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Effect of recovery technique, antioxidant addition and compositional features on lipid oxidation in protein enriched products from cod- salmon and herring backbones

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ABSTRACT

The influence of recovery technique (pH-shift processing vs mechanical separation), antioxidant addition and endogenous factors on lipid oxidation in protein-enriched products from herring, salmon and cod backbones was investigated. Salmon-derived products were very stable during both ice and -20°C storage. Contrary, peroxide value and TBA-reactive substances in herring- and cod-derived products increased rapidly during ice storage, with the pH-shift-produced protein isolates (PI) being most susceptible to oxidation in case of cod. Duralox MANC (0.5%) however largely increased the oxidation lag phase in both PI and mechanically separated meat (MSM); from <1 day to >15 days. At -20°C , mainly the herring products oxidized, and particularly the MSM. Pearson correlation tests showed that endogenous levels of Hb, total Fe, ascorbic acid and α -tocopherol correlated significantly ($p < 0.05$) with lipid oxidation development. Evaluating the role of pre-processing storage indicated that fish co-products should be processed immediately after the filleting process unless antioxidants are added.

1. Introduction

In 2018, fish production worldwide amounted to ca. 179 million tons (FAO, 2020). However, of the catches dedicated to food (87.2%), only about 50–60% ends up on people's plate, resulting in huge amounts of fish co-products being generated every year (Stevens, Newton, Tlustý, & Little, 2018). Co-products from fish-filleting operations are mainly used for the production of low-value bulk products such as silage, fish meal, and oil for subsequent feed production, resulting in low profit for the fish industry (Sajib & Undeland, 2020). However, fish co-products have been reported to be highly nutritious based on abundance of proteins having balanced amino acid profile, high levels of the fat-soluble vitamins A and D, long chained n-3 polyunsaturated fatty acids (LC n-3 PUFA), as well as essential macro- and microminerals (Jayatilakan, Sultana, Radhakrishna, & Bawa, 2012). Improved utilization of these co-products for human consumption could improve both access to, and variability of, seafood products as well as provide greater economic and environmental sustainability in the fisheries sector. With this background, the utilization of fish co-products for food production have gained increasing attention over the past twenty years along with the development of innovative processing techniques (Al Khawli et al., 2019).

Any value-adding operation is however rendered difficult by the fact that fish co-products are usually stored as mixtures of heads, fins, backbones, belly flaps and sometimes also guts. Such mixing may enhance degradation reactions such as oxidation, proteolysis/lipolysis and amine formation as there is a risk that enzymes, lipids, blood and melanin contaminate parts that *per se* are relatively clean (Wu, Sajib, & Undeland, 2021). One such co-product part is the backbone, which basically consists of the spine and inner part of the fillet. Given that it can carry up to 80% pure muscle (Abdollahi et al., 2020), we hereby hypothesize that separating and processing backbones alone increases the chances for a high-quality end product.

Among the various techniques for converting fish co-products to food ingredients, the pH-shift process has been recognized as a promising strategy for functional protein recovery (Undeland, Kelleher, & Hultin, 2002; Abdollahi & Undeland, 2018). However, with raw materials that are rich in hemoglobin (Hb) and PUFA, the pH shift process will pose a risk for lipid oxidation (Abdollahi, Olofsson, Zhang, Alminger, & Undeland, 2020) as it includes some steps (e.g. high-speed homogenization, acid/alkaline treatment, dilution), which could activate Hb as a pro-oxidant (Undeland, Kristinsson, & Hultin, 2004), and change the overall endogenous antioxidant/pro-oxidant/lipid profile of the fish co-

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products (Undeland, Hall, Wendin, Gangby, & Rutgersson, 2005; Abdollahi et al., 2020). For example, Kristinsson and Demir (2003) found that acid- (pH 2.5) or alkali (pH 11)-processed isolates from mackerel (*Scomber scombrus*) fillets were more susceptible to oxidation than conventional surimi during cold storage. Also, it has been reported that protein isolates from Atlantic croaker (*Micropogonias undulatus*) fillets produced with the acid (pH 2.5) pH-shift process version displayed poor oxidative stability during storage at 4 °C and yielded higher oxidation values than the minced raw material (Kristinsson & Liang, 2006). To date, there are, however, no systematic studies comparing how different fish species respond to the pH-shift process from a lipid oxidation perspective, which also includes how endogenous pro- and antioxidants are affected by the dilutions, pH-shifts and separation steps of the process. Further, no studies have addressed pH-shift processing of fish backbones alone, and apart from a few attempts (Undeland et al., 2005; Abdollahi et al., 2020), studies are also scarce on suitable application of antioxidants to stabilize pH-shift produced fish isolates.

The aims of this study were to: (i) investigate how the application of pH-shift processing versus classic meat bone separation to herring, salmon and cod backbones affect lipid oxidation in the resulting protein-enriched ingredients during ice and frozen storage, (ii) evaluate if the oxidative stability of the PI and MSM could be improved by adding exogenous antioxidants, and (iii) clarify which endogenous pro- and antioxidants that are the most relevant to lipid oxidation in the six types of protein-enriched ingredients.

2. Materials and methods

2.1. Preparation of fish co-products

Fresh backbones of salmon (*Salmo salar*) and cod (*Gadus morhua*) were provided by Fisk Idag AB (Gothenburg, Sweden) while herring (*Clupea harengus*) backbones were provided by Sweden Pelagic AB (Ellös, Sweden). Immediately following filleting of the fish, the backbones were covered with ice-filled plastic bags and divided into two batches. The first batch was transported to Fisk Idag AB (Öckerö, Sweden) and subjected to industrial scale mechanical meat-bone separation. The second batch was transported to the marine lab at Chalmers University of Technology within 3 h of post-processing. Upon arrival, backbones were immediately ground using a table top meat grinder (C/E22 N, Minerva Omega group, Italy) equipped with a plate with 4.5 mm holes, and thereafter stirred to complete homogeneity. The mince was then processed by the pH-shift process in order to obtain PIs (see below). In a separate trial, a new batch of herring backbones was sent from Sweden Pelagic AB to Fisk Idag AB. The batch was divided in two parts; one subjected to immediate meat-bone separation, the other subjected to meat-bone separation after 24 h pre-storage on ice.

