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Lipid oxidation in sorted herring (*Clupea harengus*) filleting co-products from two seasons and its relationship to composition

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ABSTRACT

Lipid oxidation in ice-stored sorted herring fractions (head, backbone, viscera + belly flap, tail, fillet) from spring and fall, and its association with endogenous prooxidants, antioxidants and lipid substrates were investigated. Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) had increased significantly in all fractions after 1 day, but for both seasons, the most rapid PV and TBARS development occurred in head, which also had highest hemoglobin (Hb) levels and lipoxygenases (LOX) activity. Viscera + belly flap was overall the most stable part, and also had the highest α-tocopherol content. Pearson correlation analyses across all five fractions confirmed a significant impact of Hb, LOX and α-tocopherol on the lipid oxidation susceptibility, while content of total iron, copper, lipids or polyunsaturated fatty acids provided no significant correlation. Overall, the study showed which pro-oxidants that should be inhibited or removed to succeed with value adding of herring filleting co-products and the fillet itself.

1. Introduction

The demand for high value seafood increases yearly because of, e.g., population growth and a diet shift to more protein driven by an aging population (Cai & Leung, 2017). However, it is a challenge to expand both wild fisheries and aquaculture based on endurance of our ecosystems (Alexandratos & Bruinsma, 2012). Thus, there is an increasing interest in using as much as possible of the caught or harvested fish directly for food, which comprises conversion also of non-fillet fractions to food ingredients. Currently, these fractions of the fish mainly end up as side streams (co-products, also called by-products) during the filleting operation, and are then dedicated to low value uses as fodder meals or mink feed, even if they contain significant amounts of protein, long chain (LC) n-3 polyunsaturated fatty acids (PUFA), and other nutritional components such as vitamins and minerals (Abdollahi, Wu, & Undeland, 2021).

Established and more recent techniques enabling value addition to fish filleting co-products are for example mechanical meat-bone separation using belt and drum (Wu, Abdollahi, & Undeland, 2021), the pH-shift process (Abdollahi, Wu, & Undeland, 2021), supercritical fluid extraction (Haq, Ahmed, Cho, & Chun, 2017), and enzymatic hydrolysis (Slizyte, Rommi, Mozuraityte, Eck, Five, & Rustad, 2016). However, most factories that fillet fish mix their co-products, not least when it comes to small pelagic species like herring. This practice limits use of the co-products for food production since the raw material gets very complex, and since blood, enzymes and lipids from e.g., the viscera and head parts easily contaminate the cleaner parts like the backbones and tails, accelerating e.g., their oxidative or enzymatic degradation. Also, different filleting co-products contain different amounts of nutrients and could therefore be suitable for different end uses and value addition processes. For example, Ahmmed et al. (2021) reported that head and skin had a higher lipid content compared with the roe and male gonad of Pacific blue mackerel (*Scomber australasicus*). Bechtel (2003) reported that cod skin had a higher crude protein content compared with head, viscera, and frame. We also recently identified backbones from herring, cod and salmon as protein-rich parts very promising for mince or protein isolate production (Abdollahi, Wu, & Undeland, 2021). Therefore, sorting fish filleting co-products into separate raw material fractions, and choosing the best method to add value to them individually depending on their nutritional components will be important to take co-product valorization to the next level. It is however also essential to make sure each co-product fraction gets proper stabilization, to assure that the initial quality of the raw material is retained. To date, only a few studies exist on the oxidative stability of individual fish co-product fractions, including our own previous work on salmon, cod and herring backbones (Wu, Abdollahi, & Undeland, 2021) as well as studies of

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catfish viscera (Hwang, Kim, Kang, Jung, Park, & Weller, 2004) and mackerel head-viscera mixture (Liu, Morioka, Itoh, & Obatake, 2000).

