DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

Alternative Approaches to Improve the Processing and Quality of Under-utilized Fish

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TURKU, FINLAND – 2023

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ISBN 978-951-29-9292-8 (print)
ISBN 978-951-29-9293-5 (pdf)
ISSN 2323-9395 (print)
ISSN 2323-9409 (pdf)
Painosalama, Turku, Finland 2023

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ABSTRACT

Due to the global issues related to climate change and population growth, more sustainable use of existing food resources is required. Fish and other aquatic organisms are rich in health beneficial polyunsaturated fatty acids, protein, and several vitamins and minerals. Fish and other aquatic resources are estimated to play an even more vital role in the future. While many popular fish stocks have already been overexploited, several species are currently not utilized at all or are directed to low-value, non-human uses, such as feed.

One example of such species is Baltic herring (*Clupea harengus membras*). Baltic herring is the most important commercial capture species in Finland, based on both value and quantity. However, only a small fraction of the total catch is used as food. Some factors limiting the food use of Baltic herring include the decreasing size of the caught fish, its high susceptibility to oxidation and other degradation leading to quality loss, as well as its distinct odor and flavor that are not preferred by some consumers. Roach (*Rutilus rutilus*) along with other cyprinid fish, are not commercially important fish species, and are utilized for human consumption even less than Baltic herring. In addition to providing a valuable source of proteins, lipids and other nutriens, increasing the capture of roach and other cyprinid fish could result in beneficial effects in reducing eutrophication.

This thesis focused on alternative ways of increasing utilization of under-utilized fish, with a focus on Baltic herring and roach. The two processing methods studied were the pH shift and enzymatic hydrolysis, both of which have been widely studied for other fish species but not Baltic herring or roach. Both processes have the advantage that whole fish or fish side streams without any pre-processing can be used as raw materials. Further, the addition of natural antioxidants was investigated as a means of inhibiting lipid oxidation and degradation of sensory quality during frozen and refrigerated storage of minced Baltic herring. Lingonberry juice press residue, sea buckthorn juice press residue, sea buckthorn juice press residue after supercritical CO_2 extraction of lipids, and a commercial extract mixture as natural additions were compared to conventional antioxidants ethylenediaminetetraacetic acid (EDTA) and combination of α-tocopherol and ascorbic acid.

The composition of proteins and lipids in Baltic herring and roach protein isolates and hydrolysates was significantly affected by the processing type (pH shift or enzymatic hydrolysis). Compared to enzymatic hydrolysis, the pH shift led to enrichment of essential amino acids, phospholipids, and in case of acid extraction, also polyunsaturated fatty acids such as docosahexaenoic acid (DHA). The pH shift, especially alkaline pH shift, however, induced considerable lipid

and protein oxidation. The fish protein hydrolysates (prepared using enzymatic hydrolysis) showed more moderate formation of hydroperoxides, secondary volatile oxidation products, and protein carbonyls. The incomplete elimination of fishy odors and flavors during preparation of fish protein isolates and hydrolysates is a major factor limiting their use. Based on this research, while not completely eliminating these odors, the alkaline pH shift reduced the total intensity of odor and intensity of the fishy odor in Baltic herring, which was reflected in the quantity and quality of odor-active compounds.

The natural antioxidants, lingonberry juice press residue (3%), sea buckthorn juice press residue (3% w/w), and commercial extract mixture (0.1%) retarded lipid oxidation in Baltic herring mince stored at –20 °C more effectively than EDTA or combination of α-tocopherol and ascorbic acid, as indicated by lower loss of polyunsaturated fatty acids, lower PV, and/or lower formation of secondary oxidation related volatiles, such as 1-penten-3-ol and hexanal. During refrigerated storage (1 °C), sea buckthorn juice press residue after supercritical CO_2 oil extraction and lingonberry juice press residue were effective antioxidants also at the lower concentrations (1.5%, 1%). Based on the odor and flavor profile of Baltic herring minces, particularly sea buckthorn juice press residue after supercritical oil extraction (1.5%), also prevented changes in sensory quality during a 3-day storage. This was further reflected in the odor-active compounds of raw minces. Berry press residues are side streams of berry juice and oil production, and their use as antioxidantive materials would provide added value for these currently under-utilized materials as well.

This thesis provided valuable insights related to increasing utilization of Baltic herring and other under-utilized fish. The results showed that pH-shift processing and enzymatic hydrolysis of Baltic herring and roach produced fish protein isolates and hydrolysates with high nutritional quality. However, lipid and protein oxidation during these processes and storage of Baltic herring poses a challenge for preservation of quality. Use of antioxidants may improve preservation of the nutritional and sensory quality of Baltic herring during processing and storage.

SUOMENKIELINEN ABSTRAKTI

Väestönkasvuun ja ilmastonmuutokseen liittyvät haasteet vaativat olemassa olevien resurssien hyödyntämistä elintarvikekäyttöön yhä tehokkaammin. Kala ja muut merenelävät ovat erinomaisia terveyttä edistävien monityydyttymättömien rasvahappojen, proteiinin ja useiden vitamiinien ja kivennäisaineiden lähteitä. Kalan ja muiden merenelävien merkityksen ravinnon lähteenä odotetaan kasvavan edelleen tulevaisuudessa. Vaikka monien kaupallisesti hyödynnettyjen kalalajien kannat ovat romahtaneet ylikalastuksen vuoksi, useita lajeja ei hyödynnetä lainkaan, tai ne käytetään muuksi kuin ihmisravinnoksi, kuten rehuksi.

Esimerkki vajaasti hyödynnetystä kalalajista on silakka (*Clupea harengus membras*). Silakka on sekä saalismäärältään että arvoltaan merkittävin kaupallisen kalastuksen laji Suomessa. Vain pieni osa silakkasaaliista päätyy kuitenkin elintarvikekäyttöön. Silakan elintarvikekäyttöä rajoittaa saaliskalojen pienenevä koko, alttius rasvojen hapettumiselle ja muulle laadun heikkenemiselle, sekä silakalle tyypillinen maku ja haju, jotka eivät miellytä kaikkia kuluttajia. Särki (*Rutilus rutilus*) ja muut särkikalat eivät ole kaupallisesti merkittäviä kalalajeja Suomessa, ja siksi niitä hyödynnetään elintarvikkeeksi vielä vähemmän kuin silakkaa. Ne ovat arvokkaita hyvälaatuisten proteiinien ja rasvojen lähteitä ja niiden kalastuksen lisäämisellä voi olla suotuisia vaikutuksia rehevöitymisen vähentämiseen.

Tämä väitöskirjatutkimus keskittyi uudenlaisiin tapoihin lisätä alihyödynnettyjen kalalajien, erityisesti silakan ja särjen, käyttöä elintarvikkeena. Tutkimuksessa käytettiin kahta eri prosessointimenetelmää, pH:n muunnokseen perustuvaa proteiinien uuttoa ja entsymaattista hydrolyysiä, joista on julkaistu tutkimuksia useille muille kalalajeille, mutta ei silakalle ja särjelle. Molempien menetelmien etuna on, että kokonaisia kaloja tai kalojen sivuvirtoja voidaan käyttää sellaisenaan prosessin raaka-aineena. Väitöskirjatutkimuksessa tutkittiin myös luonnollisten antioksidanttien vaikutusta silakan rasvojen hapettumiseen ja aistinvaraiseen laatuun pakkas- ja kylmäsäilytyksen aikana. Tutkimuksessa käytettyjä luonnollisia antioksidantteja olivat puolukkamehun ja tyrnimehun puristuksessa muodostuvat puristejäännökset, tyrnimehun puristejäännös, josta oli lisäksi uutettu öljy superkriittisellä hiilidioksidiuutolla, sekä kaupallinen uuteseos. Näitä luonnollisia antioksidanttilisäyksiä verrattiin etyleenidiamiinitetra-asetaattiin (EDTA) ja askorbiinihapon ja α-tokoferolin yhdistelmään.

Prosessointimenetelmä (pH:n muutokseen tai entsymaattiseen hydrolyysiin perustuva uutto) vaikutti merkittävästi proteiinien ja rasvojen koostumukseen. PH:n muutokseen perustuvalla menetelmällä valmistetut silakan ja särjen

proteiini-isolaatit sisälsivät enemmän välttämättömiä aminohappoja ja fosfolipidejä, ja happaman uuton tapauksessa myös monityydyttymättömiä rasvahappoja, kuten dokosaheksaeenihappoa (DHA), kuin entsymaattisesti valmistetut hydrolysaatit. PH:n muutokseen perustuva menetelmä, erityisesti emäksinen uutto, aiheutti kuitenkin huomattavaa rasvojen ja proteiinien hapettumista. Entsymaattisella uutolla valmistetuissa proteiinihydrolysaateissa hydroperoksidien, haihtuvien sekundääristen hapettumistuotteiden ja proteiinikarbonyylien muodostuminen oli maltillisempaa. Epätäydellinen kalaisan hajun ja maun poistuminen kalaproteiini-isolaattien ja -hydrolysaattien valmistuksessa rajoittaa merkittävästi niiden käyttöä elintarvikkeissa. Tutkimuksen perusteella silakan proteiini-isolaatti koettiin hajultaan hieman kalaiseksi, mutta merkitsevästi vähemmän kalaiseksi kuin silakka. Silakan ja silakkaproteiinin ero kalaisan hajun voimakkuudessa ja hajun kokonaisvoimakkuudessa ja näkyi myös hajuun vaikuttavien yhdisteiden laadussa ja määrässä.

Luonnolliset antioksidantit, puolukkamehun puristejäännös (3 %), tyrnimehun puristejäännös (3 %) ja kaupallinen uuteseos (0,1 %) hidastivat –20 °C:ssa säilötyn jauhetun silakan rasvojen hapettumista tehokkaammin kuin EDTA tai α-tokoferolin ja askorbiinihapon yhdistelmä vähäisemmän monityydyttymätömien rasvahappojen tuhoutumisen ja matalampien peroksidilukujen ja/tai sekundääristen hapettumistuotteiden määrän perusteella. Kylmässä (1 °C) toteutetussa kokeessa erityisesti puolukkamehun puristejäännös ja tyrnimehun ja öljyn uuttojäännös hidastivat primääristen ja sekundääristen hapettumistuotteiden muodostumista myös matalampina pitoisuuksina (1,5 % ja 1 %). Marjojen puristejäännökset, erityisesti tyrnimehun ja öljyn uuttojäännös (1,5 %) esti myös silakan hajun ja maun muutoksia kolmen päivän säilytyksen aikana, mikä havaittiin myös hajuun vaikuttavissa yhdisteissä. Marjojen puristejäännökset ovat marjamehujen ja -öljyjen valmistuksen sivuvirtoja, ja niiden hyödyntäminen antioksidatiivisina materiaaleina lisäisi myös niiden arvoa.

Tämän väitöskirjan tulokset toivat uutta tietoa silakan ja muiden vajaasti hyödynnettyjen kalalajien käytön lisäämiseen liittyen. Tutkimus osoitti pH:n muunnokseen perustuvan uuton ja entsymaattisen hydrolyysin tuottavan ravitsemuksellisesti laadukkaita proteiini-isolaatteja ja -hydrolysaatteja silakasta ja särjestä. Rasvojen ja proteiinien hapettuminen näissä prosesseissa ja silakan säilytyksen aikana on kuitenkin haaste laadun säilymisen kannalta. Antioksidanttien käytöllä voidaan parantaa silakan ravitsemuksellisen ja aistinvaraisen laadun säilymistä prosessoinnin ja säilytyksen aikana.

LIST OF ABBREVIATIONS

αT+AA	*L*-ascorbic acid & *α*-tocopherol
AA	Amino acid
AB	Antimicrobial blend
DAG	Diacylglycerol
DH	Degree of hydrolysis
DHA	Docosahexaenoic acid
EAA	Essential amino acid
EDTA	Ethylenediaminetetraacetic acid
EPA	Eicosapentaenoic acid
FD	Flavor dilution
FA	Fatty acid
FFA	Free fatty acid
FPH	Fish protein hydrolysate
FPI	Fish protein isolate
GC-O	Gas Chromatography – Olfactometry
GDA	Generic Descriptive Analysis
Hb	Hemoglobin
HNE	4-hydroxynonenal
LOX	Lipoxygenase
LR	Lingonberry juice press residue
LRI	Linear retention index
MAG	Monoacylglycerol
MDA	Malondialdehyde
Mb	Myoglobin
MUFA	Monounsaturated fatty acid
NEAA	Non-essential amino acid
NIF	Nasal Impact Frequency
PCA	Principal Component Analysis
PL	Phospholipid
PUFA	Polyunsaturated fatty acid
PV	Peroxide value
SFA	Saturated fatty acid
SPME	Solid phase microextraction
SR	Sea buckthorn juice press residue
SRO	Sea buckthorn juice press and oil extraction residue
TAG	Triacylglycerol
TBARS	Thiobarbituric acid reactive substances
UFA	Unsaturated fatty acid

LIST OF ORIGINAL PUBLICATIONS

I. Kakko, T.; Damerau, A.; Nisov, A.; Puganen, A.; Tuomasjukka, S.; Honkapää, K.; Tarvainen, M.; Yang, B. Quality of protein isolates and hydrolysates from Baltic herring (*Clupea harengus membras*) and roach (*Rutilus rutilus*) produced by pH-shift processes and enzymatic hydrolysis. *Foods* **2022**, 11(2), 230.

II. Kakko, T.; Aitta, E.; Laaksonen, O.; Tolvanen, P.; Jokela, L.; Salmi, T.; Damerau, A.; Yang, B. Baltic Herring (*Clupea harengus membras*) protein isolate produced using the pH-shift process and its application in food models. *Food Res. Int.* **2022**, 158, 111578.

III. Damerau, A.; Kakko, T.; Tian, Y.; Tuomasjukka, S.; Sandell, M.; Hopia, A.; Yang, B. Effect of supercritical CO_2 plant extract and berry press cakes on stability and consumer acceptance of frozen Baltic herring (*Clupea harengus membras*) mince. *Food Chem*. **2020**, 332, 127385.

IV. Kakko, T.; Damerau, A.; Mejia Rios, C.; Laaksonen, O.; Yang, B. Reutilization of berry press residues in minced Baltic herring (*Clupea harengus membras*) – effect on lipid oxidation and sensory characteristics during cold storage. *Submitted*.

1 INTRODUCTION

The food industry is under pressure to find alternative ways to feed the growing population in a sustainable way. The challenge includes discovery of new protein sources, but also utilization of all resources more efficiently. The vital role of fish and other marine resources as food and feed has been estimated to increase further in the future (FAO, 2022). Overfishing has led to endangerement of several fish species, such as bluefin tuna (*Thunnus thynnus*), halibut (*Hippoglossus hippoglossus*), and Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) (World Wildlife Fund, 2021). Meanwhile, many other fishes and other marine resources are currently not utilized to their full potential. Baltic herring (*Clupea harengus membras*) is an example of an under-utilized fish species, as a marine resource of significant potential. It is volume- and catch-wise the most significant fish species in Finland with its share of the total catch in 2021 being 79% (77 000 tonnes) (Natural Resources Institute Finland, 2022a). However, most of this catch is used as feed, and only 17% is utilized as food, of which 4% accounts for domestic consumption (Natural Resources Institute Finland, 2022b). On the other hand, most of the fish consumed in Finland is imported. In 2021, Finns consumed approximately 8.3 kg/person/year imported fish, while the consumption of Baltic herring was 0.4 kg/person (Natural Resources Institute Finland, 2022c).

Challenges related to low utilization of Baltic herring as food include its small size, high content of polyunsaturated lipids prone to oxidation, and abundance of dark muscle rich in pro-oxidants. Baltic herring is commonly processed into fillets, but Baltic herring under 17 cm in length are too small for commercial filleting and are mostly used as feed for farmed fish and fur animals. The high content of polyunsaturated fatty acids (PUFAs), combined with a high content of pro-oxidants makes Baltic herring susceptible to lipid oxidation, posing a challenge for preserving its nutritional and sensory quality during storage. Furthermore, Baltic herring has a distinct odor and flavor, possibly derived from lipid oxidation, which are not preferred by many consumers (Pihlajamäki et al., 2019).

Lipid oxidation is a major challenge in preserving the quality of fish, but especially in the case of dark-muscled fish (Undeland et al., 1998), such as Baltic herring. Most processing technologies aiming to improve utilization of small fish or fish co-products, such as the pH shift or enzymatic hydrolysis, include the use of high temperature or extremely acidic or alkaline conditions, that may further promote oxidation of lipids and proteins (Abdollahi et al., 2020; Halldorsdottir et al., 2013). Lipid oxidation leads to development of off-odors and off-flavors (Lindsay, 1990) that limit the application and consumption of fish and fish

products. Sufficient control of lipid oxidation, by the use of antioxidants or other methods, is therefore required to increase the use of under-utilized fish.

This thesis aimed to tackle the challenges related to the use of Baltic herring and other under-utilized fish from a food perspective. Though the focus was on regionally important fish, the same concepts may be applied to other fish with similar challenges. The literature review discusses some common challenges related to under-utilized fish. In particular, lipid oxidation and its implications on the sensory quality of fish are discussed. Finally, the use of natural antioxidants to control oxidation, as well as pH-shift processing and enzymatic hydrolysis as protein extraction mehods are reviewed. The experimental part of the thesis investigated different solutions to improve the utilization of Baltic herring and roach. The pH shift and enzymatic hydrolysis were assessed in terms of the nutritional quality and oxidative stability of the resulting protein isolates and hydrolysates. The odor profile of the protein isolate produced by alkaline pH-shift processing, as well as the underlying odor-active compounds were studied. The inhibiting effect on lipid oxidation by antioxidants, such as α-tocopherol, ascorbic acid, and side streams of berry processing were examined in frozen or refrigerated minced Baltic herring. The berry side streams were further evaluated in terms of their effect on the odor and flavor as well as odor-active compounds of Baltic herring.

2 REVIEW OF THE LITERATURE

2.1 Baltic herring and other under-utilized fish

2.1.1 Baltic herring

Baltic herring (*Clupea harengus membras*) is a subspecies of Atlantic herring (*Clupea harengus*) found in the Baltic Sea region. Baltic herring are spring-spawning fish, and spawning occurs in coastal areas of the Baltic Sea (Arula et al., 2019). Baltic herring is relatively small, with the usual length varying between 15–20 cm (NatureGate, 2022). The size of Baltic herring has however been gradually decreasing. According to a recent report (Rajasilta et al., 2022), most of the tested Baltic herring from Archipelago Sea in winter 2020 were 15–16 cm long, while fish over 17 cm were scarce.

The fatty acid (Aro et al., 2000) and lipid class (Linko et al., 1985) composition of Baltic herring fluctuate between seasons due to the fluctuation in the lipid composition of the plankton that they feed on (Linko et al., 1985; Möllmann et al., 2004).The highest feeding activity of Baltic herring occurrs during spring and summer (Möllmann et al., 2004). The total and relative amount of phospholipids (PLs) was found to be highest in June (1.0% of flesh weight, 41% of total lipids) and lowest in October (0.4% of flesh weight, 8% of total lipids) when the total lipid content was highest (3.1% of flesh weight) (Linko et al., 1985). The PUFA content, on the other hand, was seen to vary between 34% in the summer and 38% in the autumn (Aro et al., 2000). While the lipid content in the muscle of Baltic herring (caught during winter) has decreased from approximately 7–8% to 2–3% over the past decades, the relative content of *n*-3 PUFAs has increased (Rajasilta et al., 2022). Of Baltic herring caught 2020-2021, almost 40% of all fatty acids consisted of EPA and DHA. The decreasing trend in the lipid content is postulated to be due to the decreasing size of spawning Baltic herring and decreasing water salinity of the Baltic sea (Rajasilta et al., 2019). Baltic herring contains approximately 16% protein, and is also an excellent source of vitamin D (Finnish Institute for Health and Welfare, 2022).

The annual catch of Baltic herring in Finland has in most years exceeded 100 000 tonnes (Natural Resources Institute Finland, 2022a) and it is one of the most caught species in the Baltic Sea region in terms of volume, but majority of the catch ends up as feed for farmed fish or fur animals (Sarkki and Pihlajamäki, 2019). The domestic consumption of Baltic herring has decreased during the past decades (**Figure 1**). Meanwhile, most of fish consumed in Finland is imported (Natural Resources Institute Finland, 2022c). It has been estimated that the contribution of Baltic herring to food security and safety could be significantly

increased (Pihlajamäki et al., 2016). The main catch season for Baltic herring usually extends from October to May, depending on the year.

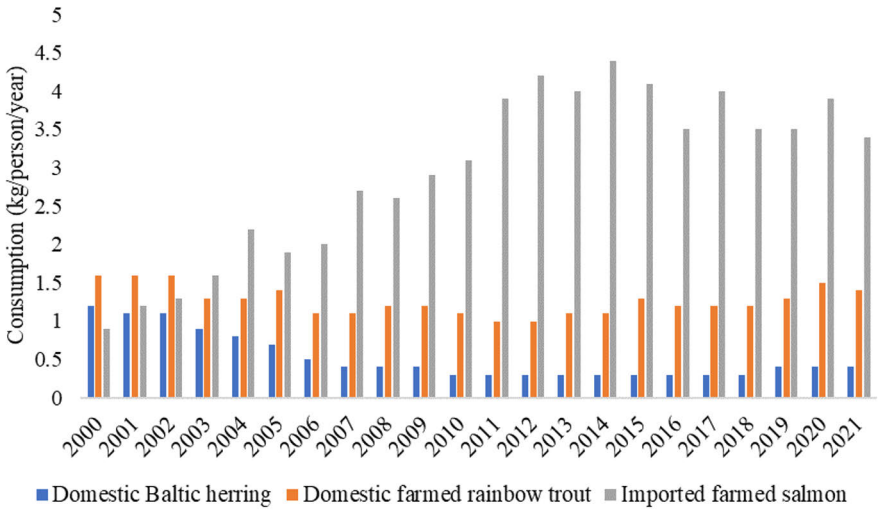

Figure 1. Consumption of domestic Baltic herring and farmed rainbow trout and imported farmed salmon in Finland (kg/person/year). Drawn based on data from the Statistics database of Natural Resources Institute Finland (Natural Resources Institute Finland, 2022c).

2.1.2 Challenges and opportunities of Baltic herring and other under-utilized fish

Fish is not only a source of high-quality lipids with an abundance of long-chain PUFAs, but also an excellent source of animal proteins (presence of all essential amino acids (EAAs)) and micronutrients (Gil and Gil, 2015). Due to the global issues related to climate change and population growth, fish and other aquatic resources have been recognized as having a vital and increasing role in food security (FAO, 2022). Fish, especially wild fish, have generally a considerably lower carbon footprint compared to other animal protein sources. For instance, roach (*Rutilus rutilus*) has a lower "global warming potential" compared to several other protein sources, including vegetal meat substitutes and pulses (Uusitalo et al., 2018). In addition, increased catching of zooplanktivorous fish, such as cyprinid fish, may help to reduce predation pressure on zooplankton and therefore enhance their grazing on phytoplankton, which has an alleviating effect on eutrophication (Gerke et al., 2021). In Finland, roach catches from commercial inland and marine fisheries were 444 tonnes and 298 tonnes, respectively, in 2021, which was only 0.7% of the total fish catch from inland and marine fisheries (Natural Resources Institute Finland, 2023a, 2023b).

While many desired or popular fish stocks have been over-exploited (Branch et al., 2011), there are several fish species and other marine resources that are currently under-utilized, or even wasted by discarding as landfill. Fish may be considered "under-utilized" for various reasons. Here, under-utilized refers to fish that are either directed to feed, waste, or other uses with low value from an economic or sustainability point of view. According to Kruijssen et al. (2020), loss in fish value chain may be caused by either physical, quality, nutritional, and market loss. Baltic herring in Finland may be considered as under-utilized as most of the catch is used as feed (Sarkki and Pihlajamäki, 2019). On the contrary, some fish are caught as by-catch, and therefore might be considered of low-value and treated only as waste. For instance, whitemouth croaker (*Micropogonias furnieri*) in Brazil is caught as by-catch of shrimp trawling, but there is currently no destined use for this by-catch (Rocha Camargo et al., 2021). Cyprinid fish are not common targets of commercial fishery, and their consumer awareness and demand is low (Dahlin et al., 2021). Though improvements have been seen in implementing more selective fishing gear, it was assessed that between 2010 and 2014, 9.1 million tonnes, 10.8% of the whole catch was discarded by global marine fisheries (Pérez Roda et al., 2019). According to the report, almost half of all discards were produced by bottom trawls, and highest discards were produced by fisheries targeting crustaceans. According to another estimation, approximately 20 million tonnes of global marine fisheries landings were destined for other purposes than direct human consumption, and most of this, approximately 90%, was food-grade or prime food grade (Cashion et al., 2017). Poor fishing practices and management procedures are the most common reasons behind the excessive amount of discards (Zeller et al., 2018). Improving utilization of fish currently destined for non-food and low-value use could in part help answer the growing demand of protein.

However, increasing the utilization of low-value fishes and their co-products is not without challenges. Fish may be considered "low-value" or "trash fish" due to having a low commercial value, low quality, small size, or lack of consumer preference (Tacon and Metian, 2009). For instance, the challenges in case of Baltic herring relate to its small size, susceptibility to oxidation, and low consumer acceptance. Baltic herring is commonly processed into fillets, but Baltic herring <17 cm in length are too small for commercial filleting and are mostly used as feed for farmed fish and fur animals. The high content of PUFAs (Aro et al., 2000) combined with a high content of dark muscle, known to be rich in pro-oxidants compared to white muscle (Park, 2013; Undeland et al., 1998), make Baltic herring susceptible to lipid oxidation, which poses a challenge for preserving its nutritional and sensory quality during storage. Baltic herring has a distinct aroma and flavor that are not preferred by many consumers. According to a study by (Pihlajamäki et al., 2019), 'bad taste' was the most common reason

for not consuming Baltic herring, among Finnish and Swedish consumers. Baltic herring is especially unpopular among younger people, which implies that novel products are needed to attract various consumer groups (Pihlajamäki et al., 2016). Dark muscle is also higher in lipids, lower in pH, higher in proteolytic activity, and higher in other sarcoplasmic prtoeins (in addition to heme proteins) (Park, 2013).

Fish is a highly perishable resource, and in addition to being prone to oxidation, microbial spoilage is an issue, emphasizing the need for maintaining low temperatures during transportation, storage, and processing. In addition, the presence of environmental contaminants may limit the use of certain fishes. In the Baltic Sea region, dioxins and polychlorinated bifenyls (PCBs) accumulate in the lipids of especially prey fish. The EU limit for dioxins, dioxins + dioxin-like PCBs, and non dioxin-like PCBs, are 3,5 pg/g, 6,5 pg/g, and 75 ng/g muscle (fresh weight), respectively (European Union, 2011). The content of these pollutants in Baltic herring have been higher than the limit set by EU, but have decreased by 80% over the past decades, and the levels in small (< 17 cm) Baltic herring have been found to be low (Airaksinen et al., 2014). According to a recent assessment, the health benefits of consuming small Baltic fish are greater than the risk posed by environmental pollutants (Tuomisto et al., 2020).

Despite the challenges, it is highly desirable or even required to increase the utilization of low-value fish and fish co-products. Potential ways to improve utilization include their fractionation to utilize fish proteins and lipids separately, as well as addition of antioxidants to limit oxidation during storage and processing and help maintain the quality.

2.2 Oxidation in fish

2.2.1 Factors effecting lipid oxidation in fish

Lipid oxidation is one of the most important factors influencing the quality of fish. In addition to degradation of sensory quality (further discussed in chapter 2.2.5) oxidation can be detrimental in terms of nutritional quality (Huang and Ahn, 2019). It is well established that the PUFAs in fish have several health benefits (Ruxton et al., 2007), but due to their unsaturated nature and susceptibility to oxidation they are easily destroyed. Oxidation can not only lead to loss of PUFAs, but ingestion of oxidized lipids can also give rise to unfavorable metabolic responses (Kubow, 1993; Turner et al., 2006). For example, oxidized fish oil was shown to have a less beneficial or even negative effect on cardiovascular disease markers compared to high-quality fish oil, despite having the same *n*-3 fatty acid concentration (Rundblad et al., 2017). In addition, primary and secondary lipid oxidation products can react with proteins,

leading to changes such as increased hydrophobicity and aggregation, which may further impact the functionality and bioavailability of the proteins (Hematyar et al., 2019).

Lipid content is not a driver of lipid oxidation, as lipid oxidation takes place even at a lipid content as low as 0.01% (Richards & Hultin, 2001). Susceptibility of different lipids to oxidation, however, varies. Highly unsaturated long-chain PUFAs are more susceptible to oxidation, as the number of bis allylic positions in the molecule have been shown to correlate with autoxidation rate (Cosgrove et al., 1987). However, there is some contradiction related to whether a high content of unsaturated lipids increases the rate of lipid oxidation. For instance, Aubourg (2001) reported that free fatty acids (FFAs) with high degree of unsaturation, compared to low degree of unsaturation, increased the rate of lipid oxidation more when added in cod liver oil. Wu et al. (2021d), on the other hand, reported that PLs promoted myoglobin mediated oxidation in washed pig muscle, regardless of whether the added PLs were from cod and had a high polyenoic index (282) or from the pig muscle and had a low polyenoic index (24). In a study by Richards et al. (2007), hemoglobin was more pro-oxidative in washed tilapia compared to washed cod muscle despite an almost 3-fold higher polyenoic index in the washed cod. However, suggested hydrolysis of PLs was hypothesized to provide an antioxidative effect in washed cod. Wu et al. (2022a) also found no correlation between lipid oxidation rate and quantity of lipid substrates (e.g. total lipids, PUFA, or long chain n-3 PUFA). Due to the presence of both pro-oxidants and antioxidants and interaction of various components in complex systems, such as the fish muscle, direct comparison of different systems in terms of lipid oxidation is challenging.

In the fish muscle, PLs are mostly present as membrane lipids and have a role in regulating the fluidity of the membranes. Compared to triacylgycerols that are storage fats present as inter- or intracellular fat droplets, PLs are thought to be more susceptible to oxidation due to their high degree of unsaturation, presence in membranes, close proximity to pro-oxidants, and large surface area (Erickson, 2002; Liang and Hultin, 2005a; Soyer and Hultin, 2000). PLs have been found to have both pro-oxidative and antioxidative effects in different systems, as reviewed by Cui and Decker (2016). The PUFAs in marine PLs are usually positioned in the sn-2 position, and different stability of PLs in different system is likely due to their different organization in these systems, such as bulk oil vs liposomes (Araseki et al., 2002). The accessibility of pro-oxidants to lipids may have a more determining effect on lipid oxidation than lipid class or degree of unsaturation (Wu et al., 2021d).

Lipases or extremely alkaline conditions induce hydrolysis of TAGs (Kim et al., 2016). Hydrolyzed lipids may be more susceptible to oxidation, and oxidized lipids may be more susceptible to hydrolysis by lipases (Erickson, 2002).

Lipolysis may have either an anti- or pro-oxidative effect on lipid oxidation (Tatiyaborworntham et al., 2022). FFAs with varying chain lengths and degrees of unsaturation were seen to increase oxidation when added in a commercial marine oil or white muscles of and hake and pout (Aubourg, 2001). On the contrary, addition of FFAs was seen to inhibit lipid oxidation in a washed turkey model, which was attributed to conversion of hemoglobin to hemichrome, which in turn was seen to eliminate the pro-oxidative effect of hemoglobin (Wu et al., 2021e). In addition, phospholipase A2, cleaving the fatty acid from the *sn*-2 position of PLs, has been reported to prevent lipid oxidation by trout hemoglobin, and the hydrolytic activity to be necessary for the antioxidant activity (Tatiyaborworntham et al., 2021).

In order to optimize utilization of fish co-products, investigations have been carried out to determine the fish parts most susceptible to oxidation. Belly flap, compared to skin and mince, was identified as having the highest rate of oxidation in silver carp (*Hypophthalmichthys molitrix*) (Kunyaboon et al., 2021). On the contrary, viscera + belly flap were identified as the most stable parts in herring, while head was the least stable towards oxidation (Wu et al., 2022a).

All processing of fish has a potential to promote oxidation. Salting (addition of NaCl) has been seen to increase lipid oxidation, due to increasing the activity of lipoxygenase (Guo et al., 2019). For instance, injection of salt prior to marinating Atlantic herring (*Clupea harengus*) fillets resulted in higher TBARS and lower α-tocopherol content compared to when fillets were only marinated (Sampels et al., 2010). Alteration of pH on the other hand has an effect on lipid oxidation due to its effect on heme proteins (Kristinsson and Hultin, 2004a; Maqsood and Benjakul, 2011a), as further discussed in chapter 2.2.3. Temperature is one of the most important factors influencing the rate of oxidation of lipids as well as proteins. Both storage (Nørrelykke et al., 2006) and cooking (Cropotova et al., 2019) temperature play an important role. Different cooking methods also show differences in promoting oxidation, and methods using milder temperatures, such as boiling or steaming have been shown to result in lower rates of lipid and protein oxidation (Hu et al., 2017). On the other hand, *sous vide* cooking European sea bass (*Dicentrarchus labrax*) fillets at 85 °C (20 min, inner temperature of fish 83 °C at the end) was seen to induce higher formation of oxidation derived volatiles compared to boiling (100 °C, 10 min, inner temperature of fish 88 °C at the end) (Nieva-Echevarría et al., 2017).

Lipid oxidation in fish can occur via different routes. Autoxidation in fish can be initiated by light, heat, presence of metal ions and radicals (Sampels, 2013). Transition metals, such as iron, are able to reduce molecular oxygen to form oxygen radicals (Welch et al., 2002). Along with endogenous pro-oxidants, the level of endogenous antioxidants, such as tocopherols, is a determining factor related to oxidation (Wu et al., 2022a). According to previous literature (Fu et

al., 2009; German et al., 1985; Medina et al., 1999; Richards and Hultin, 2002, 2001; Tolasa Yılmaz et al., 2018; Undeland et al., 1998), the most important oxidation routes in fish are hemeprotein and lipoxygenase mediated oxidation, and hence they will be further discussed in the following chapters.

2.2.2 Lipoxygenase mediated oxidation

Lipoxygenases, endogenous enzymes catalysing the inclusion of oxygen into unsaturated fatty acids, are present in various plants and animal tissues. They are metallo-oxides with iron in their active sites, and catalyze oxidation of PUFAs with cis-1,4-pentadiene structures (**Figure 2**) to form hydroperoxides (Schaich et al., 2013). The only major difference between metal and lipoxygenase catalyzed oxidation is the regio- and stereospecificity of the latter (Ghnimi et al., 2017).

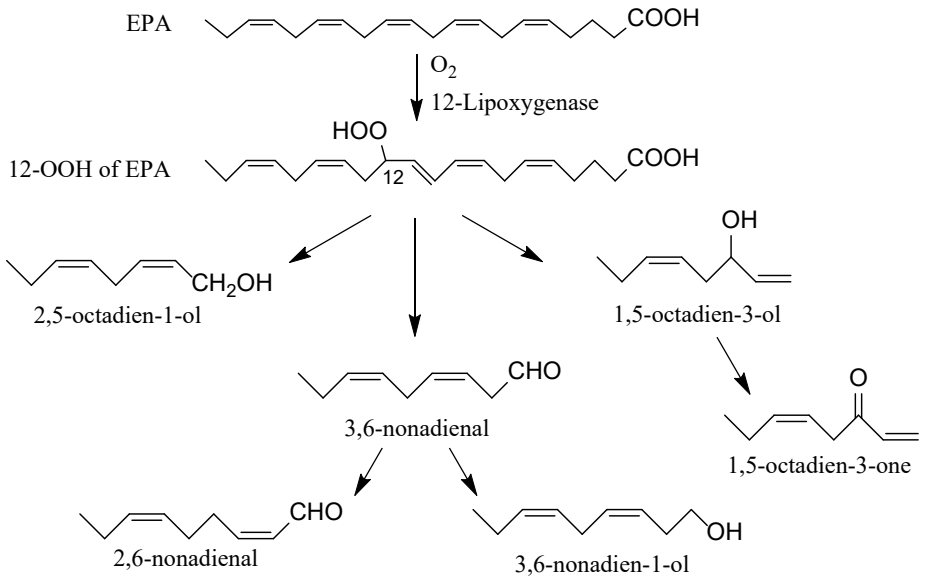

Figure 2. Oxidation of EPA via 12-lipoxygenase (adapted from Hsieh and Kinsella (1989) and Shahidi and Hossain (2022)).