2.2. pH-shift process and mechanical separation

The mechanical separation of the backbones of the three species was conducted by a Baader 600 meat-bone separator (Baader 600, Baader Fish Processing Machinery, Germany). In this process, remaining meat on the herring, salmon and cod backbones was mechanically separated by squeezing them through a perforated drum with 3 mm (herring) and 5 mm (cod/salmon) hole diameter, respectively. The mechanically separated muscle (MSM) was immediately collected, packaged in plastic zip lock bags and frozen at −80 °C until further studies. The pH-shift method was carried out as earlier described (Abdollahi & Undeland, 2018) and is schematically outlined in Supplementary Fig. 1. In short, ground backbones were homogenized with 6 volumes of cold distilled water where after the pH was adjusted to 11.5. Following separation at 8000×g, the aqueous protein containing supernatant was collected and proteins were precipitated at pH 5.5 followed by dewatering at 8000×g.

2.3. Ice and frozen storage trials

Fresh or pre-stored ground backbones (pre-stored only in the case of herring), MSM, MSM + 0.5% Duralox-MANC 213 (a mixture of rosemary extract, ascorbic acid, tocopherols and citric acid) (Kalsec, Kalamazoo, Mich., UK), PI and PI + 0.5% Duralox-MANC 213 were subjected to ice storage to monitor development of lipid oxidation. The samples were ice stored at the bottom of 250 mL screw-capped Erlenmeyer flasks according to the method of Wu, Ghirmai, and Undeland (2020). A frozen storage trial was also carried out, but here only the MSM and PI from each species was included. Around 10 g of each sample was packed and flattened out to 5 mm thickness in plastic zip lock bags and frozen stored at −20 °C for up to 12 months. The ice- and frozen storage trials were performed on at least two different batches of MSM and PI for each fish species.

2.4. Analysis of lipid oxidation

Lipid oxidation was monitored by peroxide value (PV) and TBA-reactive substances (TBARS). Both of these analyses were carried out as previously described by Sajib and Undeland (2020).

2.5. Analysis of ascorbic acid and α -tocopherol

The levels of ascorbic acid and α -tocopherol in MSM and PI were measured by HPLC based on the methods developed by Lykkesfeldt (2000) and Larsson et al. (2007), respectively.

2.6. Quantification of hemoglobin (Hb), total Fe, and Cu

The Hb content was measured following the acetone-based method originally developed by Hornsey (1956) as described in our recent study (Wu, Sajib, & Undeland, 2021). Total Fe, and Cu were analyzed by flame atomic absorption spectroscopy (AAS) according to the method of Maestre, Pazos, and Medina (2011). The heme-iron content was then calculated with the factor of 0.0882 μ g iron/ μ g heme. The non-heme Fe was calculated as the difference between total iron and heme-iron.

2.7. Analysis of total lipids and fatty acid pattern

Total lipids were extracted using chloroform and methanol and determined gravimetrically according to the method described by Bligh and Dyer (1959). The fatty acid pattern was analyzed on the extracted lipid fraction using gas chromatography and mass spectrometry (GC-MS) following methylation of the fatty acids (Larsson et al., (2007).

2.8. Statistical analysis

All analyses were performed at least on duplicate samples, and the results were reported as mean \pm standard deviation (SD) ($n \geq 2$). Data were subjected to analysis of variance (ANOVA) to detect significant differences between treatments and/or storage points. Comparison of means was carried out by Duncan's multiple range test. Simple correlations between on the one hand the content of endogenous pro-/antioxidants or PUFA and on the other hand lipid oxidation vulnerability were evaluated by Pearson coefficients based on data from all three samples types (backbone, MSM, PI) and three species (herring, cod, salmon). Lipid oxidation vulnerability was determined as the PV and TBARS index, which was defined by the development rate (increase in PV or TBARS/day) for those storage points which had a significantly higher ($p < 0.05$) PV or TBARS values than the zero-time values. Statistical analysis was performed using the SPSS software (IBM SPSS Statistics Version 22, IBM Inc., Chicago, USA). The threshold for significance for all tests was set at $p < 0.05$.

3. Results

3.1. Lipid oxidation

Fig. 1A–F shows the changes in PV and TBARS of ground backbones, MSM, and PI, from the three species during ice storage. In the MSM and PI samples, the antioxidant activity of Duralox-MANC was also investigated. For herring, the PV and TBARS of all three sample types without antioxidants added had significantly increased on day 1 and then levelled off (Fig. 1A, 1B). However, the TBARS values for the PI levelled off at a significantly lower value than for MSM and backbone (at ~ 85 $\mu\text{mol/kg}$ compared to at ~ 150 $\mu\text{mol/kg}$). Differently from the herring samples, the PV and TBARS of salmon-derived samples remained in the range of 112–167 $\mu\text{mol/kg}$ and 3.1–7.8 $\mu\text{mol/kg}$, respectively, during 7 days of storage (Fig. 1C, 1D). After 7 days, both PV and TBARS displayed a slight, but significant increase ($p < 0.05$) for the ground backbones, while the MSM and PI were stable up to 11 days. For cod, the PV and TBARS immediately increased in all three samples types, but the ground backbone and MSM increased somewhat slower than the PI; and also reached lower maximum PVs (Fig. 1E, 1F). In both MSM and PI of three fish co-products, addition of 0.5% Duralox MANC completely inhibited the increase of PV and TBARS during the whole 11–18 days ice storage period (Fig. 1A–F).

