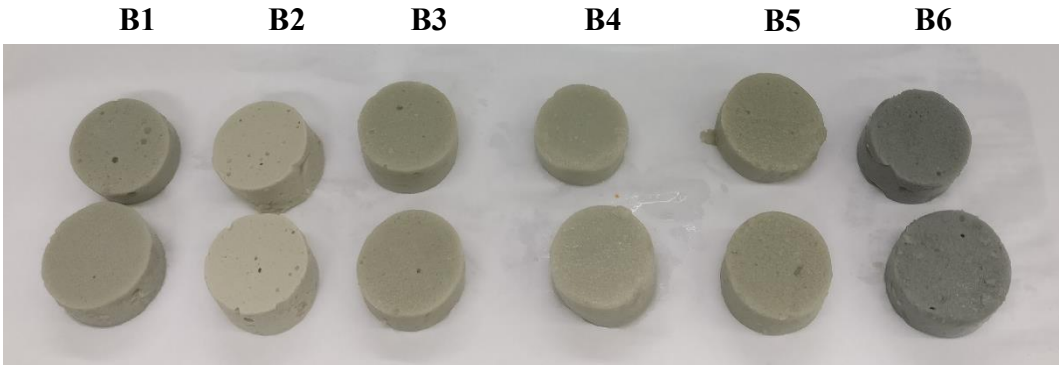

Figure 12. *Duplicate surimi samples from different pH-shift processes.* ***B1****: alkaline pH-shift process,* ***B2****: alkaline pH-shift process, 0.5% TiO_2 added before cooking,* ***B3****: isolate with bentonite used during the pH-shift process,* ***B4****: isolate with a higher precipitation pH (6.5 instead of 5.4),* ***B5****: isolate with a higher solubilisation pH (11.5 instead of 11.2) and* ***B6****: isolate with activated carbon used during the pH-shift process. Sample 2 differs significantly ($p<0.05$) from the other samples.*

