Cross-processing herring and salmon co-products with agricultural and marine side-streams or seaweeds produces protein isolates more stable

(article starts on next page)

Cross-processing herring and salmon co-products with agricultural and marine side-streams or seaweeds produces protein isolates more stable towards lipid oxidation

Jingnan Zhang [*], Mehdi Abdollahi, Marie Alminger, Ingrid Undeland [*]

Department of Biology and Biological Engineering-Food and Nutrition Science, Chalmers University of Technology, SE 412 96 Gothenburg, Sweden

ABSTRACT

Herring and salmon filleting co-products were pH-shift processed together with seven antioxidant-containing raw materials ("helpers") including lingonberry-, apple-, oat-, barley- and shrimp-co-products, and two seaweeds (*Saccharina latissima, Ulva fenestrata*) to produce protein isolates stable towards lipid oxidation. Malondialdehyde (MDA) and 4-hydroxy-(E)-2-hexenal (HHE) levels revealed that all helpers, except shrimp shells, to different extents retarded lipid oxidation both during pH-shift-processing and ice storage. The three helpers performing best were: lingonberry press-cake > apple pomace ~ *Ulva*. Color of protein isolates was affected by helper-derived pigments (e.g., anthocyanins, carotenoids, chlorophyll) and lipid oxidation-induced changes (e.g., metHb-formation, pigment-bleaching). In conclusion, combining fish co-products with other food side-streams or seaweeds during pH-shift processing appears a promising new tool to minimize lipid oxidation of protein isolates, both during their production and subsequent storage. Lingonberry press-cake was the most efficient helper but provided dark color which may narrow product development possibilities, something which requires further attention.

1. Introduction

Global fish production reached around 179 million tonnes in 2018, 88% of which was utilized for human consumption (FAO, 2020), to a large extent after conversion to fillets. The filleting operation, as well as subsequent steps such as canning and packaging lead to huge amounts of co-products, which make up 40%-60% of the fish weight (Abdollahi, Wu, & Undeland, 2021). Heads and backbones, which make up > 75% of the total co-product weight, contain large amounts of residual muscle with a high nutritional value and are thus promising sources for the production of high-quality protein ingredients (Nolsøe & Undeland, 2009). In Scandinavia, herring (*Clupea harengus*) and salmon (*Salmo salar*) co-products are particularly abundant, but these species are also highly important on a global basis. In 2018, 1820 thousand tonnes of herring (live weight) were captured, accounting for 3% of total captured finfish (FAO, 2020). For salmon, 2435.9 thousand tonnes were aquacultured in 2018, making up 4.5% of the total finfish (FAO, 2020). Both species have, beyond the proteins, high or relatively high levels of long-chained n-3 polyunsaturated fatty acids (LC n-3 PUFAs), iodine, selenium, vitamin B12 and D (Bourre & Paquotte, 2008), motivating a full

use of the raw materials for food production as opposed to only focusing on the fillets.

One method used for converting fish co-products to food ingredients is the pH-shift process, first patented by Hultin et al. in 2000 (Hultin and Kelleher, 2000). This technique has been demonstrated effective for protein recovery from complex marine food resources, such as gutted fish (Marmon & Undeland, 2010) and fish processing co-products (Abdollahi, Olofsson, Zhang, Alminger, & Undeland, 2020; Abdollahi et al., 2021). However, although the process can remove lipids and active pro-oxidants to some extents (Abdollahi, Marmon, Chaijan, & Undeland, 2016; Nolsøe & Undeland, 2009), the remaining levels were still enough to induce increased peroxide values (PV), TBA-reactive substances (TBARS) or specific aldehydes (malondialdehyde, MDA, and 4-hydroxy-(E)-2-hexenal, HHE) during production and storage of protein isolates from herring (Abdollahi et al., 2020; Undeland et al., 2005). In addition to the negative influences on color and sensory attributes, the lipid oxidation products can destroy or aid cross-linking of amino acids such as tryptophan, methionine and lysine to other compounds, making these amino acids biologically unavailable (Özyurt, Şimşek, Karakaya, Aksun, & Yeşilsu, 2015). Hence, to produce high-

quality protein isolates from fish co-products that are rich in heme-proteins, lipids and endogenous enzymes, it is necessary to prevent lipid oxidation not only during pH-shift processing, but also during subsequent storage of protein isolates.

Synthetic antioxidants such as ethylenediaminetetraacetic acid (EDTA) and sodium tripolyphosphate (STPP), were earlier employed to prevent lipid oxidation during pH-shift processing of herring fillets (Undeland et al., 2005), and were reported to significantly reduce PV and TBARS levels at the end of the process and after subsequent ice storage. However, natural antioxidants have in the past 10–20 years attracted significantly more interest than synthetic ones due to the wish for a clean label within the food industry. Plant and algae extracts rich in natural antioxidants have shown a remarkable ability to limit lipid oxidation in fish muscles during ice and frozen storage (Abdollahi et al., 2020; Bitalebi, Nikoo, Rahmanifarah, Noori, & Ahmadi Gavlighi, 2019; Damerau et al., 2020; Sánchez-Alonso, Borderías, Larsson, & Undeland, 2007; Sun, Sun, Thavaraj, Yang, & Guo, 2017). With this in mind, and with the aim to prevent lipid oxidation during pH-shift processing, our research group recently proposed a new approach of cross-processing fish co-products with seaweed (*Saccharina latissima*) or with antioxidant-rich co-products generated from the production of lingon-berry juice and peeled shrimps (Abdollahi et al., 2020). The study showed that such materials -hereafter referred to as helpers- when added on a 30% level (dry weight (dw)/dw fish) retarded lipid oxidation in herring and salmon co-products during both acid and alkaline versions of pH-shift processing. The study did however not reveal how the storage stability of the produced isolates was affected by cross-processing fish co-products with helpers.

Shrimp shells that were used as helpers in our previous study were selected based on their abundance of astaxanthin (Khumallambam, Rama, Karuppannan, & Manjabhat, 2011), and the lab-produced ling-onberry press-cake and *Sacccharina* based on documented high levels of phenolic compounds, e.g. anthocyanins/anthocyanidins, flavonols (Bujor, Ginies, Popa, & Dufour, 2018), phlorotannins and fucoxanthin (Roohinejad et al., 2017). The promising results obtained with these helpers paved the way for screening other seaweed species and underutilized food side streams that are produced in the geographic vicinity of the herring and salmon filleting industries. From a Swedish perspective, producers of apple juice, beer and oat-products (Oatly®) have been identified as attractive suppliers of sidestreams, since they generate large volumes of pomace, barley-spent grain and fiber residues, respectively, which are rich in phenolic compounds such as flavonoids, tannins and phenolic acids (Birsan, Wilde, Waldron, & Rai, 2019; Rat-nasari, Walters, & Tsopmo, 2017; Sun et al., 2017). Further, cultivation of *Ulva fenestrata* has been initiated on the Swedish West coast, in addition to the cultivation of *Saccharina latissima*, and here e.g. carot-enoids, chlorophyll and sulfated polysaccharides are expected (Peasura, Laohakunjit, Kerdchoechuen, & Wanlapa, 2015; Qi et al., 2005). Based on their antioxidant content, we hypothesized that all these five materials could be promising helpers to stabilize pH-shift-produced fish protein isolates towards oxidation during storage, something which has never been reported on before. If successful, the combined processing of several side streams or side streams with seaweeds could be a fundament for a new type of industrial symbiosis providing added value to poorly utilized, or even wasted, raw materials.

The present study thus aimed to further study the cross-processing concept by broadening the selection of helpers from lingonberry press-cake, shrimp shells and *Saccharina latissima* to also include apple pomace, oat fiber residues, barley-spent grain and *Ulva fenestrata*. The fish co-products were still from herring and salmon, but for the latter focus was on heads alone since salmon backbones currently are subjected to manual scaping of residual muscle. The specific aim was to evaluate whether cross-processing the selected fish co-products and helpers could improve the oxidative stability of produced protein isolates both directly after the alkaline/acid pH-shift process and during subsequent ice storage. The level of lipid oxidation in protein isolates

was evaluated as the formation of three specific aldehydes including MDA, HHE and 4-hydroxy-(E)-2-nonenal (HNE). Changes in surface color were also measured with the focus of L*-, a*- and b*-values to evaluate if oxidation-related phenomena such as met-hemoglobin (Hb) formation, pigment bleaching or Schiff base polymerization (Wetterskog & Undeland, 2004; Zamora & Hidalgo, 2005) would correlate with the aldehyde formation.

2. Materials and methods

2.1. Chemicals and reagents

Hydrochloric acid, sodium chloride, trichloroacetic acid (TCA), EDTA, BHT, DNPH, and streptomycin sulfate were from Sigma-Aldrich Co. (USA). Sodium hydroxide and methanol were from Honeywell Riedel-de Haën AG (Germany). All of the other chemicals used were reagent grade.

2.2. Fish co-products

Herring (*Clupea harengus*) heads and backbones were provided by *Sweden Pelagic AB* (Ellös, Sweden). Salmon (*Salmo salar*) heads were provided by *Fisk Idag AB* (Gothenburg, Sweden). The fresh herring and salmon filleting co-products were transported to Chalmers University of Technology on ice, and ground by a tabletop meat grinder using a 4.5 mm hole plate (C/E22 N, Minerva Omega Group, Italy). The minces were stored at −80 °C.