In fish, 12- and 15-lipoxygenases, producing 12- and 15-hydroperoxides, respectively, are most important. Activity of lipoxygenase isoforms is species dependent. Metal ions can increase the activity of lipoxygenase (Samson and Stodolnik, 2001). Also sodium chloride has been seen to increase lipoxygenase activity at moderate concentrations, while high concentrations have an inhibitory effect (Jin et al., 2011). The redox state of the metal is essential for enzymatic activity. In the inactive state, iron is as Fe^{2+}, but hydroperoxides are able to activate the enzyme by oxidizing the iron to Fe^{3+}, which is again reduced as the

fatty acid ligand is oxidized (Ghnimi et al., 2017; Waller et al., 2008) Lipoxygenase alone is not sufficient to facilitate oxidation since no radicals are formed, but the hydroperoxides produced by it are decomposed by other oxidizing agents, such as light, heat, or metal ions. Especially in some conditions, lipoxygenase may lead to a high accumulation of hydroperoxides which can then lead to rapid oxidation once the hydroperoxides decompose and autoxidation takes over (Schaich et al., 2013).

Stodolnik and Samson (2000) studied the lipoxygenase activity of different Baltic herring tissues from four different catch times and three stages of gonad maturity. Lipoxygenase activity was highest in fins (96 nmol AM/mg), followed by muscle tissue (70), roe (67), skin (54), milt (54) and gills (34). Season and gonad maturity were seen to effect activity of lipoxygenase. They also studied the affinity of Baltic herring lipoxygenase against different substrates, of which lipoxygenase had the highest activity towards α-linolenic acid 18:3(*n*-3) (102 nmol AM/mg), followed by extracted Baltic herring muscle lipids (47), and linoleic acid (19). Wu et al. (2022a) reported that in sorted herring, lipoxygenase activity was almost 10 times higher in head compared to other parts, i.e. viscera, belly flap, fillet, backbone, and tail.

Storage temperature plays a key role in the activity of lipoxygenase. According to Tolasa Yılmaz et al. (2018), increase of fillet temperature from 0 to 10 °C significantly increased lipoxygenase activity in sardine mince. Also different preservation methods have an effect on the activity of lipoxygenase. Samson and Stodolnik (2001) studied the effect of freezing and salting on the lipoxygenase activity of Baltic herring muscle and roe. The enzyme retained 78 and 70% of its activity after 6 months of frozen storage in muscle and roe, respectively. Salting had either a catalyzing or inhibiting effect, depending on the salt type and concentration, as well as the tissue (muscle or roe).

2.2.3 Hemoglobin, myoglobin, and iron mediated oxidation

Heme proteins are responsible for oxygen transportation and storage and are thought to be the most significant endogenous pro-oxidants in fish. Hemoglobin is mostly found as a tetramer, each monomer containing one heme group, while myoglobin is a monomer. The heme consists of an iron ion inside a porfyrin ring formed by four pyrrol molecules (Maqsood et al., 2012). The protein part in hemoglobin and myoglobin has an important function in preventing the auto-oxidation of heme iron from Fe^{2+} to Fe^{3+}, since autoxidation of free hemin iron is much faster (Brantley et al., 1993). A significant amount of blood, and therefore hemoglobin, remains in the muscle tissue of fish after bleeding (Richards and Hultin, 2002). In fact, removal of blood has been shown to reduce

the release of non-heme iron and control lipid oxidation during storage (Harrysson et al., 2020; Maqsood and Benjakul, 2011b).

Several mechanisms for heme protein mediated oxidation have been suggested, but auto-oxidation of hemoglobin and myoglobin, i.e., oxidation of heme iron (**Figure 3**) is a crucial step regarding its pro-oxidativity. Autoxidation occurs via two mechanisms, depending on the availability of oxygen (Brantley et al., 1993). At high O_2 concentrations, oxyhemoglobin/oxymyoglobin dissociates to methemoglobin/metmyoglobin and a neutral superoxide radical ($HOO^{\cdot}$). This reaction requires H^+ and is therefore catalyzed by low pH. At low O_2 concentrations, deoxyhemoglobin reacts with free O_2 to produce methemoglobin and a superoxide anion radical ($O_2^{\cdot -}$), which is readily converted to hydrogen peroxide H_2O_2 (Aranda et al., 2009; Brantley et al., 1993; Richards et al., 2002). Methemoglobin may further react with H_2O_2 or preformed lipid hydroperoxides to create a prooxidative ferryl protein radical (Aranda et al., 2009). Released iron can catalyze the breakdown of lipid hydroperoxides leading to production of alkoxyl radicals (Maqsood et al., 2012).

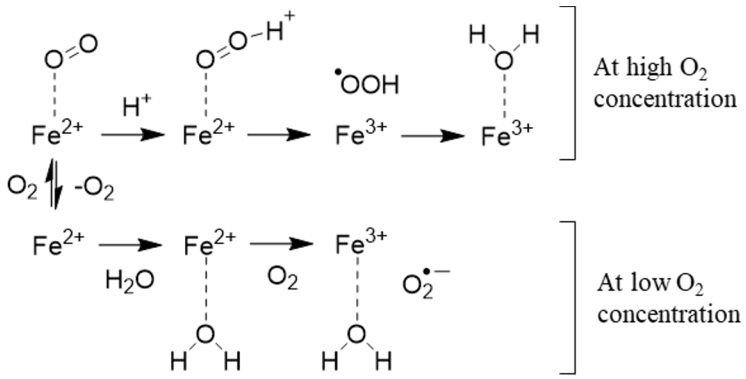

Figure 3. Autoxidation of heme iron (adapted from Aranda et al. (2009)).

Hemoglobin and myoglobin have been seen to exhibit pseudolipoxygenase activity (Kühn et al., 1981; Rao et al., 1994). For instance, myoglobin from sperm whale oxidized linoleic acid in the heme pocket (crevice) by ferryl oxygen and not the protein radical (Rao et al., 1994). In a study by Grunwald and Richards (2006) hemin release was shown to drive oxidation, whereas hemin degradation and iron release had an opposite effect. After death, heme iron is mostly in the ferrous Fe^{2+} state, but due to the postmortem changes auto-oxidation is accelerated. The decrease in oxygenation of heme proteins due to decreasing pH is called the Bohr effect, whereas a further decrease in oxygenation below pH 6.5 is called the Root effect (Richards et al., 2002). The pH has a determining role in the pro-oxidativity of hemoglobin. In addition to

the decrease in oxygenation, a low pH can promote hemin release, autoxidation, solubility of released iron, and H_2O_2 formation from the superoxide anion radical (Maqsood et al., 2012).

The reason behind the high susceptibility of fish to lipid oxidation compared to other muscles may in part be due to differences in heme proteins. Despite high sequence homology of avian, mammalian and fish hemoglobins, the last have been seen to autooxidize much faster (Jensen, 2001; Richards and Dettmann, 2003). Compared to bovine hemoglobin, perch and trout hemoglobin have been found to autooxidize 30–80 fold more rapidly (Aranda et al., 2009). Further, biological, genetical, and environmental differences may play a role in pro-oxidative differences observed between fish species (Maqsood et al., 2012; Undeland et al., 2004). For instance, autoxidation of hemoglobin from cold-water species has been found to be faster compared to those species adapted to warmer temperature (Maqsood and Benjakul, 2011a; Wilson and Knowles, 1987). Richards et al. (2007) observed that trout (cold water fish) Hb promoted more lipid oxidation compared to tilapia (warm water fish) Hb. The findings of the study indicated that trout Hb exhibited a stronger Root effect (decrease in oxygen affinity at decreased pH) at pH 6.3. At pH 7.4 oxygenation of both Hbs was at a similar level, but trout Hb auto-oxidized more and promoted more lipid oxidation compared to tilapia Hb. The results indicated that trout Hb had a more flexible structure that tilapia Hb, which was likely the reason for the differences in pro-oxidativity.

Also the content of heme proteins varies between fish species (Thiansilakul et al., 2010; Wu et al., 2021a). Hemoglobin content is higher in dark muscle compared to light muscle (Undeland et al., 1998). Hemoglobin content was also shown to vary between spring and fall in herring fillets and tail, and non-heme iron content was significantly higher in the head of spring fish compared to fall herring (approx. 40 vs <10 mg/kg) (Wu et al., 2022a). The hemoglobin or myoglobin contents in Baltic herring muscle have not been reported.

2.2.4 Connection between lipid and protein oxidation

Not only lipid, but also protein oxidation may be detrimental to the quality of fish, since oxidative damage induces changes in protein conformation, functionality, solubility, color, enzymatic activity, and changes in nutritive value and bioavailability (Zhang et al., 2013). Unlike lipid oxidation, protein oxidation has not been studied as extensively as it is more challenging to measure. The reaction products are even more diverse due to a higher amount of reactive targets (Hematyar et al., 2019). Lipid oxidation and protein oxidation, both driven by transition metals and formation of oxygen radicals, can be provoked by one another or occur independently, but often they are somehow connected.

Transition metals, such as iron, may bind to metal-binding sites in the protein and produce oxygen radicals that react with amino acid side chains to produce carbonyl derivatives, among other modifications (Stadtman, 1990). Besides modification of amino acid residues, oxidative damage to proteins induce their cross-linkage and increase their susceptibility to proteolytic enzymes (Stadtman, 1990). Both primary and secondary oxidation products of lipids may react with proteins (Viljanen et al., 2004). Protein oxidation in biological systems, such as muscles, may occur more rapidly than lipid oxidation, since proteins are in the aqueous phase where many radicals are formed (Soyer and Hultin, 2000). Proteins may also "compete" for formed radicals with membrane lipids and therefore provide an antioxidative effect from the perspective of lipids (Soyer and Hultin, 2000). Vice versa, PUFAs have been seen to reduce oxidative damage to proteins (Méndez et al., 2013). Some proteins are more susceptible to oxidation (Nørrelykke et al., 2006), and certain amino acid residues, such as proline, histidine, arginine, lysine, and cysteine are most sensitive to oxidative damage (Stadtman, 1990).

As in the case of lipid oxidation, temperature is an important factor in the development of protein oxidation. Nørrelykke et al. (2006) showed that rainbow trout muscle stored at −20 °C vs. had a protein carbonyl content of approx. 4.5 mmol carbonyls/kg protein, which was twice the amount observed in samples stored at lower temperatures (−30 or −80 °C). PV also increased faster at the higher temperature; after 2 years of storage at −20 °C, −30 and −80 °C, PV was 12.2, 1.8, and 0.1 mequivalents/kg oil, respectively. Hu et al. (2017) investigated the effect of different cooking methods on protein and lipid oxidation in sturgeon (*Acipenser gueldenstaedtii*) fillets, and observed that all cooking methods, but especially roasting and frying, induced protein oxidation indicated by increase carbonylation, formation of Schiff's bases, and decrease in free thiols, and was characterized by oxidative damage to aromatic amino acids and lysine. TBARS content, however, was lower in fried fillets, compared to the uncooked samples, which indicated their further reaction with other compounds. A closer analysis of modified proteins revealed that 4-hydroxynonenal (HNE) -modified and malondialdehyde (MDA) -modified peptides were present in all cooked fillets, clearly demonstrating the connection between protein and lipid oxidation. Most studies have also reported simultaneous inhibition of lipid and protein oxidation by addition of antioxidants (Farvin et al., 2012; Özalp Özen and Soyer, 2018; Viljanen et al., 2004).

2.2.5 Formation of secondary volatile oxidation compounds and their impact on odor and flavor of fish

Secondary volatile oxidation products are formed during decomposition of hydroperoxides (Gómez-Cortés et al., 2015). They have been widely used as indicators of fish freshness (Duflos et al., 2006) and lipid oxidation (Kunyaboon et al., 2021), due to their correlation with oxidation derived off-odors (Fu et al., 2009; Jónsdóttir et al., 2007; Venkateshwarlu et al., 2004a). Hydroperoxides are decomposed by multiple pathways to form numerous volatile and non-volatile compounds. Unsaturated aldehydes may also further react with other compounds, and secondary non-volatile oxidation compounds may further decompose to form new volatile compounds (Frankel, 1983). For instance, (*Z*)-4-heptenal is produced from (*E,Z*)-2,6-nonadienal through water addition to form 3-hydroxy-(*Z*)-6-nonenal, which is then in turn transformed through retro-aldol condensation to (*Z*)-4-heptenal (Josephson and Lindsay, 1987) (**Figure 4**). Propanal and hexanal, as products of *n*-3 and *n*-6 fatty acids, respectively, have been widely used as oxidation indicators due to their stability (lack of double bonds) and especially hexanal usually shows high formation compared to some other oxidation products (Barriuso et al., 2013). Hexanal is produced from the 13-hydroperoxide (13-OOH) of linoleic acid (18:2*n*-6) (Frankel, 1983), and has been shown to be a good indicator of lipid oxidation in fish (Alghazeer et al., 2008).

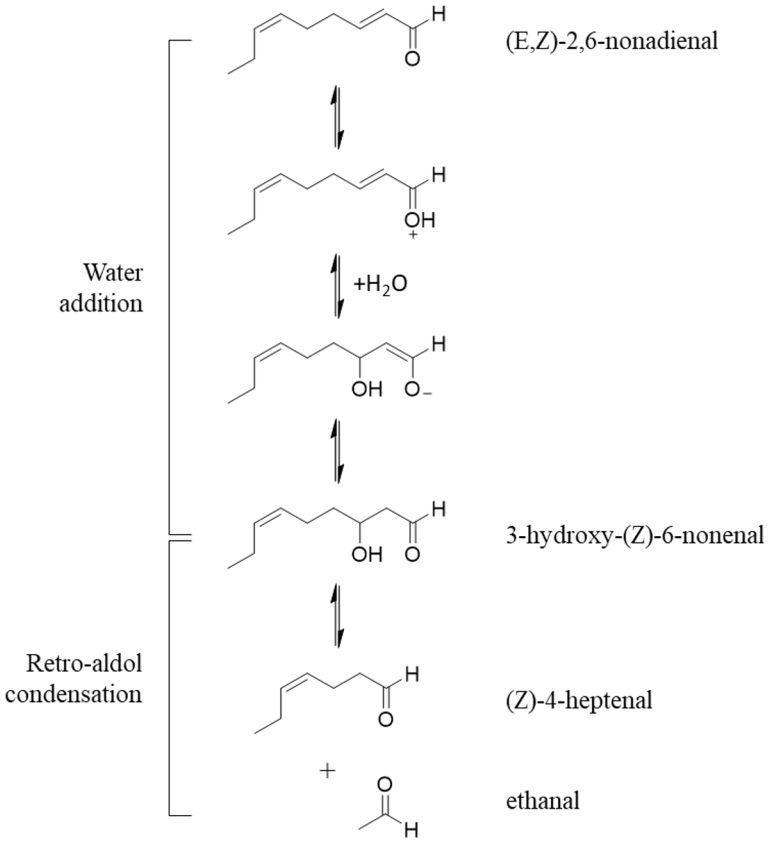

Figure 4. Formation of (*Z*)-4-heptenal from (*E,Z*)-2,6-nonadienal, adapted from Josephson and Lindsay (1987).

According to Lindsay (1990) the classes of fish flavors can be classified to six categories 1) very fresh fish and seafood-like, 2) oxidized, stale, and stored flavors, 3) spoiled and putrid, 4) species-related characterizing flavors, 5) derived or processing flavors, and 6) environmentally derived. Although some microbial-derived compounds such as geosmin and 2-methylisoborneol or trimethylamine contribute to earthy (Frank et al., 2009; Liu et al., 2017; Phetsang et al., 2021a) and fishy, ammonia-like, crab-like, or fishhouse-like odors (Lindsay, 1990; Wu et al., 2014), respectively, majority of the odor and flavor compounds in fish are lipid oxidation derived. Fresh fish is found palatable by consumers, but the loss of freshness and development of fishiness often leads to rejection (Lindsay, 1990). Human senses may detect oxidation derived compounds when their concentrations are too low for instrumental measurements. For instance, off-odors have been detected in fish oil with extremely low PV (Hamilton et al., 1998).

The term “rancid” is used in many foods to describe off-odors or flavors formed due to lipid oxidation. However, since various secondary oxidation products with sensory significance may be formed depending on the food,

"rancid" may present a different sensory perception in different products (Jacobsen, 1999). Also, oxidation reactions are influenced by the processing and storage conditions, which leads to variation in the development of rancidity within the same fish raw material (Refsgaard et al., 1998). Some sensory attributes used to describe fish off-odors and off-flavors formed due to lipid oxidation are presented in **Table 1**.

Thiansilakul et al. (2010) reported that there was a prominent increase in fishy, and to some extent also rancid odor, in sea bass and red tilapia during 15 days of storage on ice. Sea bass showed higher fishy and rancid odor scores, which was in line with higher formation of TBARS in sea bass compared to red tilapia. In another study, bleeding was shown to decrease fishy odor formation, PV and TBARS in sea bass stored on ice for 15 days (Maqsood and Benjakul, 2011b). Farmed hybrid catfish was shown to develop fishy, rancid and overall off-odor intensity, which correlated with volatile lipid oxidation products, but also trimethylamine and total volatile base-nitrogen (TVB-N). Wen et al. (2023) characterized oxidized fish oil odors as rancid, fishy, grassy, painty, and metallic, and of these attributes, fishy odor increased fastest and most during oxidation. Painty odor was also used as an indicator of oxidation in a washed cod mince with added hemoglobin from different species (Undeland et al., 2004).

Table 1. Sensory attributes used to describe oxidation related off-odors and off-flavors in fish and fish products.

Descriptor	Definition	Fish or fish product	Reference
Odor			
Rancid	Oxidized fish oil, painty	frozen herring	Hyldig et al. (2012)
	n.d.	herring, salmon, and cod protein isolates	Abdollahi and Undeland (2018)
	Rancid odor	Atlantic mackerel fillet	Sveinsdóttir et al. (2020)
	n.d.	farmed hybrid catfish muscle	Phetsang et al. (2021a)
	n.d.	washed cod mince	Jónsdóttir et al. (2007)
	An aromatic reminiscent of odor or flavor of highly oxidized oils containing high amounts of linoleic acid such as sunflower, cottonseed, or peanut	oxidized fish oils	Wen et al. (2023)
Fishy	Odor associated with the chopped silver carp mince stand at 25 °C for 0.5 h	washed silver carp mince, silver carp slices	Fu et al. (2009), Fu et al. (2015)
	n.d.	fish oils and microencapsulated fish oils	Serfert et al. (2010)
	n.d.	Asian sea bass muscle	Maqsood and Benjakul (2011a), Maqsood and Benjakul (2011b)
	n.d.	fish protein hydrolysate	Yarnpakdee et al. (2012c)
	n.d.	sea bass skin	Sae-leaw and Benjakul (2014)
	n.d.	farmed hybrid catfish muscle	Phetsang et al. (2021a)
	An aromatic reminiscent of cod liver oil	oxidized fish oils	Wen et al. (2023)
Fish oil	n.d.	herring, salmon, and cod protein isolates	Abdollahi and Undeland (2018)
Oxidized oil	Odor associated with rancid pork fat	washed silver carp mince, silver carp slices	Fu et al. (2009), Fu et al. (2015)

Descriptor	Definition	Fish or fish product	Reference
Painty	n.d.	washed cod mince with added hemoglobin from different species	(Undeland et al., 2004)
	An aromatic reminiscent of oils containing linolenic acid such as linseed or rapeseed (canola) oil.	oxidized fish oils	Wen et al. (2023)
Grassy/green	Odor associated with fresh grass	washed silver carp mince	Fu et al. (2009)
	n.d.	fish oils and microencapsulated fish oils	Serfert et al. (2010)
	An aromatic reminiscent of the green character of mowed grass	oxidized fish oils	Wen et al. (2023)
Train-like	n.d.	boiled cod	Milo and Grosch (1995)
Metallic	n.d.	fish oils and microencapsulated fish oils	Serfert et al. (2010)
	An aromatic associated with metal coins.	oxidized fish oils	Wen et al. (2023)
Pungent	n.d.	fish oils and microencapsulated fish oils	Serfert et al. (2010)
Frying	Characteristic aroma of oil during frying	oxidized fish oils	Wen et al. (2023)
Flavor			
Rancid	Oxidized fish oil, painty	frozen herring	Hyldig et al. (2012)
	n.d.	herring, salmon, and cod protein isolates	Abdollahi and Undeland (2018)
Fishy	n.d.	fish protein hydrolysate	Yarnpakdee et al. (2012c)
Fish oil	n.d.	herring, salmon, and cod protein isolates	Abdollahi and Undeland (2018)
Train oil	n.d.	farmed Atlantic salmon	Refsgaard et al. (1998)
Metallic	n.d.	farmed Atlantic salmon	Refsgaard et al. (1998)

n.d. = not defined

Lipid oxidation is a complex combination of different reactions and may thus lead to highly diverse sensory outputs depending on the conditions and precursors (fatty acids) present. Some volatile compounds associated with off-odors in fish are shown in **Table 2**. Hammer and Schieberle (2013) investigated the degradation products of n-3 fatty acids, formed via to autoxidation by copper, or lipoxygenase action. The autoxidation products of EPA were found intensely fishy, with metallic, fatty, and pungent notes, and the most significant volatile compounds contributing to these odors were (*Z*)-1,5-octadien-3-one (flavor dilution, FD≥8192), trans-4,5-epoxy-(*E,Z*)-2,7-decadienal (FD≥8192), (*Z*)-3-hexenal (FD=1024), (*Z,Z*)-2,5-octadienal (FD=1024), (*Z,Z*)-3,6-nonadienal (FD=1024), butanoic acid (FD= 1024), and (*E,E,E*)-2,4,7-decatrienal (FD=1024). Compared to autoxidation, the distillate of lipoxygenase-oxidized EPA was found less fishy and more pungent and metallic, with volatiles such as 1-penten-3-one (FD≥8192) and (*Z*)-3-hexenal (FD≥8192) being more pronounced. Likewise, autoxidation of DHA produced a more intense pungent odor and slightly less fishy odor compared to autoxidation of EPA. However, even in fish oil, several other fatty acids than EPA and DHA exist and their degradation products contribute to formation of off-odors (Wen et al., 2023). When taking into account the number of different precursors and oxidizing agents present in fish, as well as different storage or processing conditions, it can be considered that a vast range of volatile combinations can be produced during lipid oxidation of fish. It has also been shown that the diet of fish strongly influences the volatile profile and sensory properties, due to influencing that fatty acid composition of the muscle (Sérot et al., 2002).

Fu et al. (2009) investigated the effect of added hemoglobin or lipoxygenase on the off-odor formation of washed silver carp mince. Lipoxygenase produced a prominent fishy odor, whereas hemoglobin addition gave rise to a strong oxidated oil odor. Nonanal and hexanal had a higher concentration in the hemoglobin treated mince and were suggested to contribute to the oxidized oil odor, whereas (*E,E*)-2,4-heptadienal was suggested to contribute to the fishy odor in lipoxygenase treated mince.

Table 2. Secondary volatile oxidation compounds that have been identified as most significant odorants in fish and/or associated with lipid oxidation derived fishy off-odors.

Compound	Description	Associated with	Reference(s)
Alcohols			
(*E*)-2-penten-1-ol	mushroom, raw fish, metallic	fishy flavor	Sérot et al. (2002)[a], Frank et al. (2009)[a]
(*Z*)-2-penten-1-ol	musty, compost-like		Hartvigsen et al. (2000)[a]
(*E*)-2-hexen-1-ol	moss, green		Sérot et al. (2002)[a]
1-octen-3-ol	pungent, soil, fruity		Hartvigsen et al. (2000)[a], Jiarpinijnun et al. (2022)[b]
(*E,Z*)-3,6-nonadien-1-ol	plastic	fishy odor	Frank et al. (2009)[a]
Aldehydes			
propanal	sweet		Milo and Grosch (1995)[a], Milo and Grosch (1996)[b]
(*E*)-2-pentenal	green, grass		Sérot et al. (2002)[a]
hexanal	grassy	oxidized oil odor, rancid odor, train-like odor	Hartvigsen et al. (2000)[a], Jiarpinijnun et al. (2022)[b], Milo and Grosch (1996)[b], Fu et al. (2009)[a], Jónsdóttir et al. (2007)[a]
(*Z*)-3-hexenal	sour, old cheese, green	train-like odor	Milo and Grosch (1995)[a]
heptanal	green, floral	Seawater flavor	Selli et al. (2006)[a], Frank et al. (2009)[a]
(*Z*)-4-heptenal	boiled potato, fishy, sweet, biscuit-like, fatty-fishy, fish oil	rancid odor, train-like odor, fishy odor, fishy flavor, fishy aftertaste, pungent odor	Hartvigsen et al. (2000)[a], Milo and Grosch (1995)[a], Jónsdóttir et al. (2007)[a], Venkateshwarlu et al. (2004a)[c], Triqui (2006)[a], Triqui and Bouchriti (2003)[a], Frank et al. (2009)[a]
2,4-heptadienal	sweet, citrus	rancid odor	
(*E,E*)-2,4-heptadienal	fishy, green, cucumber, green, fatty	fishy odor, rancid odor, fishy flavor	Hartvigsen et al. (2000)[a], Sérot et al. (2002)[a], Fu et al. (2009)[a], Venkateshwarlu et al. (2004a)[c]

Compound	Description	Associated with	Reference(s)
(*E,Z*)-2,4-heptadienal	fishy, fatty, burnt, savory	fishy aftertaste	Hartvigsen et al. (2000)[a], Frank et al. (2009)[a]
octanal	sweet, orange, floral	seawater odor	Frank et al. (2009)[a]
(*E*)-2-octenal	savory, fatty	fishy flavor	Frank et al. (2009)[a]
(*E,E*)-2,4-octadienal	sweet, cucumber	fishy odor	Frank et al. (2009)[a]
nonanal	oxidated oil, green, floral, fatty, grassy	oxidized oil odor	Fu et al. (2009)[a], Selli et al. (2006)[a], Mahmoud and Buettner (2017)[a]
(*E*)-2-nonenal		rancid odor	Triqui and Bouchriti (2003)[d]
(*E,Z*)-2,6-nonadienal	cucumber, floral	train-like odor, fishy odor, metallic odor, fishy flavor, metallic flavor, seawater flavor	Venkateshwarlu et al. (2004a)[c], Frank et al. (2009)[a]
(*E,E*)-2,6-nonadienal	cucumber-like		Mahmoud and Buettner (2017)[a]
(*E,E*)-3,6-nonadienal	fatty, green	train-like odor	Milo and Grosch (1995)[a], Milo and Grosch (1996)[b]
(*E,E*)-2,4-decadienal	cucumber, green, plastic	low sensory score	Selli et al. (2006)[a], Mahmoud and Buettner (2017)[a]
(*E,Z*)-2,4-decadienal	fatty, green, plastic	fishy odor	Mahmoud and Buettner (2017)[a], Frank et al. (2009)[a]
Ketones			
2,3-butanedione	Buttery, sweet, caramel	prawn flavor, prawn odor, prawn aftertaste	Milo and Grosch (1995)[a], (Frank et al., 2009)[a]
1-penten-3-one	pungent, rancid, green, glue	fishy odor, metallic odor, fishy flavor, metallic flavor	Venkateshwarlu et al. (2004a)[c]
2,3-pentanedione	caramel	fishy odor	Frank et al. (2009)[a]
1-octen-3-one	mushroom-like	train-like odor	Milo and Grosch (1995)[a]

Compound	Description	Associated with	Reference(s)
(*Z*)-1,5-octadien-3-one	geranium-like, metallic	fishy, train-like odor, pungent odor	Hartvigsen et al. (2000)[a], Milo and Grosch (1995)[a], Triqui (2006)[a], Triqui and Bouchriti (2003)[a], Hammer and Schieberle (2013)[a]
(*E,Z*)-3,5-octadien-2one		fishy flavor	Frank et al. (2009)[a]
(*E,E*)-3,5-octadien-2one		fishy odor	Frank et al. (2009)[a]
2-undecanone	sweet, peach-like		Mahmoud and Buettner (2017)[a]
Others			
(*E*)-4,5-epoxy-(*E*)-2-decenal	metallic		Mahmoud and Buettner (2017)[a]
(*E*)-4,5-epoxy-(*E,Z*)-2,7-decadienal	metallic	fishy	Hammer and Schieberle (2013)[a]

[a]Odor significance determined based on GC-O

[b]Odor significance determined based on OAVs

[c]Odor significance determined based on recombinant studies

[d]Odor significance determined only based on correlation with sensory data

Milo and Grosch (1995) observed that trout mince stored for 26 weeks at –13 °C prior to boiling, exhibited a strong train-like odor, which correlated with a higher intensities of (*Z*)-1,5-octadien-3-one, 1-octen-3-one, hexanal, (*Z*)-3-hexenal, (*Z*)-heptenal, (*Z,Z*)-3,6-nonadienal, and (*Z,Z*)-2,6-nonadienal compared to trout mince stored at –60 °C. (*Z*)-1,5-octadien-3-one seems to be one of the most potent volatiles in fish and fish-oil emulsions (Hartvigsen et al., 2000; Milo and Grosch, 1996; Triqui, 2006; Venkateshwarlu et al., 2004b), and due to its low threshold is usually observed already when the fish is still fresh (Triqui, 2006).

(*Z*)-4-heptenal has been suggested as one of the most significant volatiles contributing to fishy odor (Hartvigsen et al., 2000; McGill et al., 1977, 1974). On the other hand, when hybrid catfish muscle was stored refrigerated for 15 days, the concentration of (*Z*)-4-heptenal remained relatively constant, but a prominent increase in fishy odor was observed (Phetsang et al., 2021a). It is likely that other volatile compounds modify the fishy odor or flavor induced by (*Z*)-4-heptenal. For example, Venkateshwarlu et al. (2004b) used sensory analysis and different multivariate models to investigate the contribution of (*E,Z*)-2,6-nonadienal, 1-penten-3-one, (*Z*)-4-heptenal, and (*E,E*)-2,4-heptadienal to fishy and metallic off-odors and off-flavors in a milk system. The former two were seen as most important for both off-flavors but had significant interactions with (*Z*)-4-heptenal. (*E,Z*)-2,6-nonadienal, which is also a precursor of (*Z*)-4-heptenal, showed a synergistic effect with (*Z*)-4-heptenal on fishy flavor. Presence of all four volatiles was needed to reach the maximal intensity of fishy odor and flavor. On the other hand, Triqui (2006) found that (*E,Z*)-2,6-nonadienal, which as its own is described as cucumber-like, was associated with the fresh odor of hake. During storage, its odor intensity decreased, likely due to its conversion to (*Z*)-4-heptenal. Interestingly, (*Z*)-4-heptenal and (*E,E*)-2,4-heptadienal were seen to have compensating effects on fishy odor and fishy flavor – low or high concentration of both volatiles simultaneously resulted in a lower intensity compared to having one low and the other one at high concentration (Venkateshwarlu et al., 2004a).

In addition to contributing to fishy and rancid odors, lipid oxidation products such as hexanal or 1-octen-3-ol may enhance the earthy and muddy odors, common to freshwater fish and caused by 2-methylisoborneol and geosmin (Liu et al., 2017). Based on previous literature, it can be concluded that lipid oxidation derived off-odors, such as fishy odor, are never a result of one volatile, but a combination of several volatiles (Venkateshwarlu et al., 2004a). It is challenging to estimate the contribution of individual volatiles on fish off-odors based on concentration only, since they may have somewhat unexpected interactions with each other (Venkateshwarlu et al., 2004a). However, some volatiles, such as (*Z*)-1,5-octadien-3-one, (*E,Z*)-2,6-nonadienal, (*Z*)-4-heptenal, and (*E,E*)-2,4-

heptadienal have been identified in most studies and are likely key odorants contributing to fishy odors.

2.3 Strategies to improve utilization of low-value fish

2.3.1 Natural antioxidants

Since lipid oxidation is a significant factor influencing the quality of fish, and the sensory quality can in many cases be a factor limiting utilization, use of antioxidants might provide beneficial effects in terms of increasing the food use of under-utilized fish. In addition, when it comes to utilization of fish side streams, that may contain even more pro-oxidants than the fillet (Wu et al., 2022a), limiting oxidation is of even higher importance. In addition, many technologies aiming to improve utilization of low-value fish, such as the pH-shift or enzymatic hydrolysis, may further accelerate oxidation, which in turn emphasizes the need for antioxidants.

Antioxidants can be classified as primary, i.e. type 1 antioxidants, and secondary, type 2 antioxidants, according to their mechanism of action (Lorenzo et al., 2018). Primary, i.e. chain-breaking antioxidants convert radicals into stable molecules and thus help prevent iniation or interrupt propagation. Secondary, i.e. preventing antioxidants act in various ways to bind or deactivate pro-oxidants, or provide synergy for primary antioxidants (Lorenzo et al., 2018). Some key factors affecting the efficiency of antioxidants are their solubility, reducing potential, chelation ability, stability, as well as the pH of the media/matrix, and most of these may also determine whether the their effect is antioxidative of pro-oxidative (Decker, 1997).

Plant-based compounds with antioxidative activity can be present in any plant part, e.g. grains (Adom and Liu, 2002), fruits (Ganhão et al., 2013; Määttä-Riihinen et al., 2005; Zheng and Wang, 2003), nuts (Pycia et al., 2019), seeds (Sancho et al., 2011; Vuorela et al., 2005), leaves (Capecka et al., 2005; Sancho et al., 2011), roots and tubers (Kähkönen et al., 1999; Mattje et al., 2019), arils (Kulkarni and Aradhya, 2005), and barks (Vuorela et al., 2005), and are usually phenolic compounds such as tocopherols, flavonoids, and phenolic acids (Kumar et al., 2015). For instance, lingonberry and cranberry catechins and procyanidins have been seen to be efficient radical scavengers (Määttä-Riihinen et al., 2005). The antioxidative effect of sea buckthorn berry residue in mechanically deboned chicken and turkey was mainly attributed to flavonols (Püssa et al., 2008). Anthocyanins were postulated to account for the high radical scavenging activity of black currant press residue extract (Puganen et al., 2018). Quercetin has been shown to inhibit 12-lipoxygenase (Hsieh et al., 1988) as well as act as an effective hydroxyl radical scavenger and reduce metHB formation (Wu et al.,

2022b). Quercetin was also suggested to contribute most to inhibition of Hb-mediated lipid oxidation by cranberry polyphenols in washed cod muscle (Lee et al., 2006). However, synergy among different compounds present in natural sources is likely to have a strong impact on the antioxidative effect (Capecka et al., 2005). Most commonly, a high total content of phenolics is associated with high antioxidativity measured *in vitro* (Puganen et al., 2018; Wojdyło et al., 2007). Investigation of suitable doses is important, since too high concentrations might result in pro-oxidative effects (Alghazeer et al., 2008; Silveira Alexandre et al., 2022).