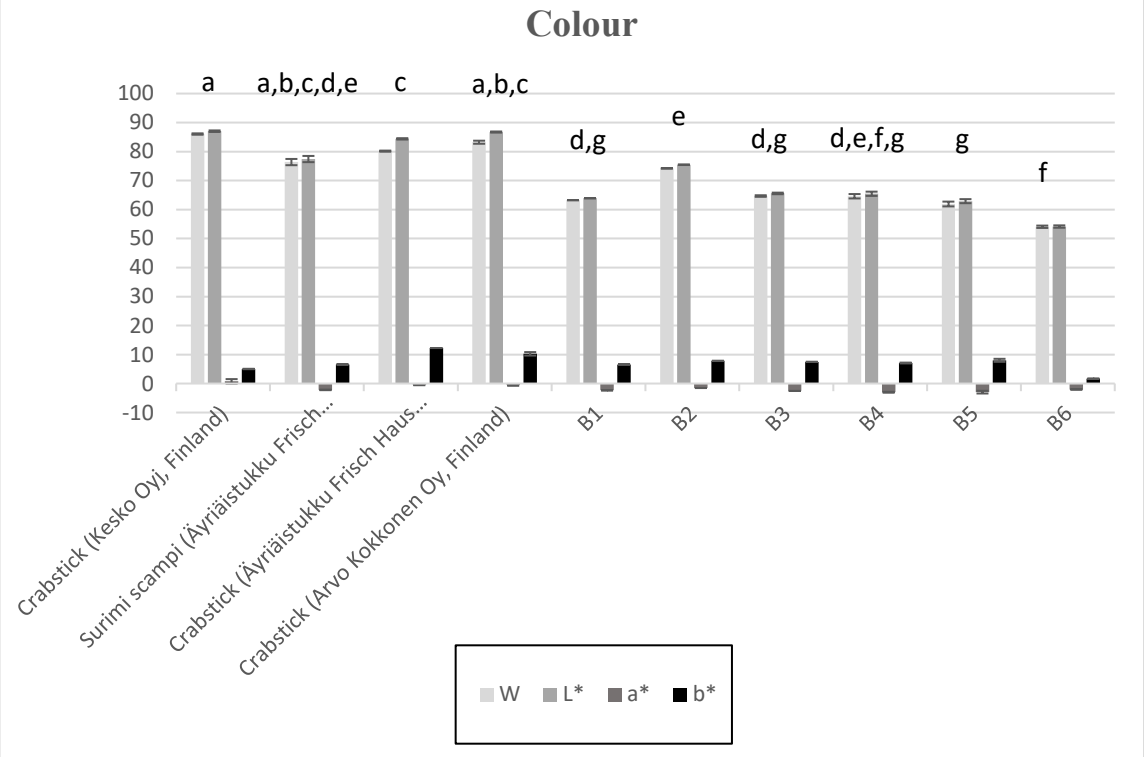

Figure 13. *Colour measurement results of four commercial surimi products and six samples made of Baltic herring protein isolate. The samples are;* ***B1****: alkaline pH-shift process,* ***B2****: alkaline pH-shift process with 0.5% TiO_2 added before cooking,* ***B3****: isolate with bentonite used during the pH-shift process,* ***B4****: isolate with a higher precipitation pH (6.5 instead of 5.4),* ***B5****: isolate with a higher solubilisation pH (11.5 instead of 11.2) and* ***B6****: isolate with activated carbon used during the pH-shift process.* ***W****: whiteness,* ***L****: lightness,* ***a****: red-green axis &* ***b****: yellow-green axis. Significant differences ($p<0.05$) in whiteness are indicated with different letters.*

4. Conclusions

The alkaline pH-shift method using precipitation and solubilisation values of 11.2 and 5.4, respectively, is a suitable method for solubilising and extracting proteins from Baltic herring. The FPI yield was 82.5% of the amount of raw material whereas the protein yield was 53.6%. The produced FPI with 4% of added cryoprotectants had a moisture content of 85.3%, lipid content of 3.7% and protein content of 9.2%. The protein and lipid contents of the FPI were 62.3% and 25.2% on dry basis. The isolate had good gelling properties, resulting in surimi with a texture close to commercial surimi scampi. The pH shift method led to a lipid reduction of 18% based on dry weights, and a 61% reduction based on wet weights. The reduction in the lipid content is also likely to lead to the reduction of lipophilic environmental toxins, such as dioxins and heavy metals, contents of which were however not analysed in this study.

In this study, the use of different solubilisation of precipitation pH-values or the additions of bentonite or activated carbon did not increase the whiteness of the isolate. In contrast, the addition of activated carbon decreased the whiteness significantly. Additionally, the changing of the solubilisation pH-value from 11.2 to 11.5 did not affect the texture of the surimi. However, the changing of the precipitation pH-value from 5.4 to 6.5 caused significant softening of the surimi due to an increased moisture content of the isolate. The addition of 0.5% of TiO_2, however, increased the whiteness of the Baltic herring surimi to the level of the commercial surimi scampi used as a reference product.

The addition of FPI improved the texture or fish balls by increasing the moisture content, as well as increasing the hardness closer to the level of the commercial fish products. Furthermore, using 50% of FPI lowered the fat content of the fish balls by replacing other ingredients with a higher fat content without affecting the texture adversely. In conclusion, FPI from Baltic herring would be a good substitute of minced fillets or other ingredients in the fish ball mixture due to its good gelling properties. The use of FPI could also lower the price of the product if the industrial pH-shift processing becomes feasible. Most importantly, this would open possibilities for large-scale FPI production, utilising the Baltic herring which is now mostly processed into animal feed.

In this study, beheaded and gutted Baltic herring was used as the raw material, but the method could be applied to whole fish or fish by-products, which would lower the price of the isolate, and thereof products made of it. Using whole fish might pose a microbial

threat, which should be analysed further. The processing conditions should also be optimised for a better protein yield, but the method is effective in removing scales and bones while producing a smooth paste which is suitable for many industrial applications. The use of Baltic herring produces a grey isolate, which might be rejected in products where light colour is typical, such as surimi. Different whitening techniques during the pH-shift processing should be developed, unless the isolate is used in products, such as fish balls, where the darker colour does not have such a big impact. Furthermore, the lipid content of the isolate was too high based on the general guidelines for fish protein concentrates described by FAO (Windsor, 1969). Based on the results presented here, the FPI produced by alkaline pH-shift processing is best suited to replace other products in fish balls and patties. The addition of FPI would also allow marketing the product with a higher fish content, if it replaces ingredients such as starch, egg, cream etc.

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