The MSM and PI samples from the three species of backbones were also stored up to 12 months at -20°C to compare stability towards lipid oxidation. Between 0 and 2 months, the PV of MSM from herring displayed a more rapid increase (from 224.3 to 642.8 $\mu\text{mol/kg}$) compared to PI (from 271.7 to 355.6 $\mu\text{mol/kg}$) (Fig. 2A). All PVs for the MSM were also significantly higher than those of PI during 12 months of storage. For TBARS, there was no significant difference between MSM and PI at 4 and 6 months. However, from 8 to 12 months, TBARS in the MSM was significantly higher than in the PI (Fig. 2B). In the salmon samples, the PV of PI displayed a slight increase ($p < 0.05$) from month 2 to 12, while the PV for the MSM was stable up to 8 months and then rapidly increased between month 10 and 12 (Fig. 2C). There was no significant difference between the PVs in the MSM and PI at month 12. For TBARS, both MSM and PI from salmon did not display a significant increase during the storage (Fig. 2D). For cod samples (Fig. 2E), the PV of MSM slightly, but significantly, increased ($p < 0.05$) between month 2 and 4, while the PI was stable until month 10, followed by a slight increase. There was no significant difference between MSM and PI up to 8 months of storage. However, between month 8–10, the TBARS of PI showed a slight increase which rendered the TBARS values significantly higher than those in MSM (Fig. 2F).

Under industrial settings, it is expected that the co-product raw material may not be value-added immediately as there is often a higher capacity of the filleting lines than the extra process lines to produce e.g. MSM. With this background, we also investigated how pre-storing herring backbones on ice affected subsequent lipid oxidation in the minced backbone raw material or MSM produced thereof. Supplementary Fig. 2A show that 24 h pre-storage resulted in significantly higher starting PV both in the minced backbone (210.0 vs. 76.7 $\mu\text{mol peroxide/kg}$) and MSM (263.9 vs. 50.1 $\mu\text{mol peroxide/kg}$) compared to samples from fresh herring backbones. However, samples from fresh and pre-stored backbones reached the same maximum PV. Regarding TBARS, there was no significant difference between samples from fresh and pre-stored backbones at start of the storage, however, the mince and MSM from pre-stored backbones showed a higher onset of TBARS development rate during ice storage and also reached significantly higher TBARS levels after 2.8 days (Supplementary Fig. 2B).

3.2. Initial antioxidant levels in raw materials and protein-enriched products -ascorbic acid and α -tocopherol

Endogenous antioxidants have been reported to influence the onset of lipid oxidation in fish raw materials (Undeland, Ekstrand, & Lingnert,

1998; Maestre, Pazos, & Medina, 2011). Ascorbic acid, which is quickly lost during storage and processing of fish, is important for maintaining the redox balance of the fish muscle, e.g. by reducing tocopheroxyl radicals generated during oxidation of tocopherol to regenerate vitamin E (Niki, 1987; Undeland, Ekstrand, & Lingnert, 1998). Thus, initial ascorbic acid levels of fish muscle will contribute to its oxidative susceptibility. Fig. 3A shows the contents of ascorbic acid in ground backbones, MSM and PI from the three species of fish backbones. In salmon, the level of ascorbic acid in ground backbones and MSM were 25.9 and 23.7 mg/kg mince, respectively, which were by far the highest ($p < 0.05$) levels among the three species and the two process methods. In contrast, the cod displayed the lowest initial levels of ascorbic acid in the ground raw material, and herring was in between. For both herring and salmon, the ground backbones had significantly higher levels of ascorbic acid compared to MSM and PI. It was clear that in the pH-shift process, the aqueous nature of ascorbic acid made it severely diluted, and while it reached low levels in the salmon PI, it was not even detected in the PI samples of both herring and cod.

Among the endogenous antioxidants of fish muscle, α -tocopherol is one of the most important lipid-soluble compounds (Maestre, Pazos, & Medina, 2011); and as described above, it acts in synergy with the aqueous ascorbic acid. It was observed that the backbone raw material and MSM of salmon had the highest α -tocopherol levels (14.3, 15.9 mg/kg mince, respectively) of all samples (Fig. 3B) and overall, the content of α -tocopherol decreased in the following order during processing: salmon > cod > herring, regardless of processing method. Further, the α -tocopherol levels were significantly lower in PI compared to the corresponding values of backbone and MSM in all three species.

3.3. Initial pro-oxidant levels in raw materials and protein-enriched products - Hb, Fe and Cu

Heme proteins and transition metals are the primary endogenous components with the ability to promote radical-mediated oxidative chain reactions in fish (Richards, 2010). Fig. 4A shows the level of Hb in three species of fish backbones before and after the two processing methods. As expected, the Hb content was highest in herring, followed by cod and salmon, regardless of processing methods. In herring and salmon, the PI had significantly lower Hb levels than the backbone and MSM. However, for cod, the level of Hb in PI samples was similar to, or slightly higher, than the levels in backbone and MSM. Also, the total Fe content in herring-derived samples was significantly higher than in samples from salmon and cod (Fig. 4B). For herring and cod, processing had no significant effect on total Fe levels. However, for salmon, the PI showed significantly lower total Fe content compared to backbone or MSM. Herring PI had significantly ($p < 0.05$) more non-heme Fe than raw material and MSM, while for cod, the raw material had significantly higher levels than the two products (Fig. 4C). Total Cu content was significantly higher in PI than MSM, regardless of the fish species.