Several natural and synthetic antioxidants have been investigated on their efficacy to inhibit lipid oxidation in fish, other muscle foods, fish oil (Sekhon-Loodu et al., 2013), or fish oil emulsions (Farvin and Jacobsen, 2015; Let et al., 2005; M. Pazos et al., 2005a). Most of the synthetic antioxidants used in the food, such as synthethic phenolic antioxidants butylhydroxytoluene E321 (BHT), butylated hydroxyanisole E320 (BHA), tertiary butylhydroquinone E319 (TBHQ), and their metabolites, may have several negative health implications, especially if misused (Xu et al., 2021). Due to increased concerns related to synthetic antioxidants and additives in general, as well as consumer demand for "naturalness", natural sources have been the focus of antioxidant research during the past two decades (Babakhani et al., 2016; Özalp Özen and Soyer, 2018; M. Pazos et al., 2005a; Sánchez-Alonso et al., 2007). **Table 3** presents studies where natural antioxidants were investigated during refrigerated or frozen storage of fish. Most of the literature has focused on small pelagic fish species such as mackerels and herring. Most common strategy for antioxidant inclusion in previous studies has been their addition into mince (Babakhani et al., 2016; Joaquin et al., 2008; Özalp Özen and Soyer, 2018; M. Pazos et al., 2005a; Tarvainen et al., 2016), although marinating (Cropotova et al., 2019; Sampels et al., 2010; Tarvainen et al., 2015), brining (Shi et al., 2014), and dipping (Chaijan et al., 2020; Sveinsdóttir et al., 2020; Wu et al., 2021b) treatments have also been studied.

While most of the research has focused on plant-based antioxidants, also animal-based antioxidants have been studied. For instance, milk protein concentrate was seen to reduce the activity of lipoxygenase and decrease TBARS formation in sardine mince (Tolasa Yılmaz et al., 2018). Milk protein concentrate also reduced lipid oxidation and fishy odor formation in herring mince during frozen storage (Joaquin et al., 2008). Chaijan et al. (2020) studied the protective impact of coating sea bass steaks with mixtures of whey protein isolate (WPI) and extracts of green tea, lemongrass, and ginger. The coating with any of the extracts or only WPI retarded lipid and protein oxidation and prevented decrease in odor likeness score during storage at 4 °C, but the WPI +

ginger extract was the most efficient treatment, especially in terms of reducing protein carbonyl formation.

Vinification and juice manufacturing produce large quantities of co-products that have been investigated as antioxidants. Grape antioxidant dietary fiber significantly delayed lipid oxidation in minced horse mackerel during first 3 months of frozen storage (Sánchez-Alonso et al., 2007). Pazos et al. (2005a) studied different polyphenol fractions of grape pomace (after pressing and maceration) as antioxidants in fish oil, fish oil-in-water emulsions, and frozen minced mackerel muscle. Out of the tested fractions, flavanol oligomers (proanthocyanidins) with intermediate degree of polymerization were most efficient in delaying lipid oxidation of frozen minced muscle and fish oil-in-water emulsions. Grape proanthocyanidins were also shown to protect washed horse mackerel mince from hemoglobin mediated oxidation (Maestre et al., 2009).

It is important to assess the effect of antioxidants on sensory quality as well, as in some cases the improvements observed in instrumental measurements such as PV or TBARS are not reflected in the sensory quality (Hamilton et al., 1998; Harrysson et al., 2020; Sveinsdóttir et al., 2020). In a study by Hamilton et al. (1998), refined fish oil with 2% δ-tocopherol, 0.1% ascorbyl palmitate, and 0.5% lecithin added showed no increase in PV when stored at 20°C over a period of 6 months, but their antioxidant effect did not improve flavor stability, and off-flavors developed already within 3 weeks. On the contrary, dipping Atlantic mackerel fillets into a sodium erythorbate solution increased the shelf-life of frozen fillets from less than 2.5 months (control) to up to 15 months according to sensory analysis, while differences in PV and TBARS between dipped and control fillets were less clear (Sveinsdóttir et al., 2020). On the other hand, antioxidants may influence the sensory quality in a negative way and reduce consumer acceptance (Mattje et al., 2019). Polyphenols may be perceived as bitter or astringent (Ares et al., 2009), and may react with other compounds during processing, resulting in changes in odor, flavor and color (Han et al., 2022). Plant-based extracts or ingredients with antioxidant activity also contain other compounds with odor and flavor activity which may be a challenge in terms of their incorporation into food. For instance, addition of ginger essential oil or ginger supercritical CO_2 extract had a negative impact on the liking of tilapia fish burger, due to the strong ginger flavor (Mattje et al., 2019).

Table 3. Studies evaluating the antioxidative effect of natural antioxidants during frozen or refridgerated storage of fish.

Fish	Storage conditions	Antioxidant(s)	Way of addition	Main outcomes	Reference
Atlantic mackerel (*Scomber scombrus*), minced white muscle	−10 °C, 6 months	Various phenolic fractions, extracted from grape (Vitis vinifera) press residue, and synthetic propyl gallate, 0.01% w/w	Mixed into mince	All antioxidants: TBA index, peroxides, conjugated dienes & trienes, aldehydes, fluorescent compounds ↓ vs control Total extract and propyl gallate: 22:6 n−3 ↑ vs control Propyl gallate showed lowest amount and highest induction period for most oxidation products	Pazos et al. (2005a)
Atlantic mackerel (*Scomber scombrus*), minced white muscle and horse mackerel (*Trauchurus trauchurus*), minced fillets with skin	−10 °C, 138 d	Phenolic extract from grape (Vitis vinifera) press residue (OW), a purified fraction of procyanidins (IV), and synthetic propyl gallate. 0.01% w/w	Mixed into mince	Antioxidants inhibited the depletion of endogenous antioxidants, α-tocopherol depletion followed the order control>OW>IV>propyl gallate The depletion of α-tocopherol was highly correlated with the increase in peroxides and TBARS	Pazos et al. (2005b)
Horse mackerel (Trachurus trachurus), minced	−20 °C, 180 d	Grape antioxidant dietary fiber, 2% or 4%	Mixed into mince	Highest inhibition of conjugated diene and triene formation was observed after 30 days TBA-index was lower in the samples with dietary fibre up to 90 days	Sánchez-Alonso et al. (2007)
Atlantic mackerel (*Scomber scombrus*), minced fillets	−10 °C, 26 weeks	Instant green tea, 250 or 500 ppm	Mixed into mince	Both concentrations reduced hydroperoxide formation, 250 ppm more than 500 ppm Inhibition of TBARS formation was almost equal between 250 ppm and 500 ppm, whereas hexanal formation was lower in mince with 500 ppm	Alghazeer et al. (2008)
Atlantic herring (*Clupea harengus*) fillets	–18 °C, 4 months	Milk protein concentrate (MPC), 2, 4, or 6%	Mixed into mince	4 and 6% MPC resulted in 33% and 50% reduction of TBARS, respectively and better preservation of EPA and DHA after 4 months MPC reduced formation of fishy odor and volatiles associated with fishy odor, propanal and 1-penten-3-ol were major volatiles	Joaquin et al. (2008)

Fish	Storage conditions	Antioxidant(s)	Way of addition	Main outcomes	Reference
Atlantic herring (*Clupea harengus*) fillets	on ice for 7 days, then at –20 °C 6 months	Spray-dried elderberry, cranberry, and blackcurrant powder, 50 g/L marinade	As marinade	TBARS and volatile lipid compounds were lowest in fillets marinated in black currant, 1-penten-3-ol was the most abundant oxidation derived volatile Protein carbonyls were lowest in black currant sample, but difference to control was not statistically significant	Sampels et al. (2010)
White hake (*Cynoscion* ssp) fish balls	−18 °C, 120 d + cooking (95 °C, 30 min)	annatto seeds (0.1 g/100g) and/or coriander leaves (0.5 g/100g)	Mixed into mince prior to preparation of fish balls	No significant differences were observed in formation of conjugated dienes, but TBARS were lower in fish balls with annatto and annatto + coriander After 120 d, UFAs were most preserved in the order annatto + coriander > annatto > coriander > control	Sancho et al. (2011)
Chub mackerel (*Scomber japonicus*), fillets with skin	−18 °C, 3 months	Pomegranate seed extract (PSE) and grape seed extract (GSE), 2%	Mixed into mince	Total phenolic content and antioxidant activity were higher in GSE vs PSE Formation of lipid hydroperoxides was significantly inhibited by GSE addition, while PV was higher in PSE sample compared to control Formation of TBARS was significantly inhibited by both extracts	Özalp Özen et al. (2011)
Horse mackerel (*Trachurus trachurus*)	5 °C, 96 h	water extract (WE) and ethanol extract (EE) of potato peel (2.4 and 4.8 g/kg)	Mixed into mince	After 96 h, tocopherol content was significantly higher, and protein carbonyls, peroxide values and lipid oxidation derived volatiles (1-penten-3-ol and 2,4-heptadienal) lower in minces with potato peel EEs EEs were more effective antioxidants than WEs	Farvin et al. (2012)
Silver carp (*Hypophthalmichthys molitrix*)	4 °C, 18 d	Clove bud extract (CBE20), grape seed extract (GSE), brining mixture 1.0% salt + 2.0% extract (/w water and fillet), fillet to water ratio 10:1	Fillets brined with antioxidants	Both extracts had high Fe^{2+}-chelating and DPPH radical scavenging activity Sensory score declined faster in control compared to fillets brined with CBE20 and GSE PV and TBA index remained lowest in fillets with CBE20 throughout the study period	Shi et al. (2014)

Fish	Storage conditions	Antioxidant(s)	Way of addition	Main outcomes	Reference
				The antioxidant effect of GSE and CBE20 on protein oxidation was less pronounced compared to effect on lipid oxidation	
Atlantic herring (*Clupea harengus*) and Atlantic mackerel (*Scomber scombrus*)	High pressure processing (HPP) →5 °C, 14 d	Annatto powder (500mg/kg) or bixin (1.0 mg//kg)	Mixed into mince	In mackerel, both antioxidants inhibited cholesterol oxidation during HPP and storage, in herring, bixin inhibited during HPP and both during storage Annatto, but not bixin inhibited DHA loss in herring	Figueirêdo et al. (2015)
Atlantic salmon (*Salmo salar*) fillets	Cooking → 6 °C, 26 days	Commercial supercritical CO_2 extracts; rosemary (R), oregano (O), and antimicrobial blend (AB), 30, 60, or 120 mg extract/mLmarinade	As marinade	AB delayed peroxide formation the most; PV was lowest after 7, 14, and 26 days compared to all other the samples AB was also the most efficient in delaying TAG oxidation, highest concentration was the most efficient	Tarvainen et al. (2015)
Atlantic mackerel (*Scomber scombrus*), deskinned fillets	5 °C, 8 days	Absolute ethanol, 50% etanol, and water extracts of *Fucus serratus* and *Polysiphonia fucoides*, 0.5 g/kg, or BHT 0.2 g/kg	Mixed into mince	Mince with 50 % ethanol extract of P. fucoides and mince with BHT showed greater resistance towards oxidation (low PV and lipid oxidation derived volatiles throughout the storage period) Based on PVs, absolute ethanol extracts of both species showed a pro-oxidative tendency Water extract with lowest phenolic content showed no antioxidant effect	Babakhani et al. (2016)
Atlantic salmon (*Salmo salar*) deskinned fillets	Cooking → 4 °C, 14 days	Commercial supercritical CO_2 extracts; rosemary (R), oregano (O), and antimicrobial blend (AB), 1 or 3 g/kg fish	Mixed into mince	All tested CO_2 plant extracts were effective in reducing cholesterol oxidation during cooking Atfer 7 days all samples with extracts had less cholesterol oxidation products (COPS) compared to both raw and cooked control AB and O samples at 3g/kg had the lowest amounts of COPs at 14 days.	Tarvainen et al. (2016)

Fish	Storage conditions	Antioxidant(s)	Way of addition	Main outcomes	Reference
Atlantic mackerel (*Scomber scombrus*), deskinned fillets	−18 °C, 6 months	Water extracts of green tea extract (GTE), grape seed (GSE), and pomegranate rind (PRE) (100 ppm equivalent phenolics), BHT (0.01%)	Mixed into mince	BHT was most efficient in reducing PV and TBARS formation, PRE was the second most efficient GTE and GSE were less effective than PRE, but were able to reduce TBARS formation significantly compared to control, after 6 months All additions retarded protein oxidation equally (lower carbonyl values and higher sulphydryl content) compared to control	Özalp Özen and Soyer (2018)
Atlantic mackerel (*Scomber scombrus*)	Sous vide-cooking → 4 °C, 9 days	commercial antioxidants TR25 (rosemary extract and tocopherols) (1000 ppm) and RPT40 (rosemary extract, α-tocopherol and ascorbyl palmitate) (2000 ppm)	As marinade	Samples treated with antioxidants before *sous vide* cooking at 70 °C, had significantly lower PV and TBARS compared to untreated samples PV and TBARS were higher in mackerel fillets subjected to sous-vide treatment at 70 vs 80 °C	Cropotova et al. (2019)
Asian sea bass (*Lates calcarifer*) steak	4 °C, 20 days	WPI (8%) coating with or without crude extracts (200 ppm) of green tea, ginger, or lemongrass	Dipping (2 min)	TBARS were lower in all coated samples compared to the control, no difference was observed between only WPI vs with WPI + extracts At the end of the storage period, WPI + ginger extract, had least protein carbonyls, lowest propanal content, and highest odor liking score Heme iron loss and metmyoglobin formation were highest in the control	Chaijan et al. (2020)
Tilapia, mechanically separated meat	−18 °C, 180 days	0.50% sodium tripolyphosphate (TPP) + one of the following; 0.25% sodium erythorbate (SE), 0.05% ascorbic acid (Asc), 0.05% commercial green tea powder extract, 0.10% aqueous propolis extract	Mixed into mince	TPP+SE and TPP+Asc efficiently retarded lipid oxidation as indicated by lower TBARS values, whereas addition of TPP alone or with green tea or propolis extract had no antioxidative effect Additions with green tea or propolis extract had a higher TBARS compared to the control at the end of the 180-d storage period	Silveira Alexandre et al. (2022)

Several natural antioxidants have shown their potential in inhibiting lipid (Alghazeer et al., 2008; M. Pazos et al., 2005a; Tarvainen et al., 2015) and protein (Chaijan et al., 2020; Özalp Özen et al., 2011) oxidation in fish. In addition to showing antioxidative effects, the natural additions have to be safe to consume. Extracts are considered as novel foods that have to be accepted by food authorities to be used. Similarly, as with synthetic antioxidants, natural antioxidants may have toxic properties if used excessively. As many natural antioxidants, such as extracts, fall under the novel food legislation in the EU, they need to undergo a thorough safety assessment (Regulation (EU) 2015/2283, 2015). Currently, rosemary extract (E392) is the only natural extract accepted by the European Food Safety Authority (EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), 2015).

2.3.2 Extraction of fish proteins using the pH-shift

Production of protein isolates using the pH-shift method has been suggested as a potential way to provide added value for under-utilized fish species, such as small pelagic fishes. The pH-shift process, i.e. alkaline or acidic extraction followed by isoelectric precipitation is based on pH induced changes in protein solubility (**Figure 5**). In the process, proteins are first solubilized using acid or base to increase their positive or negative net charge, inducing protein-protein electrostatic repulsion and increasing protein-water interactions (Gehring et al., 2011). The liquid phase containing the solubilized proteins is separated from the insoluble matter and lipids by decantering or centrifugation, after which the pH is adjusted to the isoelectric point (IP) of the protein where the protein has a zero net charge, causing precipitation. In most cases the pH of the isolate is finally adjusted to a neutral pH (7.0) (Abdollahi and Undeland, 2018) since the functional properties at IP are usually poor due to low solubility, and the slightly acidic pH may promote oxidation (Richards et al., 2002).

The pH shift can improve utilization of small fish and by products, since no pre-processing is needed, and during the process proteins are separated from non-protein materials due to differences in density (Undeland et al., 2002). In addition to fillets (Thawornchinsombut and Park, 2007; Undeland et al., 2005) and gutted fish (Marmon and Undeland, 2010), the pH shift has been applied to extract proteins from whole fish (Nisov et al., 2021) and side streams (Abdollahi and Undeland, 2019; Chen and Jaczynski, 2007; Chomnawang and Yongsawatdigul, 2013; Zhong et al., 2016). In addition to the proteins, other fractions obtained in the process may be utilized to collect minerals, collagen, or lipids, providing added value. For instance, the solid fraction obtained after the first separation step, containing skin and bones, was used to prepare collagen and collagen hydrolysate (Abdollahi et al., 2018). According to Abdollahi and Undeland

(2020) the yield of lipids collected during the pH shift was lower compared to traditional extraction by heating, but the n-3 PUFA content was higher with the former.

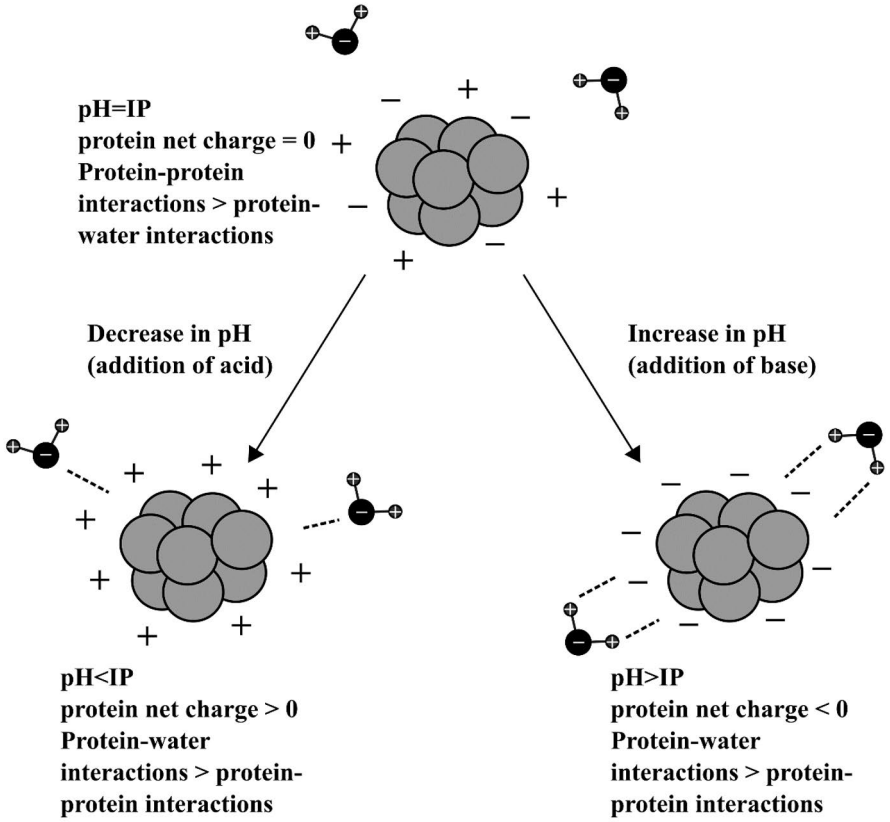

Figure 5. Principle of protein solubilization and precipitation in the pH shift. When pH is close to the isoelectric point (IP) of the protein, protein solubility as its lowest, whereas increasing or decreasing pH increases solubility.

A significant advantage of the pH-shift process is that the proteins are not significantly cleaved or do not have to be subjected to a heat treatment and hence retain certain functional properties of the muscle, such as gelation (Undeland et al., 2002; Wang et al., 2015). Due to different proteins having different IPs, the extraction can be somewhat selective. Of the muscle proteins in fish, myofibrillar proteins are typically extracted using the pH shift. Sarcoplasmic proteins are water soluble in both alkaline and acidic conditions and do not in their native state precipitate to a large extent at pH 5-6 (Gehring et al., 2011). However, the changes due to extreme pH conditions in the pH shift have been shown to alter their conformation and solubility, leading to their retention in the protein precipitate (Tadpitchayangkoon et al., 2010). For instance, the solubility of

hemoglobin at pH 5.5 was seen to be lower when it was first subjected to high (11.5) or low (2.5) pH (Abdollahi et al., 2016). In the same study it was also observed that increasing precipitation pH from 5.5 to 6.5 improved hemoglobin removal from 78% and 37% to 91% and 74% in alkaline and acid processing, respectively, which is desired in terms of limiting oxidation.

The pH shift has been seen to have favorable effects on protein and lipid composition, when compared to the raw material. For instance, the extraction process has been seen to improve the ratio of EAAs to non-essential amino acids (NEAAs), since more of the latter are removed during the process (Abdollahi and Undeland, 2018; Marmon and Undeland, 2010; Surasani et al., 2018; Zhong et al., 2016). In addition to reducing total lipid content, the pH-shift is reported to reduce particularly the content of PLs (Liang et al., 2007; Undeland et al., 2002). Further, in a study by Marmon et al. (2009), both acid and alkaline pH-shift processing, while removing majority of the lipids, resulted in 70-80% (per amount of protein) reduction in dioxin and PCB levels. Reduction of these contaminants is a significant benefit in terms of raw materials from the Baltic Sea.

One of the challenges in the pH-shift processing of fish is its accelerating effect on lipid and protein oxidation. Since pH has a vital role in the pro-oxidativity of hemoglobin (as discussed in section 2.2.3), the extreme high and especially low pH values used in the pH shift can induce lipid and protein oxidation. Also, in the process, pH is adjusted in two consecutive steps, and the pH that hemoglobin has been exposed to during the extraction, may effect its stability at the precipitation pH (Pazos et al., 2005). In addition, natural antioxidants present in fish are diluted and to some extent discarded during the process (Wu et al., 2021a). Further, homogenization and centrifugation break down the muscle structure, leading to increased contact between pro-oxidants and membrane lipids. When comparing acidic and alkaline extraction, most studies have reported the alkaline process to result in protein isolates with a lower degree of oxidation (Kristinsson & Hultin, 2004; Zhong et al., 2016), although contradictory findings have been reported (Abdollahi et al., 2020). The contradiction might be due to species related differences in content and stability of hemoglobin. For instance, Abdollahi et al. (2020) studied lipid oxidation in acid- and alkali-extracted protein isolates from salmon and herring side streams. In case of salmon, there were no significant differences between the two protein isolates, whereas herring protein isolate made with alkaline pH shift showed a higher PV and malondialdehyde content compared to acid-extracted isolate. A similar observation was also made by Zhang et al. (2022), comparing alkali- and acid-made protein isolates from herring heads and backbones. Several pretreatments and antioxidants have been seen to control lipid oxidation during the pH shift (Abdollahi et al., 2020; Zhang et al., 2022). Zhang et al. (2022)

studied co-products from lingonberry, apple, oat, barley, and shrimp, and two seaweeds as antioxidants during pH shift of herring and salmon filleting co-products. Except for the shrimp shells, all additions retarded lipid oxidation during pH-shift-processing and subsequent ice storage, lingonberry press-cake being the most efficient. Further, addition of lingonberry press cake was seen to prevent formation of lipid oxidation derived volatiles (hexanal, (*E*)-2-hexenal, heptanal, octanal, and 2,4-heptadienal) during 21 days of ice storage of herring protein isolate (Zhang et al., 2023).

While different results have been observed depending on the raw material and processing conditions, in general the alkaline process has been shown to be more favourable in terms of yield (Abdollahi and Undeland, 2019; van Berlo et al., 2023), lipid removal (Marmon and Undeland, 2010; van Berlo et al., 2023; Zhong et al., 2016), and gel forming ability (Phetsang et al., 2021b). Most of the differences in functional properties have been attributed to higher degree of denaturation in the acidic conditions (Tang et al., 2020).

Despite the abundant literature on the alkaline/acidic solubilization and isoelectric precipitation, few studies have investigated the pH-shifted protein isolates in regard to their sensory properties or compounds responsible for them (Abdollahi and Undeland, 2018; Nisov et al., 2021; Phetsang et al., 2021b). Also, research on potential uses or food concepts including pH-shifted fish protein isolates (FPIs) has mostly been conducted only during the recent years. **Table 4** presents studies in which the sensory quality of FPIs produced using the pH shift, or model foods including them, has been investigated. The pH shift has commonly been suggested as an alternative for traditional production of surimi (Nguyen et al., 2022; Phetsang et al., 2021b; Rawdkuen et al., 2009). FPIs have been successfully added in fish-based foods, such as fish sausages, in which up to 50% of mince have been replaced with FPI (Surasani et al., 2022a, 2022b, 2020) and fish balls (Shaviklo et al., 2010). However, 2% FPI from lantern fish could also be added in meat sausages, without significantly reducing the pleasantness compared to control sausages with soy protein isolate (Moosavi-Nasab et al., 2018). Also inclusion in pasta has been suggested (Surasani et al., 2019). While 2.5–10% FPI did not affect the liking of appearance, color, or texture of pasta, especially levels above 5% decreased flavor acceptability and increased the intensity of fishy flavor (Singh et al., 2021). Since complete elimination of fishy odor and flavor during the protein extraction is challenging, the odor and flavor of the protein isolates is likely to limit their application. In a study by Phetsang et al. (2021b) both alkaline and acidic pH shift, as well as surimi processing reduced the fishy and earthy off-odors of hybrid catfish mince. Acid-made protein isolate was, however, perceived as having the most rancid odor, which was in accordance with TBARS values.

Table 4. Studies on sensory properties of protein isolates prepared using the pH shift, or fortified foods including them. FPI denotes fish protein isolate, SPI denotes soy protein isolate.

Raw material	Process	Sensory profiling	Conclusion	Reference
Filleting co-products of cod (*Gadus morhua*), salmon (*Salmo salar*), and herring (*Clupea harengus*)	Solubilization pH 11.5 for cod and 12 for salmon and herring, precipitation pH 5.5, final pH adjustment to 7.0 →freeze-drying	Quantitative descriptive analysis of the three FPIs and SPI as 1% suspensions in water	Compared to SPI, FPIs had higher intensities in most odor and flavor attributes Of the 3 FPIs, herring FPI had most intense fish oil flavor, rancid flavor, dried fish flavor, bitter taste, off-flavor, fish oil odor, and dried fish odor compared to other FPIs, while cod protein was mildest in most attributes	Abdollahi and Undeland (2018)
Baltic herring (*Clupea harengus membras*) and roach (*Rutilus rutilus*)	Solubilization pH 11.5 or 2.5, precipitation pH 6 and 5.2 for alkaline and acidic, respectively → freeze-drying	Generic descriptive analysis	All FPIs were considered more rancid compared to enzymatically produced protein hydrolysates of the same raw material Alkali-produced FPI was more bitter compared to other roach samples, which was likely linked to higher degree of hydrolysis	Nisov et al. (2021)
Hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*)	Solubilization pH 11.2 or 2.7, precipitation pH 5.5	Fishy, earthy, and rancid odor on a scale from 0 to 4	Both alkali- and acid-processed FPIs had lower fishy and earthy odor compared to hybrid catfish mince Acid-processed FPI had a higher intensity of fishy odor compared to the alkali-processed	Phetsang et al. (2021b)

Raw material	Process	Sensory profiling	Conclusion	Reference
Haddock (*Melanogrammus aeglefinus*) cut-offs	Alkaline pH shift Wet (moisture content 80%) FPI was used to replace 0%, 25% or 50% haddock mince in fish balls	Quantitative descriptive analysis of fish balls	There were no significant differences between most odor and flavor attributes between fish balls with 0%, 25% or 50%, except for "frozen storage" flavor, which was higher in FPI 50% vs FPI 0% Addition of 50% FPI increased graininess compared to control when evaluated immediately after preparatation, and increased softness and decreased juiciness, but only after frozen storage of 4 and 8 weeks, respectively	Shaviklo et al. (2010)
Lantern fish (*Benthosema pterotum*)	Extraction pH 12, precipitation pH 5 → Freeze-drying FPI was added in minced meat sausage at 0%, 2%, or 4% level (containing 4%, 2%, or 0% SPI, respectively)	Color, odor, flavor, overall acceptability, and texture on a 5-point hedonic scale	Sausage with 2% fish protein isolate was considered more pleasant compared to control (4 % SPI) in terms of texture, color and general acceptability, equal in other attributes Sausage with 4% FPI was considered least liked in all attributes	Moosavi-Nasab et al. (2018)
Pangas (*Pangasius Pangasius*) co-products	Solubilization pH 13, precipitation pH 5.5 0, 5%, 10%, 25%, or 50% of pangasius mince was replaced with pangasius FPI in sausages	Appearance, odor, hardness, chewability, juiciness, stickiness and overall acceptability on a 9-point scale	Replacing pangasius mince with any amount of pangasius FPI did not significantly affect the pleasantness of sausages	Surasani et al. (2020)
Pangas (*Pangasius pangasius*)	Solubilization and precipitation pH not specified Pasta was supplemented with 0, 2.5%, 5.0%, 7.5% or 10.0% FPI	Appearance, color, texture, flavor and overall acceptability on a 9 point hedonic scale, overall flavor acceptability and fish flavor intensity	Increasing FPI level decreased liking of flavor and overall acceptability, and the difference to control was significant already at concentrations of 5% and 7.5%, respectively FPI addition increased fishy flavor in line with the amount added	Singh et al. (2021)

Raw material	Process	Sensory profiling	Conclusion	Reference
Rohu (*Labeo rohita*) co-products	Solubilization pH 13, precipitation pH 5.5, final pH adjustment to 7 0, 5%, 20%, 25%, or 50% of pangasius mince was replaced with rohu FPI in sausages	Appearance, odor, hardness, chewability, juiciness, stickiness, and overall acceptability were evaluated on 9-point scale	Replacing pangasius mince with any amount of rohu FPI did not significantly affect the pleasantness of sausages	Surasani et al. (2022b)
Pangas (*Pangasius pangasius*) co-products	Solubilization pH 13, precipitation pH 5.5 0, 2.5%, 5.0%, and 10% of rohu mince was replaced with pangasius FPI in sausages	Appearance, odor, hardness, rancid flavor, juiciness, stickiness, and overall acceptability were evaluated on 9-point hedonic scale	Sausages with 2.5% FPI had slightly, but significantly, lower overall acceptability compared to the control, but at other added concentrations the difference was not statistically sgnificant	Surasani et al. (2022a)

2.3.3 Enzyme-aided fish protein extraction

Enzymatic hydrolysis (enzyme-aided protein extraction) has been widely used to recover proteins from several fish (Aspevik et al., 2021; Idowu et al., 2019; Rocha Camargo et al., 2021) but also several other foods (Lamsal et al., 2007; Severin and Xia, 2006), and was studied for fish long before the pH-shift process was first developed (Adler-Nissen, 1976; Hale, 1972; Hultin and Kelleher, 2001). Since then, several different endogenous and exogenous commercial and non-commercial proteases, including pepsin, trypsin, α-chymotrypsin, Alcalase, pancreatin, Flavourzyme, Neutrase, Protamex, and papain, have been applied (**Table 5**). Proteases vary in their function and specifity, and therefore yield different peptides. Proteases either act on the N- or C-terminus of the polypeptide chain (exopeptidases) or cleave the chain from the middle (endopeptidases), and the specificity of the protease determines at which residue the peptide bond is cleaved (Tavano, 2013). For instance, Alcalase in an endopeptidase with a broad specificity and with preference to AAs with hydrophobic side chains, hence the use of Alcalase often leads to high degree of hydrolysis (DH) and formation of hydrophobic peptides (Tacias-Pascacio et al., 2020)

As in the case of pH shift, whole fish and side streams may be used, making enzymatic hydrolysis a suitable way to extract proteins (Aspevik et al., 2021; Jafarpour et al., 2020; Nisov et al., 2021) or lipids (Aitta et al., 2021; Mbatia et al., 2010) from under-utilized fish resources. However, the enzymatic extraction of proteins is fundamentally different compared to the pH shift, regarding the protein fractions obtained. Enzymatic extraction is based on hydrolyzing the proteins to increase their solubility (Adler-Nissen, 1976), after which the aqueous phase with hydrolyzed proteins (peptides) can be separated from insoluble materials and lipids by decantering or centrifugation, contrary to the pH shift, where mostly intact proteins are collected as a precipitate (Gehring et al., 2011). Due to the different degree of protein hydrolysis with the use of these methods, and therefore different functional properties, fish protein hydrolysates (FPHs) produced by enzymatic hydrolysis are suitable for different applications compared to FPIs.

Table 5. Enzymes and enzyme preparations studied for preparation of FPHs.

Enzyme or enzyme preparation	Raw material	References
Alcalase®	tra catfish (*Pangasius hypophthalmus*) side streams, whitemouth croaker (*Micropogonias furnieri*), banded croaker (*Paralonchurus brasiliensis*), serra spanish mackerel (*Scomberomorus brasiliensis*) side streams, sardinella (*Sardinella sindensis*), turbot (*Scophthalmus maximus*) side streams, cod (*Gadus morhua*) frames, rainbow trout (*Oncorhynchus mykiss*) side streams, salmon (*Salmo salar*) frames, Nile tilapia (*Oreochromis niloticus*), freshwater carp (*Catla catla*), yellow stripe trevally (*Selaroides leptolepis*) muscle, Pacific whiting (*Merluccius productus*) muscle, bigeye tuna (*Thunnus obesus*) dark muscle, herring (*Clupea harengus*), Pacific whiting side streams	Nguyen et al. (2022), Rocha Camargo et al. (2021), Lima et al. (2021), Amini Sarteshnizi et al. (2021), (Vázquez et al., 2020), Jafarpour et al. (2020), Nikoo et al. (2019), Idowu et al. (2019), Yarnpakdee et al. (2015), Silva et al. (2014), Elavarasan et al. (2014), Klompong et al. (2009), Pacheco-Aguilar et al. (2008), Qian et al. (2007), Sathivel et al. (2003), Benjakul and Morrissey (1997)
Brauzyn®	serra spanish mackerel (*Scomberomorus brasiliensis*) side streams	Lima et al. (2021)
Bromelain	rainbow trout (*Onchorhynchus mykiss*) heads, salmon (*Salmo salar*) heads and backbones, mackerel (*Scomber scombrus*) heads and backbones, herring (*Clupea harengus*) heads and backbones, freshwater carp (*Catla catla*)	Kvangarsnes et al. (2021), Aspevik et al. (2021), Elavarasan et al. (2014)
Corolase 7089®	serra spanish mackerel (*Scomberomorus brasiliensis*) side streams, Baltic herring (*Clupea harengus membras*), roach (*Rutilus rutilus*)	Lima et al. (2021), Nisov et al. (2021)
Flavourzyme®	serra spanish mackerel (*Scomberomorus brasiliensis*) side streams, Nile tilapia (*Oreochromis niloticus*), freshwater carp (*Catla catla*), Argentine croaker (*Umbrina canosai*) fillet, yellow stripe trevally (*Selaroides leptolepis*) muscle	Lima et al. (2021), Yarnpakdee et al. (2015), Elavarasan et al. (2014), Centenaro et al. (2014), Klompong et al. (2009)
Food Pro PNL®	salmon (*Salmo salar*) heads and backbones, mackerel (*Scomber scombrus*) heads and backbones, herring (*Clupea harengus*) heads and backbones	Aspevik et al. (2021)
Neutrase®	cod (*Gadus morhua*) frames, bigeye tuna (*Thunnus obesus*) dark muscle, Pacific whiting side streams, Baltic herring (*Clupea harengus membras*), roach (*Rutilus rutilus*)	Jafarpour et al. (2020), Qian et al. (2007), Benjakul and Morrissey (1997), Nisov et al. (2021)
Papain	rainbow trout (*Onchorhynchus mykiss*) heads, sardinella (*Sardinella sindensis*), salmon (*Salmo salar*) frames, Nile tilapia (*Oreochromis niloticus*), bigeye tuna (*Thunnus obesus*) dark muscle	Kvangarsnes et al. (2021), Amini Sarteshnizi et al. (2021), Idowu et al. (2019), Yarnpakdee et al. (2015), Qian et al. (2007)

Enzyme or enzyme preparation	Raw material	References
Pepsin	sardinella (*Sardinella sindensis*), bigeye tuna (*Thunnus obesus*) dark muscle	Amini Sarteshnizi et al. (2021), Qian et al. (2007)
Protamex®	whitemouth croaker (*Micropogonias furnieri*), banded croaker (*Paralonchurus brasiliensis*), Nile tilapia (*Oreochromis niloticus*), cape hake (*Merluccius capensis*) side streams, freshwater carp (*Catla catla*), Baltic herring (*Clupea harengus membras*), roach (*Rutilus rutilus*)	Rocha Camargo et al. (2021), Yarnpakdee et al. (2015), (Pires et al., 2009), Elavarasan et al. (2014), Nisov et al. (2021)
Protease N "Amano"®	mackerel (*Scomber austriasicus*) fillets	Wu et al. (2003)
Protease P "Amano"®	cod (*Gadus morhua*) bone mince, cod muscle mince	Halldorsdottir et al. (2014), (Halldorsdottir et al., 2013)
Trypsin	bigeye tuna (*Thunnus obesus*) dark muscle	Qian et al. (2007)
α-Chymotrypsin	Argentine croaker (*Umbrina canosai*) fillet, bigeye tuna (*Thunnus obesus*) dark muscle	Centenaro et al. (2014), Qian et al. (2007)
Intestinal extract from Nile tilapia (*Oreochromis niloticus*)	Nile tilapia (*Oreochromis niloticus*) side streams	Silva et al. (2014)
Pyloric caeca extract from brownstripe red snapper (*Lutjanus vitta*)	brownstripe red snapper (*Lutjanus vitta*) muscle	Khantaphant et al. (2011)

The hydrolysis of fish muscle proteins may provide several benefits and challenges. A long recognized issue of bitterness development due to formation of bitter peptides (Adler-Nissen, 1976) has been a challenge in terms of the sensory quality of FPHs. However, it has been shown that formation of bitterness can be controlled by a careful selection of enzyme(s) used (Yarnpakdee et al., 2015). The selection of enzyme has a role in altering the sensory (Yarnpakdee et al., 2015) and bioactive properties (Elavarasan et al., 2014) of the hydrolysates. Further, the DH is an important factor determining the properties of FPHs. Yarnpakdee et al. (2015) reported that increasing hydrolysis time and DH led to an increase in antioxidant activity of FPH from Nile tilapia *(Oreochromis niloticus*). On the other hand, DH did not affect the emulsifying capacities of FPHs from Pacific whiting (*Merluccius productus*), prepared using Alcalase (Pacheco-Aguilar et al., 2008). The raw material (fish species) is likely to be a determining factor on the FPH quality (Aspevik et al., 2021). For instance, in a study by Aspevik et al. (2021) FPHs were prepared from mackerel, salmon, or herring, from either backbone or head side streams, and using Bromelain or Food Pro as enzymes. Regardless of the enzyme or raw material fraction, herring FPHs were characterized as having the most intense sensory properties, such as fishy flavor, rancid flavor, acidic taste, and total flavor intensity.