2.3. Marine and agricultural helpers

Shrimp shells including cephalothorax, legs, peels, and tails were provided by *Räkor och Laxgrossisten AB* (Gothenburg, Sweden) in March 2018 after storing at −25 °C for three months. Brown seaweed (*Saccharina latissima*) and green seaweed (*Ulva fenestrata*) were tank-cultivated at the *Sven Lovén Centre for Marine Infrastructure* (Tjärnö, Sweden). *Saccharina* and *Ulva* were harvested in June 2018 and November 2019, respectively. The fresh-harvested seaweeds were transported on ice to Chalmers University of Technology within one day. Lingonberry (*Vaccinum vitis-idaea*) press-cake including peels, leftover flesh, seeds, and stems was obtained from Grangärde in Sweden in November 2019 after storing at −20 °C for five months. Lingonberry press-cake, shrimp shells, *Saccharina* and *Ulva* were ground according to the same procedure as fish co-products. Apple (*Malus domestica)* pomace including peels, leftover flesh, core with seeds and stems was obtained from *Kiviks Musteri AB* (Kivik, Sweden) in October 2019 after storing at −20 °C for four weeks. Barley-spent grain including pale malt with small percentages of caramel malts, flaked oats, and rice hulls was provided by Beerbliotek (Majorna, Sweden) in September 2019 after storing at −20 °C for four days. Oat fiber residues were provided by *Oatly ® AB* (Lund, Sweden) in June 2018. All helpers were stored at −80 °C until the production of protein isolates in March-May 2020.

2.4. Hemoglobin (Hb) content of fish co-products

Frozen herring and salmon co-product minces were milled into small frozen pieces by a chopper after mixing with liquid nitrogen. The determination of Hb content followed a modified version of Hornsey acid hematin method described by Wu et al. (2020).

2.5. Production of protein isolates

Protein isolates were produced as described by Abdollahi et al. (2020) with a few modifications to render as high as possible protein yields (Zhang et al., 2022). In short, the amount of ice-cold distilled water which was originally adjusted to keep the same ratio of dry matter to moisture as the herring controls, was increased to six times the wet

weight of the raw materials; a more efficient mechanical homogenizer, Silverson Rotor-stator mixer (L5M-A, Silverson, UK) was used to homogenize raw materials at 8000 rpm for 90 s; and, the solubilization pH's and precipitation pH's applied to different combinations of raw materials were optimized (see **supplementary** Table 1). The helpers were added to the fish co-products in an amount that corresponded to 30% of fish co-products' dry weight (see **supplementary** Table 2 for moisture content of raw materials). The exact amount of fish co-products, helper and distilled water are shown in **supplementary** Table 3 together with the initial pH of fish co-products without/with the addition of helper. The whole process was carried on ice. Herring controls, i.e. without helpers, and combinations with lingonberry press-cake were subjected to both alkaline and acid pH-shift processing based on the promising results with a lab-produced lingonberry press-cake used in our previous study (Abdollahi et al., 2020). For the same reason, salmon heads were only co-processed with lingonberry press-cake. Protein solubility and protein yield during the solubilization and precipitation steps were recorded using a modified version of the Lowry protein quantification method described by Markwell et al. (1978); finally also total protein yield was calculated (**supplementary** Table 4). The pellet (i.e. protein fractions) was manually stirred on ice with a stainless-steel spatula until homogeneous. Extra centrifugations ($8500 \times g$, 10–40 min, 4 °C, see **supplementary** Table 1) were employed until the protein fractions had reached 78%-80% of moisture content; the latter was estimated by a moisture balance (HA 300, Precisa, Switzerland). Isolates were then adjusted to pH=7.00 ± 0.05 by dropwise addition of 2 M NaOH or 2 M HCl after which their moisture contents were determined again (see **supplementary** Table 1) by heating at 105 °C overnight. The protein isolates were then stored at −80 °C for 2-3 months until the ice storage trials in June-July 2020.

2.6. Ice storage of protein isolates

For each sample, 33 g of frozen protein isolate was thawed in a tight plastic bag under cold running water. Different amount of cold distilled water was added to equalize their moisture contents to the same as the isolate having the highest moisture (the one from herring co-products and shrimp shells had 83.4% of moisture). Streptomycin (200 ppm on a moisture basis) was added to inhibit bacterial growth. After stirring evenly on ice, 27.6 ± 0.3 g of each sample was transferred to a screw-capped 250 ml glass Erlenmeyer flask and flattened out at the bottom. The thickness of the sample was 5–6 mm. The caps of flasks were screwed tightly and the whole flask was wrapped by an aluminum film to avoid light. The flasks were then stored on ice in insulated cooler boxes in a 4 °C cold room for up to 16 days. Samples were taken from the flasks regularly; daily for the first 6 days, then the intervals were extended to 2 days until day 10, followed by 3 days until the end of the trial. The whole storage trial was conducted twice.

2.7. Proximate composition of fish co-products, helpers and protein isolates

Crude protein content was determined by a LECO nitrogen analyzer (TruMac-N, LECO Corp., USA) using a modified version of the Dumas method as explained by Marco et al. (2002). The nitrogen-to-protein conversion factor used was 5.58 for fish co-products and protein isolates, 5.45 for barley-spent grain, 5.34 for oat fiber residues, 5.4 for lingonberry press-cake and apple pomace (Mariotti, Tomé, & Mirand, 2008), 3.88 for shrimp shells (Rødde, Einbu, & Vårum, 2008) and 5 for seaweeds (Angell, Mata, de Nys, & Paul, 2015). Crude lipid content was analyzed by a modified version of Lee's method using extraction with chloroform and methanol described by Abdollahi et al.(2020). Ash content was determined by placing a 1.0 g sample in a crucible and heating at 550 °C for 6 hrs. Moisture content analysis is described in 2.5.

2.8. Total phenolic content (TPC) of protein isolates

For extraction of phenolic compounds, two grams of each sample was mixed with 10 ml of extraction solution (70% methanol containing 1% trifluoroacetic acid) and homogenized (T18 digital Ultra-Turrax, IKA, Germany) on ice at 12000 rpm for 30 s. Another 10 ml of extraction solution was used to wash the probe of the homogenizer and added to the first 10 ml. After vortexing for 15 s, the sample solution was sonicated for 5 min twice with a manual shake in the middle. The sonicated sample solution was incubated in a water bath (60 °C, 100 rpm) for 30 min, then quickly cooled and centrifuged ($5000 \times g$, 5 min, 4 °C). The supernatant was collected. Five ml of extraction solution was added to dissolve the pellet followed by sonication (2×5 min) and centrifugation ($5000 \times g$, 5 min, 4 °C). The second supernatant was collected and added to the first supernatant. Total phenolic content was measured by a modified version of the Folin-Ciocalteu colorimetric method described by Oliveira et al. (2014). The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/100g sample, dw.

2.9. Analysis of aldehydes (MDA, HHE and HNE) in protein isolates

Protein isolate samples (0.7–0.8 g) taken during storage trials were thawed under cold running water, weighed and homogenized (T18 digital Ultra-Turrax, IKA, Germany) on ice with 4 volumes of ice-cold Milli Q water at 20000 rpm for 30 s. Five hundred μl of homogenate was then transferred to a 1.5 ml Eppendorf tube. The contents of MDA, HHE, and HNE were measured by the method explained by Tullberg et al. (2016) using DNPH-derivatization followed by LC-MS analysis.

2.10. Color measurements

The surface color of protein isolates during storage was daily measured (n=3) as described by Abdollahi et al. (2016), using a Minolta colorimeter (CR-400, Konica Minolta Sensing, Japan) in the CIE *L*a*b** color space. The color changes caused by inducing different helpers to alkali-produced herring protein isolates were quantified by ΔE_{2000}, which were calculated by CIEDE2000 color-difference formula using *L*-, a*-,* and *b*-* values (Sharma, Wu, & Dalal, 2005).

2.11. Statistical analysis

The ice storage trials were conducted twice. One sample of 0.7–0.8 g was collected from each E-flask each time during each ice storage trial and used for aldehyde analysis. Compositional analyses were done on at least duplicate samples. Significant differences between sample groups were determined by one-way ANOVA followed by Duncan's multiple range test (SPSS Statistics version 27, IBM Corp. NY, USA), which were also used to determine whether the time had a significant influence on the changes of aldehyde content and color within a specific sample group at specific time points. The significance level was set at 0.05, below which the differences were considered significant.

3. Results and discussion

3.1. Proximate composition of fish co-products, helpers and protein isolates

As shown in Table 1, the increase in protein content measured in protein isolates compared to their corresponding fish co-products indicated that the pH-shift processing, with or without helpers, was able to concentrate proteins. The alkaline process version yielded isolates with a significantly ($p<0.05$) higher protein content than the acid version when doing pH-shift processing of herring co-products, without or with the addition of lingonberry press-cake. These two findings were in agreement with our earlier reported study (Abdollahi et al., 2020). Different helpers had different effects on the protein contents of protein

Table 1

Proximate composition of herring and salmon co-products, helpers and protein isolates produced thereof in the absence or presence of helpers. Protein, lipid, and ash data are given as g/100 g on the dry weight with data showing mean values ± standard deviation (n ≥ 2).