3.4. Moisture, total lipid content and PUFA

The moisture content, total lipid content, and PUFA of all samples are presented in Table 1. The PI had the highest moisture content, followed by MSM and backbone in all three fish species. The ground salmon backbone contained the highest total lipid level (218 g/kg mince) of all samples, and for both PI and MSM, salmon had significantly higher total lipid content than corresponding products from herring and cod. For salmon and herring, the total lipid contents followed the order: backbone > MSM > PI while cod had significantly higher total lipid content than backbone and MSM (Table 1). Similarly, for total PUFA, herring and salmon backbone and MSM had higher levels than PI, while the opposite order was observed in cod where the PUFA contents in backbone, MSM and PI was 1.89, 2.55 and 6.42 g/kg mince, respectively.

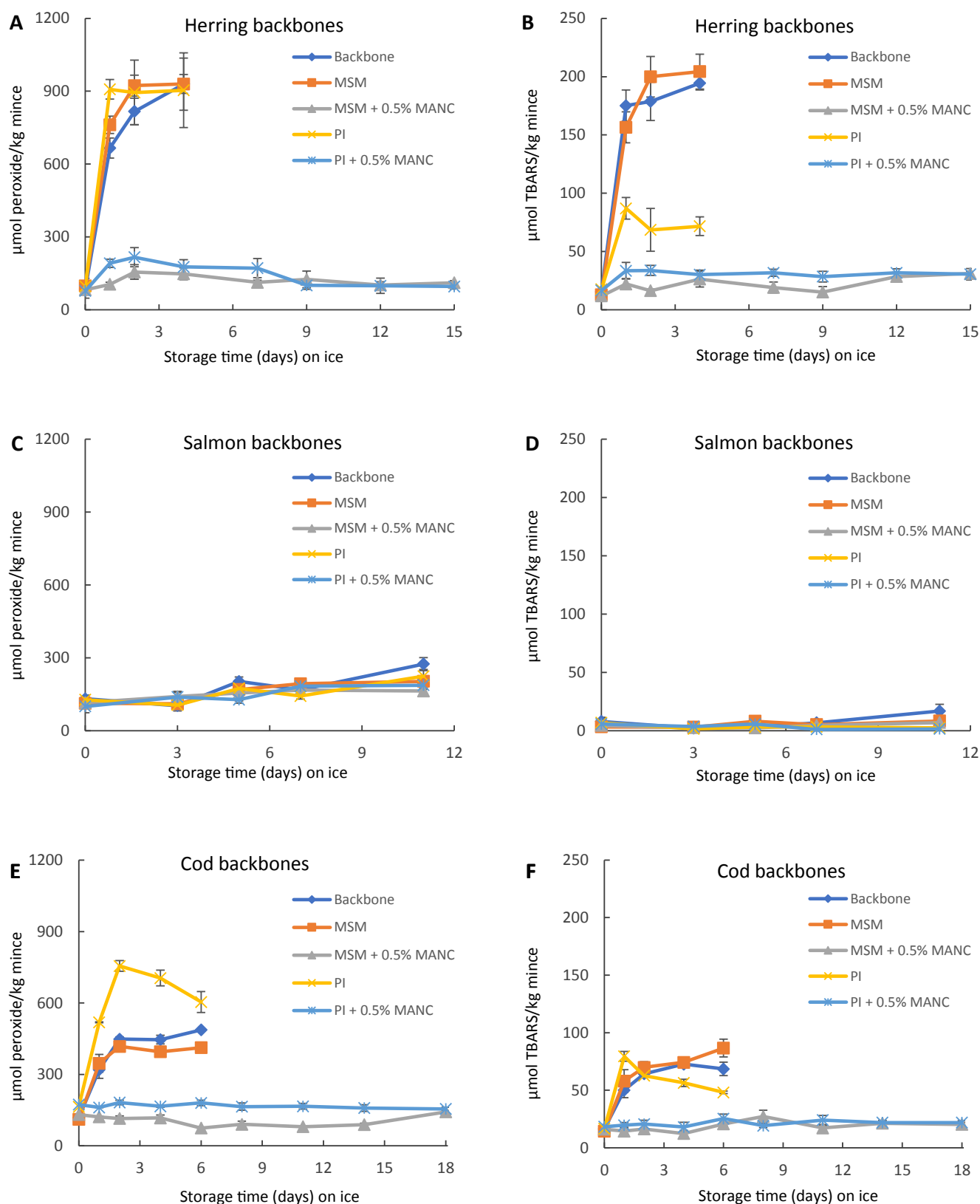


Fig. 1. Lipid oxidation TBARS and PV in herring (A, B), salmon (C, D) and cod (E, F) backbones as well as mechanically separated meat (MSM) and pH-shift produced protein isolates (PI) thereof, with and without addition of 0.5% Duralox-MANC, during ice storage.