Enzymatically fractionated FPHs as food ingredients can provide improved water-holding capacity, emulsification and foaming (Halim et al., 2016; Pacheco-Aguilar et al., 2008; Pires et al., 2015). Hydrolysis has been shown to result in peptides with several bioactivities, such as antioxidative effects (Centenaro et al., 2014; Khantaphant et al., 2011; Sathivel et al., 2003; Wu et al., 2003) and angiotensin converting enzyme (ACE) -inhibition (Je et al., 2004; Pires et al., 2015; Qian et al., 2007). For instance, FPH from rainbow trout side streams was seen to inhibit oxidation during refrigerated storage of a raw fish emulsion (Nikoo et al., 2019). FPH from skipjack tuna roe was also seen to retard hydroperoxide and TBARS formation in fish emulsion sausages (Intarasirisawat et al., 2014).

Since the optimal temperatures for many enzymes are high and after hydrolysis even higher temperatures are used for inactivation, and in many cases also pH adjustments are made, oxidation of lipids and proteins may also pose a challenge on the stability and quality of FPHs (Halldorsdottir et al., 2013). The lipids and heme proteins remaining in the FPH induce oxidation and promote formation of fishy odor (Yarnpakdee et al., 2014), which is a major issue limiting their commercialization (Halldorsdottir et al., 2014). Washing and/or pre-treatment with calcium chloride and citric acid prior to hydrolysis have been seen to reduce heme protein content (Khantaphant et al., 2011), PL content (Khantaphant et al., 2011; Pires et al., 2015), and lipid oxidation (Khantaphant et al., 2011; Nikoo et al., 2019). In addition, antioxidants, such as EDTA and

Trolox (Yarnpakdee et al., 2012c), pistachio green hull extract (Nikoo et al., 2019), and brown algae (Halldorsdottir et al., 2014), have been used to limit lipid oxidation in FPHs. According to Amini Sarteshnizi et al. (2021), addition of pistachio green hull extract during hydrolysis also enhanced the bioactive properties of the FPHs. FPHs prepared from cod bone mince with brown algae showed a lower intensity of rancid, soapy, and fish oil flavor, as well as bitterness (Halldorsdottir et al., 2014).

Some studies have combined the pH shift and enzymatic hydrolysis (Nikoo et al., 2019; Pires et al., 2015; Yarnpakdee et al., 2012a, 2012b). In a study by Pires et al. (2015), cape hake co-products were first solubilized using alkaline extraction and then subjected to hydrolysis by Protamex, which was seen to lead to higher lipid removal. Yarnpakdee et al. (2012a) used prewashing and alkaline or acid solubilization to extract proteins from Nile tilapia, which were then subjected to hydrolysis using Alcalase. Pretreatment including alkaline solubilization led to lower peroxide values (PVs), TBARS, and non-heme iron contents in the hydrolysates, and a higher likeness score when the hydrolysate was added to milk.

2.4 Concluding remarks

Fish is highly nutritious and an excellent source of micronutrients, proteins, as well as beneficial lipids. Improving utilization of fish resources that are currently discarded or directed to non-food uses, is vital in the context of global food security. Small dark-muscled fish, such as Baltic herring may be difficult to utilize by traditional processing methods (such as filleting), but technologies such as enzymatic hydrolysis and the pH-shift may enable the valorization of proteins and lipids from small fish and fish co-products.

The pH-shift process has been suggested, for instance, as an alternative for traditional production of surimi, since it can be used to extract the muscle proteins as relatively intact, retaining most of their functional properties. The protein isolates produced by the pH shift have been incorporated into fish-based food products, such as fish balls and fish sausages, but also to other foods, such as pasta. However, despite removing a majority of the lipids as well as pro-oxidants, the pH shift has been seen to induce protein and lipid oxidation, leading to development of off-odors and off-flavors that may limit the use of the protein isolates. Compared to other literature regarding the pH shift, there is still relatively little information on the sensory properties and underlying chemistry of the protein isolates.

Similarly to the pH shift, enzymatic hydrolysis has been proven a successful technology for extracting proteins, as well as lipids, from under-utilized fish resources. Fish protein hydrolysates obtained via hydrolysis using proteases have

generally high solubility, foaming, and emulsifying capacities. In addition, fish protein hydrolysates from several raw materials have been shown to have bioactivities, such as antioxidation and inhibition of angiotensin converting enzyme, showing their potential in biomedical applications as well. However, fish protein hydrolysates are also limited by the development of fishy odor and flavor, induced by lipid oxidation.

Oxidative enzymes, mainly lipoxygenases, as well as heme proteins are important factors inducing lipid oxidation in fish. Degradation of the cellular structures in fish muscle (e.g. during mincing or homogenization) leads to increased exposure of polyunsaturated fatty acids to both lipoxygenases and heme proteins. Increasing temperature or adjusting pH increases the rate of oxidative reactions. Oxidation gives rise to secondary compounds, especially aldehydes and ketones, which contribute to development of fishy off-odors and off-flavors during processing and storage.

Several synthetic and natural antioxidants have been investigated for their effect during frozen or refrigerated storage of fish muscle, as well as during the pH shift or enzymatic hydrolysis. Antioxidants are not only able to inhibit oxidation as indicated by a lower content of primary and secondary oxidation products, but to also limit degradation of sensory quality. Investigation of the antioxidant effect on sensory quality is important, as it is not always well reflected by instrumentally measured oxidation markers. Previous literature has shown that several natural antioxidants to be as or more effective compared to synthetic or conventional antioxidants. However, the use of natural antioxidants, such as extracts, may be challenging regarding their sensory properties and legislative restrictions.

3 AIMS OF THE STUDY

The overall aim of the thesis was to increase the use of under-utilized fish, with a focus on Baltic herring and roach, using two approaches. The first approach was to study the pH-shift and enzymatic hydrolysis as potential processes to improve the utilization of especially small Baltic herring and roach. The second approach was to investigate the addition of natural antioxidants to retard lipid oxidation of Baltic herring, and therefore help preserve its nutritional and sensory quality. The overall aim was divided to sub aims:

The first aim of the study was to assess pH-shift processing and enzymatic hydrolysis as protein extraction methods for roach and Baltic herring to compare their effects on the composition of proteins and lipids, as well as protein and lipid oxidation (**I**).

The second aim was to compare the effects of conventional and natural antioxidants, such as berry press residues, on the loss of EPA and DHA, formation of hydroperoxides, and formation of secondary lipid oxidation products in Baltic herring mince stored at –20 °C or 1 °C (**III**, **IV**).

The third aim was to investigate the effects of pH-shift processing or berry press residue addition on the sensory quality and odor-active compounds of Baltic herring (**II**, **IV**).

4 MATERIALS AND METHODS

4.1 Outline of studies I-IV

The outline of studies **I–IV** is presented in **Figure 6**. Studies **I** and **II** focused on investigation of protein isolates and hydrolysates produced using the pH-shift or enzymatic hydrolysis, respectively, whereas studies **III** and **IV** examined the effect of natural antioxidants in minced Baltic herring.

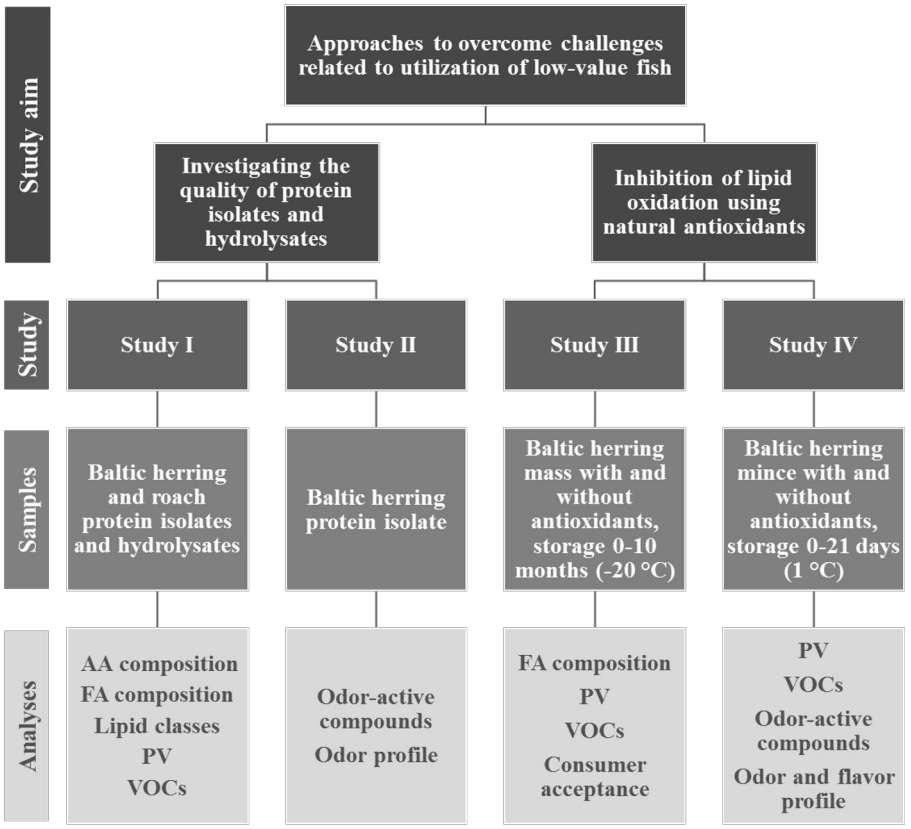

Figure 6. Outline of studies **I-IV**. Abbreviations: AA= amino acid, FA= fatty acid, PV= Peroxide value, VOC= secondary volatile oxidation compound.

4.2 Materials

Freeze-dried protein isolates and hydrolysates from roach (*Rutilus rutilus*) and Baltic herring (*Clupea harengus membras*), investigated in study **I**, were prepared by VTT Technical Research Centre in Finland (Espoo, Finland). Detailed processes are described by (Nisov et al., 2021). Briefly, whole

unprocessed Baltic herring and descaled roach (AK Foods Arvo Kokkonen Oy, Finland) were subjected to acidic (solubilization pH 2.5, precipitation pH 5.2) and alkaline (solubilization pH 11.5, precipitation pH 5.2). Enzymatic hydrolysis of same raw materials was conducted using three endoproteases (Protamex, Neutrase, and Corolase 7089).

Freshly caught (within 24 hours) Baltic herring were purchased as fillets without skin (**III**), fillets with skin (**III**, **IV**), or as gutted and beheaded (**II**, **III**) from Martin Kala Oy (Turku, Finland). In study **IV**, fresh fillets with skin, caught and filleted in the morning of the same day, were kindly provided by Kalaset Oy (Uusikaupunki, Finland), and were used for sensory analysis. Fish were always brought to the laboratory on ice, and immediately frozen or processed, as discussed in 4.2.1.

Berry press residues, investigated in studies **III** and **IV**, were side streams of commercial production of berry juice and/or oil. Lingonberry juice press residue (LR) was kindly gifted by Kiantama Oy (Suomussalmi, Finland). Sea buckthorn juice press residue (SR) in study **III** was purchased from Polarforma Oy (Tornio, Finland), whereas SR and sea buckthorn juice press residue, from which oil had been extracted through supercritical CO_2 extraction (SRO) in study **IV** were provided by Aromtech Oy Ltd (Tornio, Finland). All press residues were provided by the companies as dried (dried using fluid bed drying, moisture content <10%) coarse flakes, and were further ground to a smaller particle size to ensure uniform distribution to the mince. The press residues were added to the mince as such, i.e. without any further extraction of bioactive compounds. The polyphenol, tocopherol, and carotenoid content of LR and SR were reported by (Damerau et al., 2020b). The berry press residues in study **IV** were collected from batches that contained less seeds. 'Antimicrobial blend' (AB), a mixture of supercritical CO_2 extracts from sage (*Salvi fruticosa* M.), hop (*Humulus lupulus* L.), licorice root (*Glycyrrhizia uralensis* F.), temulawak (*Curcuma xanthorrhiza* R.), clove bud (*Syzygium aromaticum* L.), oregano (*Origanum vulgare* L.) leaves, and 5% ajowan fruit (*Trachyspermum ammi* (L.) Sprague ex Turrill), was purchased from Flavex (Flavex Naturextrakte GmbH, Rehlingen, Germany). *L*-Ascorbic acid and *α*-tocopherol were bought from Sigma-Aldrich (Sigma-Aldrich Co, St. Louis, Missouri, U.S.A).

4.3 Processing methods

4.3.1 Fish processing and storage tests

In study **III**, fillets with or without skin were processed into mince using industrial scale machinery at a fish processing facility (Kolvaan Kala Oy, Säkylä, Finland). In studies **II** and **IV** gutted and beheaded Baltic herring (**II**) or Baltic

herring fillets with skin (**IV**) were minced using a food processor with a meat grinder attachment (Chef Titanium, Kenwood Limited, Havant, United Kingdom) with a 4 mm hole plate installed. In studies **III** and **IV**, antioxidant additions (**Table 6**) were mixed into the mince immediately after mincing. Storage tests were conducted by storing the minces frozen at –20 °C for 0–10 months (**III**) or refridgerated at 1 °C for 0–21 days (**IV**), after which they were frozen at –80 °C until being analysed. Mince samples used for volatile analysis were stored in 20 mL glass vials, while the minces for other analyses were stored in plastic boxes. Air was not excluded to allow the presence of oxygen in vials and boxes. For the sensory analysis in study **IV**, minces with and without LR and SRO were stored refridgerated at 1 °C for 0 or 3 days, after which they were subjected to sensory analysis as raw or after cooking *sous vide* in a 70 °C water bath for 20 minutes. Prior to cooking, 0.65% NaCl was added to the minces.

Table 6. Antioxidative additions in studies **III** and **IV**.

Addition	Concentration (g/100g mince)	Study
Ethylenediaminetetraacetic acid (EDTA)	0.0075	III
L-ascorbic acid & *α*-tocopherol (αT+AA)	0.2 & 0.01	III, IV
Antimicrobial blend (AB)	0.1	III
Lingonberry juice press residue (LR)	1.0 (IV), 1.5 (IV), 3.0 (I, IV)	III, IV
Sea buckthorn juice press residue (SR)	1.0 (IV), 1.5 (IV), 3.0 (I, IV)	III, IV
Sea buckthorn juice press and oil extraction residue (SRO)	1.0, 1.5, 3.0	IV

4.3.2 The pH-shift

Baltic herring (gutted and beheaded) were processed using alkaline pH shift in study **II** (**Figure 7**). Alkaline pH shift instead of acidic pH shift was used since most previous literature has reported the former more optimal in terms of yield (Abdollahi and Undeland, 2019; van Berlo et al., 2023), lipid removal (Marmon and Undeland, 2010), and gel forming ability (Phetsang et al., 2021b). The latter was particularly important, since the same protein isolate was used as an ingredient in surimi type gels and fish balls (Kakko et al., 2022). Degutted and beheaded fish were homogenised with water (100 g fish + 800 g water), and proteins were solubilized at pH 11.2. The homogenate was centrifuged and the proteins in the supernatant were precipitated at pH 5.4. The sediment containing the precipitated proteins was collected and 2% fructose and 2% sorbitol were added as cryoprotectants. The combination of fructose and sorbitol was chosen based on previous experiments, and while it might have been beneficial to use a non-reducing sugar instead of fructose, no optimization was conducted regarding

the type and concentration of cryoprotectants. Finally, the pH of the protein isolate was adjusted to 7.0 using 4 M NaOH, and it was stored frozen at -80 °C.

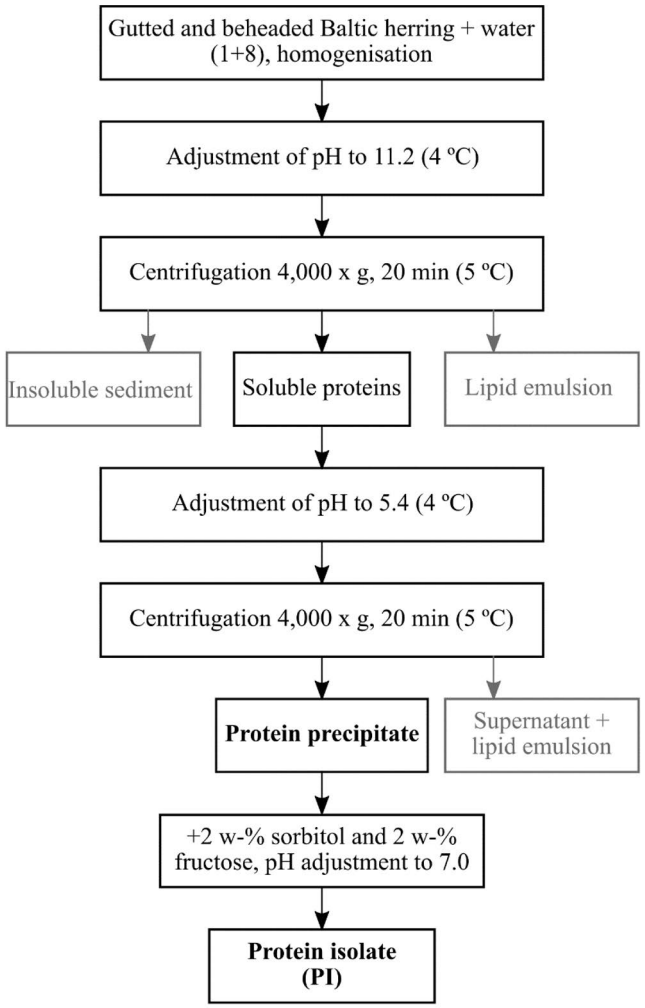

Figure 7. The alkaline pH-shift processing of gutted and beheaded Baltic herring (**II**).

4.4 Chemical analyses

4.4.1 Lipid extraction and analysis of primary lipid oxidation, fatty acids, and lipid classes

Two different lipid extraction methods were used. In studies **I**–**III**, extraction and gravimetrical determination of lipid content was conducted according to Folch et al. (1957). In studies **III** and **IV**, lipids for PV or lipid composition

analyses were extracted according to Lee et al. (1996). In study **IV** a modified version of this method, including the use of 0.05% butylated hydroxytoluene (BHT) (Sigma-Aldrich) in the extraction solvent, as described by Cavonius and Undeland (2017), was used to prevent lipid oxidation during extraction.

PVs of the lipids extracted from Baltic herring minces with or without antioxidants (**III**, **IV**) and Baltic herring and roach protein isolates and hydrolysates (**I**) were analyzed using a ferric thiocyanate method according to Lehtonen et al. (2011). Fatty acids from Baltic herring minces (**III**) and roach and Baltic herring protein isolates and hydrolysates (**I**) were analyzed using gas chromatography (GC) with a flame ionization detector (FID) as fatty acid methyl esters (FAMEs) prepared with an acid-catalyzed method (Damerau et al., 2020a). The peaks were identified using external standards (37 Component FAME mix, 68D, and GLC-490, Supelco, St. Louis, MO, USA) and quantified using an internal standard (PC19:0; 1,2-dinonadecanoyl-*sn*-glycero-3-phosphatidylcholine, Larodan, Solna, Sweden) and correction factors determined with the standard mixtures.

Lipid classes of Baltic herring and roach protein isolates and hydrolysates in study **I** were determined semiquantitatively using ultra-high-performance liquid chromatography with electrospray ionization and mass spectrometer (UHPLC–ESI–MS) as previously described by (Damerau et al., 2020a). Prior to the analysis, the concentration of extracted lipids was adjusted to 0.5 mg/mL in chloroform-methanol (2:1, *v/v*). Data was presented as total peak areas of lipids belonging to a certain lipid class.

4.4.2 Analysis of amino acid composition and protein carbonyls

In study **I**, Baltic herring and roach protein isolates and hydrolysates were analyzed for their amino acid composition and protein carbonyl content. Amino acids were determined using reverse-phase HPLC and fluorescence detection. Alkaline (quantification of tryptophan) and acidic (quantification of other amino acids) hydrolysis of the samples were performed according to Dai et al. (2014) prior to their analysis. Hydrolyzed samples were derivatized using iodoacetic acid, ortho-phthalaldehyde, and 9-fluorenyl methoxycarbonyl chloride (Sigma-Aldrich), and chromatographic separation was achieved using the method by Henderson et al. (2021). Correction by internal standards norvaline and sarcosine, and standard curves of external *L*-amino acid standards (Sigma-Aldrich) were used to quantify amino acids.

Protein carbonyls, as indicators of protein oxidation, were analyzed spectrophotometrically after labelling with 2,4-diphenylhydrazine (Sigma-Aldrich). The protein carbonyls of protein isolates (prepared using the pH-shift) were analyzed using a method by Levine et al. (1994). The carbonyls in the

protein hydrolysates (prepared using enzymatic hydrolysis) were quantified using the method by Mesquita et al. (2014) that does not require precipitation of the labelled proteins or peptides.

4.4.3 Analysis of volatile compounds

Volatile compounds in studies **I**–**IV** were analyzed using headspace-solid phase microextraction (HS–SPME)–GC–MS. The instrument used was by Thermo Fisher Scientific (Waltham, MA, USA), consinsting of a TriPlus autosampler, Trace 1310 GC and TSQ8000 or ISQ7000 mass spectrometer. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 µm film thickness, Supelco, St. Louis, MO, USA) was used for extraction of volatiles. In studies **II** and **IV** for identification of odor-active compounds a 2 cm fiber was used, but in other cases a fiber with 1 cm length was used. In studies **I**–**III**, equilibration and subsequent extraction of volatiles was continued for 20 and 30 min, respectively, at a 40 °C temperature. In study **IV**, for analysis of odor-active compounds, an equilibration and extraction temperature of 35 °C was used, and extraction time was extended to 35 minutes.

In case of raw or cooked fish mince, 3.0 g ± 0.05 g was weighed in 20 mL glass vials, but in case of protein isolates and hydrolysates (**I**), 0.5 ± 0.01 g of sample was weighed, and 3 mL of water was added. In most cases, headspace of vials was flushed with nitrogen to limit oxidation during analysis, but not in the storage tests in studies **III** and **IV**, since the storage was conducted in the vials. Sample tray was cooled at 5 °C.

A semipolar column, SPB-624 (30 or 60 m × 0.25 mm i.d., 1.4 µm film thickness; Supelco, St. Louis, MO, USA) was used in all studies, but in addition a polar column, DB-WAX (60 m, 0.25 mm, 0.25 µm; Agilent Technologies, Santa Clara, California, USA) column was used in studies **II** and **IV** (**Table 7**). The GC temperature gradient depended on the polarity and length of the column and exact conditions are presented in **Table 7**. Temperatures of front inlet, transfer line, and ion source were 240 °C, 220 °C, and 250 °C, respectively. The MS was operated in electron ionization mode (at a voltage 70 eV), and spectra was collected in the mass range of 40–300 amu. Volatile data were processed using Xcalibur or Chromeleon (Thermo Scientific), and compounds were identified by comparison of the MS spectra to NIST MS library (version 2.3, National Institute of Standards and Technology, Gaithersburg, Maryland, USA) or to commercial standard compounds. Calculation of linear retention indices (LRIs) and their comparison to the NIST library and previous literature was also used to identify volatiles.

Table 7. Gas chromatographic conditions for volatile analyses (**I**–**IV**).

Study	Column	Oven programme	Helium flow (mL/min)
I, II & III	SPB-624, 60 m × 0.25 mm, 1.4 µm film thickness	40 °C for 5 min, increase 5 °C/min to 200 °C, 200 °C for 10 min	1.4
II	SPB-624, 30 m × 0.25 mm, 1.4 µm film thickness	40 °C for 3 min, increase 8 °C/min to 150 °C, increase 10 °C/min to 220 °C, 220 °C for 10 min	1.4
II	DB-WAX, 60 m × 0.25 mm, 0.25 µm film thickness	40 °C for 3 min, increase 7 °C/min to 220 °C, 220 °C for 10 min	1.4
IV	SPB-624, 30 m × 0.25 mm, 1.4 µm film thickness	40 °C for 3 min, increase 10 °C /min until 220 °C, 220 °C for 10 min	1.4
IV	DB-WAX, 60 m × 0.25 mm, 0.25 µm film thickness	40 °C for 3 min, increase 10 °C /min until 220 °C, 220 °C for 10 min	1.6

4.5 Sensory analyses and gas chromatography olfactometry

4.5.1 Consumer test of Baltic herring fish minces with and without antioxidants

In study **III**, consumer acceptance of Baltic herring mince with and without antioxidants was investigated. The consumer studies were carried out in a sensory laboratory complying with ISO 8589, at the Functional Foods Forum in the University of Turku, Finland. The volunteer participants (n=55) were recruited from the Aistila Consumer Register of the University of Turku. Most of the participants (75%–85%) were frequent fish eaters (at least 1–2 times/week). For the consumer test, minces with and without added antioxidants were prepared into fish loaves (67% fish mince, 15% egg, 3% toast crumb, 13% heavy cream and 1% salt, cooked in an oven for 35 min at 200° C). Liking of odor, appearance, color, texture, taste, and overall appeal were rated on a 9-point hedonic scale (1 = dislike extremely to 9 = like extremely).

4.5.2 Odor and flavor profiling

In studies **II** and **IV**, panelists for odor and flavor profiling were recruited from the students and staff of the Food Sciences unit in the University of Turku (Turku, Finland). Informed consent of the panelists to participate was acquired. All sensory analyses were conducted using Compusense Cloud (Compusense

Inc., Guelph, Ontario, Canada). Evaluations were carried out in a sensory laboratory following the ISO8589 standard.

In study **II**, 6 panelists (four women, two men, age 24–38) evaluated the odor of Baltic herring and Baltic herring protein isolate using check-all-that-apply (CATA). After CATA, panelists were asked to rate the intensities of odor attributes found in the samples. Panelist participating in odor profiling had previously participated in gas chromatography – olfactometry (GC-O) analysis of the same samples. The odor attributes in CATA were chosen from the most frequently mentioned descriptions in the GC-O analyses. The intensities of the selected odor attributes were rated on a scale 0–4 (0 = not detected, 1 = very mild, barely noticeable, 2 = mild, 3 = fairly strong, and 4= strong). The samples were presented to the panelists in glass bottles (5 g in 30 mL bottle) covered with aluminum foil and coded with random three-digit codes.

In study **IV**, generic descriptive analysis (n=9) was used to determine the odor, flavor, and taste profile of Baltic herring minces with and without LR and SRO, as fresh (0 d) or after 3-d storage. Nine panelists (4 men, 5 women, age 25–56) took part in generic descriptive analysis (GDA) focusing on odor and flavor. All the panelists had prior experience in sensory analysis and were further trained for the study purposes in 4 sessions. Training included creating the vocabulary and agreeing on the descriptors and reference samples and their intensities. Samples of raw minces (6 g) were weighed in 30 mL brown glass bottles, and the samples were allowed to reach room temperature prior to evaluation. *Sous vide* cooked minces were tempered on a 60 °C hot plate immediately after being cooked and served in approximately 12 g portions in glass bowls with lids. Samples were presented one sample at a time in randomized order, but raw and cooked minces were evaluated separately from each other. A list of the attributes (in the order or evaluation), their descriptions and possible reference samples are presented in **Table 8**. Attributes were evaluated on a scale 0–10 (0= not at all, 10= very intense).

Table 8. Evaluated odor, flavor, and taste attributes as well as possible reference samples used in study **IV**.

Attribute	Description	Reference (*intensity*)
Total intensity of odor	The intensity of all odors in the sample	-
Fishy odor	The intensity of odor(s) characteristic to fish	-
Marine odor	Marine-like odor, that can consist of e.g. fresh fish odor, seaweed odor and scents of ocean and salty water	Wakame seaweed, 0.4 g soaked in 4 ml water, served in 20 mL plastic cups (***6.5***)
Fish-oil odor	Oily or fatty odor, typical to fatty fish	Fish oil (Möller), 2 mL served in 20 mL plastic cups (***7.0***)
Rancid odor	Odor of rancid (fish) oil	Fish oil (Möller), oxidized by microwaving (600W, 3.5 min) and after stored at 4 for 2-3 d, 2 mL in 20 mL plastic cups (***6.0***)
Musty odor	Musty, stale, or stuffy odor, may be the opposite of fresh	Broth of canned mushrooms (K-menu), 5 mL in 30mL glass bottles (***4.0***)
Berry-like/fruit like odor	Odor characteristic to either a specific berry or fruit, or berry-/fruit like in general	-
Total intensity of flavor	The intensity of all flavors in the sample	-
Fishy flavor	The intensity of flavor(s) characteristic to fish	-
Marine flavor	Marine-like flavor, that can consist of e.g. fresh fish flavor and seaweed flavor	Wakame seaweed, 0.4 g soaked in 4 mL water, served in 20 mL plastic cups (***6.5***)
Fish oil flavor	Oily or fatty flavor typical to fatty fish	Fish oil (Möller), 2 mL served in 20 mL plastic cups (***7.0***)
Berry-like/fruit like flavor	Flavor characteristic to either a specific berry or fruit, or berry-/fruit like in general	-
Umami	Taste that can also be described as savory, and can be found in e.g. meat, broth, mushroom, and soy	0.1% Monosodium glutamate monohydrate, 5 mL in 20mL plastic cups (***6.0***)
Saltiness	A basic taste caused by e.g. table salt	0.3% Sodium chloride, 5 mL in 20mL plastic cups (***6.0***)
Metallic flavor/taste	Flavor of metal, for example the metallic flavor caused by blood in the mouth	-

4.5.3 GC-O

Odor-active compounds of Baltic herring protein isolate and Baltic herring minces with and without SRO (1.5%) were investigated in studies **II** and **IV**, respectively, using GC-O. HS-SPME was used for extraction of volatiles and was conducted similarly as described for GC-MS analysis of volatiles in chapter 4.2.6. A 2 cm DVB/CAR/PDMS fiber was used for the extraction, which took place in a 90 mL erlenmeyer flask with 10 g sample (minced Baltic herring or Baltic herring protein isolate). The sample was incubated for 20 minutes in a 35 °C (study **IV**) or 40 °C (study **II**) water bath prior to extraction for 30 min (**II**) or 35 min (**IV**) at the same temperature. The instrument used was a HP 6890 Series GC with a flame ionization detector (FID) (Hewlett Packard, Palo Alto, California, USA). The column eluent was split 1:1 between the FID and an olfactometry port (Gerstel, Linthicum, Maryland, USA), except in case of study **II** when using the SPB-624 column, when the eluent was directed to either FID or olfactometry port. The columns and oven temperature programmes were the same as in **Table 7**. The panelist used a button connected to a microphone, and the durations, descriptions, and intensities were recorded as an audio file.

As in the case of odor and flavor profiling, panelists for GC-O were recruited from the students and staff of the Food Sciences unit in the University of Turku, and all the panelists had prior experience from GC-O and/or sensory analysis. Informed consent of the panelists to participate was acquired. In study **II**, 6 panelists (four women, two men, age 24–38) evaluated the odor-active compounds of Baltic herring and Baltic herring FPI after two training sessions. The first session included familiarization of one of the samples on the instrument, while the second training was a group session that included describing and rating the intensity of standard compounds identified in fish. In study **IV**, 6 panelists (4 women, 2 men, age 25–33) assessed Baltic herring minces with and without SRO (1.5%), using GC-O. Training for the GC-O included 3 sessions, involving a group training with dilutions of standard compounds, pipetted on a 1 cm x 1 cm paper in a brown glass bottle (30 mL). The second and third training sessions included evaluation of a standard compound mix and one of the four Baltic herring mince samples on the GC-O.

Panelists evaluated the samples in a randomized order. In study **II**, 6 panelists evaluated the samples twice using the SPB-624, and 2 panelists evaluated samples once using the DB-WAX. In study **IV**, 6 panelists evaluated the samples once on SPB-624 and once on DB-WAX. Detection frequency and direct intensity methods were used. The intensity of odors in both studies, **II** and III, was evaluated on a scale of 0–4 (1 = very mild, barely noticeable, 2 = mild, 3 = fairly strong, and 4= strong; 0= not detected). Recorded audio files were processed using Audacity 3.0.2 (The Audacity Team). Average odor intensity for each compound was calculated by only including the intensities given by the

assessors who detected the odor (i.e. null values were not included). Odorants with Nasal Impact Frequency (NIF) ≥ 33% were considered as significant odor contributors. Odorants were identified by HS-SPME-GC-MS analyses and comparison of ms spectra to the NIST library, as described in section 4.2.6, by comparison of their linear retention indices to previous literature and RI libraries, and by comparison of odor descriptors to previous literature and databases.

4.6 Statistical analyses

In most cases the samples were compared using one-way ANOVA and Tukey's HSD test or independent-samples t test in SPSS (IBM SPSS Statistics, version 25.0.0.1, IBM, New York, USA). In study **II**, GC-O detection frequencies and selection frequencies of CATA attributes of Baltic herring and FPI were compared using McNemar's test. In study **III**, DHA and EPA content and PV at 0 months and 10 months were compared using the paired samples t-test (within same sample type) and two-way ANOVA (EPA and DHA content as dependent variables, time point and sample type as fixed factors). In study **IV**, odor and flavor attributes of Baltic herring minces, within the same sample type at different time points, were compared using paired samples's t test. Differences were considered statistically significant if *p*-value was below 0.05. Principal Component Analysis (PCA) and Partial Least Squares (PLS) regression with the Unscrambler version 10.4.1 (Camo Process AS, Oslo, Norway) were used in studies **I**, **III**, and **IV**. In study **IV**, the agreement, sensitivity, and reproducibility, of the panel in GDA was evaluated according to Tomic et al. (2009) using PanelCheck software (version 1.4.2, Nofima, Tromsø, Norway).