Type	Source	pH-shift version	Protein	Lipid	Ash
Fish co-products	Herring heads and backbones		48.73 ± 1.15^g	32.65 ± 1.51^b	14.48 ± 0.20^d
	Salmon heads		34.26 ± 0.70^i	56.42 ± 3.01^a	9.44 ± 0.74^e
Helper	Lingonberry press-cake		5.57 ± 0.10^l	14.37 ± 2.09^{efg}	2.04 ± 0.16^n
	Apple pomace		2.34 ± 0.03^m	4.22 ± 0.63^k	2.73 ± 0.47^{mn}
	Barley-spent grain		14.76 ± 0.33^j	5.68 ± 0.18^j	3.60 ± 0.04^{klm}
	Oat fiber residues		36.12 ± 0.44^i	10.66 ± 0.45^h	5.79 ± 0.02^{gh}
	Shrimp shells		39.77 ± 0.12^h	7.71 ± 0.49^i	48.05 ± 0.77^a
	Saccharina		11.78 ± 0.06^k	3.17 ± 0.14^k	23.78 ± 0.06^c
	Ulva		15.40 ± 0.07^j	7.02 ± 0.12^{ij}	28.30 ± 0.06^b
Protein isolate	Herring heads and backbones	Alkaline	79.85 ± 1.43^a	13.99 ± 0.24^{fg}	3.22 ± 0.12^{lm}
		Acid	70.40 ± 0.51^{de}	18.37 ± 0.93^d	4.94 ± 0.26^{hij}
	Herring + Lingonberry press-cake	Alkaline	74.51 ± 0.73^b	13.31 ± 0.53^g	3.03 ± 0.10^m
		Acid	71.39 ± 1.53^{cd}	15.75 ± 0.08^e	4.04 ± 0.11^{jkl}
	Herring + Apple pomace	Alkaline	68.72 ± 0.04^{ef}	14.12 ± 0.09^{fg}	4.04 ± 0.02^{jkl}
	Herring + Barley-spent grain	Alkaline	78.81 ± 0.14^a	15.36 ± 0.16^{ef}	3.38 ± 0.01^{klm}
	Herring + Oat fiber residues	Alkaline	78.74 ± 1.16^a	15.19 ± 0.22^{ef}	3.49 ± 0.04^{klm}
	Herring + Shrimp shells	Alkaline	78.94 ± 0.35^a	15.71 ± 0.28^e	4.25 ± 0.20^{ijk}
	Herring + *Saccharina*	Alkaline	73.80 ± 0.84^b	11.79 ± 0.08^h	5.90 ± 0.12^{fg}
	Herring + *Ulva*	Alkaline	72.74 ± 0.15^{bc}	11.88 ± 0.04^h	4.84 ± 0.02^{ij}
	Salmon heads	Alkaline	69.44 ± 0.19^{def}	23.30 ± 1.19^c	6.66 ± 0.19^f
	Salmon + Lingonberry press-cake	Alkaline	68.24 ± 0.13^f	14.03 ± 0.05^{fg}	5.00 ± 0.06^{hi}

Data within the same column carrying a different superscript letter are significantly different (p < 0.05).

isolates. For the alkali-produced herring protein isolates, the ones produced with the addition of lingonberry press-cake, apple pomace, *Saccharina* and *Ulva* showed significantly (p<0.05) lower protein content than herring control, i.e., without adding helper, which could be caused by co-precipitation of non-protein compounds from these helpers, e.g. carbohydrates and phenolics, with the proteins (Czubinski & Dwiecki, 2017; Dhouafli et al., 2018; Soares, Mateus, & De Freitas, 2012). Oat fiber residues, barley-spent grain or shrimp shells had no significant (p<0.05) effect on the protein content of isolates compared to the control.

Table 1 also shows that salmon co-products contained more lipids than herring co-products, which yielded higher lipid contents in salmon-derived protein isolates compared to herring-derived ones. The acid version also rendered isolates with higher lipid content than the alkaline version, which agreed with our earlier study (Marmon and Undeland, 2010). The addition of different helpers showed different effects on the lipid contents. This is because the lipid removing capacity of the pH-shift process not only depends on the density and polarity of the ingoing lipids but also on emulsifying properties of proteins and polysaccharides existing in the system, as well as on the polarity of the aqueous phase (Vareltzis & Undeland, 2008). For example, the addition of seaweeds (i.e. *Saccharina* and *Ulva*) rendered a significantly (p<0.05) lower lipid content in isolates than the control sample, which could be explained by the increase of polarity in the system due to the salts brought in by the seaweeds (Roohinejad et al., 2017). In contrast, by adding shrimp shells, the lipid content of protein isolates significantly (p<0.05) increased. The higher lipid contents could be related to the fragile floating lipid layer formed after the first centrifugation in these samples, which resulted in inefficient removal of lipids. Similarly, in our previous study (Abdollahi et al., 2020), when employing the alkaline pH-shift process version, adding *Saccharina* to herring/salmon heads and backbones rendered lower content of lipids in the final protein isolate while when adding shrimp shells, higher lipid contents were achieved.

Although the ash content of the herring heads plus backbones was higher than in salmon heads, the ash content in herring-derived protein isolates was lower than in salmon-derived ones, i.e., the relative ash removal was larger (59–79% compared to 29–47%). Further, alkali-produced protein isolates showed higher ash content than acid-produced protein isolates, which was in agreement with the study of Foh et al. (Foh, Xia, Amadou, & Jiang, 2012). The ability of the pH-shift process to reduce ash is mainly explained by the removal of mineral-

containing parts like bones and scales during the first centrifugation step (Abdollahi et al., 2020). However, since acid may dissolve some minerals into the aqueous phase, a certain removal into the second supernatant can also be expected during acid-aided pH-shift processing. The addition of *Saccharina, Ulva* or shrimp shells significantly increased the ash content of herring protein isolates, which agrees with the high ash content of seaweeds and shrimp shells (Table 1). The highest ash contents were detected in the isolates produced with *Saccharina*. Opposite, the addition of lingonberry press-cake yielded 25% lower ash content of salmon protein isolates, compared to the salmon control. This could be due to simple dilution of the mineral-rich fish co-products with a low-ash material; lingonberry press-cake contained only about 2g ash /100 g dw.

This study did not include analyses of carbohydrates, but from a simple addition of protein, lipid and ash contents, it is estimated that up to 13% of carbohydrates ended up in the cross-processed protein isolates on a dw basis. For protein isolates produced with the addition of lingonberry press-cake, *Saccahrina*, and *Ulva*, about 10 g carbohydrates/ 100 g dry protein isolates are estimated, while the highest level (13 g/ 100 g dw) was estimated for isolates made with apple pomace. The carbohydrate profiles in these samples will be subject for separate studies to create a more comprehensive understanding of the effects of cross-processing on the composition and techno-functional properties of protein isolates.

3.2. Formation of aldehydes in herring and salmon co-products during pH-shift processing with/without helpers, and during subsequent storage of protein isolates on ice

The formation of MDA, HHE (Fig. 1), and HNE was used to monitor the level of lipid oxidation. The results of HNE-analyses showed extremely low values, and are therefore only presented in supplementary Fig. 1. In short, the HNE results aligned with the conclusions generated from the results of MDA and HHE, except for that the salmon-derived control isolate obtained significantly higher HNE-values than that from herring. Since HNE is a specific product of n-6 PUFA-oxidation (Tullberg et al., 2016), this is due to the higher level of n-6 fatty acids in salmon than herring co-products (Abdollahi et al., 2021), caused by the presence of vegetable oils in salmon feed. Significant increases (p<0.05) in MDA and HHE levels were detected in freshly made herring and salmon protein isolates compared with their corresponding raw
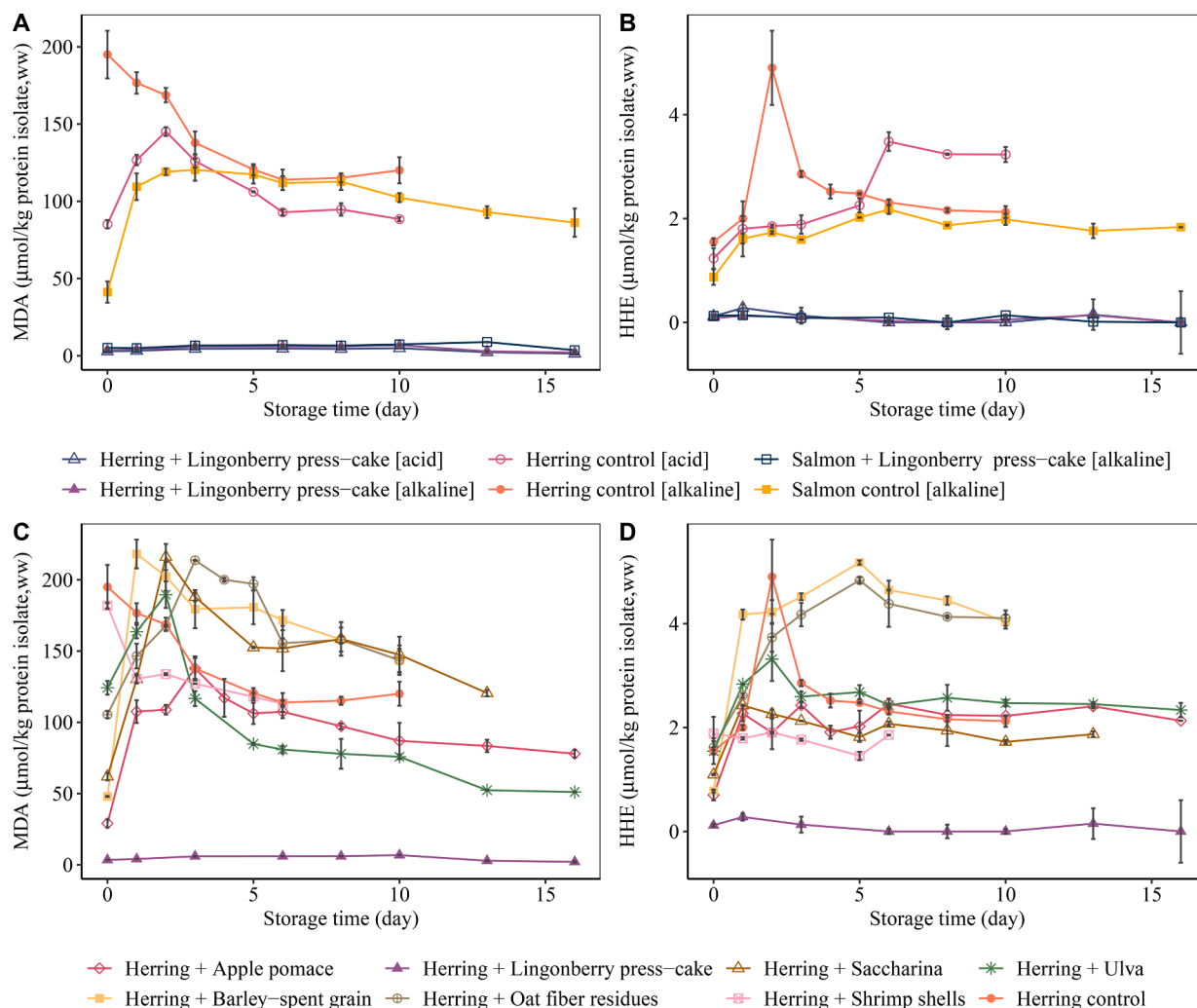
Fig. 1. MDA and HHE measured in protein isolates during extended storage on ice. Panels A and B compare the isolates produced from herring and salmon co-products by the alkaline or acid version of pH-shift process, in the absence or presence of lingonberry press-cake; panels C and D compare the herring protein isolates produced by the alkaline version of pH-shift process in the presence of all different helpers.