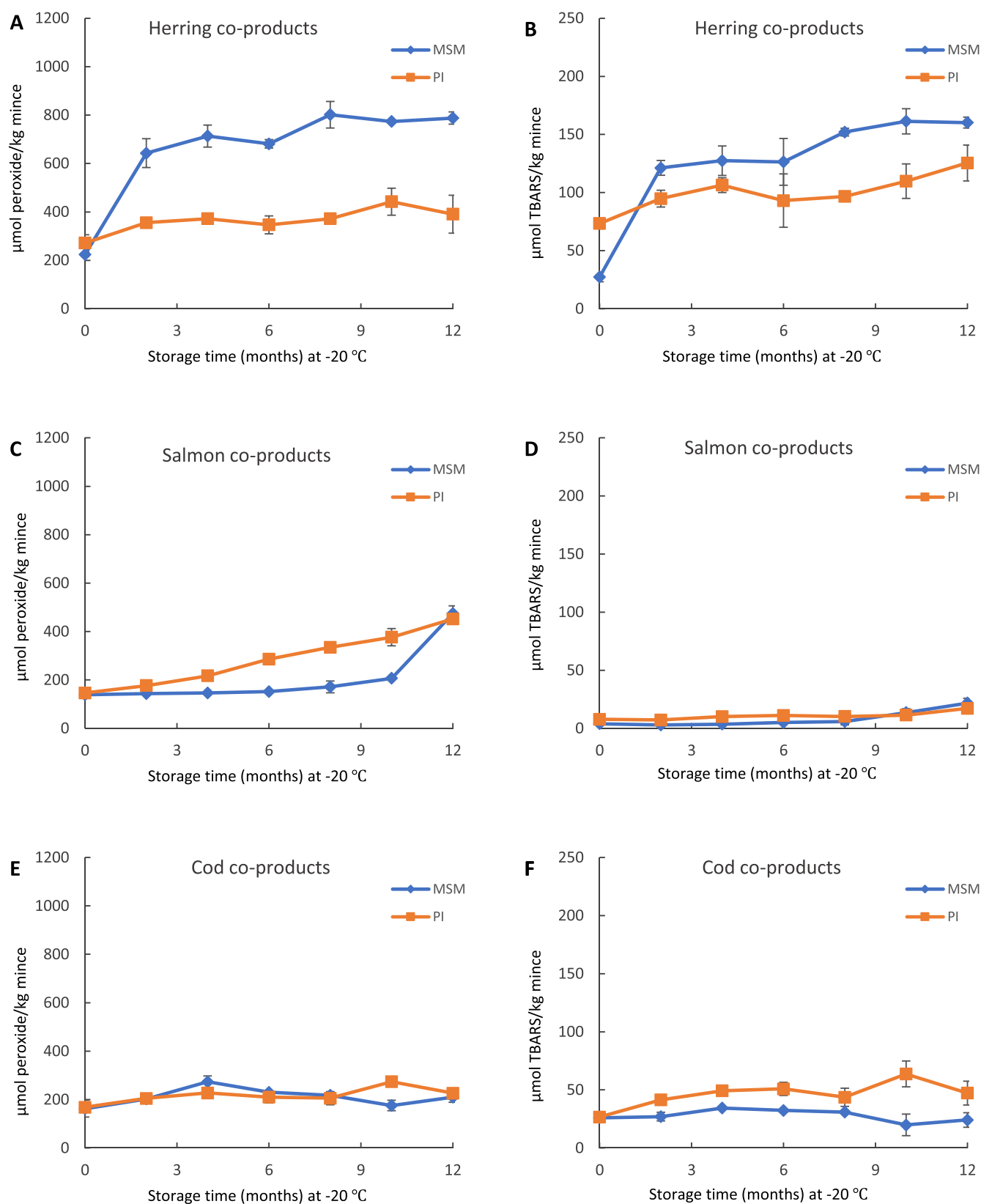


Fig. 2. TBARS and PV in mechanically separated meat (MSM) and pH-shift produced protein isolates (PI) from herring (A, B), salmon (C, D) and cod (E, F) backbones during storage at -20°C .

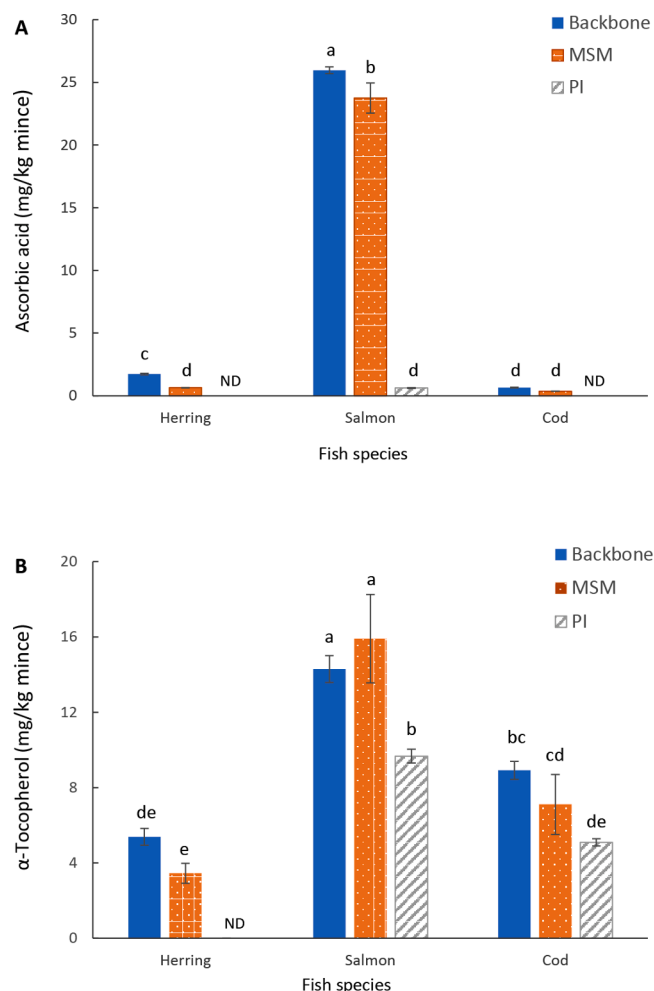


Fig. 3. Ascorbic acid (A) and α -tocopherol (B) content of herring, salmon and cod backbones, and their mechanically separated meat (MSM) and protein isolates.

3.5. Correlation between initial composition and lipid oxidation

Pearson correlations were used to analyze the correlation between initial composition and the PV as well as TBARS index (see section 2.8) during the ice storage trials (Table 2). Also, the correlation coefficient (r) between PV and TBARS was determined and the outcome 0.982 indicated a strong correlation. The PV index exhibited a significant positive correlation with the level of Hb ($r = 0.963$) and the total Fe content ($r = 0.943$), and a significant negative correlation with ascorbic acid ($r = -0.740$) and α -tocopherol ($r = -0.925$). The correlation to the TBARS index revealed similar results. Correlations between the two oxidation indexes and PUFA, total lipids, non-heme Fe and Cu were non-significant, and in many cases very low, e.g. -0.28 , -0.39 , -0.075 and 0.016 , respectively, for the PV-index.