5 RESULTS AND DISCUSSION

5.1 Composition

5.1.1 Protein, lipid, and moisture content

The protein, lipid, and moisture content of the raw materials (whole roach, whole Baltic herring, and gutted and beheaded Baltic herring) as well as FPIs and FPHs in studies **I** and **II** are presented in **Table 9**. When preparing FPIs and FPHs, it is desired to remove as much lipids as possible for several reasons. Firstly, the fish lipids extracted by the pH shift are rich in *n*-3 PUFAs and can be utilized as another value-added fraction (Abdollahi and Undeland, 2020), and therefore their efficient separation from the proteins is beneficial. Secondly, lipids may have a negative effect on the stability and functional properties of the FPIs and FPHs. Further, as most compounds contributing to fishy off-odor are lipid derived (Lindsay, 1990; Venkateshwarlu et al., 2004a), their removal may help in eliminating fishy odor.

Table 9. Protein, lipid, and moisture contents (%, i.e. g/100g "as is" or on dry matter basis) of protein isolates and hydrolysates from roach and Baltic herring (**I**, **II**).

Fish	Process	Protein (w-%, as is)	Lipids (w-%, as is)	Moisture	Protein, (w-%, d.w.)	Lipids (w-%, d.w.)
Roach (study **I**)*	Raw material	16.1±0.1	4.2±0.0	75.1±0.0	64.9±0.3	17.0±0.1
	Acidic pH shift	80.1±1.0	10.7±0.5	3.5±0.7	83.0±1.6	11.1±0.4
	Alkaline pH shift	70.8±1.7	18.8±0.9	3.0±0.4	73.0±2.0	19.4±0.9
	Protamex	80.4±2.2	5.5±0.1	4.3±1.0	84.0±1.5	5.8±0.1
	Neutrase	81.3±1.6	5.7±0.2	4.4±0.7	85.1±1.5	6.0±0.2
	Corolase	79.9±1.6	6.0±0.1	4.4±0.3	83.6±1.5	6.3±0.1
Baltic herring	Raw material	15.0±0.0	7.2±0.1	76.3±0.0	63.1±0.0	29.9±0.4
(Study **I**)*	Acidic pH shift	79.4±0.7	16.5±1.0	1.2±0.3	80.4±0.8	16.7±1.0
	Alkaline pH shift	76.0±0.9	16.4±1.8	2.3±0.4	77.9±0.7	16.8±1.8
	Protamex	87.2±1.0	3.1±0.1	4.2±0.7	91.1±1.2	3.2±0.1
	Neutrase	84.1±1.3	3.1±0.2	4.4±1.0	87.9±0.7	3.3±0.2
	Corolase	85.0±1.4	3.4±0.2	4.2±0.3	88.7±1.2	3.5±0.2
Baltic herring	Raw material	14.7±0.8	6.1±0.1	80.2±0.0	74.4±3.9	30.8±0.7
(Study **II**)	Alkaline pH shift	9.2±0.3	3.7±0.0	85.3±0.0	62.3±2.4	25.1±0.0

*Data from Nisov et al. (2021)

When comparing to the lipid content (on dry weight basis) of FPIs in study **I**, reported by Nisov et al. (2021), the alkaline pH shift in study **II** led to slightly

lower removal of lipids, as the lipid content in Baltic herring FPI was 25% (d.w.), while the lipid content of alkali extracted FPIs from roach and Baltic herring were 19% and 17% (d.w.), respectively. The protein content of the FPI in study **II**, on the other hand was only 9% on fresh weight basis and 62% on dry weight basis, since the FPI contained 85% moisture, and approximately 4% cryoprotectans (fructose and sorbitol). The mass yield of the isolation process in study **II** was 82%.

The lipid contents of Baltic herring raw materials (caught in April 2018) in study **III** were 5.02 ± 0.10% in gutted and beheaded Baltic herring, 5.22 ± 0.14% in fillets with skin, and 4.18 ± 0.03% (fresh weight basis) in fillets without skin. The lipid content of 5.0% gutted and beheaded Baltic herring in study **III** was slightly lower compared to the same raw material in study **II**, caught in March 2019 (6.1%), but slightly higher than the lipid content of whole Baltic herring (**I**), caught in September 2018. Nevertheless, the lipid content in Baltic herring has been seen to fluctuate between seasons (Aro et al., 2000) and all lipid contents were within the range previously observed for Baltic herring (Aro et al., 2000; Rajasilta et al., 2019).

5.1.2 Amino acid composition

Amino acids (**Table 10** and **Table 11**) in study **I** were analysed to assess the nutritional quality of the prepared FPHs and FPIs and to determine how it is affected by the processing type (enzyme-aided extraction or pH shift). Both raw materials, whole Baltic herring and whole roach, were abundant in EAAs, which is common to most fish species. Little variation has been observed between AA composition of different fish species (Njaa and Utne, 1982), but so far the AA composition has not been reported for Baltic herring and roach.

Only minor differences in amino acid composition between FPIs and FPHs prepared by the same process (pH shift or enzymatic hydrolysis) were observed. In case of Baltic herring (**Table 10**), the acid extracted FPI prepared contained less arginine but more glutamine + glutamic acid compared to alkali extracted FPI. However, in case of roach FPIs (**Table 11**), acid extracted FPI contained slightly more arginine and similar amount of glutamine + glutamic acid compared to alkali-made isolate. In case of different enzymes, even fewer significant differences were observed.

Processing type, on the other hand, had more pronounced effects. In case of both raw materials, the FPIs had less glycine and hydroxyproline, and in case of Baltic herring also proline, compared to the FPHs and fish raw materials. Lower content of these amino acids in FPIs prepared using the pH shift (compared to respective raw materials) has been observed in previous studies (Abdollahi and Undeland, 2018; Marmon and Undeland, 2010; Surasani et al., 2018).

Hydroxyproline is exclusively found in collagen, and lycine and proline have been reported as the most abundant amino acids in collagen (Abdollahi et al., 2018). Collagen is effectively removed during the first centrifugation step of the pH shift (Abdollahi et al., 2018), whereas, based on the results of the present study, the collagen was solubilized and/or hydrolyzed by the heating and enzymatic action during the hydrolysis. Also Aspevik et al. (2021) and Idowu et al. (2019) reported considerable amounts of glycine, proline, and/or hydroxyproline in FPHs prepared using bromelain or commercial enzyme 'Food Pro', or alcalase and papain, respectively.

Due to higher removal of NEAAs compared to EAAs in the pH-shift process, the EAA to NEAA ratio was significantly higher in the FPIs compared to raw materials or FPHs (0.85 vs 0.67–0.77 in case of Baltic herring, and 0.84–0.85 vs 0.65–0.79 in case of roach). Similar observations have been made in other studies on FPIs (Abdollahi and Undeland, 2018; Chen et al., 2007; Surasani et al., 2018). For instance, Abdollahi and Undeland (2018) reported that pH-shift processed FPIs from salmon, cod and herring by-products all had EAA to NEAA ratios of 0.47 (not including cysteine, tryptophan or hydroxyproline), which was higher than the respective raw materials (0.38, 0.40, 0.41, respectively). In regard to FPHs, some studies have reported increased content of EAAs after hydrolysis using Alcalase and Flavourzyme (Klompong et al., 2009) and pepsin (Amini Sarteshnizi et al., 2021). Other studies have, however, reported losses in tryptophan and other EAAs (Benjakul and Morrissey, 1997; Shahidi et al., 1995). The observed decrease in EAA to NEAA ratio in FPHs compared with raw materials and FPIs in the present study could be explained by loss of hydrophobic EAAs, valine, leucine, isoleucine, phenylalanine, methionine, and tryptophan to the sediment during centrifugation.

Table 10. Amino acids (mg/1000 mg protein) in Baltic herring raw material, protein isolates (alkaline and acidic pH shift), and protein hydrolysates (hydrolyzed using Protamex, Neutrase, or Corolase) (**I**)[a].

AA	Raw material	Acidic pH shift	Alkaline pH shift	Protamex	Neutrase	Corolase	FAO/WHO/ UNU (2007)[b]
Essential							
His	21.10±0.42[c]	24.35±1.33[d]	24.59±0.76[d]	18.37±0.65[a]	19.86±0.48[bc]	19.38±0.38[ab]	15
Ile	35.77±0.81[b]	46.03±1.74[c]	45.03±0.77[c]	24.43±0.63[a]	25.08±0.32[a]	25.10±0.26[a]	30
Leu	63.53±1.11[b]	81.66±2.97[c]	79.75±2.15[c]	53.46±0.82[a]	55.54±0.49[a]	55.32±0.66[a]	59
Lys	74.05±4.93[ab]	79.92±5.18[ab]	83.87±3.86[b]	70.67±7.81[a]	74.04±5.71[ab]	74.68±5.08[ab]	45
Met	24.62±0.51[b]	29.94±1.01[c]	30.01±0.86[c]	20.71±0.41[a]	21.02±0.11[a]	21.31±0.20[a]	22[c]
Phe	32.73±0.91[b]	37.99±1.56[c]	38.27±0.76[c]	22.91±0.59[a]	23.14±0.50[a]	23.75±0.38[a]	38[d]
Thr	36.24±0.64[b]	42.14±1.59[c]	42.20±1.06[c]	31.25±0.70[a]	31.91±0.68[a]	31.83±0.46[a]	23
Trp	9.04±0.34[b]	11.84±0.16[c]	12.30±0.33[c]	6.06±0.42[a]	5.96±0.27[a]	6.35±0.02[a]	6
Val	39.39±0.72[b]	45.46±1.77[c]	46.25±1.22[c]	30.05±0.70[a]	30.66±0.41[a]	30.72±0.51[a]	39
Non-essential							
Ala	47.59±0.43[a]	54.22±2.11[c]	53.90±1.51[c]	47.45±0.72[a]	50.03±0.72[b]	49.00±0.81[ab]	
Arg	59.32±0.33[b]	59.74±2.51[b]	63.79±1.96[c]	49.28±1.24[a]	50.55±1.03[a]	51.32±1.07[a]	
Asn+Asp	72.24±1.33[b]	86.84±2.78[c]	87.96±3.09[c]	67.77±1.35[a]	69.40±0.70[ab]	69.37±1.17[ab]	
Cys[e]	2.77±0.19[c]	1.95±0.82[b]	1.97±0.39[b]	0.50±0.20[a]	0.36±0.07[a]	0.47±0.21[a]	
Gln+Glu	104.98±1.56[a]	131.52±4.16[d]	125.50±3.78[c]	114.16±1.63[b]	119.02±1.07[b]	117.60±1.80[b]	
Gly	42.43±2.43[b]	28.52±1.20[a]	30.97±0.73[a]	44.78±1.86[bc]	46.71±1.54[c]	47.02±2.89[c]	
Hyp	6.74±0.85[b]	1.95±0.04[a]	2.05±0.01[a]	8.70±0.50[c]	9.46±0.54[c]	9.07±0.31[c]	
Pro	37.54±1.34[b]	33.61±1.78[a]	34.78±1.18[a]	33.66±1.12[a]	33.19±0.86[a]	35.20±1.15[ab]	
Ser	33.74±0.30[b]	37.04±1.54[c]	37.80±1.02[c]	31.61±0.61[a]	32.46±0.38[ab]	32.42±0.57[ab]	
Tyr	29.59±0.49[b]	36.41±1.41[c]	35.10±1.38[c]	18.41±0.43[a]	17.99±0.41[a]	18.54±0.29[a]	
EAA : NEAA	0.77±0.02[b]	0.85±0.01[c]	0.85±0.01[c]	0.67±0.01[a]	0.67±0.01[a]	0.67±0.01[a]	

[a]Mean and standard deviation (n=6), different letters within the same row indicate a statistically significant difference ($p < 0.05$) between samples
[b]Nutritional requirements for adults (FAO/WHO/UNU, 2007)
[c]Methionine + cysteine (16+6)
[d]Phenyalanine + tyrosine
[e]Cysteine was only partially recovered with the method used

Table 11. Amino acids (mg/1000 mg protein)[a] in roach raw material, protein isolates (alkaline and acidic pH shift), and protein hydrolysates (hydrolyzed using Protamex, Neutrase, or Corolase) (**I**).

AA	Raw material	Acidic pH shift	Alkaline pH shift	Protamex	Neutrase	Corolase	FAO/ WHO/ UNU (2007)[b]
Essential							
His	27.11±2.72[b]	22.47±2.77[a]	21.60±2.78[a]	23.99±1.93[ab]	24.26±1.72[ab]	22.93±2.15[a]	15
Ile	39.95±2.47[b]	46.20±0.71[c]	47.11±1.58[c]	32.54±0.62[a]	32.90±1.02[a]	31.12±0.74[a]	30
Leu	70.55±3.48[b]	79.43±0.74[c]	80.91±2.53[c]	60.67±1.00[a]	61.00±1.43[a]	59.86±1.42[a]	59
Lys	78.58±14.00	85.99±5.95	82.22±12.93	77.61±6.77	81.71±5.54	73.94±13.92	45
Met	25.76±1.24[b]	28.28±0.45[c]	29.01±0.98[c]	21.66±0.43[a]	21.93±0.48[a]	21.25±0.50[a]	22[c]
Phe	36.63±2.95[b]	40.51±0.61[c]	41.81±1.78[c]	29.62±0.79[a]	29.84±1.12[a]	29.19±1.06[a]	38[d]
Thr	38.14±1.80[b]	41.28±0.44[c]	42.36±1.47[c]	33.93±0.81[a]	34.01±0.90[a]	33.71±0.97[a]	23
Trp	10.03±0.24[b]	11.71±0.33[c]	13.51±0.35[d]	7.20±0.14[a]	7.42±0.27[a]	6.73±0.29[a]	6
Val	39.49±1.88[b]	43.64±0.34[c]	43.74±1.54[c]	32.07±0.78[a]	32.73±0.67[a]	31.78±0.76[a]	39
Non-essential							
Ala	48.70±1.91[a]	50.12±1.00[ab]	48.77±1.71[a]	50.03±1.38[ab]	49.32±1.44[ab]	51.18±1.40[b]	
Arg	55.06±2.82[a]	57.69±0.73[b]	55.02±2.38[a]	54.00±1.41[a]	53.86±0.96[a]	55.03±1.18[a]	
Asn+Asp	85.34±3.86[b]	91.71±1.67[c]	92.25±3.68[c]	79.01±1.87[a]	79.29±1.87[a]	78.87±1.99[a]	
Cys[e]	2.28±1.10[c]	3.25±0.46[d]	2.11±0.43[bc]	1.25±0.51[a]	1.44±0.31[ab]	1.05±0.15[a]	
Gln+Glu	124.56±5.34[a]	134.62±2.46[b]	133.14±5.63[b]	123.09±3.15[a]	122.62±3.18[a]	125.50±3.26[a]	
Gly	38.03±1.46[b]	30.95±1.27[a]	30.31±2.44[a]	49.72±2.02[c]	50.14±2.25[c]	51.89±2.11[c]	
Hyp	5.29±0.41[b]	1.31±0.02[a]	1.27±0.04[a]	10.87±0.93[c]	10.63±0.58[c]	11.91±0.68[c]	
Pro	35.33±1.53[b]	31.77±0.58[a]	32.23±1.26[a]	38.44±1.45[c]	39.08±0.82[c]	40.20±0.73[c]	
Ser	36.88±1.74[bc]	37.35±0.49[c]	37.23±1.47[bc]	34.43±0.89[a]	35.16±0.82[a]	35.69±0.98[ab]	
Tyr	32.55±1.96[c]	37.14±1.14[d]	38.40±1.90[d]	24.40±0.52[ab]	25.26±0.78[b]	23.26±0.64[a]	
EAA : NEAA	0.79±0.03[c]	0.84±0.02[d]	0.85±0.03[d]	0.69±0.02[ab]	0.70±0.01[b]	0.65±0.03[a]	

[a]Mean and standard deviation (n=6), different letters within the same row indicate a statistically significant difference ($p < 0.05$) between samples
[b]Nutritional requirements for adults (FAO/WHO/UNU, 2007)
[c]Methionine + cysteine (16+6)
[d]Phenyalanine + tyrosine
[e]Cysteine was only partially recovered with the method used

5.1.3 Fatty acid composition

The fatty acid composition of Baltic herring and roach FPIs and FPHs (**Table 12** and **Table 13**) was analyzed, as the composition of lipids in FPIs and FPHs has seldomly been reported in previous studies. Fatty acid composition of lipids remaining in FPIs and FPHs after the extraction is not only of interest from a nutrition point-of-view, but in addition, fatty acids are important precursors for odor-active compounds contributing to fishy odors and flavors, and changes in fatty acid composition have been shown to affect the composition of odorants in fish (Sérot et al., 2002). Unlike with amino acids, fatty acid composition differed considerably between different processes (pH-shift or enzymatic hydrolysis), but also between different variations of the same process. In case of Baltic herring, both FPIs contained a higher proportion of SFAs and less MUFAs compared to FPHs and Baltic herring raw material (34.1–35.3 vs 25.5–29.6% and 32.1–37.5 vs 40.6–41.6%, respectively) (**Table 12**). However, while acid extracted FPI contained the highest relative content of PUFAs (33.8%), the content of PUFAs in the alkali extracted FPI was the lowest (27.2%).

Interestingly, the relative DHA content was particularly high in the FPI extracted by acidic pH shift, in which the relative content of DHA was 14.4%, compared to 10.5% in Baltic herring, and 8.6–9.2% in FPHs. Despite having the lowest proportion of total PUFAs, the alkali extracted FPI was also abundant in DHA (11%). DHA is mostly found in membrane PLs, whereas only a small proportion of depot triacylglycerols and flesh PLs contain DHA. Enrichment of DHA in the FPIs indicates that pH shift led to accumulation of the membrane lipids, though in general the opposite has been observed (Undeland et al., 2002). Other studies have also reported increases in the relative content of total PUFAs and long chain PUFAs (Abdollahi et al., 2021; Wu et al., 2021a) during the pH shift. For instance, Abdollahi et al. (2021) reported that alkaline pH shifting increased the relative content of DHA considerably from 9.7% in herring backbones to 28.6% in the FPI.

The composition of FAs in FPHs were relatively similar compared to the raw material, though FPHs had a slightly higher content of SFAs (26.6–29.6 vs 25.5). The FPH prepared using Neutrase had a significantly lower proportion of PUFAs (29.1%) compared to FPHs prepared using Protamex (31.8%) and Corolase (31.7%) and Baltic herring (33.1%). The difference could be due to a higher degree of oxidation and therefore higher loss of PUFAs in the FPH prepared using Neutrase.

Table 12. Fatty acids (% of total fatty acids, w/w)[a] in Baltic herring protein isolates and hydrolysates (**I**).

	Whole B. herring	Acidic pH shift	Alkaline pH shift	Protamex	Neutrase	Corolase
Saturated						
14:0	5.37±0.02[a]	5.51±0.18[ab]	6.01±0.21[d]	5.70±0.03[bc]	5.89±0.04[cd]	5.71±0.05[c]
16:0	17.39±0.06[a]	24.80±0.13[e]	25.43±0.27[f]	18.02±0.26[b]	20.36±0.11[d]	18.87±0.21[c]
18:0	1.65±0.01[a]	2.69±0.03[e]	2.72±0.01[e]	1.78±0.07[b]	2.14±0.04[d]	1.93±0.04[c]
others[b]	1.13±0.01[ab]	1.15±0.02[bc]	1.19±0.05[c]	1.10±0.02[a]	1.16±0.03[bc]	1.13±0.01[ab]
Σ SFA	25.54±0.07[a]	34.15±0.28[e]	35.34±0.52[f]	26.60±0.35[b]	29.56±0.12[d]	27.64±0.24[c]
Monounsaturated						
16:1(n-7)	9.45±0.11[e]	6.16±0.27[a]	7.50±0.21[b]	8.86±0.10[d]	8.54±0.04[c]	8.33±0.06[c]
18:1(n-7)	2.85±0.04[a]	3.23±0.04[c]	3.69±0.04[d]	2.80±0.05[a]	3.06±0.06[b]	2.79±0.07[a]
18:1(n-9)	25.62±0.14[c]	19.73±0.79[a]	22.84±0.75[b]	26.34±0.22[c]	26.17±0.23[c]	25.98±0.18[c]
20:1(n-9)	1.92±0.02[c]	1.36±0.07[a]	1.60±0.08[b]	2.02±0.01[d]	1.93±0.02[c]	1.93±0.03[c]
24:1(n-9)	0.96±0.01[a]	1.12±0.01[d]	1.26±0.02[e]	1.00±0.02[b]	1.08±0.00[c]	0.99±0.02[b]
others[b]	0.54±0.01[ab]	0.50±0.02[a]	0.56±0.03[abc]	0.59±0.01[bc]	0.62±0.04[c]	0.61±0.07[bc]
Σ MUFA	41.35±0.25[c]	32.10±1.15[a]	37.45±1.07[b]	41.62±0.27[c]	41.39±0.24[c]	40.63±0.28[c]
Polyunsaturated						
n-3						
18:3(n-3)	1.47±0.02[c]	1.26±0.02[b]	1.02±0.03[a]	1.54±0.01[d]	1.47±0.03[c]	1.66±0.03[e]
18:4(n-3)	1.63±0.00[d]	0.96±0.01[b]	0.74±0.03[a]	1.66±0.02[de]	1.40±0.04[c]	1.69±0.03[e]
20:3(n-3)	0.79±0.01[c]	0.73±0.01[b]	0.60±0.01[a]	0.89±0.01[e]	0.84±0.01[d]	0.88±0.01[e]
20:4(n-3)	1.16±0.01[d]	0.93±0.02[b]	0.70±0.04[a]	1.21±0.01[e]	1.03±0.02[c]	1.15±0.01[d]
20:5(n-3)	7.22±0.04[c]	7.03±0.31[c]	5.50±0.46[a]	6.46±0.04[b]	5.74±0.05[a]	6.56±0.02[b]
22:4(n-3)	1.14±0.02[e]	0.73±0.02[b]	0.52±0.02[a]	1.22±0.01[f]	0.99±0.02[c]	1.10±0.02[d]
22:5(n-3)	0.86±0.03[d]	0.73±0.03[b]	0.51±0.04[a]	0.83±0.01[cd]	0.70±0.02[b]	0.81±0.01[c]
22:6(n-3)	10.45±0.05[b]	14.39±1.08[c]	11.03±1.02[b]	9.13±0.14[a]	8.63±0.25[a]	9.19±0.21[a]
24:4(n-3)	1.10±0.01[d]	0.72±0.02[b]	0.52±0.01[a]	1.17±0.01[e]	0.95±0.03[c]	1.07±0.02[d]
24:5(n-3)	0.61±0.06[b]	0.37±0.09[a]	0.28±0.12[a]	0.71±0.11[b]	0.58±0.12[b]	0.73±0.07[b]
Σ n-3	26.44±0.17[c]	27.87±1.45[c]	21.44±1.52[a]	24.82±0.19[b]	22.34±0.17[a]	24.83±0.07[b]
n-6						
18:2(n-6)	3.80±0.02[c]	3.26±0.05[a]	3.39±0.03[b]	4.01±0.03[e]	3.92±0.03[d]	4.05±0.02[e]
20:2(n-6)	1.29±0.01[c]	1.06±0.03[b]	1.02±0.01[a]	1.39±0.01[e]	1.32±0.01[d]	1.33±0.01[d]
20:4(n-6)	0.74±0.00[b]	0.89±0.05[c]	0.76±0.07[b]	0.66±0.01[a]	0.67±0.01[a]	0.68±0.01[a]
22:2(n-6)	0.71±0.01[c]	0.56±0.04[b]	0.52±0.02[a]	0.76±0.01[d]	0.69±0.02[c]	0.71±0.01[c]
others[b]	0.13±0.00[d]	0.11±0.00[b]	0.08±0.00[a]	0.13±0.01[d]	0.12±0.00[c]	0.13±0.00[d]
Σ n-6	6.67±0.04[c]	5.89±0.06[b]	5.77±0.08[a]	6.95±0.04[d]	6.71±0.03[c]	6.90±0.03[d]
Σ PUFA	33.10±0.20[cd]	33.75±1.39[d]	27.20±1.59[a]	31.78±0.19[c]	29.05±0.14[b]	31.73±0.05[c]
Ratios						
UFA:SFA	2.91±0.01[f]	1.93±0.02[b]	1.83±0.04[a]	2.76±0.05[e]	2.38±0.01[c]	2.62±0.03[d]
n-3:n-6	3.97±0.02[c]	4.74±0.29[d]	3.71±0.21[bc]	3.57±0.04[ab]	3.33±0.04[a]	3.60±0.03[b]

[a]Mean and standard deviation (n=6), different letters within the same row indicate a statistically significant difference ($p < 0.05$) between samples
[b]Others include 12:0, 15:0, 17:0, 20:0, 22:0, 14:1(n-5), 15:1, 22:1(n-9), 20:3(n-6), and 22:3(n-6)

Similarly, as with Baltic herring, the FPI prepared by acidic pH shift had the highest relative DHA content (10.9%), followed by alkaline pH shift (8.9%)

(**Table 13**). The lowest relative DHA content (6.4%) as well as total PUFA content (25.4%) were observed in the FPH prepared using Corolase. Again, the difference observed between the three enzymes could be due to oxidative deterioration. There are few reports about the fatty acid composition of FPHs. Silva et al. (2014) reported the fatty acid contents of FPHs prepared from tilapia processing co-products using Alcalase or extracted intestinal enzymes at two different concentrations (100 or 600 mg/L). FPHs prepared by Alcalase or extracted enzymes at 100 or 600 mg/L, contained 101, 70 and 138 g PUFAs/ kg dry FPHs, corresponding to 22, 14, and 18 % of total fatty acids, respectively. DHA was not detected at all in the FPH prepared by intestinal enzymes at the lower concentration. In another study, only the FA composition of oil fraction separated from FPH during hydrolysis of salmon heads (using bromelain and papain) was analyzed, and was concluded to be comparable to the FA composition of the raw materials (Kvangarsnes et al., 2021), indicating that different lipids were separated in similar ratios between the FPH and oil phase.

Table 13. Fatty acids (% of total fatty acids, w/w)[a] in roach protein isolates and hydrolysates (**I**).

	Whole roach	**Acidic pH shift**	**Alkaline pH shift**	**Protamex**	**Neutrase**	**Corolase**
Saturated						
14:0	2.26±0.02^{b}	1.94±0.03^{a}	2.32±0.13^{bc}	2.69±0.01^{e}	2.39±0.03^{cd}	2.44±0.03^{d}
16:0	16.54±0.11^{a}	18.28±0.02^{b}	17.99±0.24^{b}	17.84±0.16^{b}	19.59±0.49^{c}	20.02±0.46^{c}
18:0	3.23±0.06^{a}	4.00±0.04^{c}	3.76±0.14^{b}	3.23±0.04^{a}	3.86±0.17^{bc}	3.99±0.11^{c}
others	1.13±0.02^{bc}	1.08±0.02^{ab}	1.06±0.04^{a}	1.12±0.01^{b}	1.17±0.05^{c}	1.17±0.03^{c}
Σ SFA	23.16±0.15^{a}	25.30±0.05^{b}	25.14±0.27^{b}	24.88±0.21^{b}	27.02±0.73^{c}	27.62±0.63^{c}
Monounsaturated						
16:1(n-7)	13.25±0.10^{a}	12.30±0.06^{a}	17.96±1.59^{b}	17.85±0.12^{b}	17.14±0.59^{b}	17.88±0.52^{b}
18:1(n-7)	5.22±0.07^{a}	5.49±0.04^{b}	5.53±0.10^{b}	5.39±0.02^{ab}	5.19±0.16^{a}	5.21±0.27^{a}
18:1(n-9)	22.88±0.22^{d}	21.72±0.20^{b}	20.67±0.38^{a}	21.75±0.18^{b}	22.42±0.14^{c}	22.38±0.23^{b}
20:1(n-9)	0.87±0.02^{ab}	0.97±0.01^{d}	0.87±0.05^{a}	0.91±0.01^{bc}	0.95±0.02^{cd}	0.93±0.01^{cd}
others	0.61±0.02^{b}	0.71±0.02^{c}	0.78±0.02^{d}	0.57±0.02^{ab}	0.60±0.07^{ab}	0.54±0.01^{a}
Σ MUFA	42.83±0.17^{b}	41.20±0.25^{a}	45.81±1.10^{c}	46.47±0.11^{cd}	46.29±0.39^{cd}	46.94±0.42^{d}
Polyunsaturated						
n-3						
18:3(n-3)	1.87±0.05^{d}	1.37±0.03^{c}	1.03±0.11^{ab}	1.15±0.03^{ab}	1.18±0.14^{b}	1.01±0.11^{a}
18:4(n-3)	0.61±0.02^{b}	0.48±0.01^{a}	0.60±0.04^{b}	0.68±0.02^{c}	0.63±0.02^{b}	0.62±0.02^{b}
20:4(n-3)	0.72±0.02^{b}	0.66±0.01^{a}	0.73±0.01^{b}	0.83±0.01^{c}	0.75±0.04^{b}	0.73±0.03^{b}
20:5(n-3)	8.30±0.12^{c}	7.40±0.04^{b}	6.46±0.16^{a}	7.07±0.06^{b}	6.67±0.31^{a}	6.41±0.27^{a}
22:5(n-3)	2.31±0.04^{e}	1.98±0.03^{c}	1.95±0.01^{bc}	2.17±0.03^{d}	1.91±0.01^{ab}	1.88±0.02^{a}
22:6(n-3)	8.63±0.17^{c}	10.89±0.23^{d}	8.92±0.14^{c}	7.47±0.07^{b}	6.62±0.26^{a}	6.38±0.31^{a}
others	0.73±0.01^{c}	0.71±0.01^{c}	0.55±0.04^{a}	0.64±0.01^{b}	0.56±0.04^{a}	0.52±0.03^{a}
Σ n-3	23.15±0.07^{c}	23.50±0.26^{c}	20.25±0.38^{b}	20.01±0.03^{b}	18.31±0.80^{a}	17.55±0.76^{a}
n-6						

	Whole roach	Acidic pH shift	Alkaline pH shift	Protamex	Neutrase	Corolase
18:2(n-6)	4.85±0.10^{d}	3.70±0.04^{c}	3.32±0.12^{b}	3.72±0.05^{c}	3.24±0.28^{b}	2.93±0.26^{a}
20:2(n-6)	1.12±0.02^{cd}	1.17±0.00^{d}	0.95±0.10^{a}	1.02±0.02^{ab}	1.07±0.02^{bc}	1.03±0.01^{ab}
20:4(n-6)	4.11±0.07^{c}	4.37±0.07^{d}	3.77±0.23^{b}	3.09±0.02^{a}	3.21±0.05^{a}	3.10±0.05^{a}
others	0.77±0.01^{a}	0.77±0.00^{a}	0.77±0.05^{a}	0.80±0.02^{ab}	0.84±0.03^{b}	0.84±0.03^{b}
Σ n-6	10.85±0.14^{d}	10.01±0.04^{c}	8.80±0.48^{b}	8.64±0.11^{b}	8.37±0.32^{ab}	7.90±0.29^{a}
Σ PUFA	34.00±0.09^{c}	33.50±0.29^{c}	29.05±0.86^{b}	28.65±0.11^{b}	26.69±1.11^{a}	25.44±1.05^{a}
Ratios						
UFA:SFA	3.32±0.03^{c}	2.95±0.01^{b}	2.98±0.04^{b}	3.02±0.03^{b}	2.70±0.10^{a}	2.62±0.08^{a}
n-3:n-6	2.13±0.03^{a}	2.35±0.02^{c}	2.30±0.08^{c}	2.32±0.03^{c}	2.19±0.03^{ab}	2.22±0.03^{b}

[a]Mean and standard deviation (n=6), different letters within the same row indicate a statistically significant difference ($p < 0.05$) between samples
[b]Others include 12:0, 15:0, 17:0, 20:0, 22:0, 14:1(n-5), 15:1, 22:1(n-9), 20:3(n-6), and 22:3(n-6)

In study **III**, FA composition of different minces were compared at the beginning of the study to establish the effect of the additions on composition. Main differences in the FAs were due to the added lipids from AB, LR and SR. Compared to the control mince, mince with SR contained more 16:0 (23.1 vs 18.6% of total FAs), 16:1(*n*-7) (15.5 vs 11.1%), and 18:1(*n*-7) (4.0 vs 3.4%), which are the most abundant FAs in sea buckthorn pulp and peel (Yang and Kallio, 2001). Mince with LR contained more 18:3(*n*-3) (2.9 vs 1.4%) and 18:2(*n*-6) (5.6 vs 4.1%), which were previously reported to be the most abundant FAs in lingonberry pomace oil (Kitrytė et al., 2020).

The changes in EPA and DHA content (**Table 14**) due to oxidative degradation during 10 months of storage at –20 °C were investigated in study **I**. EPA and DHA were chosen as indicators due to their importance and high susceptibility to oxidation compared to shorter FAs with less double bonds (Cosgrove et al., 1987). The content of EPA and DHA (mg/100 mg lipids) in Baltic herring minces with and without antioxidants differed statistically significantly ($p < 0.05$) at the beginning of storage. Compared to the control, DHA and EPA content were lower in minces containing LR and SR, and in case of DHA also AB, since all of these contain fatty acids other than EPA or DHA. On the contrary, minces containing αT+AA or EDTA, and mince containing EDTA, in case of DHA and EPA, respectively, contained significantly more of these FAs compared to the control, which is likely due to lipid oxidation occurring in the control mince during sample preparation prior to the first measurement. Minor differences could have also occured through sampling of minces for lipid extraction.

Table 14. EPA and DHA content (mg/100 mg lipids)[a] in Baltic herring mince (from fillets without skin) with and without antioxidants after 0 and 10 months of storage at –20 °C (**III**).

Baltic herring mince	EPA 0 months	EPA 10 months	DHA 0 months	DHA 10 months	EPA loss	DHA loss
Gutted & beheaded	5.98±0.01^{efB}	5.63±0.05^{eA}	9.36±0.02^{eB}	8.65±0.15^{eA}	5.75 %	7.63 %
Fillet, with skin	5.41±0.01^{bB}	5.05±0.01^{bA}	7.79±0.01^{aB}	6.61±0.01^{aA}	6.62 %	15.25 %
Fillet, skinless (C)	5.93±0.04^{deB}	5.36±0.00^{cA}	8.87±0.06^{cB}	7.78±0.01^{bA}	9.64 %	12.20 %
EDTA	6.03±0.05^{fB}	5.61±0.01^{eA}	9.21±0.09^{dB}	8.22±0.03^{cA}	7.02 %	10.80 %
αT+AA	5.88±0.04^{dB}	5.45±0.06^{cdA}	9.49±0.07^{efB}	8.59±0.06^{deA}	7.35 %	9.45 %
AB	5.96±0.01^{efB}	5.56±0.05^{deA}	9.54±0.01^{fB}	8.42±0.07^{dA}	6.87 %	11.69 %
LR	5.60±0.00^{cB}	5.37±0.05^{cA}	9.11±0.01^{dB}	8.51±0.05^{deA}	4.06 %	6.64 %
SR	4.90±0.01^{aB}	4.71±0.02^{aA}	8.47±0.01^{bB}	7.84±0.03^{bA}	3.82 %	7.51 %

[a]Mean and standard deviation (n=3), different lower case letters within the same row indicate a statistically significant difference ($p < 0.05$) between samples, while different capital letters indicate a significant difference between 0 and 10 months.