materials, implying the occurrence of lipid oxidation during the actual pH-shift processing. The minced herring co-products contained 8.31±0.36 µmol MDA/kg wet weight (ww) and 0.18±0.01 µmol HHE/kg ww, while salmon co-products contained 7.65±0.05 µmol/kg ww of MDA and 0.09±0.00 µmol/kg ww of HHE. The contents of MDA and HHE measured in herring protein isolates produced by the alkaline pH-shift processing were 195.01±15.44 and 1.55±0.07 µmol/kg ww, respectively, which were significantly ($p<0.05$) higher than the MDA and HHE levels measured in the alkali-produced salmon protein isolate (41.21±6.91 µmol/kg ww of MDA and 0.87±0.15 µmol/kg ww of HHE), indicating that herring heads and backbones were more prone to lipid oxidation during pH-shift processing than salmon heads. This could be explained both by the presence of astaxanthin in salmon muscle (Abdollahi et al., 2020), and by the higher hemoglobin (Hb) content of herring heads and backbones (270.61±0.87 µmol/kg dw) compared to salmon heads (144.35±1.77 µmol/kg dw). Hb is a strong pro-oxidant which according to several mechanisms such as the decomposition of lipid hydroperoxides into free radicals which can stimulate lipid oxidation. Our previous study (Abdollahi et al., 2020) reported similar results as were found here when comparing peroxide value (PV) and aldehydes (MDA and HHE) in minced herring and salmon co-products (heads plus backbones) to their corresponding protein isolates recovered by alkaline or acid pH-shift processing. However, in that study, no significant increase in MDA level was found during pH-shift processing

of salmon co-products, which may be due to the lower Hb content in the used heads and backbones (84.99±1.01 µmol/kg, dw) compared to separated salmon heads used here (144.35±1.77 µmol/kg, dw); not least the gills are a large source of blood. In addition, the higher starting level of oxidation products in the salmon heads used here (7.65±0.05 µmol/kg ww of MDA and 0.09±0.00 µmol/kg ww of HHE) than in the heads plus backbones used in our previous study (5.72 µmol/kg ww of MDA and 0.07 µmol/kg ww of HHE) (Abdollahi et al., 2020) could have stimulated more oxidation during the process.

The major increases in MDA and HHE during pH-shift processing of herring and salmon co-products in this study could be related to several different features of the pH-shift process. Firstly, endogenous aqueous antioxidants such as ascorbic acid are diluted into the water added at the start of the processing, something which we earlier documented as critical for lipid oxidation (Harrysson et al., 2020). Secondly, the high-speed homogenization applied to the raw materials may disrupt the highly organized fish muscle microstructure and remove the surrounding connective tissues (endomysium), leading to better exposure of membranal phospholipids to pro-oxidants as Hb, stimulating lipid oxidation (Marmon, Krona, Langton, & Undeland, 2012). Third, α-tocopherol is partly removed along with the removal of membranes during the first centrifugation step (Wu, Abdollahi, & Undeland, 2021). Fourth, and maybe most important, the low pH (5.5) used during the protein precipitation step could induce Hb-deoxygenation and metHb-

formation that increases the prooxidative activity of Hb via heme group exposure and hemin loss, respectively (Kristinsson & Hultin, 2004; Undeland, Kristinsson, & Hultin, 2004). Also, it has been found that sarcoplasmic proteins like Hb can co-precipitate with myofibrils and/or membranes at pH 5.5, creating close proximity between Hb/hemin and the phospholipids (Abdollahi et al., 2016).

During the ice storage, as shown in Fig. 1A, the MDA content in protein isolates recovered from herring co-products declined in the beginning and then stabilized from day 5 on an MDA level of around 115 μmol/kg ww. Opposite, the MDA content of salmon protein isolate initially increased and had achieved its maximum (109.44±8.63 μmol/kg ww) on day 2. It then stayed steady until day 8 before the MDA levels decreased slowly. As presented in Fig. 1B, the HHE content in herring protein isolates showed a maximum level on day 2 (4.90±0.71 μmol/kg ww) whereafter it continuously decreased. For salmon protein isolates, the level of HHE increased from the start of the storage and reached its maximum of 2.18±0.09 μmol/kg ww on day 6 after which it decreased with some fluctuations. That HHE peaked during the storage of alkali-produced herring protein isolates, but already during the protein isolate production for MDA, reveal that either the conditions for HHE-formation were more favorable during the storage than during processing, or HHE formation simply have slower kinetics. It can here be stressed that while MDA derives from any PUFA, HHE derives from n-3 PUFA alone (Tullberg et al., 2016). Fig. 1A and B also illustrate that less MDA was formed during the acid than alkaline pH-shift processing, which was in agreement with the findings of Abdollahi et al. (2020). For both MDA and HHE contents, the acid version also postponed the storage-induced development and rendered lower maximum levels. We previously discussed how this could be due to potential membrane aggregation at extremely acidic pHs, i.e. ≤ pH 3.5 (Pazos, Medina, & Hultin, 2005), retarding oxidation, despite activation of Hb as a prooxidant at slightly acidic pH's (i.e. pH 5.7–7) (Pazos et al., 2005; Undeland et al., 2004); a range that is passed in acid and alkaline processing. Fig. 1 A and B further reveal that there was a remarkable antioxidant capacity of lingonberry press-cake when integrated both in the alkaline and acid pH-shift process versions and both with herring and salmon co-products. In isolates from herring co-products, maximum MDA and HHE levels were reduced to 4.90–6.83 and 0.12–0.28 μmol/kg ww, respectively, and in salmon isolates to 8.87±0.29 and 0.14±0.01 μmol/kg ww, respectively.

Fig. 1 C and D show the levels of MDA and HHE in alkali-produced herring protein isolates, with and without adding helpers. Clearly, lingonberry press-cake had the best effect in preventing lipid oxidation both during pH-shift processing and during ice storage. Compared to the others, the protein isolate produced with lingonberry press-cake also presented the highest total phenolic content (TPC) (827.98±16.07 mg GAE/100 g protein isolate dw), which was significantly (p<0.05) higher than the control (224.62±6.92 mg GAE/100 g dw), and higher than all other alkali-produced protein isolates produced with the other six helpers. A remarkable antioxidant capacity of dried lingonberry press-cake generated from industrial juice production was recently also seen when added directly to minced herring fillets without skin (3%,w/w) (Damerau et al., 2020). In that study, the lingonberry press-cake considerably prevented the formation of PV and volatile oxidation products during 10-month frozen storage. The excellent antioxidant capacity of lingonberry press-cake is most likely attributed to its abundance of proanthocyanidins, hydroxycinnamic acid derivatives (HCA), flavonols, and arbutin derivatives in leaves, as well as anthocyanins and benzoic acid (e.g. benzoyl glucose) in the fruits (Bujor et al., 2018). The anthocyanins profile of lingonberry press-cake, which had a berry to leaf ratio of about 91:9 (ww), was analyzed and attached in **supplementary Table 5.** Since these phenolic compounds are water-soluble, they were easily extracted from lingonberry press-cake during the pH-shift process, and partitioned into the final isolate in aqueous form or as a precipitate, thereby limiting lipid oxidation both during the actual processing and during the extended storage on ice.