However, to the best of our knowledge, there is no study systematically comparing the development of lipid oxidation and its relationship to pro-/antioxidants in different fractions of sorted fish co-product fractions side by side, and then compare it with the fillet part. The aims of this study were to: (i) quantify prooxidants, antioxidants and lipid substrates of sorted herring (*Clupea harengus*) co-product fractions as well as the fillet from two catching occasions (spring and fall), (ii) investigate the development of lipid oxidation in the individual herring fractions during ice storage in a minced state, and (iii) identify the most relevant endogenous factors that affect lipid oxidation in the different herring fractions.

2. Materials and methods

2.1. Chemicals

Streptomycin sulfate, glutathione, Tween-20, linoleic acid, ammonium thiocyanate and ferrous sulfate were obtained from Sigma Chemical A/S (St. Louis, MO, USA). Chloroform, methanol, potassium ferricyanide, and tris(hydroxymethyl) aminomethane (Tris) were obtained from Fisher Scientific (Pittsburgh, PA, USA). All other (not mentioned above) chemicals were of reagent grade.

2.2. Preparation of fish co-products

A filleting machine from Baader (Model Baader 36, Nordisher Maschinenbau Rudolf Baader Gmbh, Lubeck, Germany) placed at Sweden Pelagic AB (Ellös, Sweden), was re-built to allow sorting of herring filleting co-products into 4 separate fractions; head, backbone (also called frame), viscera + belly flap, and tail. Fresh herring caught in April and October 2020 were subjected to filleting and sorting according to the new procedure. The four co-product fractions plus skin-on fillets were covered with ice-filled plastic bags and transported within 3 h to the marine lab at Chalmers University of Technology. Upon arrival, sorted herring fractions 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 pooled and mixed to complete homogeneity. Fifty grams mince of each fraction were directly subjected to ice storage while 80–100 g mince were packed in Polynova plastic bags (89 mm × 114 mm, 50 my) from which the air was manually squeezed out by flattening the packages. Then these samples were stored at −80 °C until subsequent chemical analysis. In the Supplementary material, Figure S2 shows the weight distribution of the sorted fractions and photo documentation.

2.3. Ice storage

The process of storage and sampling was conducted according to the method of Wu, Ghirmai, and Undeland (2020). After manual stirring in 200 ppm streptomycin into the fresh minces to prevent bacterial growth, 25 g mince of each sample was transferred into two 250 mL screw-capped Erlenmeyer flasks. The mince was flattened out in the bottom of the flasks, giving rise to a sample thickness of ~5 mm. The capped sample bottles were then stored on ice for up to 4 days. Samples for oxidation analysis (~1 g "plugs") were taken out from the Erlenmeyer flask using a hollow cylinder to obtain a constant surface-to-volume ratio between different samplings. The plug was wrapped in aluminum foil and kept at − 80 °C until analysis.

2.4. Analysis of lipid oxidation

Peroxide value (PV) and TBA-reactive substances (TBARS) were used to monitor the development of lipid oxidation in minced sorted herring filleting co-products during ice storage. The lipids were extracted from 1 g-samples with 10 mL of chloroform/methanol (2:1). Sample and solvents were homogenized using a polytron (T18 digital Ultra-Turrax, IKA, Germany) for 15 s at 12,000 rpm. Sodium chloride (3.08 mL, 0.5%) was then added, and the sample was vortexed for 30 s followed by centrifugation at 2,000 × g for 10 min. The lower phase (chloroform) was collected for PV analysis as described by Larsson et al. (2007). Briefly, 1.33 mL ice-cold chloroform/methanol (1:1) was mixed with 2.0 mL of the chloroform extract, and hereafter ammonium thiocyanate (8.76 M) and iron(II) chloride (18 mM) (33.4 μL of each) were added with 2–4 s vortexing between each addition. The sample was incubated for 20 min at room temperature, and the absorbance was read at 500 nm by using a spectrophotometer (Cary 60 UV–vis, Agilent technologies, USA). The upper phase (water–methanol) from the lipid extraction was used to determine TBARS (Schmedes & Hølmer, 1989). An aliquot (2.5 mL) of the water-methanol extract was mixed with 2.5 mL TBA reagent (including 5.0% thiobarbituric acid and 0.5% trichloracetic acid) in a screw capped test tube. All samples were then heated in boiling water for 30 mins and then the tubes were cooled in tap water. The absorbance was measured at 532 nm. PV and TBARS results were expressed as μmol peroxides and μmol TBARS/ kg of minced co-product, respectively.