4. Discussion

4.1. Effect of pH-shift processing vs mechanical meat-bone separation on lipid oxidation

This study was undertaken to systematically investigate how lipid oxidation was affected by applying pH-shift processing vs mechanical meat-bone separation on three species of fish backbones. The pH-shift processing method involves severe dilution in water, high speed homogenization and two major pH changes, as shown in Supplementary Fig. 1. These processes could potentially change the antioxidant/pro-

oxidant balance as well as the composition and physical structure of the lipid substrate, thereby affecting the onset of lipid oxidation during subsequent storage. Specifically, the pH-shift process could induce structural changes to Hb during the precipitation step at pH 5.5 of the pH-shift process, which could stimulate co-precipitation of Hb with myofibrils and increase the prooxidative activity of Hb in the storage trials (Abdollahi, Marmon, Chaijan, & Undeland, 2016). Undeland et al. (2004) reported a higher catalytic activity of fish Hb at pH 6 compared to pH 7.2 in washed cod muscle. The mechanism by which low pH can increase the prooxidative activity of Hb is attributed to unfolding (Kristinsson & Hultin, 2004), exposing the heme group and, therefore, resulting in more rapid autooxidation and heme loss. In our previous studies, we observed that autooxidation and heme loss play crucial roles in Hb-mediated lipid oxidation in washed cod muscle (Wu, Yin, Zhang, & Richards, 2017). Our findings thus indicate that it is the precipitation step of the pH-shift process which is critical for the onset of oxidation.

Secondly, the pH-shift process comprises a dilution of the muscle aqueous fraction, with subsequent discarding of the entire aqueous phase resulting from the second centrifugation. This resembles the washing steps in surimi production, although with a less extensive dilution, 8.5–9.5-fold compared to the > 14 -fold dilution taking place when muscle mince is washed 3 times with 3 volumes of solution, as in classic surimi processing. Given the importance of endogenous antioxidants in maintaining the redox balance of fish muscle (Maestre, Pazos, & Medina, 2011), the drastic reduction in ascorbic acid during pH-shift processing (Fig. 3A) is thought to play a fundamental role. Also, the loss of α -tocopherol along with the removal of membranes during pH-shift processing is identified as an important reason to explain why this process can lead to a more rapid onset of lipid oxidation (Fig. 3B). Our result was consistent with some of our previous studies, showing that washing of minced herring fillets (one time with 5 volumes) removed substantial amounts of endogenous antioxidants and significantly increased lipid oxidation compared to unwashed samples during subsequent frozen storage at -20°C (Undeland, Ekstrand, & Lingnert, 1998). Harrysson et al. (2020), also recently showed how a limited dilution of minced fish muscle can stimulate oxidation more than an extensive surimi process-like dilution, as the former gives a larger net removal of antioxidants compared to pro-oxidants.

Thirdly, pH-shift processing includes high speed homogenization, which could disrupt the highly organized microstructure of fish muscle mince and thereby stimulate lipid oxidation. Fish muscle has a continuous network of connective tissue through its attachment to the myocommata, which is tightly packed and surrounded by connective tissue (endomysium) (Wu, Xiao, Yin, Zhang, & Richards, 2021). However, it has been reported that the connective tissue is removed after high speed homogenization using a Polytron during pH-shift processing (Hultin & Kelleher, 2000). This would disrupt the microstructure and the membranes would become more exposed compared to in the non-processed backbone or MSM samples. This hypothesis was confirmed in our previous studies (Marmon, Krona, Langton, & Undeland, 2012), where an irregular and porous microstructure was observed in pH-shift processed samples compared to muscle minces of herring. Furthermore, the more exposed muscle cell membranes in pH-shift produced isolates could be more accessible to the prooxidants (e.g. Hb) compared to the folded membrane in non-processed fish materials. In our previous study, we found that added myoglobin (Mb) could not oxidize the endogenous phospholipids (PL) in a washed pig muscle system, but PV and TBARS increased significantly more after adding pure PL isolated from washed pig muscle (Wu et al., 2021). This result indicated that the muscle microstructure plays an important role in lipid oxidation.

4.2. The effect of exogenous antioxidants on lipid oxidation

Although pH-shift processing is an innovative technology to obtain functional proteins from fish co-products, the high susceptibility of the PI to lipid oxidation, accompanied by rancidity, calls for stabilization of