According to 2-way ANOVA, sample type, storage time, and sample type*storage time were all statistically significant ($p < 0.001$) in case of both EPA and DHA, i.e. the storage time effected the EPA and DHA content differently depending on whether antioxidants were added. The loss of EPA and DHA during the 10 months of frozen storage was lower in all minces containing additions when compared to the control. Mince containing LR had the lowest decrease in EPA content, while SR had the lowest decrease in DHA, indicating that these berry press residues were able to inhibit oxidation of PUFAs. Previously, Joaquin et al. (2008) observed decreases up to 45% in the concentrations of EPA and DHA in minced herring during 4 months of frozen storage, but treatment of the mince with milk protein concentrate retained these PUFAs better compared to the untreated mince. In another study, vitamin C and tea polyphenols helped retain EPA and DHA during during microwave-drying of silver carp (Fu et al., 2015). In the present study, vitamin C (ascorbic acid) in combination with α-tocopherol, was able to protect EPA and DHA, but not as efficiently as LR and SR.

5.1.4 Lipid class composition

Lipid classes of the FPIs and FPHs were analysed semiquantitatively in study **I** (**Figure 8** and **Figure 9**). Regarding the effect of different processes (pH shift or

enzymatic hydrolysis), similar trends were seen in case of both Baltic herring and roach. The relative content of phospholipids (PLs) in all FPIs was significantly ($p < 0.05$) higher compared to the FPHs of the same fish. Except for the alkali extracted FPI from Baltic herring, FPIs also contained a significantly higher relative amount of PLs compared to the raw material. The results are in line with the higher relative content of DHA observed in FPIs (**Table 12** and **Table 13**), especially acid extracted FPI, having both highest relative DHA and PL content in case of both raw materials. Polar PLs can be separated to the sediment during the first centrifugation of the pH shift, while neutral storage lipids float to the top (Kristinsson et al., 2005). Efficient removal of membranes, however, requires high speed centrigugation (>10000 x *g*) or pretreatments using agents such as Ca^{2+} and citric acid (Liang and Hultin, 2005a, 2005b). However, based on the present data and previous literature, it seems that without specific pretreatments to remove PLs, an equal or higher amount of total lipids compared to PLs are removed, leading to increase in the relative content of PLs. Chanarat and Benjakul (2013) reported that Indian mackerel mince contained 9.1% total lipids and 0.99% PLs (on a dry weight basis), while alkali extracted FPI without and with prewashing, contained 1.6% and 1.4% total lipids and 0.23% and 0.18% PLs, respectively, corresponding to 11%, 15%, and 13% relative content of PLs in mince and FPIs without and with washing, respectively. In a another study, 70% and 45% reductions of total lipids and PLs (on a lipids /g of protein basis), respectively, were observed during centrifugation following acid solubilization of herring, meaning that the relative content of PLs increased (Undeland et al., 2002).

Both Baltic herring and roach FPIs had significantly lower relative contents of triacylglycerols (TAGs) and higher content of monoacylglycerols (MAGs) compared to FPHs and raw materials (**Figure 8** and **Figure 9**). The lower relative content of TAGs could be attributed to higher removal of neutral lipids compared to PLs, but especially in case of alkali extracted FPI from Baltic herring, it was also accompanied by a significantly higher relative content of free fatty acids (FFAs), indicating lipid hydrolysis. In case of roach, both FPIs showed high relative contents of FFAs. The pH-shift processing was previously seen to increase the susceptibility of microalgae fatty acids to lipolysis by digestive enzymes, especially when solubilization was conducted at pH 10 instead of 7 (Cavonius et al., 2016). Alkaline conditions are known to induce hydrolysis of TAGs through saponification, which may explain higher rate of lipid oxidation in oil-water systems in alkaline conditions (Kim et al., 2016). Increased hydrolysis is likely to explain also higher relative content of MAGs in FPIs.

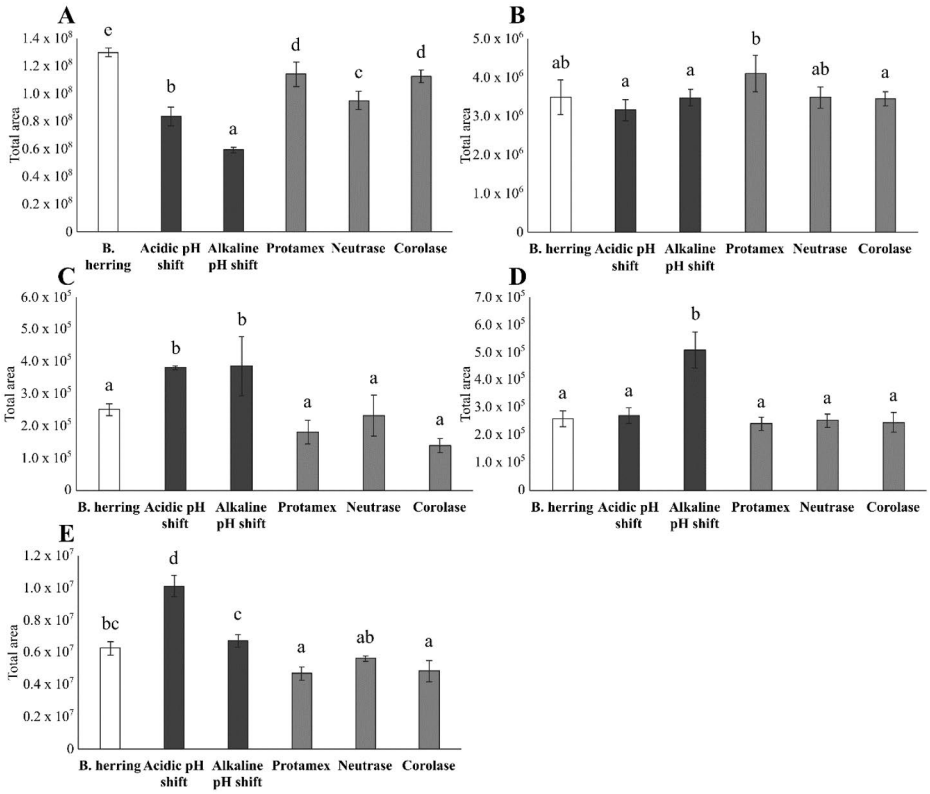

Figure 8. Total peak areas of triacylglycerols (A), diacylglycerols (B), monoacylglycerols (C), free fatty acids (D), and phospholipids (E) in Baltic herring raw material, protein isolates (prepared using acidic or alkaline pH shift), and protein hydrolysates (prepared by hydrolysis using Protamex, Neutrase, or Corolase) (**I**) based on semiquantitative UHPLC–ESI–MS analysis. Different letters indicate a statistically significant ($p < 0.05$) difference between samples.

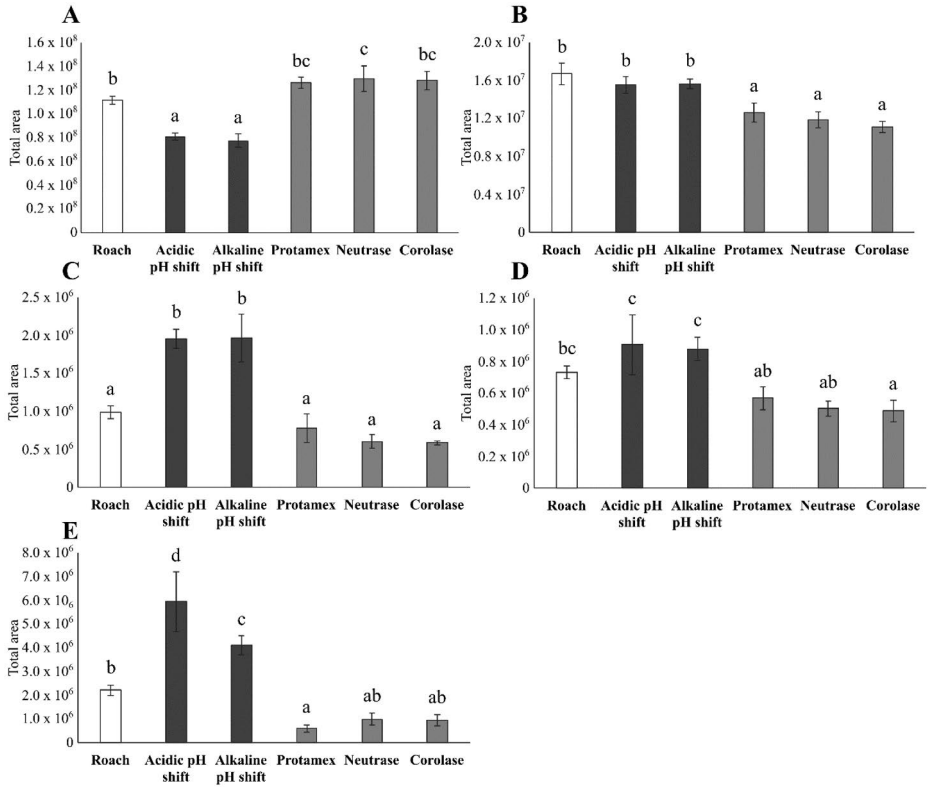

Figure 9. Total peak areas of triacylglycerols (A), diacylglycerols (B), monoacylglycerols (C), free fatty acids (D), and phospholipids (E) in roach raw material, protein isolates (prepared using acidic or alkaline pH shift), and protein hydrolysates (prepared by hydrolysis using Protamex, Neutrase, or Corolase) (**I**) based on semiquantitative UHPLC–ESI–MS analysis. Different letters indicate a statistically significant ($p < 0.05$) difference between samples.

The FPHs, on the other hand, had mostly a similar lipid class composition compared to the raw material and to each other. In case of Baltic herring, all FPHs, but especially the one prepared with Neutrase, contained a significantly lower relative content of TAGs compared to the raw material. The lower relative content of TAGs may be related to losses through oxidation, as the Baltic herring FPH prepared using Neutrase had the lowest relative content of PUFAs as well. On the other hand, all roach FPHs contained more TAGs and less PLs compared to the raw material, though in case of TAGs the difference were always not statistically significant Previous literature on the content of different classes of lipids in FPHs is scarce. Some studies have reported the content of PLs or free fatty acids of the fish raw materials prior to hydrolysis (Khantaphant et al., 2011; Kvangarsnes et al., 2021; Yarnpakdee et al., 2012c), but not in the prepared FPHs.

The results of the present work suggest that the distribution of lipid classes during hydrolysis was more dependent on the raw material than the enzyme used.

5.2 Oxidation

5.2.1 Protein oxidation

Carbonyl contents of FPIs and FPHs were similar between the two fish species (**Figure 10**). Acid extracted FPI from roach and Baltic herring contained 14.8 and 15.3 nmol carbonyls/ mg protein, respectively, while the content in alkali extracted FPIs was higher, 31.5 and 29.4 nmol/mg in case of roach and Baltic herring, respectively. The values were considerably higher than previously reported for herring FPI (Marmon and Undeland, 2013). In a study by Marmon and Undeland (2013), protein carbonyls in alkali extracted FPI were found at a level of 2.5 nmol/mg protein, which was not significantly higher compared to the raw material. In the present study, the FPIs were subjected to a brief pasteurization treatment (Nisov et al., 2021), as well as freeze-drying, both of which may have induced denaturation and oxidation of proteins, and may in part explain the higher content of protein carbonyls observed. The protein carbonyl content in alkali extracted FPIs was twice the content observed in the acid extracted FPI.

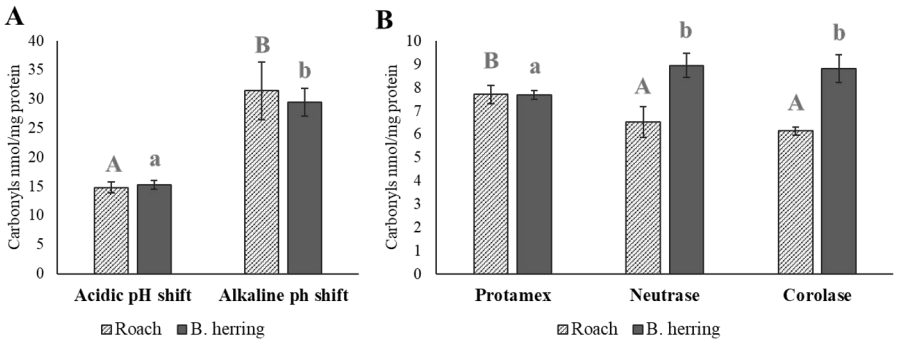

Figure 10. Protein carbonyls (nmol/mg protein) in roach and Baltic herring protein isolates (prepared using acidic or alkaline pH shift) (A), and protein hydrolysates (prepared by hydrolysis using Protamex, Neutrase, or Corolase) (B) (**I**). Different capital and small letters indicate a statistically significant ($p < 0.05$) difference between roach and Baltic herring samples, respectively.

The FPHs showed more moderate levels of protein carbonyls, ranging 7.7–9.0 and 6.1–7.7 nmol/mg protein for Baltic herring and roach FPHs, respectively. Interestingly, FPH hydrolyzed using Protamex had the lowest carbonyl content of Baltic herring FPHs, but highest content of roach FPHs. The degree of

hydrolysis, reported by Nisov et al. (2021), was lowest in case of FPH prepared by Protamex in case of Baltic herring, but highest in case of roach, though differences were not statistically significant. The higher degree of hydrolysis could explain higher degree of protein carbonylation, as more amino acids are exposed to pro-oxidants. Nikoo et al. (2019) reported the effect of different washing treatments or an antioxidant on protein carbonyl values after 1, 2, or 3 h hydrolysis of rainbow trout by-product proteins using Alcalase. The lowest carbonyl value, < 3 nmol/mg protein, was detected after the shortest hydrolysis time, 1 h, with prior washing of the raw material with only distilled water.

5.2.2 Primary lipid oxidation

In case of both roach and Baltic herring FPIs and FPHs (**I**), the FPI extracted by alkaline pH shift had the highest PV of all samples (**Figure 11**). Though the difference in PV (as meq/kg FPI) can partly be explained by the considerably high lipid content in alkali extracted FPIs (**Table 9**), they also had the highest PVs even expressed as meq/kg oil. Acid extracted FPIs from both roach and Baltic herring, however, showed more moderate hydroperoxide formation. Most previous studies comparing acidic and alkaline pH shift have reported higher rates of oxidation for acidic pH shift (Kristinsson and Hultin, 2004a; Phetsang et al., 2021b; Zhong et al., 2016). This has been considered to be due to unfolding of hemoglobin in acidic conditions, leading to an increase in its pro-oxidativity (Kristinsson and Hultin, 2004b), whereas as alkaline treatment has even been seen to have a protective effect compared to native hemoglobin (Kristinsson and Hultin, 2004a). However, Abdollahi et al. (2020) also reported that alkali-extracted FPI from herring by-products had a higher PV and higher malondialdehyde content compared to the acid process. The pro-oxidativity of heme proteins from different fish species have been shown to be influenced differently by varying pH (Maqsood and Benjakul, 2011a) and may explain contradictory findings of studies comparing lipid oxidation in acid and alkali extracted FPIs. Roach FPIs and FPHs had lower PVs compared to the respective Baltic herring FPIs and FPHs, which is likely related to the content of pro-oxidants, as roach is a white fish while Baltic herring is a dark-muscled fish. Similarly, Abdollahi et al. (2020) reported considerably higher PVs for FPIs from herring compared to salmon by-products, despite similar PVs in the raw materials.

Roach FPHs prepared using Protamex, Neutrase or Corolase had similar PVs, but in case of Baltic herring, FPH prepared using Neutrase had the highest PV (3.0 meq/kg FPH) compared to the other two FPHs (0.7–1.2 meq/kg FPH). While this difference of PV as meq/kg FPH was not statistically significant ($p > 0.05$), the PV of Baltic herring FPH produced with Neutrase expressed per kg oil (96

meq/kg oil) was significantly higher to other FPHs (21–37 meq/kg oil) and also the acid-extracted FPI (37 meq/kg oil). The Baltic herring FPH prepared with Neutrase had also the lowest content of PUFAs (**Table 12**) and TAGs (**Figure 8**) compared to FPHs prepared using Protamex or Corolase).

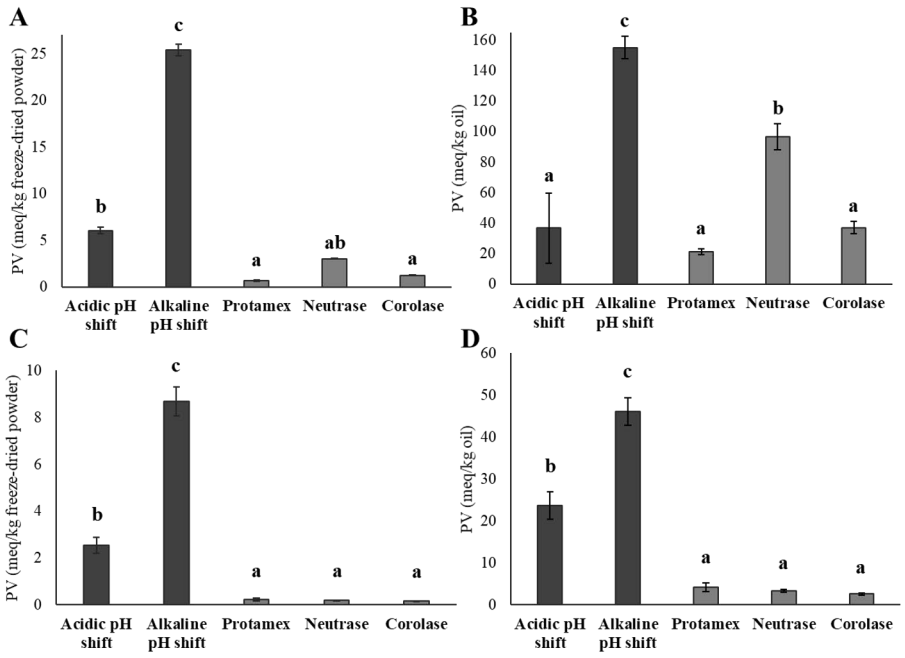

Figure 11. Peroxide values in Baltic herring (A-B) and roach (C-D) FPIs and FPHs, both as meq/kg freeze-dried protein isolate or hydrolysate (A and C) and meq/kg oil (B and D) (**I**). Different letters indicate a statistically significant ($p < 0.05$) difference between roach and Baltic herring samples, respectively.

PVs of Baltic herring minces with and without antioxidants after up to 10 months storage at –20 °C (study **III**) or 21 days at 1 °C (study **IV**) were analyzed as indicators of primary lipid oxidation (**Table 15**). The PVs of fresh minces (at the 0-month (study **III**) or 0-day (study **IV**) timepoints ranged between 0.3–1.3 and 0.1–0.9 meq/kg mince, respectively. At the end of frozen storage for 10 months, the lowest PV was measured in AB (1.3 meq/kg mince), followed by SR3 (1.8 meq/kg mince, both having a significantly ($p < 0.05$) lower PV compared to the control (3.0 meq/kg). In study **III**, PV of all minces was higher after 10 months of frozen storage compared to the beginning of storage, but as the PV was not analyzed at any point in between, it is difficult to estimate to what extent the added antioxidants were able to retard hydroperoxide formation. Hydroperoxides are unstable, and are rapidly transformed into volatile and non-volatile secondary

oxidation products (Frankel, 1983). For instance, mince with 3% LR (LR3) had the highest PV at 0 months (1.3 meq/kg mince) and second highest PV at 10 months (1.3 and 4.1 meq/kg mince). In case of refridgerated minces, LR3 also had a significantly higher PV compared to the control at the beginning, 0 d (1.1 vs 0.1 meq/kg mince), and end of storage, 21 d (5.9 vs 3.6 meq/kg mince), but comparing their PVs throughout the storage period, it can be seen that mince with 3% LR maintained a low PV longer than the control.

In the control mince (from fillets with skin) in study **IV**, the highest PV measured was at day 10, 6.5 meq/kg. At day 3, the PV in the control was already 3.6 meq/kg, which was significantly higher compared to other minces, except for αT+AA, SR1.5 and SRO1. Most of the antioxidants delayed and reduced the total extent of hydroperoxide formation. For instance, the highest PV observed for SRO3 was 4.4 meq/kg mince, which was detected at 14 days. LR3 had a similar PV at 14 days, 4.6 meq/kg, but was still increasing at 21 days (5.9 meq/kg). In a previous study, minced mackerel stored at 5 °C for up to 8 days showed a rapid increase between days 2 and 4 (to approx. 20 meq/kg oil), after which the PV increased slowly during the rest of storage, while minces with added ethanol extract of *P. fucoides* or BHT maintained a low PV (< 3 meq/kg oil) throughout storage (Babakhani et al., 2016).

When comparing different concentrations of added berry press residues (1%, 1.5%, and 3%) throughout the storage period of 21 days, SR had more diverse effects at different concentrations, while in case of LR there were few statistical differences between different concentrations. Previously lingonberry press residue (81% moisture) at a 30% concentration/dry weight of fish was seen to inhibit hydroperoxide formation during pH-shift processing of herring and salmon by-products (Abdollahi et al., 2020). Dried lingonberry press residue at a concentration of 15% was seen also seen to retard hydroperoxide formation in vegetable oil spreads (Baltuonytė et al., 2022). The higher PVs observed in SR3 and αT+AA could be due to pro-oxidative effects or stabilization of hydroperoxides. Ascorbic acid and α-tocopherol are both endogenous antioxidants in the fish muscle, and they have a synergy due to the ability of ascorbic acid to regenerate α-tocopherol. Both α-tocopherol and ascorbic may, however also act as pro-oxidants (Jayasinghe et al., 2013; Jerzykiewicz et al., 2013).

Table 15. Peroxide values[a] of Baltic herring minces stored at –20 °C or 1 °C with and without antioxidants. In study **III**, antioxidants were added in minced fillets without skin, in study **IV** into minced fillets with skin.

	–20 °C (**III**)		1 °C (**IV**)					
	0 months	**10 months**	**0 days**	**3 days**	**7 days**	**10 days**	**14 days**	**21 days**
Gutted and beheaded	1.01±0.01^{c}	2.76±0.02^{d}						
Fillet with skin (control **IV**)	1.20±0.04^{d}	2.98±0.03^{d}	0.10±0.00^{a}	3.59±0.24^{c}	4.37±0.26^{cde}	6.52±0.33^{e}	5.96±0.25^{ab}	3.57±0.86^{ab}
Fillet without skin (control **III**)	0.51±0.07^{b}	2.79±0.01^{d}						
EDTA	0.40±0.02^{ab}	2.44±0.01^{c}						
αT+AA	0.26±0.03^{a}	6.73±0.18^{f}	0.10±0.09^{a}	2.87±0.19^{bc}	8.17±0.05^{f}	10.50±0.20^{d}	14.66±1.24^{e}	8.93±0.47^{de}
AB	0.30±0.01^{a}	1.32±0.01^{a}						
LR1			0.45±0.05^{ab}	1.33±0.26^{a}	2.87±0.28^{abc}	5.05±0.66^{cd}	5.55±0.12^{ab}	5.73±1.27^{bc}
LR1.5			0.63±0.09^{bc}	1.42±0.14^{a}	3.82±0.47^{abcd}	4.13±0.04^{bc}	5.82±0.85^{ab}	5.73±0.17^{bc}
LR3	1.29±0.08^{d}	4.08±0.01^{e}	1.09±0.15^{cd}	1.12±0.04^{a}	2.15±0.44^{ab}	2.88±0.16^{ab}	4.55±0.36^{a}	5.93±0.41^{bc}
SR1			0.74±0.29^{bc}	1.91±0.30^{ab}	4.61±0.11^{cde}	5.79±0.68^{de}	5.43±0.04^{ab}	6.81±0.09^{cd}
SR1.5			1.38±0.09^{d}	2.99±0.34^{bc}	5.52±0.56^{de}	4.74±0.48^{cd}	10.14±0.40^{d}	9.76±0.76^{e}
SR3	0.41±0.02^{ab}	1.79±0.00^{b}	1.88±0.00^{e}	3.58±0.57^{c}	5.72±0.68^{e}	6.58±0.13^{e}	8.35±0.43^{cd}	6.64±0.50^{cd}
SRO1			0.71±0.02^{bc}	2.62±0.15^{bc}	3.77±0.10^{abc}	5.02±0.22^{cd}	7.34±0.55^{bc}	5.16±0.22^{bc}
SRO1.5			0.84±0.12^{bc}	1.44±0.29^{a}	4.69±1.09^{cde}	4.83±0.04^{cd}	5.38±0.25^{ab}	2.16±0.34^{a}
SRO3			0.67±0.06^{bc}	0.85±0.15^{a}	1.42±0.24^{a}	2.52±0.35^{a}	4.35±0.23^{a}	1.79±0.41^{a}

[a]Mean and standard deviation (n=2), different letters within the same column indicate a statistically significant ($p < 0.05$) difference between samples.

5.2.3 Volatile secondary lipid oxidation products

Volatile oxidation compounds were analyzed as secondary lipid oxidation indicators in studies **I**, **III** and **IV**. The peak areas of identified volatile compounds in FPIs and FPHs from Baltic herring and roach (**I**) were used as variables in Principal Component analysis (PCA) (**Figure 12**). In case of both Baltic herring and roach, most oxidation related volatiles accounted for variation on the X axis, while 1-penten-3-ol and propanal (roach samples) or nonanal (Baltic herring samples) accounted for most of the variation on the Y axis. Alkali extracted FPIs were associated with several lipid oxidation derived volatiles, such as (*E*)-2-hexenal, 2,4-heptadienal (*E,E* and *E,Z*), 2,6-nonadienal, and hexanoic acid in Baltic herring FPI, and nonanal, 2-butanone, 1-penten-3-one, and 2,6-nonadienal in roach FPI. Acid extracted FPIs, however, were characterized by lower peak areas of all volatiles compared to alkali extracted FPIs.

Despite having lower release of most oxidation related volatiles, FPHs from Baltic herring and roach showed high peak areas of 1-penten-3-ol and nonanal (Baltic herring) or 1-penten-3-ol and propanal (roach). Propanal and 1-penten-3-ol are oxidation products of n-3 PUFAs, such as DHA (Ahonen et al., 2022). Nonanal can be produced from the 10-OOH hydroperoxide of 18:1(*n*-9) (Frankel, 1983), which was measured at a significantly higher relative content in Baltic herring FPHs (26.0–26.3%) compared to FPIs (19.7–22.8%) (**Table 12**). Among Baltic herring FPHs, FPH prepared using Neutrase had the highest peak area of most volatiles, which is in line with the higher PV, higher content protein carbonyls, and lower relative content of PUFAs compared to the FPHs prepared using Protamex or Corolase. In case of roach, Corolase had the highest peak areas of 1-penten-3-ol and propanal. Compared to roach FPHs prepared by Protamex or Neutrase, the Corolase FPH had the lowest relative content of *n*-3 PUFAs (**Table 13**).

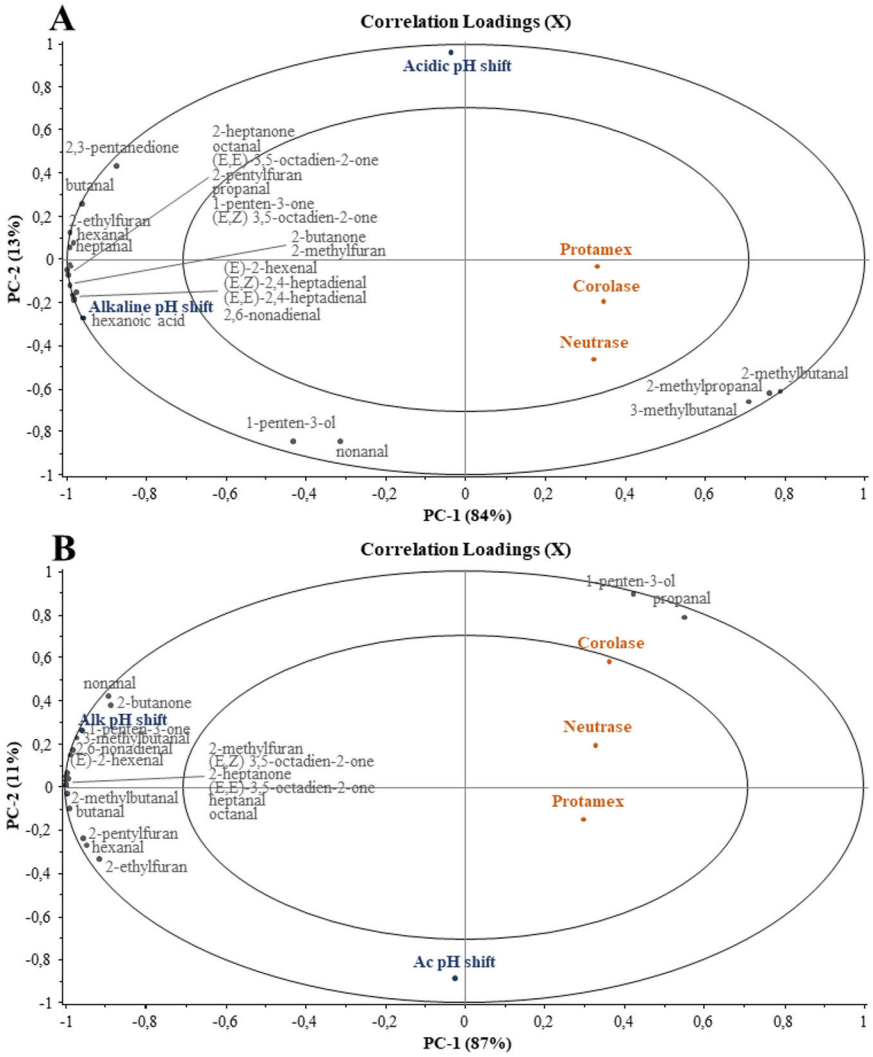

Figure 12. PCA correlation loadings of identified volatiles (as peak areas, n=19) in FPIs and FPHs (n=5) from Baltic herring (A) and roach (B) as well as sample names as dummy variables (**I**).

PCA was also used to investigate correlations of volatiles (correlation loadings, as peak areas) in Baltic herring minces (scores) in study **III** (**Figure 13**). PC1 accounted for 67% of variation between samples and was related to secondary lipid oxidation products, such as 2-ethylfuran, 1-penten-3-ol, and 2,4-heptadienal (*E,E* and *E,Z*). PC2, accounting for 26% variation, separated the minces based on peak areas of 3-methylbutanal, 2-methylbutanal, and 2-hydroxy-3-butanone. The minces after 0 and 2 months of storage were characterized by higher areas of these volatiles and lower areas of most other volatiles. 3-methylbutanal and 2-methylbutanal, branched aldehydes produced

by various reactions from amino acids, are important aroma compounds in many foodstuffs (Smit et al., 2009). They were previously seen to be the most abundant volatiles in fresh, cooked Baltic herring, while their content was greatly reduced during 8 days of storage at 6 °C (Aro et al., 2003). Similarly in the present study, the content of 2-methylbutanal and 3-methylbutanal were decreased after 2 months of frozen storage, but then increased again slightly after 6 months. The biggest increase in oxidation derived volatiles of minces occurs between 6 and 10 months.

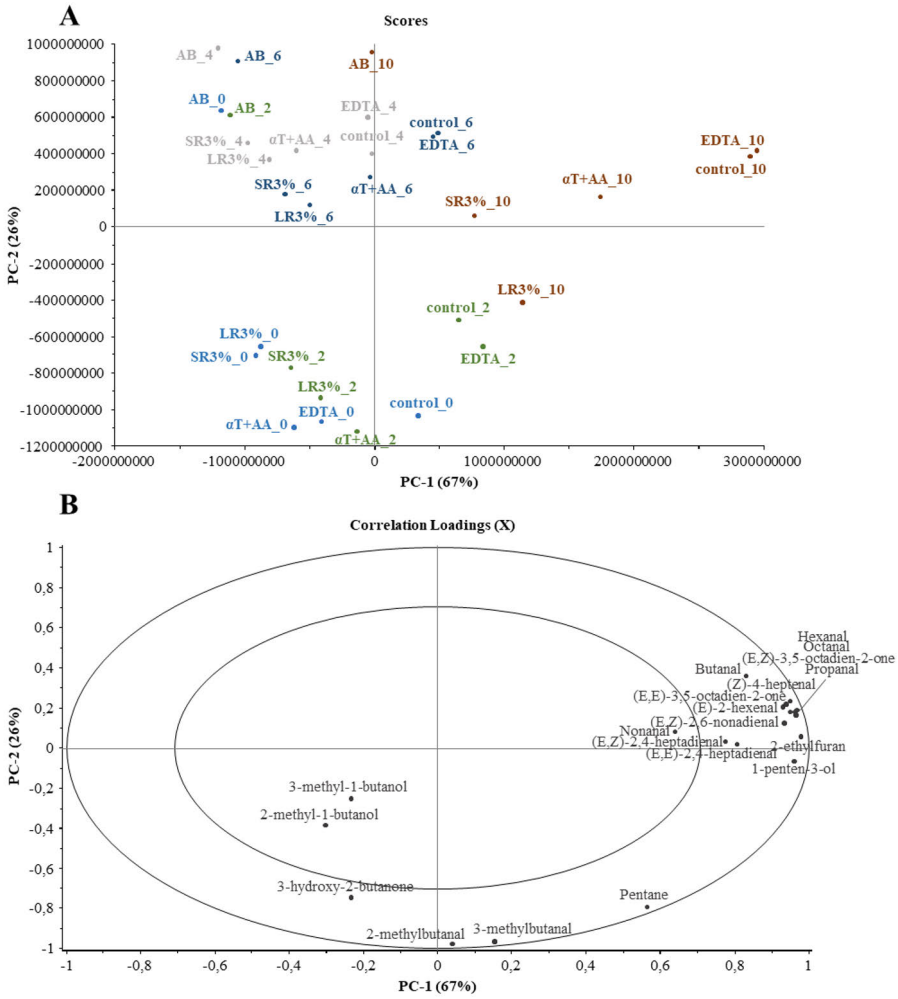

Figure 13. PCA scores (A) and correlation loadings (B) of identified volatiles (as peak areas, n=20) in Baltic herring minces with and without antioxidants, stored at –20 °C for 0, 2, 4, 6, or 10 months (n=30) (**III**). Different colors in scores refer to minces at different time points.

When comparing the positions of different minces within the same time point, the control was always located further right due to higher rate of formation of oxidation related volatiles, compared to all other minces except for EDTA. EDTA, though not strictly an antioxidant, has been seen to have a protective effect from lipid oxidation due to its metal chelating ability. At a concentration of 2 mmol/kg, EDTA was seen to have some protective effect against hemoglobin-mediated lipid oxidation in washed horse mackerel muscle stored 4 days at 4 °C, but it was less effective compared to compounds with reducing ability, and grape proanthocyanidins with both reducing and chelating properties (Maestre et al., 2009). In the present study, the concentration of EDTA was approximately 0.2 mmol/kg mince, which may have been too low to achieve a sufficient effect.

AB, consisting of seven plant CO_2 extracts, was previously shown to delay oxidation of triacylglycerols in Atlantic salmon fillets (Tarvainen et al., 2015) as well as cholesterol oxidation in Atlantic salmon patties (Tarvainen et al., 2016). In the present study, AB reduced the extent of lipid oxidation after 10 months of storage, based on better preservation of EPA (**Table 14**), lower PV (**Table 15**), and lower formation of volatile oxidation products compared to the control (**Figure 13**). Both berry juice press residues, LR and SR (3%) were effective in retarding formation of secondary oxidation products, and only showed moderate increases in oxidation related volatiles during storage (**Figure 13**). The result is in line with the EPA and DHA loss (**Table 14**), which were lowest in minces containing LR and SR.

In study **IV**, 1-penten-3-ol, hexanal, and 3,5-octadien-2-one were selected as secondary lipid oxidation indicators (**Figure 14**), since they are produced from different precursors and were seen to be good indicators based on study **III**. 3,5-octadien-2-one is produced from DHA (Ahonen et al., 2022), while 1-penten-3-ol can result from 15-OOH hydroperoxide of EPA and 17-OOH hydroperoxide of DHA (Lee et al., 2003), and hexanal is mainly formed from *n*-6 fatty acids, e.g. through the 13-hydroperoxide (13-OOH) of linoleic acid (18:2n-6) (Frankel, 1983).