2.5. Proximate composition

The moisture content of the samples was measured gravimetrically by overnight heating at 105 °C. Ash content was also determined gravimetrically by heating the samples at 550 °C in a furnace for 6 h. Total nitrogen content was measured using a LECO nitrogen analyzer (TruMac-N, LECO Corp., St. Joseph, MI, USA) according to the Dumas method as described by the method described by Abdollahi et al. (2021), and then the crude protein content in samples was calculated using a nitrogen-to-protein conversion factor of 5.58 (Mariotti, Tomé, & Mirand, 2008). Crude lipid content was measured gravimetrically following extraction with chloroform:methanol (2:1). Briefly, two grams herring minces sample were mixed with 20 mL of chloroform/methanol (2:1). Sample and solvents were homogenized using a polytron (T18 digital Ultra-Turrax, IKA, Germany) for 15 s at 12,000 rpm. Sodium chloride (6.16 mL, 0.5%) was added, and the sample was vortexed for 30 s followed by centrifugation at 2,000 × g for 10 min. Four milliliters chloroform phase (lower phase) was transferred into a glass tube and then evaporated to dryness under oxygen free nitrogen gas at room temperature following weighting of the lipid fraction for crude lipid analysis. Another 4 mL of the chloroform phase were transferred into a new glass tube with Teflon screw-caps and were stored at −80 °C for subsequent fatty acid or tocopherols analysis.

2.6. Fatty acid profile of fractions

The fatty acid composition of the lipid fraction from section 2.5 was determined according to the method described by Abdollahi, Wu, and Undeland (2021). Prior to methylation, the fatty acid heptadecanoic acid (C17:0, 5 mg/mL) was added to all samples as an internal standard based on the fact that odd numbered long-chain fatty acids (e.g C15:0, C17:0, C23:0) are unusual in most fish. The lipid samples were methylated by methanolic HCl transesterification according the method described by Cavonius et al. (2014). After methylation, the toluene was evaporated under nitrogen gas and the fatty acid methyl esters were resolubilized by adding 1.0 mL of isooctane. The samples were then diluted in isooctane and subjected to GC–MS analysis using an Agilent 7890 A GC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a J&W DB-wax column (30 m × 0.250 mm × 0.25 μm) and interfaced with an Agilent 5975C triple-axis mass spectrometric (MS) detector. Injection volume was 1 μL with a 15:1 split at an inlet temperature of 275 °C. The carrier gas was helium, with a fixed flow of 1 mL/min throughout the temperature program, which was as following, 100 °C for 0 min, ramp at 4 °C/min to 205 °C, thereafter ramp at 1 °C/min to 230 °C, hold 5 min. An example chromatogram is shown in the

Supplementary material (Figure S4). The standard GLC 463 (Nu-Chek prep, Inc., Elysian, USA), containing 52 different FAMEs (Figure S4), was used for identification of the different peaks in herring lipid samples. Fatty acids were quantified against the C17:0 internal standard.