In addition to lingonberry press-cake, apple pomace, oat fiber residues, barley-spent grain, *Saccharina,* and *Ulva* also showed antioxidant capacity during alkali-aided pH-shift processing of herring co-products, which was reflected in significantly (p<0.05) lower levels of MDA and HHE in the freshly produced protein isolates compared to control. Before storage, MDA levels increased in the order: lingonberry press-cake (3.47±0.03 μmol/kg) < apple pomace (29.09±2.80 μmol/kg) < barley-spent grain (47.98±0.34 μmol/kg) < *Saccharina* (61.96±2.65 μmol/kg) < oat fiber residues (105.42±2.53 μmol/kg) < *Ulva* (124.25±4.81 μmol/kg) (Fig. 1C). HHE ranked the samples in the same order, although the protein isolates produced with oat fiber residues and with *Ulva* had similar levels around 1.6 μmol/kg (Fig. 1D). During ice storage, aldehyde levels in protein isolates cross-processed with the mentioned five kinds of helpers, apart from oat, initially increased, and then declined. The maximum MDA levels of the protein isolates produced with barley-spent grain, oat fiber residues and *Saccharina* were similar, which were around 216 μmol/kg ww, although the maxima were observed on different days: with barley-spent grain on day 2, with *Saccharina* on day 3, while with oat fiber residues on day 4, which implied a different ability in delaying the formation of MDA during ice storage with oat fiber residues being most antioxidative. Furthermore, apple pomace and *Ulva* significantly reduced (p<0.05) the maximum level of MDA to 137.88±8.50 and 189.58±9.33 μmol/kg ww, respectively, indicating that apple pomace showed the highest ability to prevent the formation of MDA among the five helpers mentioned. The results of HHE (Fig. 1D) rendered similar conclusions but with a few differences. Firstly, the addition of barley-spent grain and oat fiber residues hardly reduced the maximum of HHE content but delayed its formation during the ice storage compared to the control. The latter achieved a maximum HHE value of 4.90±0.71 μmol/kg ww on day 2 while processing with barley-spent grain and oat fiber residues gave maximum HHE-levels on day 5 at around 5 μmol/kg ww. When adding apple pomace, *Saccharina* and *Ulva*, the maximum level of HHE was significantly reduced (p<0.05) to 2.46±0.10, 2.43±0.01, and 3.32±0.42 μmol/kg protein isolates, respectively. Although the five helpers discussed here are all rich in natural antioxidants, none of them thus showed the same strong antioxidant capacity as lingonberry press-cake did. The reasons for this could vary. Apple pomace was hypothesized to have good antioxidant potential because of its abundance in phenolic compounds including tannins, phlorizin, chlorogenic acid, epicatechin, and quercetin glycosides (Sun et al., 2017). Also, the antioxidant effects of apple and apple side streams in fish have been reported in several studies. Bitalebi et al. (2019) successfully mixed apple peel water extracts obtained by ultrasound-assisted extraction into minced rainbow trout and thereby retarded both lipid oxidation (PV and TBARS values), protein oxidation (protein carbonyls) and total sulfhydryl group losses during 96 h of refrigerated storage. Sun et al. (2017) found that adding polyphenols extracted from young apples by microporous resins with ethanol successfully retarded lipid oxidation of grass carp surimi during 7 days of refrigerated storage, which was reflected by the reduced PV and TBARS values; chlorogenic acid was shown to be the primary preservative component (Sun et al., 2017). In our study, the feature limiting the antioxidant ability of apple pomace could be related to the inefficient extraction of its phenolic compounds by water/alkali/acid, as are used in the pH-shift process. TPC measured in the protein isolate produced with apple pomace was 338.82±18.90 mg GAE/100 g protein isolate dw, which was significantly (p<0.05) lower compared to the one produced with lingonberry press-cake (827.98±16.07 mg GAE/100 g dw). For protein isolates produced with the addition of barley and oat co-products, TPC contents were even lower, 252.61±13.96 and 263.47±18.86 mg GAE/100 g dw, respectively, being in line with the substantially lower antioxidative potential of these helpers compared with apple pomace and lingonberry press-cake. Chemical and physical pretreatments could likely be applied to these helpers to pursue higher antioxidant capacity given that the homogenization during pH-shift processing was not enough to disintegrate e.g. plant cell walls and

release antioxidants. Such treatments have been applied prior to the extraction of aqueous phenolic compounds from barley and oat side-streams with examples being enzymatical hydrolysis (Ratnasari et al., 2017), maceration, ultrasound and microwave treatments (Birsan et al., 2019). The current absence of such methods and/or the less efficient pH-shift-driven extraction of antioxidants from apple, oat and barley co-products compared to those from lingonberry may have resulted in natural antioxidants being discarded into the pellets obtained from the first centrifugation step. Different ratios of polar to non-polar phenolic/non-phenolic antioxidants in the different agricultural helpers most likely also played a role in their activity, given that the pH-shift system is mainly aqueous, apart from the presence of fish lipids, which can also act as extractants. It can also not be excluded that the profile of antioxidants in apple, oat and barley co-products were less efficient towards the largely Hb-mediated oxidation during pH-shift processing of fish co-products (Abdollahi et al., 2016); something which is now subject of a separate study.

As for the seaweeds, *Saccharina is* known to contain significant levels of polyphenols e.g., phlorotannins, and fucoxanthin as the main carotenoid (Roohinejad et al., 2017). Brown seaweed phlorotannins have been shown to scavenge free radicals, peroxyl and nitric radicals, as well as to chelate ferrous ions *in vitro* (Roohinejad et al., 2017); the latter is however not a major pro-oxidant in fish where most iron is heme-bound (Wu et al., 2021 submitted) (Maestre, Pazos, & Medina, 2011). Regarding *Ulva,* its sulfated polysaccharides were reported to present good DPPH·, ABTS·+, hydroxyl and superoxide radical scavenging ability, as well as ferrous ion chelating ability *in vitro* (Peasura et al., 2015; Qi et al., 2005). However, similar as for the apple, oat and barley co-products, physical barriers due to complex cell wall polysaccharides may have limited the extraction efficiency of these active compounds from seaweeds. Compared to the cell walls of land-based plants, seaweed cell walls and cuticles are chemically and structurally more complex and heterogeneous, not least due to polysaccharides as alginates and carrageenan polysaccharides (Roohinejad et al., 2017). In addition, the high contents of salts and minerals in seaweeds likely acted as pro-oxidants in the fish/water/alkali system (Roohinejad et al., 2017), counteracting the potential antioxidant ability of seaweeds. When we recently added a pressed juice from *Saccharina latissima* in minced trout muscle, a net pro-oxidative effect on TBARS/PV-formation during ice storage was seen (Harrysson et al., non-published). The TPC of isolates produced with the addition of *Saccharina* and *Ulva* were 219.65 ± 3.28 and 179.64 ± 8.28 mg GAE/100 g dw, respectively, which were surprisingly lower than control isolates. This could reflect a very polymerized nature of the seaweed polyphenols, giving a relatively lower response with the Folin-Ciocalteu reagent than monomers/dimers, and/or, development of crosslinks with amino acids of the protein isolate, also lowering its reaction with the reagent.

The addition of shrimp shells to herring co-products during alkaline pH-shift processing did not prevent lipid oxidation during processing; rather, as for the control, MDA appeared to peak the actual processing step and then continuously decreased during the ice storage (Fig. 1D). These kinetics was also seen in our previous study on oxidation during acid pH-shift processing of herring fillets (Undeland et al., 2005). Declines in secondary oxidation products are something we have also recorded in many other ice storage studies with e.g. minced herring co-products (Wu et al., 2020); or Hb-fortified washed cod mince (Sánchez-Alonso et al., 2007; Undeland, Hultin, & Richards, 2002), and we hypothesize that it is due to the high reactivity of many carbonyls with proteins and phospholipids, forming e.g. Schiff bases (Domingues et al., 2013). The hypothesis behind adding shrimp shells to herring co-products was its abundance of astaxanthin and the knowledge that 50.6 ± 0.30 % of astaxanthin from shrimp peeling residues was earlier shown to be extracted into a protein isolate produced using the alkali-aided pH-shift method (Khumallambam et al., 2011). Exact solubilization and precipitation pH's used in that study were however different from our study; pH 10.0 and pH 4.5, respectively. Even if astaxanthin is a

lipid-soluble colorant, significant amounts are bound to proteins (Khumallambam et al., 2011). That the addition of shrimp shells gave no significant increase in the protein content of the final protein isolates revealed a poor extraction efficiency yield of shrimp proteins/shrimp protein-astaxanthin complexes. Thus, some of the astaxanthin bound to non-soluble shrimp proteins was most likely discarded along with the sediment from the first centrifugation. This was also visible from a stronger pink-red color of the first sediment than the final protein isolate when produced with shrimp shells compared to controls. Based on color, some of the astaxanthin also went into the floating emulsion layer, which was here discarded. It is however possible to break this emulsion and recover a cold-pressed oil, in this case, fortified with astaxanthin.