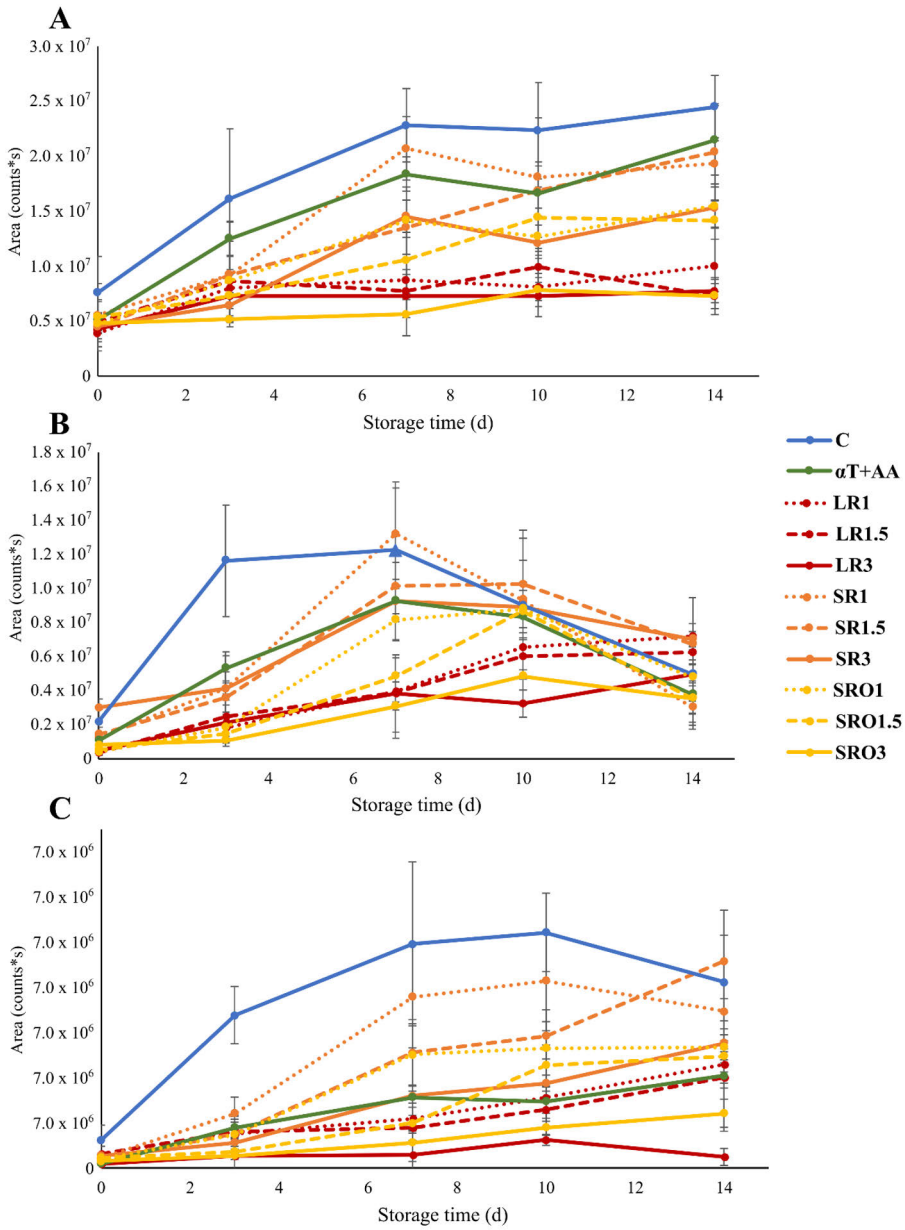

Figure 14. Changes in volatile secondary oxidation compounds, 1-penten-3-ol (A), hexanal (B), and 3,5-octadien-2-one (C) (as peak areas), in Baltic herring minces with and without antioxidants stored at 1 °C up to 14 days (**IV**).

SRO3 and αT+AA, despite showing higher or equal PVs compared to the control (**Table 15**), showed lower formation of 1-penten-3-ol, hexanal, and 3,5-octadien-2-one compared to the control. Of the berry press residues, LR and SRO were the most efficient in inhibiting volatile formation. In case of SR, only the 3%

concentration was as or more effective than αT+AA. Interestingly, SRO, especially at 1.5% and 3% had a more pronounced antioxidative effect compared to SR. SRO is the extraction residue after supercritical CO_2 extraction of SR lipids, and it is likely that most of the lipid soluble antioxidants were removed with the lipids. However, since SR is high in lipids, it is possible that their removal concentrated water-soluble antioxidants. Extraction residue of supercritical CO_2 extraction of whole dried sea buckthorn berries was previously reported to be high in flavonol glycosides, as they are not removed during the extraction (Linderborg et al., 2012). Püssa et al. (2008) reported that SR effectively reduced TBARS formation during 6-day refrigerated (6 °C) storage of mechanically separated chicken and turkey, and the antioxidative effect was suggested to be imparted by different flavonol glycosides present in SR. When comparing the different concentrations of SRO and LR in the present study, peak areas of indicator volatiles between the two higher concentrations (1.5% and 3%) were relatively similar up to 7 days at 1 °C, especially in case of LR, indicating that the concentrations of these berry press residues can be reduced without compromising their antioxidative effect.

5.3 Sensory properties and odor-active compounds

5.3.1 Liking of Baltic herring minces with and without antioxidants

The likeness scores of Baltic herring minces with and without EDTA, α-tocopherol and ascorbic acid, AB, LR (3%), or SR (3%) (**I**) (**Figure 15**), prepared into fish loaves, were assessed to evaluate the consumer acceptance of the additions. As expected, fish loaves made of minces with EDTA or αT+AA did not differ significantly compared to the control (mince from fillets with skin) in terms of likeness. The more complex additions, AB, LR, and SR, however, reduced the likeness of most attributes, and were in most attributes rated between 4–5 (dislike slightly–neither like nor dislike) on the scale of 1–9. AB was a mixture of supercritical CO_2 extracts from several aromatic plants, such as sage, hop, and licorice root, and thus possessed a strong odor. Fish loaf with AB was rated significantly ($p < 0.05$) lower in overall likeness and likeness of taste/flavor compared to the control, EDTA, and αT+AA. Similarly, supercritical CO_2 ginger extract was seen to effectively retard lipid oxidation in tilapia burgers, but the burger containing it was rated as lowest in overall liking and liking of flavor (Mattje et al., 2019).

LR, on the other hand, was rated as having a lower likeness in all attributes compared to the control. The fish loaf with LR was rated as having a significantly lower likeness of color (3.4) and appearance (3.7) compared to all other samples. Due to the pH dependence of lingonberry anthocyanins, the fish loaf with LR

had a purpleish/blueish color, which likely contributed to lower liking. In addition, the LR contained lingonberry seeds, and it was described to impart a dry and grainy mouthfeel, which may explain the lowest likeness of texture (5.0). SR received lowest liking scores for flavor/taste (4.0).

In the verbal comments of the consumers, AB, LR, and SR, were reported to mask the natural odor and flavor of fish, which was considered negative. A clear majority of consumers participating in the test were frequent fish eaters (71% reported to eat fish 1–2 times per week, 13% multiple times per week), which may in part explain the preference for traditional fish loaves, instead of the more novel combinations of fish and LR, SR, or AB. Further, the consumers were not aware of the composition of the fish loaves, and the natural additions (AB, LR, and SR) were considered weird and vague. As the natural antioxidants at the used concentrations were seen to have a negative influence on consumer acceptance, careful selection of dosage is essential to minimize negative effect on sensory quality while maintaining antioxidant effects. Based on PV (**Table 15**) and volatile secondary oxidation indicators (**Figure 14**) of Baltic herring minces with different concentrations of LR and SRO, stored at 1 °C, their concentration could be reduced to half (1.5%) or even third (1%) while still protecting the mince from lipid oxidation.

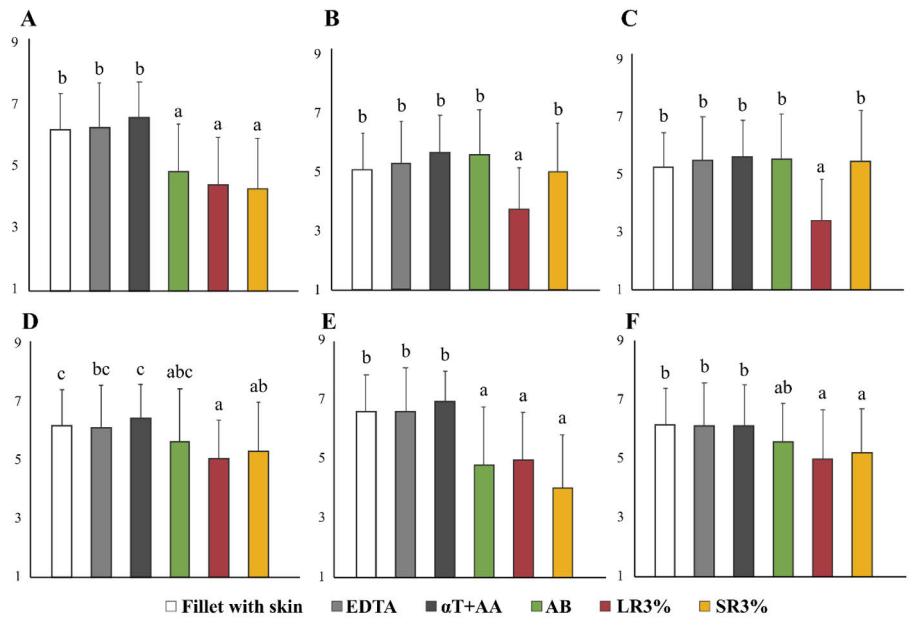

Figure 15. Overall liking (A), liking of appearance (B), liking of color (C), odor (D), taste/flavor (E), and texture (F), on a scale 1–9 (n=55), of fish loaves with or without antioxidant additions (**III**). Different letters indicate a statistically significant ($p < 0.05$) difference between samples.

5.3.2 Odor and flavor profile

In study **II**, the odor properties of raw, minced Baltic herring and alkali extracted FPI with cryoprotectants were compared using CATA (**Figure 16**). In case of Baltic herring, the most frequently chosen attributes were fish-like (92%), fish oil-like (92%), and fatty (83%). FPI was most often selected as fish oil-like (50%), sweet (42%), buttery (33%) and potato-like (33%), and hence no attribute was chosen by more than half of the panelists. Due to a small panel size, the only significant difference ($p < 0.05$) according to McNemar's test was observed in the frequency to select "fish-like" (92% in Baltic herring vs 25% in FPI).

Based on the intensities of odor attributes, FPI was evaluated as having a significantly lower total intensity of odor (1.9 vs 2.5) and intensity of fishy odor 3.3 vs 2.2). Both Baltic herring and FPI were considered to have a relatively intense (1.9 and 1.8, respectively) fatty odor. The sensory properties of FPIs prepared using the pH shift have previously been investigated by Phetsang et al. (2021b), Abdollahi and Undeland (2018), and Nisov et al. (2021). Phetsang et al. (2021b) compared the off-odor intensities (on a scale 0–4) of hybrid catfish (*C. macrocephalus* × *C. gariepinus*) mince, hybrid catfish surimi, and acid and alkali-extracted FPI from hybrid catfish. Both surimi processing and the pH shift reduced the fishy odour of the mince (3.2) to 1.3 (surimi) and 0.8 (both alkaline and acidic pH shift), and led to elimination of oxidation related volatiles, such as propanal, pentanal, hexanal, (E)-2-heptenal, and 2,3-pentanedione. On the other hand, Abdollahi et al. (2018) reported that herring protein isolate prepared by pH shift and freeze-drying was characterised by a high intensity of dried fish and fish oil odours. The Baltic herring FPI in the present study also showed a relatively intense fatty odour (1.8), and 'fatty' and 'fish oil odor' were the two most frequently chosen CATA attributes.

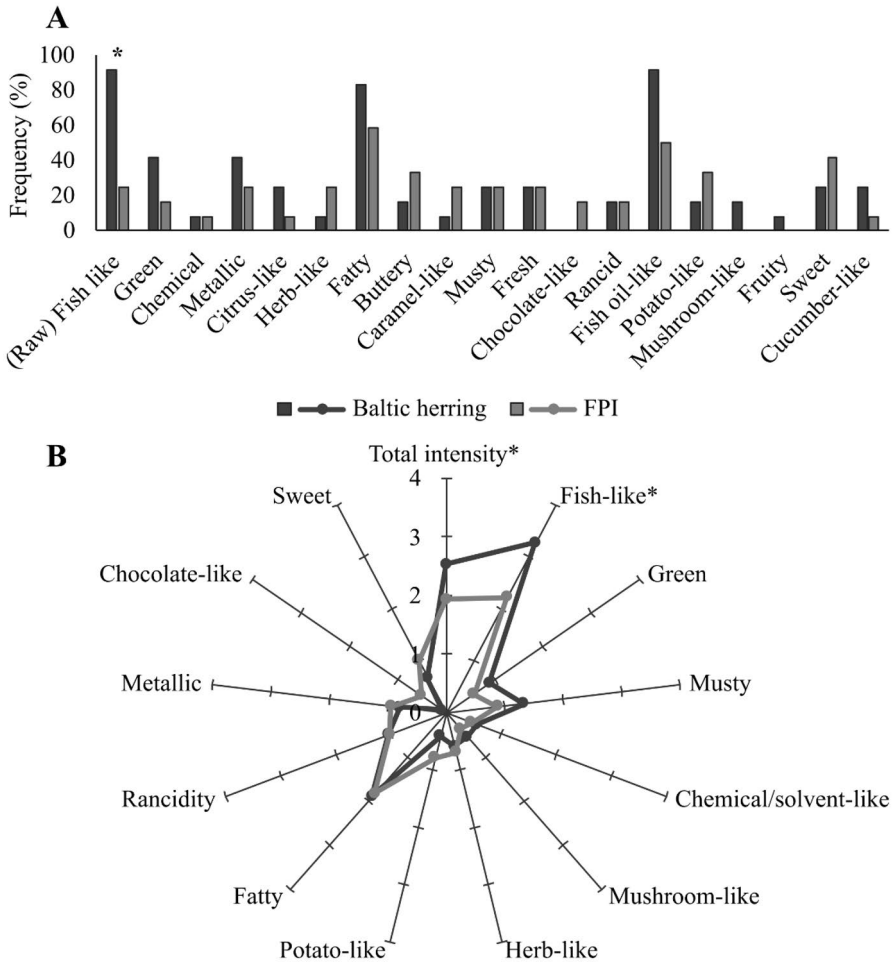

Figure 16. Selection frequencies (A) and rated intensities (B) of odor attributes in FPI and raw minced Baltic herring (n=6*2) (**II**). An asterisk (*) indicates a statistically significant ($p < 0.05$).

The sensory profiles of freeze-dried FPIs and FPHs from Baltic herring and roach (**I**) were reported by Nisov et al. (2021). Rancidity was found to be higher in the FPIs compared to FPHs from both fishes, which is line with the higher rate of oxidation in FPIs vs FPHs reported here (**Figure 11** and **Figure 12**). The perceived rancidity for alkali-extracted Baltic herring FPI in study **II** was low based on the intensity (1.0 on a scale 0–4) and selection frequency (17%), both of which were the same for the raw material. The extent of lipid oxidation in study **II** was, however, not investigated.

Reducing the fishy odor in FPIs and FPHs is vital in terms of their applications. For instance, the intensity of fishy odor in pasta supplemented with pangas FPI increased in line with the amount of added FPI (Singh et al., 2021). However,

FPIs, when added to fish-based foods, such as fish sausages (Surasani et al., 2020) or fish balls (Shaviklo et al., 2010) the addition did not cause major changes in the sensory properties or liking ratings. Baltic herring FPI (**II**) was shown to be a suitable ingredient in fish balls and surimi-type gels (Kakko et al., 2022), but the sensory properties or liking were not investigated. According to the results presented here, fishy odor was reduced during the pH shift, but the protein isolate was, nevertheless, recognized as fishy.

The odor and flavor/taste properties of Baltic herring minces with and without 1.5% LR or SRO were evaluated as raw or cooked, and as fresh or after being stored 3 days at 1°C in study **IV** (**Table 16**). As fresh (0d), the three raw minces differed significantly ($p < 0.05$) only in terms of berry-like/fruit-like odor, as LR1.5 and SRO1.5 expectedly had slightly higher intensities intensities in this attribute (4.9 and 5.0, respectively, compared to 3.3 in the control mince). However, already during the 3 days of storage at 1 °C, control exhibited a highly significant increase ($p < 0.001$) in fishy odor (3.5 vs 5.3), marine odor (2.0 vs 3.5), and fish oil odor (2.1 vs 2.7), and interestingly also a decrease in berry-like/fruit-like odor ($p = 0.002$). In the raw mince containing 1.5% LR, fishy odor increased slightly (from 3.3 to 4.0, $p = 0.02$), as well as marine odor (from 2.1 to 2.7, $p = 0.018$), but the mince with SRO (1.5%) showed no significant ($p < 0.05$) changes in any odor attributes after 3 days of storage.

In addition to storage, the *sous vide* cooking affected the the three minces differently. While in case of odor of raw fresh (0d) minces the only difference was observed in the berry-like/fruit-like odor, the fresh cooked control mince showed a significantly higher intensity in marine odor and fish-oil odor compared to SRO1.5. Cropotova et al. (2019) reported that *sous vide* cooking of Atlantic mackerel at 70 °C for 20 minutes, same conditions as used in the present study, and subsequent storage of 1 day (at 0 °C) increased the PV considerably, but not when an antioxidant was present during cooking.

Few statistically significant differences were observed in the flavor/taste attributes of cooked fish minces. Cooking induces various changes in volatile but also non-volatile compounds, and it is possible that these changes masked the changes that occurred during storage. Previously, *sous vide* cooking was seen to alter the volatile profile of European sea bass more than steaming or boiling (Nieva-Echevarría et al., 2017). However, control mince cooked after 3 d was perceived considerably fishier in flavor (5.8) compared to stored minces with LR (3.8) or SRO (3.6).

According to the results of the storage test, LR and SRO, at a concentration of 1.5%, not only retarded the formation of hydroperoxides (**Table 15**) and oxidation related volatiles (**Figure 14**) but also changes in sensory quality (odor and flavor) during storage. In case of 1.5% SRO addition, no statistically significant changes in odor and flavor attributes were during the 3-day storage.

Other studies have also reported improved preservation of sensory quality by natural antioxidants. For instance, brining silver carp fillets with clove bud and grape seed extracts was seen to retard decline in sensory score during refridgerated storage (Shi et al., 2014) and milk protein concentrate reduced the formation of fishy odor during frozen storage of herring (Joaquin et al., 2008).

Table 16. Intensities of odor, flavor, and taste attributes[a] in raw and cooked Baltic herring minces with and without 1.5% LR or SRO (**IV**).

	C-0d	LR1.5-0d	SRO1.5-0d	C-3d	LR1.5-3d	SRO1.5-3d
Raw minces - odor						
Total intensity of odor	5.3 ± 1.8^{A}	6.1±1.6	5.5±1.3	6.7 ± 1.0^{B}	6.4±1.4	5.9±1.5
Fishy odor	3.5 ± 1.9^{A}	3.3 ± 1.9^{A}	3.0±1.8	5.3 ± 1.8^{bB}	4.0 ± 2.2^{abB}	3.5 ± 2.0^{a}
Marine odor	2.0 ± 1.6^{A}	2.1 ± 1.5^{A}	2.0±1.7	3.5 ± 1.6^{bB}	2.7 ± 1.7^{abB}	2.2 ± 1.6^{a}
Berry-like/fruit-like odor	3.3 ± 2.3^{aB}	4.9 ± 1.8^{b}	5.0 ± 1.7^{b}	1.4 ± 1.4^{aA}	4.6 ± 2.4^{b}	4.7 ± 2.2^{b}
Fish oil odor	2.1 ± 1.6^{A}	2.5±1.7	2.0±1.5	3.9 ± 1.5^{bB}	2.7 ± 1.6^{a}	2.1 ± 1.7^{a}
Rancidity	1.5±1.6	1.4±1.5	1.2±1.2	1.9±1.6	1.5±1.6	1.3±1.4
Musty odor	1.7±1.4	1.4±1.2	1.3±1.1	1.3±0.9	1.6±1.6	1.8±1.8
Cooked minces - odor						
Total intensity of odor	5.6 ± 1.4^{A}	5.5±1.4	6.0±1.2	6.7 ± 1.1^{B}	5.7±1.3	5.9±1.3
Fishy odor	5.3±1.8	4.2±1.6	3.7±1.3	6.2 ± 1.4^{b}	4.5 ± 1.6^{a}	3.7 ± 1.5^{a}
Marine odor	3.8 ± 1.7^{b}	2.8 ± 1.4^{ab}	2.5 ± 1.2^{a}	3.7 ± 1.5^{b}	3.2 ± 1.2^{b}	2.1 ± 1.3^{a}
Berry-like/fruit-like odor	1.2 ± 1.5^{a}	3.0 ± 2.1^{b}	4.7 ± 1.7^{c}	1.2 ± 1.4^{a}	2.8 ± 2.1^{b}	4.2 ± 2.0^{b}
Fish oil odor	2.5 ± 1.1^{b}	1.5 ± 1.0^{a}	1.6 ± 0.9^{a}	2.4±1.3	1.9±1.6	1.5±0.9
Rancidity	1.3±1.1	1.3±1.2	1.0±0.7	1.0±1.2	1.1±1.1	0.9±0.8
Musty odor	1.5±1.1	1.9±1.3	1.5±1.1	1.2±0.8	1.9±1.5	1.7±1.2
Cooked minces - flavor						
Total intensity of flavor	5.1±1.1	5.5±1.1	5.9±1.1	5.8±1.5	5.7±1.0	5.5±1.2
Fishy flavor	5.1 ± 1.3^{b}	3.6 ± 1.4^{a}	4.1 ± 1.7^{ab}	5.8 ± 1.8^{b}	3.8 ± 1.6^{a}	3.6 ± 1.8^{a}
Marine flavor	3.0±1.2	2.0±1.4	2.3±1.6	2.6±1.6	2.1±1.4	1.9±1.2
Berry-like/fruit-like flavor	1.1 ± 1.4^{a}	3.4 ± 2.2^{b}	4.4 ± 1.9^{b}	1.0 ± 1.2^{a}	3.1 ± 2.1^{b}	3.7 ± 2.3^{b}
Umami	3.1±1.5	2.8±1.0	3.0±1.4	2.9±1.2	2.9±1.0	3.0±1.5
Saltiness	4.1±1.7	4.0±1.3	3.8±1.3	4.1±1.8	4.3±1.6	3.8±1.3
Fish oil flavor	1.9±1.1	1.8±1.5	1.8±1.2	2.3±1.2	1.8±1.1	1.6±0.9
Metallic flavor/aftertaste	1.1±1.1	1.1±1.0	1.4±1.0	1.3±1.3	1.0±0.9	1.8±1.3

[a]Mean and standard deviation (n=8*2 for cooked samples, n=8*3 for raw samples), capital letters and lower case letters indicate a statistically significant ($p < 0.05$) difference between different time points of the same mince and different minces at the same timepoint, respectively.

5.3.3 Odor-active compounds

Altogether, 24 significant odor compounds (NIF ≥ 33%, i.e. odors that at least 2 of 6 panelists detected) were identified in Baltic herring (**II**, **IV**), Baltic herring with added LR and SRO (**IV**), and Baltic herring FPI (**II**) on the SPB-624 column (**Table 17**). Most of the identified compounds were alcohols, aldehydes and ketones derived from degradation of lipids. In study **II**, more odor-active

compounds were detected, as the column flow was directed completely to the olfactometry port, while in study **IV** the column flow was split 1:1 between olfactory port and FID. However, the most significant odor-active compounds (high NIF and/or intensity values) detected in raw Baltic herring in study **II** were also detected in Baltic herring minces from fillets with skin in study **IV**. For instance, 3-methylbutanal, 2,3-pentanedione, (*Z*)-4-heptenal, (*Z*)-1,5-octadien-3-ol, and (*Z*)-1,5-octadien-3-one were detected (at ≥33% detection frequency) in all samples in both studies.

Table 17. Significant (NIF ≥ 33%) odor-active compounds identified in studies **II** and **IV**. The "x" indicates that the odorant had a detection frequency of at least 33% in the given sample.

Compound	$LRI_{SPB-624}$		Identification[a]	Study II*		Study IV			
	Study II	Study IV		Gutted and beheaded B. herring	FPI	Fresh (0 d) control mince	Stored (3 d) control mince	Fresh (0 d) SRO1.5	Stored (3 d) SRO1.5
Acids									
2-methylpropanoic acid	864		O, RI		x				
Alcohols									
2-methyl-1-propanol	681		O, RI	x	x				
1-penten-3-ol	735		std, ms, O, RI	x	x				
(*Z*)-2-penten-1-ol	830	834	std, O, RI		x		x		
(*E*)-1,5-octadien-3-ol	1039		std, O, RI	x	x				
(*Z*)-1,5-octadien-3-ol	1045	1031	std, O, RI	x	x	x	x	x	x
Aldehydes									
Propanal	<600		std, ms, O, RI	x	x				
2-methylpropanal	<600	600	std, ms, O, RI	x		x	x	x	x
3-methylbutanal	699	701	std, ms, O, RI	x	x	x	x	x	x
2-methylbutanal	707		std, ms, O, RI	x					
Hexanal	850	849	std, ms, O, RI	x	x	x	x		x
Heptanal		942					x		
(*Z*)-4-heptenal	958	954	std, ms, O, RI	x	x	x	x	x	x
(*E,E*)-2,4-hexadienal	992	986	std, ms, O, RI	x	x		x	x	x
(*E,Z*)-2,4-heptadienal	1073	1070	std, ms, O, RI	x	x		x		
(*E,Z*)-2,6-nonadienal		1228	std, O, RI				x		

Compound	$LRI_{SPB-624}$		Identification[a]	Study II*		Study IV			
	Study II	Study IV		Gutted and beheaded B. herring	FPI	Fresh (0 d) control mince	Stored (3 d) control mince	Fresh (0 d) SRO1.5	Stored (3 d) SRO1.5
(*E,E*)-2,4-nonadienal	1296		std, O, RI	x	x				
Ketones and esters									
2,3-butanedione	636	640	std, ms, O, RI	x	x	x	x	x	x
2,3-pentanedione	744	741	std, ms, O, RI	x	x		x		
Ethyl 3-methylbutanoate	881	882	std, ms, O, RI				x	x	x
1-octen-3-one	1004		ms, O, RI	x	x				
(*Z*)-1,5-octadien-3-one	1055	1042	std, O, RI	x	x	x	x	x	x
(*E,Z*)-3,5-octadien-2-one	1154	1136	ms, RI	x	x		x		
(*E,E*)-3,5-octadien-2-one	1172	1156	ms, RI	x	x		x		

*In study II, the eluent was directed to the olfactory port, while in study IV the eluent flow was split 1:1 between olfactory port and FID

[a]Identification was based on comparison to a commercial reference compound (std), comparison of ms spectra match (recorded by HS-SPME-GC-MS analysis) to the NIST library (ms), similarity of odour description to previous literature (O), and/or observed RI close to RI reported by previous literature or NIST. Comparison to previous literature and databases included Ahonen et al. (2022); Aitta et al. (2021); An et al. (2020); Hartvigsen et al. (2000); Martínez-Arellano et al. (2016); Selli et al. (2006); Sérot et al. (2002), NIST Chemistry WebBook, https://webbook.nist.gov, and Flavornet database, https://www.flavornet.org

Since alkaline pH-shift processing was seen to influence the odor profile of Baltic herring (**Figure 16**), detection frequencies and intensities of odorants in Baltic herring and Baltic herring FPI were compared to investigate the compounds underlying these changes (**Table 18**). A total of 33 compounds with NIF ≥ 33% were detected in raw minced Baltic herring, while only 29 were detected in the FPI. In addition, all odor-active compounds that differed significantly in their detection frequencies were lower in FPI compared to Baltic herring. This is in line with the significantly ($p < 0.05$) lower total intensity of odor observed for FPI (**Figure 16**).

Hexanal, (*Z*)-4-heptenal, (*E,Z*)-2,4-heptadienal, 2,3-butanedione, and 2,3-pentanedione, appeared to be the most pronounced odorants in both Baltic herring and FPI, as their detection frequencies in both were 92% or 100%. Most of these aldehydes and ketones have been previously reported in Baltic herring (Aro et al., 2003, 2002) and/or other fishes (Jónsdóttir et al., 2007; Phetsang et al., 2021a). Based on the average intensities of odorants weighed by their detection frequencies (NIF*intensity), the highest values observed in FPI were (*Z*)-4-heptenal (3.5), hexanal (3.3), and 2,3-pentanedione (3.0). (*Z*)-4-heptenal, described as fish oil-like and rancid, and 2,3-pentanedione described as having buttery and fatty notes, may have contributed to the 'fatty' and 'fish oil-like' attributes, which were the most frequently selected odor attributes for the FPI (**Figure 16**). (*Z*)-4-heptenal is produced from (*E,Z*)-2,6-nonadienal (Josephson and Lindsay, 1987), and has been associated with fishy odor and flavor (Joaquin et al., 2008; Triqui and Bouchriti, 2003), especially in combination with other aldehydes, such as its precursor (*E,Z*)-2,6-nonadienal (Venkateshwarlu et al., 2004b).

Based on the detection frequencies, the most prominent differences between Baltic herring and FPI were in 2-methylpropanal, 3-methylbutanal, 2-methylbutanal, and an unidentified compound ($LRI_{SPB\text{-}624}$ 985) described as sweat, burnt, and dry. The detection frequencies of 2-methylpropanal, 3-methylbutanal, and 2-methylbutanal were 83%, 92%, and 67%, respectively, in Baltic herring, but only 8%, 50%, and 0%, respectively, in FPI. Though none of the three branched aldehydes, 2-methylpropanal, 3-methylbutanal, and 2-methylbutanal, were described as fishy as such, it is possible that in combination with other compounds they contribute to the fishy odor in Baltic herring, which was less intense in the FPI. 3-methylbutanal, which is widely present in aquatic organisms (Jones et al., 2022), was previously reported to have a high odor activity value (OAV) in fish sauce (Lapsongphon et al., 2015) and to be one of the most important odorants in dry-cured tuantou bream (*Megalobrama amblycephala*) (Chen et al., 2023).

Previous GC-O studies on fish protein isolate produced using the pH shift were not found. Zhou et al. (2016), however studied the effect of different

washing processes on odourants on surimi from silver carp (*Hypophthalmichthys molitrix*) mince. The OAVs of most of the odor-active compounds were decreased by the washing processes, with the saline and mildly alkaline washing solution being more effective than water. The content and release of odour-active volatile compounds can be affected by several factors in the pH-shift process. For instance, the proteins undergo significant conformational changes due to the changes in both pH and ionic strength, both of which affect their ability to bind volatiles (Damodaran and Kinsella, 1983; Gu et al., 2020; Pérez-Juan et al., 2006). Therefore, it is likely that some of the differences observed between the odor-active compounds in Baltic herring and FPI were related to their altered release due to changes in the proteins and/or lipids present. Differences in the water content (**Table 9**) may also have influenced the release of volatiles (Damerau et al., 2014).

Table 18. Descriptions, detection frequencies (NIF), intensities, and intensities weighed by NIF (NIF*intensity)[a] of odor-active compounds (NIF ≥ 33%) detected in Baltic herring and Baltic herring FPI (**II**).

Compound	$LRI_{SPB-624}$	LRI_{DB-WAX}	Odour description	NIF		Intensity		NIF*Intensity	
				B. herring	FPI	B. herring	FPI	B. herring	FPI
unknown	<600		solvent, pungent, stale	42 %	42 %	1.2	1.6	0.5	0.7
unknown	<600		musty, fish, cheese, chemical	50 %	25 %	1.7	1	0.9	0.3
propanal	<600	797	chemical, pungent, solvent, glue	67 %	92 %	1.4	1.8	0.9	1.7
2-methylpropanal	<600	816	chocolate, cognac, almond, musty	83 %	8 %	2.2	1	1.8	0.1
2,3-butanedione	636	978	caramel, butter, sweet, cream	92 %	92 %	2.9	2.3	2.7	2.1
2-methyl-1-propanol	681	n.d.	solvent, sweet, pungent, cognac	67 %	58 %	1.6	1.7	1.1	1.0
3-methylbutanal	699	920	musty, chocolate, fatty, pungent	92 %	50 %	3.2	1.5	2.9	0.8
2-methylbutanal	707	n.d.	sweat, chemical, musty, chocolate	67 %	0 %	2.4	n.d. (0.0)	1.6	0.0
1-penten-3-ol	735	n.d.	pungent, solvent, chemical, green	75 %	83 %	2.3	2.1	1.7	1.7
2,3-pentanedione	744	1058	butter, caramel, sweet, popcorn	92 %	92 %	3.1	3.3	2.9	3.0
unknown	779		solvent, glue, wax, plastic	83 %	75 %	2.1	2.1	1.7	1.6
(*Z*)-2-penten-1-ol	830	n.d.	solvent, musty	25 %	50 %	1	1	0.3	0.5
hexanal	850	1085	grass, green	100 %	100 %	2.9	3.3	2.9	3.3
2-methylpropanoic acid	864	n.d.	stale, rancid, grass, boiled potato	17 %	42 %	1.5	2	0.3	0.8
ethyl-3-methylbutanoate	881	1071	fresh, citrus, fruity, sweet	42 %	42 %	1.6	1.4	0.7	0.6
(*Z*)-4-heptenal	958	1242	fish, fish oil, rancid, stale	92 %	100 %	3.3	3.5	3.0	3.5
unknown	985		sweat, burnt, dry	50 %	0 %	2.5	n.d. (0.0)	1.3	0.0
(*E,E*)-2,4-hexadienal	992	n.d.	mushroom, potato, musty, rancid	92 %	67 %	2.5	1.5	2.3	1.0
1-octen-3-one	1004	1303	mushroom, metallic	67 %	50 %	2.1	1.7	1.4	0.9

Compound	$LRI_{SPB-624}$	LRI_{DB-WAX}	Odour description	NIF		Intensity		NIF*Intensity	
				B. herring	FPI	B. herring	FPI	B. herring	FPI
1,5(*E*)-octadien-3-ol	1039	1358	pungent, rose, spicy, fatty	50 %	83 %	2.3	2.7	1.2	2.2
1,5(*Z*)-octadien-3-ol	1045	1486	mushroom, forest, green, stale	100 %	83 %	3	2.8	3.0	2.3
1,5(*Z*)-octadien-3-one	1055	1377	metal, green, forest, pelargonium	83 %	92 %	3.3	3.2	2.7	2.9
(*E,Z*)-2,4-heptadienal	1073	1467	citrus, lemon, green, mushroom	92 %	92 %	2.3	2.5	2.1	2.3
unknown	1114		grass, sawdust, sprig	33 %	25 %	1.5	2	0.5	0.5
(*E,Z*)-3,5-octadien-2-one	1154	n.d.	mushroom, forest, plastic, pungent	42 %	58 %	1.8	1.3	0.8	0.8
(*E,E*)-3,5-octadien-2-one	1172	n.d.	mushroom, soil, roasted	33 %	42 %	1.5	1.2	0.5	0.5
unknown	1187		dust, mushroom, malt, fresh	42 %	75 %	1.8	1.1	0.8	0.8
unknown	1197		fresh, citrus, sweet, chemical	17 %	75 %	1	1.7	0.2	1.3
unknown	1246		sawdust, hay, malt, flour	58 %	25 %	1.4	1.7	0.8	0.4
unknown	1281		cucumber, green, fruity	25 %	42 %	2.3	2	0.6	0.8
(*E,E*)-2,4-nonadienal	1296	1680	cucumber, green, fresh, nature	83 %	83 %	2.6	2.6	2.2	2.2
unknown	1368		musty, pungent, sweat, fruity	50 %	67 %	2.3	2.3	1.2	1.5
unknown	1412		sawdust, mushroom, sauna, forest	50 %	33 %	1.3	2	0.7	0.7
unknown	1438		soil, fish, roasted	42 %	25 %	1.8	1.7	0.8	0.4
unknown	1470		sweet, barn, pungent, acidic	67 %	50 %	2	1.7	1.3	0.9
unknown	1534		plant, green, musty, wholegrain	50 %	25 %	1.8	1.3	0.9	0.3
unknown	1581		forest, sawdust, spoiled	33 %	33 %	1.5	1.3	0.5	0.4

[a]assessed by 6 panelists
n.d. = odour not detected

The odor-active compounds in raw Baltic herring minces with and without SRO (1.5%) were analyzed prior (0 d) and after (3d) storage at 1 °C (**IV**). Odor-active compounds were analyzed using GC-O on the semipolar SPB-624 column (**Table 19**) and polar DB-WAX column (**Table 20**). Considerably more odor-contributing compounds (NIF ≥ 33%) were detected in the stored control mince (C-3d), compared to the other minces. On the SPB-624 column, 8, 23, 12, and 11 compounds were detected by ≥ 33% of the panelists in fresh control mince, stored control mince, fresh SRO1.5, and stored SRO1.5, respectively. On the polar DB-WAX, 11, 20, 8, and 10 compounds, respectively, had a NIF equal to or exceeding 33%. The number of odor-active compounds is in line with the total intensities of odor of the minces (**Table 16**), as there was a significant ($p < 0.05$) increase in the total intensity in the control mince during the 3-day storage, but not in the SRO1.5.