2.7. Analysis of alpha, beta, delta, and gamma tocopherols of fractions

The amounts of alpha, beta, delta, and gamma tocopherols in sorted herring filleting co-products and fillet were measured by the lipid fraction retrieved during total lipid analyses, followed by high-performance liquid chromatography (HPLC) with fluorescence detection according to Larsson, Almgren, and Undeland (2007). Briefly, 1 mL chloroform phase extract (see section 2.5) was evaporated to dryness under nitrogen gas, and then diluted in 0.5 mL methanol. Samples were then vortexed for 1 min and centrifuged at 1600 × g for 5 min. The supernatant (100 μL) was transferred to vials for HPLC analysis. The chromatographic separation of tocopherol isomers was performed on a C18 column (Kromasil, 150 mm × 2.1 mm, 5 μm) using 98% methanol as mobile phase under isocratic conditions at a flow rate of 0.4 mL/min, and 5 μL injection volumes. The detection was performed with a fluorescence detector (Shimadzu RF-551, Kyoto, Japan) using 295 and 330 nm as excitation and emission wavelength, respectively. The concentration was quantified using external alpha-, beta-, delta-, and gamma-tocpherol standards.

2.8. Measurement of hemoglobin, total Fe, Cu, and lipoxygenase (LOX) activity of fractions

Small portions of the mince were taken from at least 10 different sections of the original bulk mince using a plastic spatula. The portions, together weighing about 100 g, were carefully placed one by one in a porcelain mortar containing liquid nitrogen. Additional liquid nitrogen was slowly added in between the mince portions to completely freeze them. The frozen mince was then pounded using a pestle to break it into smaller pieces. Then these small pieces were ground into a fine powder by using a Waring blender (LB20E* Variable speed laboratory blender, 400 W, Waring Commercial, USA) at 6000 rpm. (Wu, Ghirmai, & Undeland, 2020). The powdered samples were assayed for total heme and lipoxygenase (LOX) activity. Total heme was measured following the acetone-based method as described in our recent study (Wu, Abdollahi, & Undeland, 2021). The difference between the absorbance values at 640 and 700 nm was used for calculating the heme concentration. Bovine hemoglobin (0.1, 0.5, 1, 5 and 10 μM) was used to prepare a standard curve and results were expressed as μmol Hb/kg mince.

LOX activity of was measured according to Sae-Leaw et al. (2013) with some modifications. Two grams powder was suspended in 18 mL of PBS buffer (50 mM dipotassium hydrogen phosphate, pH 7.0, 1 mM glutathione, 0.04% Tween-20). The mixture was homogenized at 12,000 rpm for 30 s (T18 digital Ultra-Turrax, IKA, Germany) where after the suspension was centrifuged at 16,000g for 15 min at 4 °C to remove tissue debris. The supernatant was filtered through a 0.2-μm syringe filter (Whatman, Florham Park, NJ, USA) and used as 'LOX extract'. The substrate solution was prepared by mixing 157.2 μL linoleic acid, 157.2 μL Tween 20, and 5 mL MilliQ water. One mL of 1.0 M NaOH was slowly added into the substrate solution. Finally, the solution was diluted to 50 mL with same PBS buffer, giving a 10 mM final concentration of linoleic acid. Linoleic acid substrate (0.1 mL) was mixed with 0.8 mL of same PBS buffer. To initiate the reaction, 100 μL of LOX extract was added. If the reaction system was turbid and had precipitates after the LOX extract addition, this extract was diluted by a factor of 10–50. After incubation at 25 °C for 3 min, the absorbance at 234 nm was recorded at 0 and 5 min of reaction. One unit was defined as LOX causing an increase in absorbance at 234 nm of 0.001/min under the specified condition.

The herring samples were freeze-dried at −53 °C and 0.01 hPa pressure for 24 h by using a freeze dryer (Heto LyoPro 3000, Heto/Holten A & S, Allerød, Denmark). The dried samples were ground to powder using a coffee mill (2393 OBH Nordica, Stockholm, Sweden) for 30 s before detection of total iron and copper. The powder samples were subjected to acidic microwave digestion according to the method of Larsson et al. (2007). The digested samples were diluted properly and used for measurement of Fe and Cu by inductively coupled plasma mass spectrometry (ICP-MS) (Ek, Morrison, Lindberg, & Rauch, 2004). The nonheme iron (non-heme-Fe) was determined as the difference between total iron and heme iron.