3.3. Color of protein isolates

3.3.1. Initial colors of protein isolates

Salmon and herring co-products provided different colors of the protein isolates. As shown in Fig. 2 **A-C**, salmon-derived protein isolates showed significantly higher ($p<0.05$) L^*-, a^*-, and b^*-values than those from herring, which was primarily attributed to the presence of astaxanthin in the residual muscle of salmon heads. However, a higher level of lipid oxidation products from start in herring protein isolates most likely also corresponded with higher levels of met-Hb, or even heme-ring destruction, and thus lower a^*-values, something which has been documented in numerous studies (Cai, Grunwald, Park, Lei, & Richards, 2013; Wetterskog & Undeland, 2004). Fig. 2 **A-C** further shows the color differences when applying the alkaline or acid version to herring co-products without helpers. Acid-produced protein isolates had higher initial a^*-values compared to alkali-produced ones, which could be explained by a better removal of heme-proteins during alkaline than acid pH-shift processing (Abdollahi et al., 2016). Fig. 2**A-C** also show that the alkali-produced herring protein isolates made with lingonberry press cake had lower L^*-values than their acid-produced counterparts, which could be explained by oxidation of phenolic compounds into quinones under alkaline conditions with subsequent transformation into dark/browning compounds (Zhao et al., 2020).

Fig. 2 **D-F** presents colors of all alkali-produced herring protein isolates with and without helpers and in Fig. 3, their visual appearances are shown. The color changes of protein isolates due to the addition of helper were quantified by the ΔE_{2000} values, which were calculated from L^*-, a^*- and b^*-values. The addition of lingonberry press-cake led to the largest changes ($\Delta E_{2000}=8.99$), followed by *Ulva* ($\Delta E_{2000}=6.85$), barley-spent grain ($\Delta E_{2000}=4.19$), *Saccharina* ($\Delta E_{2000}=3.65$), oat fiber residues ($\Delta E_{2000}=3.34$) and shrimp shells ($\Delta E_{2000}=3.01$). The addition of apple pomace rendered the least changes ($\Delta E_{2000}=2.35$). The color changes of protein isolates with the addition of helpers were expected because of the co-extraction of pigments together with proteins as well as the likely alkali-induced oxidation of phenolic compounds mentioned above (Zhao et al., 2020). For example, lingonberry press-cake brought anthocyanins to the system, which can change color from reddish to bluish when going from low pH (1–3) to higher pH (6–8), resulting in a protein isolate varying in color at different pHs. Overall protein isolates made with lingonberry press-cake were very dark, reflected by reduced L^*-, and their neutral pH's explain the b^*-reduction. The addition of *Ulva* rendered a green color, i.e., reduced a^*-value, which was attributed to the presence of chlorophyll (Martins et al., 2021). A parallel increased b^*-value could be explained by the presence of xanthophyll (Martins et al., 2021). *Saccharina latissima* has fucoxanthin as the major pigment (Roohinejad et al., 2017) and therefore rendered a more yellow color reflected by a higher b^*-value. Addition of shrimp shells led to a slightly more pinkish-red color explained by a small extraction of astaxanthin. The apple pomace used in this study consisted of reddish-green apple peels, seeds and a little flesh. Delgado-Pelayo et al. (2014) reported that green apples with red areas were abundant in carotenoids (orange-yellow) but with a limited amount of chlorophylls (green) (Delgado-Pelayo et al., 2014), which could explain the increased a^*- and b^*-values of
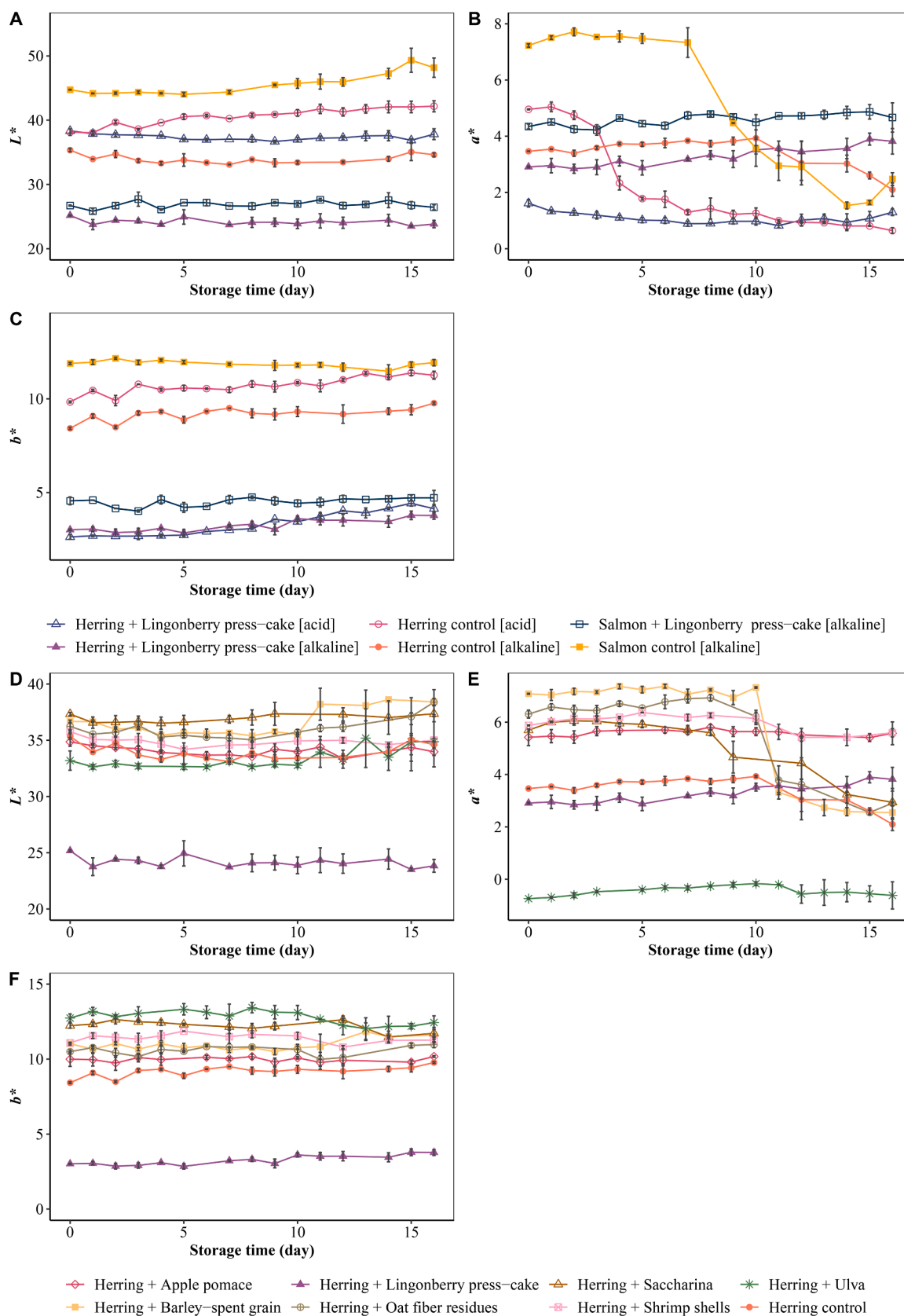
Fig. 2. L^*-, a^*- and b^*- values measured in protein isolates during extended storage on ice. Panels A, B and C compare the isolates produced from herring and salmon co-products by the alkaline or acid version of pH-shift process, in the absence or presence of lingonberry press-cake; panels D, E and F compare the herring protein isolates produced using the alkaline version of pH-shift process in the presence of all different helpers.

Fig. 3. Appearance of protein isolates produced from herring co-products in the absence and presence of helpers by the alkaline version of pH-shift process.

protein isolate with the addition of apple pomace in our study. For the protein isolates produced with barley-spent grain and oat fiber residues, carotenoids were thought to be responsible for the more orange-yellow color.

3.3.2. Color changes of protein isolates during ice storage

As shown in Fig. 2 **A-C**, the L^*-values of salmon-derived alkali-produced protein isolates significantly increased ($p<0.05$) while a^*-values significantly declined ($p<0.05$) after 7 days of storage. This was in agreement with the findings of Sathivel (2005) and Harrysson et al. (2020), and is ascribed astaxanthin bleaching as well as the loss of oxy-Hb in favor of the brownish-grey met-Hb, which both can occur along with lipid oxidation (Belitz, Grosch, & Schieberle, 2004; Wetterskog & Undeland, 2004). For alkali-produced protein isolates from herring co-products, decreased a^*-values were seen after 10 days of storage along with slightly increased b^*-values. The former did not correlate with the immediate and early MDA/HHE-formation and are thus hard to explain. The increases in b^*-values most likely reflected the formation of tertiary lipid oxidation products (e.g. Schiff bases), which can further polymerize into yellow pigments (Zamora & Hidalgo, 2005). Fig. 2 **A-C** also illustrates differences in color changes between acid- and alkali-produced herring protein isolates. Acid-produced isolates showed slight increases in L^*- and b^*-values, and from day 2–5, significant decreases ($p<0.05$) in a^*-values which strongly correlated with the onset of MDA/HHE-formation. With the addition of lingonberry press-cake, the protein isolates produced by both acid and alkaline versions showed no significant storage-induced changes in L^*- and a^*-values but presented slightly increased b^*-values. The latter appeared non-connected to lipid oxidation.