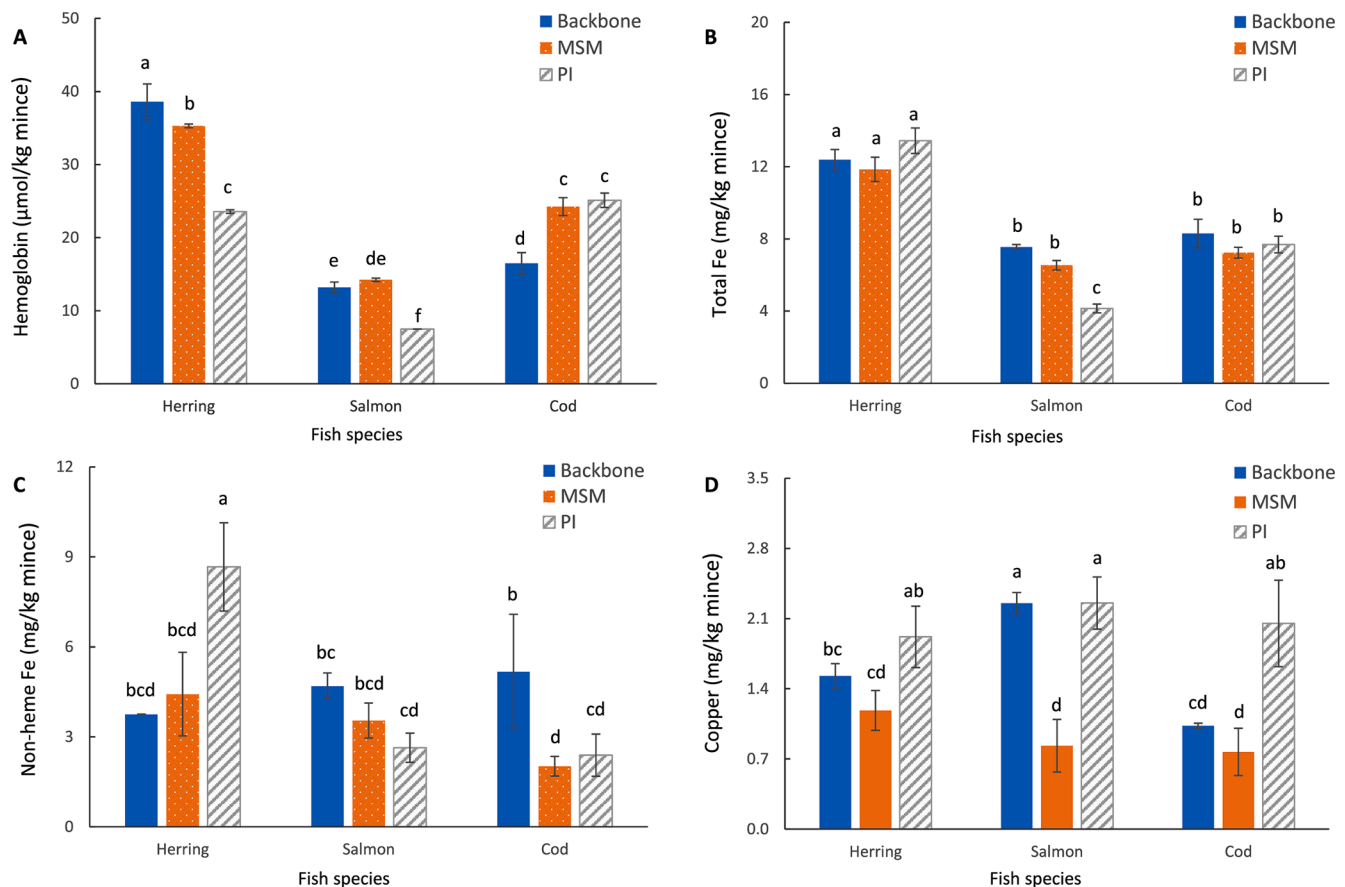


Fig. 4. Hemoglobin (A), Total Fe (B), non-heme-Fe (C) and Cu (D) content of herring, salmon and cod backbones, and their mechanically separated meat (MSM) and protein isolates.

Table 1

Moisture, lipid and polyunsaturated fatty acid (PUFA) content of herring, salmon and cod backbones, and their mechanically separated meat (MSM) and protein isolates.

Species	Materials	Moisture (%)	Lipid* (g/kg mince)	PUFA* (g/kg mince)
Herring	Backbone	72.91 ± 0.20 ^c	92.69 ± 8.32 ^c	16.45 ± 1.27 ^c
	MSM	76.58 ± 0.51 ^d	74.04 ± 8.35 ^d	15.74 ± 0.91 ^c
	Protein isolate	80.54 ± 0.08 ^{ab}	14.46 ± 1.37 ^f	3.40 ± 0.29 ^a
Salmon	Backbone	57.95 ± 0.11	218.80 ± 12.48 ^a	35.49 ± 2.65 ^a
	MSM	68.02 ± 2.43 ^f	137.87 ± 4.23 ^b	21.64 ± 0.41 ^b
	Protein isolate	79.69 ± 0.00 ^b	41.80 ± 0.49 ^e	6.10 ± 0.03 ^d
Cod	Backbone	77.77 ± 0.30 ^{cd}	13.83 ± 0.86 ^f	1.89 ± 0.07 ^a
	MSM	82.92 ± 0.55 ^a	13.35 ± 2.84 ^f	2.55 ± 0.39 ^a
	Protein isolate	81.01 ± 0.20 ^a	16.72 ± 1.06 ^f	6.42 ± 0.21 ^d

* lipid and PUFA content are presented based on g/kg mince, MSM or isolate. Results are shown as mean ± SD (n = 3). Different small letters in each column shows a significant difference (p < 0.05).

either the raw material prior to processing and/or the PI itself. The same demand is put on the MSM, which also oxidized very quickly. Based on our previous study (Wu, Ghirmai, & Undeland, 2020), where we found 0.5% Duralox MANC to completely inhibit lipid oxidation in minced herring co-product minces during 11 days on ice, we used the same antioxidant mixture for PI and MSM in this study. The outcome was a remarkable increase in the oxidation lag phase, from <1 day to >15 days. Duralox MANC contains rosemary extract, ascorbic acid,

tocopherols, and citric acid, with the former being the principal functional component for the antioxidative activity (Berdahl, Reinhout, & Schulze, 2009). It has been reported that rosemary extract's antioxidative activity is mainly attributed to carnosol and carnosic acid, which could account for over 90% of the antioxidant properties of rosemary extract (Aruoma, Halliwell, Aeschbach, & Löliger, 1992). However, also isoprenoid quinones could be involved, which act as chain terminators of free radicals, and as scavenger of reactive oxygen species (ROS) (Nieto, Ros, & Castillo, 2018). That there are synergistic effect between the rosemary extract and e.g. the ascorbic acid, tocopherols and citric acid of Duralox-MANC was confirmed by Wada and Fang (1992), who observed that the antioxidant activity of rosemary extract plus tocopherols in frozen sardine muscle was significantly higher than the activity exhibited by the individual components.