2.9. Statistical analysis

All statistical analysis was conducted with SPSS software (IBM SPSS Statistics Version 22, IBM Inc., Chicago, USA). The results were reported as mean ± standard deviation (SD) ($n \geq 2$). Duncan's multiple range test was used to compare the means. Variance (ANOVA) was used to analyze the significant differences between treatments and/or storage points. Simple correlations between endogenous factors (e.g. pro-/antioxidants or lipid substrates) and lipid oxidation were evaluated by Pearson coefficients based on the data from all fractions (head, backbone, viscera + belly flap, tail, fillet) and two season (spring and fall). Lipid oxidation susceptibility was determined as the TBARS index, which was defined by the development rate (increase in TBARS/day) for storage points that had significantly higher TBARS values than the zero-time values. The threshold for significance for all tests was set at $p < 0.05$.

3. Results

3.1. Proximate composition

Table 1 shows the proximate composition of herring filleting co-products (backbone, head, viscera + belly flap, tail) and the fillet collected in April and October. Data are expressed on a wet weight basis. As expected, in all fractions, total lipid was substantially higher in fall than in spring samples, a difference that was most pronounced in the viscera + belly flap fraction (17.3 > 5.6%), followed by the fillet and the backbone fractions. In the fall, the most lipid-rich co-product fraction was the viscera + belly flap followed by fillet > head > backbone > tail. This reflected the heavy feeding of herring in summer/fall. In the spring, the head was most lipid-rich, followed by fillet > tail > viscera + belly flap > backbone. All fractions contained a substantial amount of protein (12.8–19.2%), and the protein amounts were similar in spring and fall samples. The most protein-rich fraction was the viscera + belly flap followed by backbone, which was similar to the fillet. The tail and head were ranked last. Ash content, which partly reflects the amounts of bones, i.e., calcium, was highest in the tail (up to 7.2%), followed by the head, backbone, viscera + belly flap, and then the fillet. Seasonal differences in ash, showing higher levels in fall, could possibly be related to higher bone density, or to a lower muscle-to-bone ratio. Overall, the compositional data revealed that all the co-product fractions were as valuable sources of lipids and protein, in many cases comparable to, or higher, than the fillet, which provided a strong incentive for further value addition.

3.2. Fatty acid composition

Table 2 shows the absolute content of total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) in the different fractions as well as their percent of total fatty acids, i.e., relative content. In all fractions, the content of SFA, MUFA, PUFA and LC n-3 PUFA was substantially higher in fall than in spring samples. Compared with other fractions, the most notable difference between fall and spring was in the viscera + belly flap. Here, SFA was 52 mg/g wet weight (ww) in fall and 13 mg/g in spring; MUFA was 31 vs. 9 mg/g; PUFA was 90 vs. 24 mg/g and LC n-3 PUFA was 84 vs. 23 mg/g,

Table 1
Proximate composition (g/100 g of wet tissue) of sorted herring filleting co-products and fillet from spring and fall[A].