As shown in Fig. 2 **D-F**, protein isolates produced with barley-spent grain and oat fiber residues remarkably lost a^*-values during ice storage from day 10 along with slightly increased L^*-values; the former also showed increased b^*-values. Slightly decreased a^*-values were also found in the protein isolates produced with *Saccharina* and shrimp shells, from day 8 and day 10, respectively. As for alkali-produced herring isolates, a^*-value losses in these samples did however not follow lipid oxidation development, and appear linked to other phenomena, as co-oxidation/bleaching of helper-derived pigments (e.g. carotenoids). For the protein isolate produced with lingonberry press-cake, slightly increased a^*- and b^*-values were observed during ice storage.

4. Conclusions

The pH-shift process efficiently concentrated proteins from herring and salmon co-products, with and without adding helpers. Its ability to remove lipids and ash was dependent on the combination of raw materials and the version of pH-shift-processing; alkaline or acid. For example, alkaline solubilization as well as presence of *Saccharina* and *Ulva* stimulated significant lipid removal from the herring co-products, while acid solubilization as well as presence of lingonberry press-cake stimulated significant ash removal from the salmon co-products. MDA and HHE levels revealed that all helpers, except shrimp shells, retarded lipid oxidation both during cross-processing and subsequent ice storage, although to different extents. Lingonberry press-cake presented the best antioxidant ability, and limited the formation of MDA and HHE to a very low level throughout the whole storage, followed by apple pomace and *Ulva* which reduced the maximum levels of MDA and HHE. Cross-processing with *Saccharina* reduced the maximum level of HHE, and barley-spent grain and oat fiber residues delayed the production of both MDA and HHE. For isolates produced with agricultural helpers, their level of total phenolics followed the oxidative stability. Indeed, the addition of helpers also changed the color of protein isolates. This was most evident with lingonberry press-cake and *Ulva fenestrata* which rendered dark purple and green isolates, respectively, while isolates produced with apple pomace, barley-spent grain, oat fiber residues, shrimp shells and *Saccharina* turned more yellow. During ice storage, a^*-values decreased in several isolates, which for the alkali-produced salmon control and acid-produced herring control was ascribed co-oxidation of heme-proteins and astaxanthin along with lipid oxidation progression. Small increases in b^*-values were seen in alkali/acid-produced herring controls, and in herring isolates with barley-spent grain, most likely reflecting tentative Shiff-base polymerization.

In summary, the results of this study showed clear beneficial effects from cross-processing fish co-products with antioxidant-rich plant-based co-products or seaweed to minimize lipid oxidation both during and after pH-shift processing in a clean-label manner. This creates a basis for new types of industrial symbiosis where side streams are value-added according to a new concept. The promising results from using lingonberry press-cake will be subject to further studies e.g. on techno-functional properties of the produced protein isolates. The new colors obtained during cross-processing may be an advantage or disadvantage depending on the final product to be produced. However, given the large focus on various pigmented plant-based protein sources in recent years, it is expected that consumer acceptance of color is increasing along with increased awareness of sustainable eating habits.

CRediT authorship contribution statement

Jingnan Zhang: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Mehdi Abdollahi:** Writing – review & editing. **Marie Alminger:** Resources, Writing – review & editing. **Ingrid Undeland:** Conceptualization, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132314.

References

Abdollahi, M., Marmon, S., Chaijan, M., & Undeland, I. (2016). Tuning the pH-shift protein-isolation method for maximum hemoglobin-removal from blood rich fish muscle. *Food Chemistry, 212*, 213–224. https://doi.org/10.1016/j.foodchem.2016.05.165

Abdollahi, M., Olofsson, E., Zhang, J., Alminger, M., & Undeland, I. (2020). Minimizing lipid oxidation during pH-shift processing of fish by-products by cross-processing with lingonberry press cake, shrimp shells or brown seaweed. *Food Chemistry, 327*, Article 127078. https://doi.org/10.1016/j.foodchem.2020.127078

Abdollahi, M., Wu, H., & Undeland, I. (2021). Impact of processing technology on macro- and micronutrient profile of protein-enriched products from fish backbones. *Foods, 10*(5), 950. https://doi.org/10.3390/foods10050950

Angell, A. R., Mata, L., de Nys, R., & Paul, N. A. (2015). The protein content of seaweeds: A universal nitrogen-to-protein conversion factor of five. *Journal of Applied Phycology, 28*(1), 511–524. https://doi.org/10.1007/S10811-015-0650-1

Belitz, H.-D., Grosch, W., & Schieberle, P. (2004). Meat. In. *Food Chemistry.*. https://doi.org/10.1007/978-3-662-07279-0_13

Birsan, R. I., Wilde, P., Waldron, K. W., & Rai, D. K. (2019). Recovery of Polyphenols from Brewer's Spent Grains. *Antioxidants, 8*(9), 380. https://doi.org/10.3390/antiox8090380

Bitalebi, S., Nikoo, M., Rahmanifarah, K., Noori, F., & Ahmadi Gavlighi, H. (2019). Effect of apple peel extract as natural antioxidant on lipid and protein oxidation of rainbow trout (Oncorhynchus mykiss) mince. *International Aquatic Research, 11*(2), 135–146. https://doi.org/10.1007/s40071-019-0224-y

Bourre, J. M., & Paquotte, P. (2008). Seafood (wild and farmed) for the elderly: Contribution to the dietary intakes of iodine, selenium, DHA and vitamins B12 and D. *Journal of Nutrition, Health and Aging, 12*(3), 186–192. https://doi.org/10.1007/BF02982617

Bujor, O. C., Ginies, C., Popa, V. I., & Dufour, C. (2018). Phenolic compounds and antioxidant activity of lingonberry (Vaccinium vitis-idaea L.) leaf, stem and fruit at different harvest periods. *Food Chemistry, 252*, 356–365. https://doi.org/10.1016/j.foodchem.2018.01.052

Cai, H., Grunwald, E. W., Park, S. Y., Lei, B., & Richards, M. P. (2013). Lipid oxidation in trout muscle is strongly inhibited by a protein that specifically binds hemin released from hemoglobin. *Journal of Agricultural and Food Chemistry, 61*(17), 4180–4187. https://doi.org/10.1021/jf4006142

Czubinski, J., & Dwiecki, K. (2017). A review of methods used for investigation of protein-phenolic compound interactions. *International Journal of Food Science & Technology, 52*(3), 573–585. https://doi.org/10.1111/ijfs.13339

Damerau, A., Kakko, T., Tian, Y., Tuomasjukka, S., Sandell, M., Hopia, A., & Yang, B. (2020). Effect of supercritical CO2 plant extract and berry press cakes on stability and consumer acceptance of frozen Baltic herring (Clupea harengus membras) mince. *Food Chemistry, 332*, Article 127385. https://doi.org/10.1016/j.foodchem.2020.127385

Delgado-Pelayo, R., Gallardo-Guerrero, L., & Hornero-Méndez, D. (2014). Chlorophyll and carotenoid pigments in the peel and flesh of commercial apple fruit varieties. *Food Research International, 65*(PB), 272–281. https://doi.org/10.1016/j.foodres.2014.03.025

Dhouafli, Z., Cuanalo-Contreras, K., Hayouni, E. A., Mays, C. E., Soto, C., & Moreno-Gonzalez, I. (2018). Inhibition of protein misfolding and aggregation by natural phenolic compounds. *Cellular and Molecular Life Sciences, 75*, 3521–3538. https://doi.org/10.1007/s00018-018-2872-2

Domingues, R. M., Domingues, P., Melo, T., Pérez-Sala, D., Reis, A., & Spickett, C. M. (2013). Lipoxidation adducts with peptides and proteins: Deleterious modifications or signaling mechanisms? *Journal of Proteomics, 92*, 110–131. https://doi.org/10.1016/j.jprot.2013.06.004

FAO. (2020). The State of World Fisheries and Aquaculture 2020. *In The State of World Fisheries and Aquaculture, 2020*. https://doi.org/10.4060/ca9229en

Foh, M. B. K., Xia, W., Amadou, I., & Jiang, Q. (2012). Influence of pH Shift on Functional Properties of Protein Isolated of Tilapia (Oreochromis niloticus) Muscles and of Soy Protein Isolate. *Food and Bioprocess Technology, 5*(6), 2192–2200. https://doi.org/10.1007/s11947-010-0496-0

Harrysson, H., Swolin, B., Axelsson, M., & Undeland, I. (2020). A trout (Oncorhynchus mykiss) perfusion model approach to elucidate the role of blood removal for lipid oxidation and colour changes in ice-stored fish muscle. *International Journal of Food Science and Technology, 55*(6), 2462–2471. https://doi.org/10.1111/ijfs.14497

Hultin, H.O., & Kelleher, S.D. (2000). Patent No. US6136959.