4.3. Relationship between endogenous factors and lipid oxidation

It is known that the lipid oxidation reaction depends on number of endogenous factors, including heme proteins, iron, copper, lipooxygenase, ascorbic acid, tocopherol and unsaturated fatty acids (Richards, 2010); all which are heavily affected by processing when it comes to form and contents (Hultin, 1994). To clarify which endogenous factors that are the most relevant to lipid oxidation in MSM and PI, we used the Pearson correlation test.

As expected, the Hb content displayed a significant positive correlation with lipid oxidation (Table 2). Also Grunwald & Richards (2006b) reported that an increased Hb level lead to higher maximal TBARS values. Specifically, the transfer of the heme reactant from Hb to lipid substrates has been proposed as a key step in Hb-mediated lipid oxidation (Grunwald & Richards, 2006a). However, at the same time,

Table 2

Linear correlations as shown by Pearson's correlation coefficient between the initial composition and the onset of lipid oxidation (PV and TBARS index) during ice storage of three fish species co-products.

	PV index	TBARS index	AC	α -TO	Hb	Total Fe	Non-heme Fe	Cu	Moisture	Lipid	PUFA
PV index	1	0.982**	-0.740*	-0.925**	0.963**	0.943**	0.016	-0.075	0.481	-0.394	-0.282
TBARS index		1	-0.671*	-0.869*	0.982**	0.970**	0	0.005	0.387	-0.3	-0.195
AC			1	0.914**	-0.682	-0.567	0.152	0.446	-0.888**	0.883**	0.827*
α -TO				1	-0.876*	-0.794*	0.101	0.196	-0.701	0.638	0.542
Hb					1	0.913**	-0.184	-0.061	0.447	-0.333	-0.231
Total Fe						1	0.226	0.191	0.21	-0.155	-0.049
Non heme Fe							1	0.482	-0.426	0.282	0.295
Cu								1	-0.783*	0.754*	0.771*
Moisture									1	-0.974**	-0.949**
Lipid										1	0.992**
PUFA											1

* was shown for significant correlation (* $p < 0.05$, ** $p < 0.01$). AC = ascorbic acid, α -TO = α -tocopherol, Hb = hemoglobin, PUFA = polyunsaturated fatty acids.

the alkoxy and peroxy radicals resulting from lipid oxidation could degrade the heme group, therefore terminating the catalytic cycle (Richards, 2010). This was supported by Richards and Hultin (2002), who reported that the rate of TBARS formation increased with an increase in the amount of Hb added to washed cod muscle. The higher PV index of herring-derived materials compared to cod- and salmon-based materials could thus be linked to their higher levels of Hb. However, the Hb-level does not explain the fact that PI from herring, containing lower Hb than minced backbone and MSM (Fig. 4A) displayed a more rapid onset of oxidation than these samples (Fig. 1A). This could rather be attributed to the change in ascorbic acid and tocopherol during pH-shift processing (Table 2); both these compounds showed a significant negative correlation with lipid oxidation, and as can be seen in Fig. 3A and 3B, the ascorbic acid and tocopherol levels in the PI samples were significantly lower than in the backbone or MSM samples (Fig. 3). We also believe the strong impact from endogenous ascorbic acid and tocopherol-levels on lipid oxidation contributed to the significantly higher TBARS levels in MSM from pre-stored herring backbones (Supplementary Fig. 2).

It is important to note that we did not find a significant correlation between total lipid content or total PUFA with lipid oxidation (Table 2), which is in agreement with our previous study (Undeland, Hultin, & Richards, 2002), showing that addition of up to 15% neutral lipids did not affect Hb-mediated lipid oxidation in washed cod mince. Richards and Hultin (2001) also reported that trout blood, containing only approximately 0.01% lipids, generated a strong rancid odor and significantly increased TBARS values during 2 days storage at 2 °C. Furthermore, the same study found that having at least six times more membrane PL present did not enhance the rate or extent of rancidity development during ice storage of washed cod mince containing added blood. This view is also consistent with the results from the storage of salmon samples (Fig. 1C and 1D). Although the salmon samples had higher total lipid content and PUFA content compared to herring and cod, they were not more susceptible to oxidation. Also, the documented high levels of ascorbic acid and α -tocopherol in salmon materials could be an important reason for their stability, further to the well-known high levels of the carotenoid antioxidants e.g. astaxanthin and canthaxanthin (Torrissen, Christiansen, Struksnæs, & Estermann, 1995).

In summary, the correlation analysis provided indicators which can be used to predict the process of lipid oxidation in MSM and PI, or to develop an effective protective strategy; it is clear that Hb/total Fe needs to be targeted.

5. Conclusion

The susceptibility of backbones emerging from fish filleting to lipid oxidation followed the general order: herring > cod > salmon, regardless of processing method. However, during ice storage, MSM and PI from both cod and herring backbones developed lipid oxidation very fast. For cod, the PI was more sensitive than MSM to oxidation while the

opposite was seen for herring; the latter which was even more pronounced during frozen storage. Duralox largely increased the oxidation lag phase for ice stored MSM and PI; from <1 day to >15 days. Levels of Hb, total Fe, ascorbic acid, and α -tocopherol were the most relevant factors controlling lipid oxidation in the two backbone-derived products as well as in the raw material. Given the strong impact from ascorbic acid and α -tocopherol, backbones should thus be value-added as soon as possible after the filleting process unless they are subjected to some type of antioxidant treatments to compensate for losses of endogenous antioxidants.

CRediT authorship contribution statement

Haizhou Wu: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **Mehdi Abdollahi:** Investigation, Writing - review & editing. **Ingrid Undeland:** Conceptualization, Resources, Supervision, Writing - review & editing, Project administration, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2021.129973>.

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