3-methylbutanal (musty, chocolate, solvent) and (*Z*)-1,5-octadien-3-one (green, waxy, metal, pelargonium) were detected in all samples by all panelists and on both columns. (*Z*)-1,5-octadien-3-one is known to be produced through autoxidation of EPA (Hammer and Schieberle, 2013). The NIFs and intensities of other odorants differed between samples. On the semipolar column, (*Z*)-4-heptenal had a NIF value of 100% and average intensity of 3.1 in the stored control mince, while the NIFs in fresh control mince and fresh and stored SRO1.5 were 33% and intensities 1.0-2.0. Triqui and Bouchriti (2003) reported that (*Z*)-1,5-octadien-3-one, due to its low threshold, was one of the most potent odorants in fresh sardine, but along with (*Z*)-4-heptenal and methional, its increase was suggested to impart fishy odor to sardine stored on ice for 2 days. During the first two days of storage of sardine, also 2,3-pentanedione, (*E,Z*)-3,5-octadien-2-one, and (*E,E*)-2,4-decadienal among other secondary lipid oxidation were seen to increase in their impact. According to the present results, the NIF values for 2,3-pentanedione and (*E,Z*)-3,5-octadien-2-one in Baltic herring control mince increased from 17% to 100% and from 17% to 67%, respectively, when stored for 3 days at 1 °C. The NIF values for these compounds in SRO1.5 mince remained low (≤ 17%).

(*Z*)-4-heptenal has been associated with fishy odor/flavor in several studies (Hartvigsen et al., 2000; Joaquin et al., 2008; McGill et al., 1974), especially in combination with other volatiles, such as (*E,Z*)-2,6-nonadienal due to a synergistic effect on fishy flavor (Venkateshwarlu et al., 2004b). As both (*Z*)-4-heptenal and (*E,Z*)-2,6-nonadienal were detected at higher frequencies in the stored Baltic herring control mince compared to SRO1.5 as fresh or stored, it is possible that they were at least partially responsible for the higher intensity of fishy odor and flavor observed in the former (**Table 16**).

A PLS model (with the weighed intensities, i.e. NIF*intensity values of odor-active compounds on both columns as X variables, and intensities of odor

intensities from GDA as Y variables) was created to evaluate the contribution of detected odorants on the odor attributes of raw minces (**Figure 17**). In case of both columns, most of the odorants, separating the samples on Factor-1, were associated with the total intensity of odor, fishy odor, fish oil odor, marine odor, and the stored control mince (C-3d). These odorants included several lipid oxidation derived compounds, such as propanal, hexanal, (*Z*)-3-hexenal, (*E,Z*)-2,6-nonadienal, (*Z*)-4-heptenal, and (*Z*)-1,5-octadien-3-ol, 2,3-pentanedione, and (*E,Z*)-3,5-octadien-2-one. In a previous study lipid oxidation derived fishy odor in sea bass skin which was associated with an increase in several volatiles, such as hexanal, heptanal, and 1,5-octadien-3-ol (Sae-leaw and Benjakul, 2014). In **Figure 17**, the other three minces were positioned on the right side of the plot due to lower weighed intensities of lipid oxidation derived odorants. The close proximity of SRO1.5_0d and SRO1.5_3d on the plot (in relation to Factor-1) shows that the odor, flavor, and odor-active compounds of mince containing SRO was not considerably affected by the storage.

Table 19. Descriptions, detection frequencies (NIF), intensities, and intensities weighed by NIF (NIF*intensity)[a] of odor-active compounds (NIF ≥ 33%) detected on the SPB-624 column in raw C and SRO1.5 minces before (0 d) and after (3 d) storage at 1°C (**IV**).

Code	Compound	$LRI_{SPB-624}$	Description	NIF				Intensity				NIF*Intensity			
				C_0d	C_3d	SRO1.5_3d	SRO1.5_0d	C_0d	C_3d	SRO1.5_3d	SRO1.5_0d	C_0d	C_3d	SRO1.5_3d	SRO1.5_0d
A1	unknown	505	stale, musty	17 %	33 %	33 %	17 %	1.0	1.0	1.0	2.0	0.2	0.3	0.3	0.3
A2	unknown	526	citrus, bilberry, flowery	0 %	0 %	17 %	33 %	0.0	0.0	1.0	1.8	0.0	0.0	0.2	0.6
A3	2-methylpropanal	600	solvent, chocolate, musty, green	50 %	67 %	50 %	50 %	1.7	2.6	1.5	2.0	0.8	1.8	0.8	1.0
A4	2,3-butanedione	640	butter, caramel, fatty	67 %	50 %	67 %	67 %	2.0	1.7	1.5	1.1	1.3	0.8	1.0	0.8
A5	unknown	683	sweet, fruity, creamy	17 %	50 %	33 %	33 %	1.0	2.2	1.3	1.0	0.2	1.1	0.4	0.3
A6	3-methylbutanal + 2-methylbutanal	701	musty, green, chocolate, solvent	100 %	100 %	100 %	100 %	2.6	3.1	2.1	2.7	2.6	3.1	2.1	2.7
A7	2,3-pentanedione	741	caramel, butter, fatty	17 %	100 %	17 %	0 %	1.0	2.3	1.0	0.0	0.2	2.3	0.2	0.0
A8	unknown	765	fruity	0 %	17 %	33 %	17 %	0.0	1.0	1.0	1.0	0.0	0.2	0.3	0.2
A9	unknown	781	rancid, wax, soap	17 %	83 %	0 %	0 %	1.0	2.5	0.0	0.0	0.2	2.1	0.0	0.0
A10	(*Z*)-2-penten-1-ol	834	no common descriptor	0 %	33 %	0 %	0 %	0.0	1.3	0.0	1.0	0.0	0.4	0.0	0.0
A11	Hexanal	849	grass, leaf, fresh	33 %	50 %	17 %	33 %	1.0	1.7	1.0	1.3	0.3	0.8	0.2	0.4
A12	ethyl-3-methylbutanoate	882	sweet, fruity, candy, citrus	17 %	33 %	100 %	83 %	1.5	2.3	2.0	2.2	0.3	0.8	2.0	1.8
A13	Heptanal	942	potato-like	17 %	33 %	17 %	17 %	1.5	1.0	1.0	2.0	0.3	0.3	0.2	0.3
A14	(*Z*)-4-heptenal	954	fish, rancid, green, oil	33 %	100 %	33 %	33 %	1.0	3.1	1.0	2.0	0.3	3.1	0.3	0.7
A15	(*E,E*)-2,4-hexadienal	986	musty, potato, green, wax	0 %	83 %	33 %	50 %	0.0	1.8	1.0	1.3	0.0	1.5	0.3	0.7
A16	(*Z*)-1,5-octadien-3-ol or (*E*)-1,5-octadien-3-one	1031	musty, mushroom, green	67 %	50 %	67 %	33 %	1.7	2.7	1.5	1.5	1.1	1.3	1.0	0.5
A17	(*Z*)-1,5-octadien-3-one	1042	green, waxy, soil, metal, pelargonium	100 %	100 %	100 %	100 %	2.9	3.3	2.3	2.7	2.9	3.3	2.3	2.7

Code	Compound	$LRI_{SPB-624}$	Description	NIF				Intensity				NIF*Intensity			
				C_0d	C_3d	SRO1.5_3d	SRO1.5_0d	C_0d	C_3d	SRO1.5_3d	SRO1.5_0d	C_0d	C_3d	SRO1.5_3d	SRO1.5_0d
A18	(*E,Z*)-2,4-heptadienal	1070	green, cucumber	17 %	33 %	0 %	0 %	2.0	2.5	0.0	0.0	0.3	0.8	0.0	0.0
A19	unknown	1097	no common descriptor	0 %	0 %	33 %	17 %	0.0	0.0	1.0	1.0	0.0	0.0	0.3	0.2
A20	(*E,Z*)-3,5-octadien-2-one	1136	fruity, musty	17 %	67 %	17 %	0 %	1.0	1.3	1.0	0.0	0.2	0.8	0.2	0.0
A21	(*E,E*)-3,5-octadien-2-one	1156	soap, cucumber, cooked rice	17 %	50 %	17 %	0 %	1.0	1.3	2.0	0.0	0.2	0.7	0.3	0.0
A22	unknown	1208	no common descriptor	0 %	33 %	0 %	0 %	0.0	1.0	0.0	0.0	0.0	0.3	0.0	0.0
A23	(*E,Z*)-2,6-nonadienal	1228	cucumber, fatty	0 %	33 %	0 %	17 %	0.0	2.5	0.0	1.0	0.0	0.8	0.0	0.2
A24	unknown	1260	no common descriptor	0 %	33 %	0 %	17 %	0.0	1.0	0.0	1.0	0.0	0.3	0.0	0.2
A25	unknown	1361	musty, grain-like/cereal-like	0 %	50 %	0 %	0 %	0.0	1.3	0.0	0.0	0.0	0.7	0.0	0.0
A26	unknown	1384	no common descriptor	33 %	33 %	0 %	0 %	1.5	2.0	0.0	0.0	0.5	0.7	0.0	0.0

[a]assessed by 6 panelists

Table 20. Descriptions, detection frequencies (NIF), intensities, and intensities weighed by NIF (NIF*intensity)[a] of odor-active compounds (NIF ≥ 33%) detected on the DB-WAX column in raw C and SRO1.5 minces before (0 d) and after (3 d) storage at 1°C (**IV**).

Code	Compound	LRI_{DB-WAX}	Description	NIF				Intensity				NIF*Intensity			
				C_0d	C_3d	SRO1.5_0d	SRO1.5_3d	C_0d	C_3d	SRO1.5_0d	SRO1.5_3d	C_0d	C_3d	SRO1.5_0d	SRO1.5_3d
B1	propanal	801	no common descriptor	0 %	33 %	0 %	0 %	0.0	1.0	0.0	0.0	0.0	0.3	0.0	0.0
B2	2-methylpropanal	817	chocolate, solvent, coffee	50 %	67 %	50 %	67 %	2.5	2.0	2.0	2.1	1.3	1.3	1.0	1.4
B3	3-methylbutanal +2-methylbutanal	912	solvent, cacao, musty	100 %	100 %	100 %	100 %	2.2	3.0	2.5	2.6	2.2	3.0	2.5	2.6
B4	unknown	926	musty	0 %	50 %	0 %	0 %	0.0	2.0	0.0	0.0	0.0	1.0	0.0	0.0
B5	unknown	958	fresh, sweet	0 %	0 %	0 %	50 %	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.7
B6	2,3-butanedione	974	caramel, sweet, butter	100 %	83 %	67 %	0 %	1.8	2.0	1.5	0.0	1.8	1.7	1.0	0.0
B7	unknown	1011	solvent, rancid oil, wax	0 %	100 %	17 %	33 %	0.0	2.3	1.5	2.0	0.0	2.3	0.3	0.7
B8	2,3-pentanedione + unknown	1051	caramel, butter, fresh, fruity	33 %	100 %	100 %	100 %	1.0	2.8	1.7	1.9	0.3	2.8	1.7	1.9
B9	ethyl-3-methylbutanoate	1066	pineapple, fresh, fruit	33 %	0 %	33 %	67 %	1.0	0.0	2.5	2.1	0.3	0.0	0.8	1.4
B10	hexanal	1078	green, fresh	0 %	33 %	0 %	0 %	0.0	2.3	0.0	0.0	0.0	0.8	0.0	0.0
B11	(*Z*)-3-hexenal	1131	green, grass	17 %	33 %	17 %	33 %	2.0	1.5	2.0	1.0	0.3	0.5	0.3	0.3
B12	heptanal	1172	no common descriptor	17 %	33 %	0 %	17 %	1.0	1.3	0.0	2.0	0.2	0.4	0.0	0.3
B13	unknown	1219	pungent, musty/stuffy	50 %	0 %	17 %	33 %	2.0	0.0	2.0	2.3	1.0	0.0	0.3	0.8
B14	(*Z*)-4-heptenal	1237	fish, stale, pungent, sea	50 %	100 %	17 %	33 %	2.3	3.3	2.0	2.5	1.2	3.3	0.3	0.8
B15	(*Z*)-2-penten-1-ol + 1-octen-3-one or 2-octanone	1300	mushroom, stale	50 %	100 %	100 %	100 %	2.7	2.3	1.7	2.5	1.3	2.3	1.7	2.5
B16	unknown	1349	bergamot, pelargonium	0 %	67 %	0 %	0 %	0.0	1.5	0.0	0.0	0.0	1.0	0.0	0.0
B17	(*Z*)-1,5-octadien-3-one	1377	green, raw, metal, pelargonium	100 %	100 %	100 %	100 %	2.8	3.1	2.8	3.3	2.8	3.1	2.8	3.3

Code	Compound	LRI_{DB-WAX}	Description	NIF				Intensity				NIF*Intensity			
				C_0d	C_3d	SRO1.5_0d	SRO1.5_3d	C_0d	C_3d	SRO1.5_0d	SRO1.5_3d	C_0d	C_3d	SRO1.5_0d	SRO1.5_3d
B18	(*E*)-1,5-octadien-3-ol	1453	no common descriptor	0 %	33 %	0 %	17 %	0.0	2.0	0.0	1.0	0.0	0.7	0.0	0.2
B19	2,4-heptadienal (*E,Z* or *E,E*)	1473	potato	0 %	33 %	33 %	50 %	0.0	2.0	1.5	1.8	0.0	0.7	0.5	0.9
B20	(*Z*)-1,5-octadien-3-ol	1485	green, mushroom	33 %	83 %	0 %	0 %	1.0	1.7	0.0	0.0	0.3	1.4	0.0	0.0
B21	(*E*)-2-octenol	1621	green, grass, cucumber	0 %	67 %	0 %	17 %	0.0	2.1	0.0	1.0	0.0	1.4	0.0	0.2
B22	Unknown	1643	no common descriptor	0 %	33 %	0 %	0 %	0.0	2.5	0.0	0.0	0.0	0.8	0.0	0.0
B23	unknown	1740	no common descriptor	0 %	33 %	0 %	0 %	0.0	2.0	0.0	0.0	0.0	0.7	0.0	0.0
B24	unknown	1770	no common descriptor	33 %	17 %	17 %	50 %	1.0	2.0	2.0	1.7	0.3	0.3	0.3	0.8

[a]assessed by 6 panelists

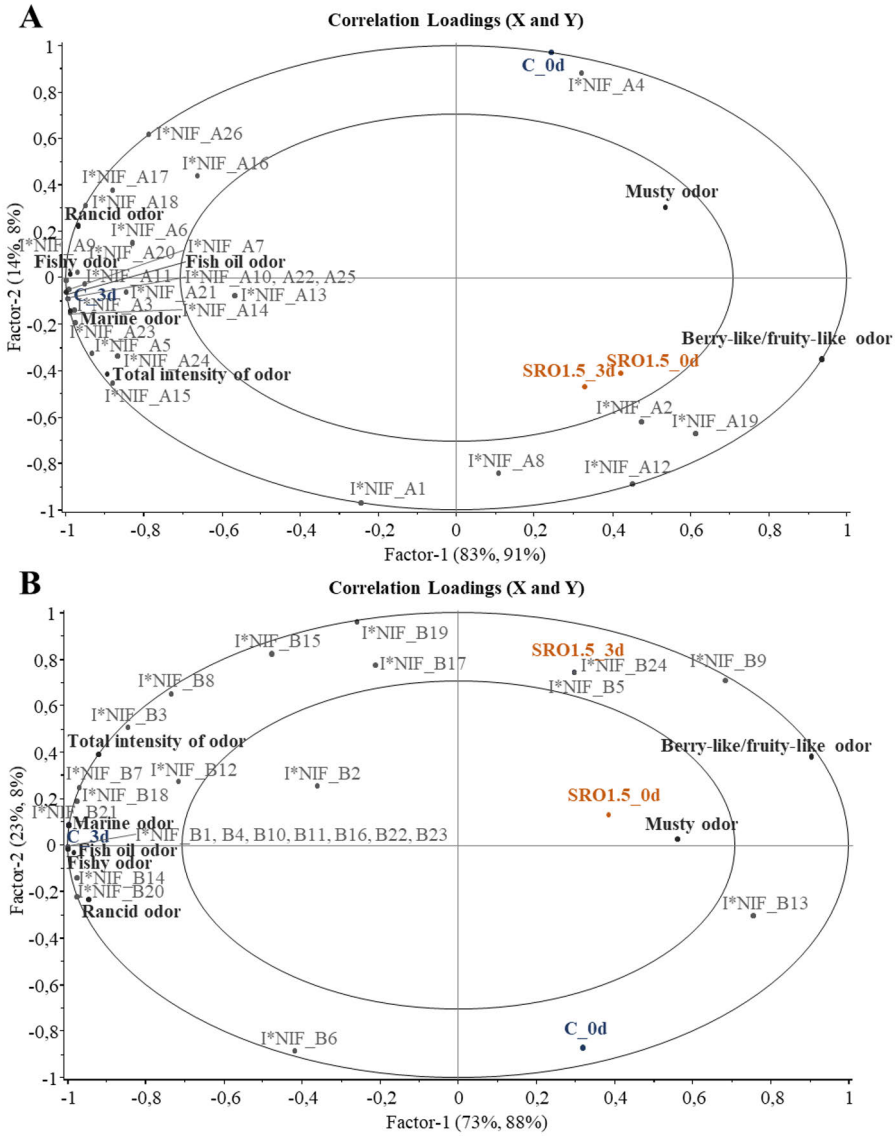

Figure 17. PLS model of odor-active compounds (X variables, as NIF*intensity, n=26 (A) or n=24 (B)) and rated intensities of odor attributes (Y variables, n=7) in raw fresh (0 d) and stored (3 d) Baltic herring minces with and without SRO (1.5%) (**IV**). Codes A1–A26 and B1–B24 refer to **Table 19** and **Table 20**, respectively.

5.4 General discussion

The pH-shift processing and enzymatic hydrolysis, as potential ways of valorizing Baltic herring and roach, were investigated in study **I**. While lipid oxidation in FPIs and FPHs is extensively studied, much less attention has been paid on investigation of their lipid composition and protein oxidation, though both may play an important role in the nutritional, sensory, and functional properties of FPIs and FPHs.

The FPIs and FPHs from Baltic herring and roach differed considerably in the measured compounds or indicators. Acid and alkali extracted FPIs from both Baltic herring and roach were associated with a high content of EAAs and high EAA:NEAA ratios, while FPHs were associated with hydroxyproline and glycine, which are abundant in collagen. Alkali extracted FPIs were characterized by oxidation indicators PV, propanal, hexanal, and protein carbonyls, while *n*-3: *n*-6 ratio was highest in acid extracted isolates. FPHs had higher relative content of TAGs compared to FPIs. Further, in FPIs and FPHs there was a negative correlation between the relative content of *n*-3 FAs and total PUFAs to 1-penten-3-ol, which is likely due to oxidative degradation of *n*-3 FAs since 1-penten-3-ol is known to be an oxidation product of *n*-3 FAs, such as DHA (Ahonen et al., 2022). The results presented here showed that the pH shift, compared to enzyme assisted extraction of Baltic herring and roach proteins/peptides, was more favorable in terms of nutritional quality, but challenging in terms of oxidation.

Alkaline extraction has often been considered as more favorable compared to acidic pH shift, as it has been reported better in terms of protein yield (Abdollahi and Undeland, 2019; van Berlo et al., 2023), lipid removal (Marmon and Undeland, 2010; van Berlo et al., 2023; Zhong et al., 2016), and gel forming ability (Phetsang et al., 2021b), and therefore only alkaline pH-shift extraction of Baltic herring proteins was investigated in study **II**. However, according to the results of study **I**, as well as findings by others (Abdollahi et al., 2020), the acid version of the process may lead to more limited oxidation compared to the alkaline process in some fish raw materials. Further, Surasani et al. (2018) reported that acidic extraction (pH 2.0) was found to have minimal effect on proteins and to result in stronger gels, while alkaline extraction (pH 13.0) caused protein denaturation, resulting in less stable proteins and gel network.

In studies **III** and **IV**, most antioxidant additions retarded lipid oxidation in Baltic herring mince, as indicated by inhibition of oxidation related volatiles (such as 2-ethylfuran, 1-penten-3-ol, hexanal, (*E*)-2-hexenal, and (*E,Z*)-3,5-octadien-2-one). Most antioxidants showed higher inhibition at the early storage period (2 months or 3 days) compared to the end of storage (10 months or 14 days). EDTA, however, did not provide antioxidative effects during frozen storage, as volatile formation was similar or even higher compared to the control.

The combination αT+AA showed similar inhibition for formation of all volatiles during storage at –20 °C (**III**) and 1 °C (**IV**), showing a greater effect towards formation of 2-ethylfuran and 3,5-octadien-2-one, and a weaker effect towards 1-penten-3-ol. The two berry press residues investigated in both studies, SR3 and LR3, performed similarly in both storage trials. AB was effective in retarding oxidation during frozen storage of Baltic herring mince, but since AB is a mixture of supercritical CO_2 extracts that are considered as novel foods, AB cannot currently be used in the food industry. Therefore, it was not investigated further in study **IV**.

In minced Baltic herring with no added antioxidants, lipid oxidation was reflected in the odor and flavor already after 3 days at 1 °C, but these changes could be inhibited by addition of 1.5% LR and especially SRO. Their antioxidative effect could also be seen as lower intensities of lipid oxidation derived odorants, such as (*Z*)-4-heptenal, hexanal, (*E,Z*)-2,6-nonadienal, and 2,3-pentanedione that may have contributed to the development of fishy odor in the control mince. In a study by Wu et al. (2021c), lipid oxidation occurred already within one day of storage at 0 °C in herring filleting co-products, but could be inhibited by an antioxidant dipping treatment. Tolasa Yılmaz et al. (2018) on the other hand reported that a significant increase in TBARS was already observed after 20 minutes (at 0 °C) of mincing in sardine in the absence of antioxidants. Altogether, mechanical treatment further increases the rate of lipid oxidation in fish susceptible to oxidation due to increased contact of enzymes, pro-oxidants, and oxygen with lipid oxidation substrates, and therefore use of antioxidants is essential to prevent a substantial loss in quality.

Based on the present findings and previous literature, the fishy and rancid odor of FPIs and FPHs is not only determined by the extent of lipid oxidation during the process but also their capacity to bind/release (odor-contributing) volatiles. For instance, despite the lower PVs and lower formation of most oxidation related volatiles in Baltic herring FPHs compared to FPIs (**I**), FPHs had a higher intensity of fishy odor and flavor (Nisov et al., 2021). As proteins are known to bind compounds contributing to odor and flavor (Damodaran and Kinsella, 1983; Gu et al., 2020), it is likely that their hydrolysis results in a lower ability to bind these compounds. In study **II**, the difference in total odor and odor-active compounds between Baltic herring and Baltic herring FPI could not be simply attributed to e.g. removal of lipids or possible increase in lipid oxidation during the pH shift, but was likely at least partly explained the changes in the matrix (including differences in water content) and therefore altered ability to bind/release volatiles.

5.5 Limitations of the study and future prospects

While most of the important odorants in studies **II** and **IV** were identified, a considerable number remained unidentified. Due to the low threshold of many odorants present in fish, their detection and identification using HS-SPME-GC-MS was challenging. Despite the many benefits of HS-SPME extraction in the analysis of odor-active volatiles (Iglesias and Medina, 2008), the content of extracted volatiles is rather low. Higher extraction temperature than 35–40 °C used in the present study, or extraction using some other technique, such as dynamic headspace (Frank et al., 2009) could have enabled higher sensitivity and better identification. SPME-Arrow extraction was also seen to result in extraction of a wider range of compounds from fish sauce compared to HS-SPME (Song et al., 2019). In addition, in order to gain deeper knowledge on the significance of individual odor-active compounds and their interactions on odors of Baltic herring, further research including recombinant and omission studies using different combinations of identified odor-active compounds is needed.

The kinetics and significance of different lipid oxidation pathways were not studied in this thesis. Assessment of e.g. lipoxygenase activity, content of heme proteins, heme and non-heme iron, etc. in protein isolates and hydrolysates or Baltic herring mince could have provided information of progress of lipid oxidation in different stages of processing or under different storage conditions. Further research should investigate these mechanisms in Baltic herring, as well as the mechanism of antioxidative effects of berry residue additions. This work showed the protective effect of berry press residues on the sensory quality of Baltic herring mince during storage. However, further consumer tests are needed regarding the effect of these additions on the liking of Baltic herring.

In study **II**, only alkaline pH shift was used to prepare the Baltic herring FPI. Including the acid solubilized FPI would have been interesting in order to see whether the possibly lower rate of oxidation could have further reduced the fishy odor and total intensity of odor compared to alkali extracted FPI. The effect of acidic pH shift should be further studied for Baltic herring, and other fish species for which only alkaline process has been investigated. Further, in studies **I** and **II**, no antioxidants or washing processes were used during the pH-shift and enzymatic hydrolysis and a high rate of oxidation especially in case of Baltic herring FPIs was observed. Previous studies have reported the use of different pre-washing processes to improve heme protein removal (Abdollahi et al., 2016; Chanarat and Benjakul, 2013). However, washing may lead to rapid development of oxidation despite removing heme proteins, due to a decrease in endogenous antioxidants of the fish muscle (Harrysson et al., 2020). Natural antioxidants have been shown to limit oxidation during both pH shift (Abdollahi

et al., 2020; Zhang et al., 2022) and enzymatic hydrolysis of fish (Halldorsdottir et al., 2014, 2013; Yarnpakdee et al., 2012c). As SRO was an effective antioxidant in Baltic herring mince, its antioxidative effects during the pH shift processing of Baltic herring should be investigated in the future.

6 SUMMARY AND CONCLUSIONS

This thesis focused on new ways of increasing utilization of under-utilized fish. While this research focused only on Baltic herring and roach, the findings may provide valuable reference for processing of other under-utilized fish species as well. The two processing methods studied were the pH shift and enzymatic hydrolysis, both of which have been widely studied for other fish species but not Baltic herring or roach. On the other hand, the addition of natural antioxidants in Baltic herring mince during refrigerated or frozen storage was investigated as a means of inhibiting lipid oxidation and changes in sensory quality. 'Antimicrobial blend', lingonberry juice press residue, sea buckthorn juice press residue, and sea buckthorn juice press residue after supercritical CO_2 oil extraction as natural additions were compared to conventional antioxidants, EDTA and α-tocopherol in combination with ascorbic acid.

The composition of proteins and lipids was significantly affected by the processing method (pH shift or enzymatic hydrolysis). The amino acid composition was better in the alkali and acid extracted FPIs compared to FPHs from both roach and Baltic herring, as the pH shift led to enrichment of essential amino acids relative to non-essential amino acids. The pH shift also led to accumulation of phospholipids and polyunsaturated fatty acids, especially DHA, in acid extracted FPIs. Particularly alkaline pH shift, however, induced considerable lipid and protein oxidation. The FPHs showed more moderate formation of hydroperoxides, secondary volatile oxidation products, and protein carbonyls.

The incomplete elimination of fishy odors and flavors during preparation of FPIs and FPHs is a major factor limiting their use. Based on this thesis research, while not completely eliminating these odors, the alkaline pH shift reduced the total intensity of odor and the intensity of fishy odor in Baltic herring. Based on analysis of the odor-active compounds, the reduction in fishy odor could not be attributed only to lipid-derived compounds. Changes in binding/release of odor-active volatiles is likely to explain some of the differences observed in the odor of the protein isolate compared to Baltic herring as such.

The natural antioxidants LR, SR, and AB retarded lipid oxidation in Baltic herring mince stored at –20 °C more effectively than EDTA or α-tocopherol and ascorbic acid, as indicated by lower loss of EPA and DHA, lower PV, and/or lower formation of secondary oxidation related volatiles, such as 1-penten-3-ol and hexanal. The addition of AB, and 3% LR and SR, however resulted in lower likeness scores when evaluated as fish loaves. In the refrigerated storage trial, three different concentrations, 3%, 1.5%, and 1%, of berry residues were investigated to determine whether the concentration could be decreased without compromising their antioxidative effect. Based on formation of hydroperoxides

during 0–21 days, and formation of 1-penten-3-ol, hexanal, and 3,5-octadien-2-one during 0–14 days of storage at 1 °C, SRO and especially LR were efficient antioxidants also at the lower concentrations. A significant change in sensory quality was observed in the Baltic herring mince without antioxidants during the 3-day storage at 1 °C. The fishy odor, fish oil odor, and marine odor increased significantly in raw control mince, but berry press residues, particularly SRO (1.5%), prevented changes in sensory quality.

Further investigation of odor-active compounds showed that 1.5% SRO retarded the formation of lipid oxidation derived odor-active compounds, such as propanal, hexanal, (*Z*)-4-heptenal, (*E,Z*)-2,6-nonadienal, and 2,3-pentanedione, which were detected at a higher frequency and/or intensity in the stored control mince. According to the results, SRO at a concentration of 1.5% was efficient in retarding the lipid oxidation derived quality loss in Baltic herring mince, based on retarding the formation of hydroperoxides, secondary volatile oxidation products, lipid-oxidation related odorants, and formation of fishy odor and flavor. LR, SR, and SRO are side streams of berry juice and oil production, and their use as antioxidants would provide added value for these currently under-utilized materials as well.

Altogether, the result showed that the pH shift and enzymatic hydrolysis can be used to extract proteins from roach and Baltic herring and are potential methods to improve utilization of especially small fish that are too small to be filleted. Using these methods also allows collection of lipids as another valuable fraction, although it was not investigated in this study. However, special attention should be paid to controlling lipid oxidation during the processing of Baltic herring. Addition of appropriate antioxidants, such as berry press residues, may help to preserve the sensory quality of Baltic herring and retard the development of odor characteristic to stored Baltic herring.

ACKNOWLEDGEMENTS

Doing a PhD, with all its hardships and joys, has been an amazing journey. I will be eternally grateful for so many people who have somehow contributed to the experience and supported me. This work was conducted at the Food Sciences unit, Department of Life Sciences, University of Turku, and was related to a national innovation programme, "Blue Products", funded by the Finnish Operational Programme of the European Maritime and Fisheries Fund. In addition, financial support from Finnish Cultural Foundation, Jenny and Antti Wihuri Foundation, Food Research Foundation and the Doctoral Programme in Molecular Life Sciences of the University of Turku Graduate School are highly appreciated. I also want to acknowledge Kiantama Oy, AromTech Oy, and Kalaset Oy for kindly providing berry press residues and Baltic herring for my research. In addition, I am highly grateful for the time invested by all the panelists taking part in the sensory and GC-O analyses.

I deeply appreciate the support from my supervisors, Baoru Yang, Oskar Laaksonen, and Annelie Damerau. Baoru, I am grateful that you welcomed me back to our unit and gave me a chance to do a PhD in Blue Products. You helped me improve the quality of my research, and always encouraged me, even when I did not believe in myself. Oskar, I would not be here without your guidance. You have been supervising/mentoring me since my MSc studies, and have always guided me with my best interest in mind. You especially helped me with the sensory analyses of my thesis. Annelie, I really appreciate all of your help and support during my PhD. When I first started, I didn't have a lot of lab experience and you basically held my hand in the lab, but through your guidance I became an independent scientist. Thank you for all your support and your friendship. I would also like to warmly thank my advisory committee members, Anu Hopia and Saska Tuomasjukka. Thank you for all your guidance and support during the past years. Further, I am grateful for Professor Soottawat Benjakul and Professor Turid Rustad for acting as pre-examiners for my thesis and providing valuable comments to improve it. I also want to thank Professor Ingrid Undeland, it is truly an honor to have you as the Opponent.

I have been extremely fortunate to have been working in the Blue Products project since 2018. During these years, I have had the opportunity to learn a lot about the fish industry and collaborate with so many great people. Warm thanks especially to Guy Svanbäck, Mathias Högbacka, Jari Setälä, Jaakko Hiidenhovi, Pirjo Mattila, Sari Mäkinen, Anna-Liisa Välimaa, Kaisu Honkapää, Anni Nisov, Raija Lantto, Jaana Huotari, Hanna-Leena Alakomi, Heikki Aisala, Iina Jokinen, Nora Lógren, Anu Hopia, and Nanna Rintala. Sincere thanks for all the collaboration and nice moments.

I really appreciate all my co-authors who have contributed to my research; Ye Tian, Mari Sandell, Anu Hopia, Saska Tuomasjukka, Pasi Tolvanen, Tapio Salmi, Anni Nisov, Kaisu Honkapää, Marko Tarvainen, and Anna Puganen. I had the honor to supervise many students, and I am truly grateful for Claudia Mejia Rios, Lauri Jokela, Ella Aitta, and Kristiina Aarnio for your contributions to my doctoral research.

I wish to thank every past and present member of the Food Sciences unit, I wish I could thank every one of you by name. During these past five years, you have become like a family to me, and I have enjoyed working with all of you. I would like to thank Anu, Heli, Tapio and Leena for always helping with practical and technical issues. Thank you Heli also for our talks. Jukka, Kaisa, Marika, and Maaria, thank you for your help and advice during the past years. I would especially like to thank past and present doctoral researchers Niina, Ella, Mikael, Hany, Gabriele, Salla, Sari, Anna, Eija, and others for the nice coffee breaks and your peer support. Warm thanks to Niina, Ella, Mikael and Hany for the lunches, afterworks, and fun times. Hany, thank you for your support and for making all of us laugh. Ella, you started as the first student that I supervised, but from very early on I have learned a lot from you. We have co-authored one article and been working together in Blue Products. In addition, I am grateful for your friendship and our nice memories together. Warm thanks to you Niina, I have turned to you for help so many times, and have always looked up to you as a researcher. We became friends through cat owner peer support, and I hope that we will stay friends regardless of whether our careers take us.

I have been fortunate to have many friends and family members as support during this journey. I want to thank my friends Anna, Ilona, Saila and Katri for listening to me rambling about my PhD and providing the best laughs and stress relieve. Johannes, I am truly grateful for your support during these years. Many extended family members have been there for me during these years, and I would like to especially thank Leena and Suvi. My deepest gratitude goes to my mum Tarja, dad Timo and brother Tomi. Each of you has always been there for me, and I would not be here without your love and support. Finally, I want to thank Mikael for your love and for standing by me. Words cannot express how grateful I am to have been on this journey with you, as colleagues, friends, and finally, partners.

Turku, March 2023

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