Khumallambam, M., Rama, S., Karuppannan, R. R., & Manjabhat, S. N. (2011). Antioxidant activity of protein isolate obtained by the pH shift technique from hydrolyzed and unhydrolyzed shrimp processing discards. *Journal of Aquatic Food Product Technology, 20*(2), 209–221. https://doi.org/10.1080/10498850.2011.559618

Kristinsson, H. G., & Hultin, H. O. (2004). The effect of acid and alkali unfolding and subsequent refolding on the pro-oxidative activity of trout hemoglobin. *Journal of Agricultural and Food Chemistry, 52*(17), 5482–5490. https://doi.org/10.1021/jf035062y

Maestre, R., Pazos, M., & Medina, I. (2011). Role of the raw composition of pelagic fish muscle on the development of lipid oxidation and rancidity during storage. *Journal of Agricultural and Food Chemistry, 59*(11), 6284–6291. https://doi.org/10.1021/jf200686z

Marcó, A., Rubio, R., Compañó, R., & Casals, I. (2002). Comparison of the Kjeldahl method and a combustion method for total nitrogen determination in animal feed. *Talanta, 57*(5), 1019–1026. https://doi.org/10.1016/S0039-9140(02)00136-4

Mariotti, F., Tomé, D., & Mirand, P. P. (2008). Converting nitrogen into protein - Beyond 6.25 and Jones' factors. *Critical Reviews in Food Science and Nutrition, 48*(2), 177–184. https://doi.org/10.1080/10408390701279749

Markwell, M. A. K., Haas, S. M., Bieber, L. L., & Tolbert, N. E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Analytical Biochemistry, 87*(1), 206–210. https://doi.org/10.1016/0003-2697(78)90586-9

Marmon, S. K., Krona, A., Langton, M., & Undeland, I. (2012). Changes in salt solubility and microstructure of proteins from herring (clupea harengus) after pH-shift processing. *Journal of Agricultural and Food Chemistry, 60*(32), 7965–7972. https://doi.org/10.1021/jf301352s

Marmon, S. K., & Undeland, I. (2010). Protein isolation from gutted herring (Clupea harengus) using pH-shift processes. *Journal of Agricultural and Food Chemistry, 58*(19), 10480–10486. https://doi.org/10.1021/jf101057q

Martins, M., Oliveira, R., Coutinho, J. A. P., Faustino, M. A. F., Neves, M. G. P. M. S., Pinto, D. C. G. A., & Ventura, S. P. M. (2021). Recovery of pigments from Ulva rigida. *Separation and Purification Technology, 255*, Article 117723. https://doi.org/10.1016/j.seppur.2020.117723

Nolsøe, H., & Undeland, I. (2009). The acid and alkaline solubilization process for the isolation of muscle proteins: State of the art. *Food and Bioprocess Technology, 2*(1), 1–27. https://doi.org/10.1007/s11947-008-0088-4

Oliveira, A., Gomes, M. H., Alexandre, E. M. C., Almeida, D. P. F., & Pintado, M. (2014). Impact of pH on the phytochemical profile of pasteurized peach purée during Storage. *Journal of Agricultural and Food Chemistry, 62*(50), 12075–12081. https://doi.org/10.1021/jf503913t

Özyurt, G., Şimşek, A., Karakaya, B. T., Aksun, E. T., & Yeşilsu, A. F. (2015). Functional, Physicochemical and Nutritional Properties of Protein from Klunzinger's Ponyfish Extracted by the pH Shifting Method. *Journal of Food Processing and Preservation, 39*(6), 1934–1943. https://doi.org/10.1111/jfpp.12432

Pazos, M., Medina, I., & Hultin, H. O. (2005). Effect of pH on hemoglobin-catalyzed lipid oxidation in cod muscle membranes in vitro and in situ. *Journal of Agricultural and Food Chemistry, 53*(9), 3605–3612. https://doi.org/10.1021/jf0403890

Peasura, N., Laohakunjit, N., Kerdchoechuen, O., & Wanlapa, S. (2015). Characteristics and antioxidant of Ulva intestinalis sulphated polysaccharides extracted with different solvents. *International Journal of Biological Macromolecules, 81*, 912–919. https://doi.org/10.1016/j.ijbiomac.2015.09.030

Qi, H., Zhao, T., Zhang, Q., Li, Z., Zhao, Z., & Xing, R. (2005). Antioxidant activity of different molecular weight sulfated polysaccharides from Ulva pertusa Kjellm (Chlorophyta). *Journal of Applied Phycology, 17*(6), 527–534. https://doi.org/10.1007/s10811-005-9003-9

Ratnasari, N., Walters, M., & Tsopmo, A. (2017). Antioxidant and lipoxygenase activities of polyphenol extracts from oat brans treated with polysaccharide degrading enzymes. *Heliyon, 3*(7). https://doi.org/10.1016/j.heliyon.2017.e00351

Rødde, R. H., Einbu, A., & Vårum, K. M. (2008). A seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp (Pandalus borealis). *Carbohydrate Polymers, 71*(3), 388–393. https://doi.org/10.1016/j.carbpol.2007.06.006

Roohinejad, S., Koubaa, M., Barba, F. J., Saljoughian, S., Amid, M., & Greiner, R. (2017). Application of seaweeds to develop new food products with enhanced shelf-life, quality and health-related beneficial properties. *Food Research International, 99*, 1066–1083. https://doi.org/10.1016/j.foodres.2016.08.016

Sánchez-Alonso, I., Borderías, J., Larsson, K., & Undeland, I. (2007). Inhibition of hemoglobin-mediated oxidation of regular and lipid-fortified washed cod mince by a white grape dietary fiber. *Journal of Agricultural and Food Chemistry, 55*(13), 5299–5305. https://doi.org/10.1021/jf0702242

Sathivel, S. (2005). Chitosan and protein coatings affect yield, moisture loss, and lipid oxidation of pink salmon (Oncorhynchus gorbuscha) fillets during frozen storage. *Journal of Food Science, 70*(8), e455–e459. https://doi.org/10.1111/j.1365-2621.2005.tb11514.x

Sharma, G., Wu, W., & Dalal, E. N. (2005). The CIEDE2000 color-difference formula: Implementation notes, supplementary test data, and mathematical observations. *Color Research and Application, 30*(1), 21–30. https://doi.org/10.1002/col.20070

Soares, S., Mateus, N., & De Freitas, V. (2012). Carbohydrates inhibit salivary proteins precipitation by condensed tannins. *Journal of Agricultural and Food Chemistry, 60*(15), 3966–3972. https://doi.org/10.1021/jf3002747

Sun, L., Sun, J., Thavaraj, P., Yang, X., & Guo, Y. (2017). Effects of thinned young apple polyphenols on the quality of grass carp (Ctenopharyngodon idellus) surimi during cold storage. *Food Chemistry, 224*, 372–381. https://doi.org/10.1016/j.foodchem.2016.12.097

Tullberg, C., Larsson, K., Carlsson, N. G., Comi, I., Scheers, N., Vegarud, G., & Undeland, I. (2016). Formation of reactive aldehydes (MDA, HHE, HNE) during the digestion of cod liver oil: Comparison of human and porcine in vitro digestion models. *Food and Function, 7*(3), 1401–1412. https://doi.org/10.1039/c5fo01332a

Undeland, I., Hall, G., Wendin, K., Gangby, I., Rutgersson, A., Undeland, I., … Rutgersson, A. (2005). Preventing lipid oxidation during recovery of functional proteins from herring (Clupea harengus) fillets by an acid solubilization process. *Journal of Agricultural and Food Chemistry, 53*(14), 5625–5634. https://doi.org/10.1021/jf0404445

Undeland, I., Hultin, H. H. O., & Richards, M. M. P. (2002). Added triacylglycerols do not hasten hemoglobin-mediated lipid oxidation in washed minced cod muscle. *Journal of Agricultural and Food Chemistry, 50*(23), 6847–6853. https://doi.org/10.1021/jf0201982

Undeland, I., Kristinsson, H. G., & Hultin, H. O. (2004). Hemoglobin-mediated oxidation of washed minced cod muscle phospholipids: Effect of pH and hemoglobin source.

Journal of Agricultural and Food Chemistry, 52(14), 4444–4451. https://doi.org/10.1021/jf030560s

Vareltzis, P., & Undeland, I. (2008). Removal of lipids and diarrhetic shellfish poisoning toxins from blue mussels (Mytilus edulis) during acid and alkaline isolation of proteins. *Journal of Agricultural and Food Chemistry, 56*(10), 3675–3681. https://doi.org/10.1021/jf800224n

Wetterskog, D., & Undeland, I. (2004). Loss of redness (a*) as a tool to follow hemoglobin-mediated lipid oxidation in washed cod mince. *Journal of Agricultural and Food Chemistry, 52*(24), 7214–7221. https://doi.org/10.1021/jf0307907

Wu, H., Abdollahi, M., & Undeland, I. (2021). Effect of recovery technique, antioxidant addition and compositional features on lipid oxidation in protein enriched products from cod- salmon and herring backbones. *Food Chemistry, 360*, Article 129973. https://doi.org/10.1016/j.foodchem.2021.129973

Wu, H., Ghirmai, S., & Undeland, I. (2020). Stabilization of herring (Clupea harengus) by-products against lipid oxidation by rinsing and incubation with antioxidant solutions. *Food Chemistry, 316*. https://doi.org/10.1016/j.foodchem.2020.126337

Zamora, R., & Hidalgo, F. J. (2005). Coordinate contribution of lipid oxidation and Maillard reaction to the nonenzymatic food browning. *Critical Reviews in Food Science and Nutrition, 45*, 49–59. https://doi.org/10.1080/10408690590900117

Zhang, J., Ström, A., Bordes, R., Alminger, M., Undeland, I., & Abdollahi, M. (2022). High shear homogenization and ultrasound assisted cross-processing of herring co-products for maximum protein yield and functionality. *In Manuscript*.

Zhao, Y., Huang, Z. H., Zhou, H. M., Zhu, K. X., Guo, X. N., & Peng, W. (2020). Polyphenol oxidase browning in the formation of dark spots on fresh wet noodle sheets: How dark spots formed. *Food Chemistry, 329*. https://doi.org/10.1016/j.foodchem.2020.126800