Unit: g/100 g of wet tissue	Backbone		Head		Viscera + Belly flap		Tail		Fillet	
	Spring[A]	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Moisture	78.71 ± 0.02[a]	71.00 ± 0.21[d]	74.78 ± 0.08[c]	71.39 ± 0.13[d]	75.36 ± 0.14[c]	65.7 ± 0.65[f]	74.95 ± 0.35[c]	71.32 ± 0.29[d]	76.2 ± 0.72[b]	67.01 ± 0.34[e]
Dry matter	21.29 ± 0.02[f]	29.00 ± 0.21[c]	25.22 ± 0.08[d]	28.61 ± 0.13[c]	24.64 ± 0.14[d]	34.3 ± 0.65[a]	25.05 ± 0.35[d]	28.68 ± 0.29[c]	23.8 ± 0.72[e]	32.99 ± 0.34[b]
Protein	15.51 ± 0.05[c]	16.68 ± 0.18[b]	12.99 ± 0.40[de]	12.82 ± 0.81[e]	19.21 ± 0.33[a]	17.35 ± 0.25[b]	15.55 ± 0.17[c]	13.84 ± 0.15[d]	16.61 ± 0.15[b]	16.8 ± 0.64[b]
Ash	2.38 ± 0.16[de]	3.01 ± 0.05[d]	4.34 ± 0.05[c]	5.24 ± 0.08[b]	2.08 ± 0.02[e]	2.29 ± 0.09[de]	5.09 ± 0.24[b]	7.16 ± 0.02[a]	1.31 ± 0.02[f]	2.39 ± 0.98[de]
Total lipid	5.21 ± 0.42[d]	10.56 ± 0.69[c]	9.45 ± 0.21[c]	12.16 ± 0.13[b]	5.58 ± 0.03[d]	17.26 ± 1.31[a]	5.98 ± 0.02[d]	9.54 ± 0.10[c]	6.05 ± 0.18[d]	16.00 ± 0.92[a]

[A] Spring (2020–04-15), Fall (2020–10-21). Results are shown as mean ± SD (n = 2). Different small letters in each row show a significant difference ($p < 0.05$, Duncan's multiple range test).

Table 2
Content of saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acid (PUFA), and long chain n-3 PUFA (LC n-3 PUFA) in mg/g wet tissue and as % of total fatty acids of sorted herring filleting co-products and fillet from spring and fall[A].

	Backbone		Head		Viscera + Belly flap		Tail		Fillet	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
∑SFA	13.76 ± 0.69[f]	27.69 ± 1.39[e]	43.12 ± 3.94[c]	55.31 ± 3.56[a]	13.21 ± 0.19[f]	52.54 ± 4[ab]	16.33 ± 3.13[f]	47.53 ± 3.5[bc]	13.82 ± 1.18[f]	35.33 ± 4.12[d]
∑MUFA	13.63 ± 0.84 [cd]	13.97 ± 0.79 [cd]	28.22 ± 2.23[a]	30.55 ± 2.11[a]	8.52 ± 1.82[d]	30.76 ± 5.17[a]	15.63 ± 5.53 [cd]	24.67 ± 0.76[ab]	11.74 ± 1.78 [cd]	18.32 ± 3.74[bc]
∑PUFA	18.74 ± 0.66[ef]	41.32 ± 1.52[c]	13.13 ± 1.05[f]	21.8 ± 2.05[de]	24.07 ± 0.5[de]	90.06 ± 5.09[a]	24.9 ± 2.46[de]	27.79 ± 0.48[d]	23.71 ± 0.95[de]	60.54 ± 6.99[b]
∑LC n-3 PUFA	16.97 ± 0.53[d]	38.06 ± 1.33[c]	9.72 ± 0.87[e]	17.51 ± 2.15[d]	22.51 ± 0.49[d]	83.56 ± 4.64[a]	22.81 ± 2.05[d]	23.33 ± 0.78[d]	21.87 ± 0.98[d]	56.29 ± 6.42[b]
∑SFA %[B]	29.82 ± 0.08	33.36 ± 0.19	51.04 ± 0.31	51.35 ± 1.58	28.85 ± 1.12	30.29 ± 1.62	28.74 ± 0.11	47.51 ± 1.71	28.04 ± 0.16	30.89 ± 1.61
∑MUFA %	29.53 ± 0.42	16.83 ± 0.2	33.41 ± 0.22	28.36 ± 1.00	18.56 ± 3.51	17.78 ± 3.38	27.06 ± 4.44	24.67 ± 0.17	23.77 ± 1.72	16.18 ± 4.32
∑PUFA %	40.65 ± 0.50	49.81 ± 0.39	15.55 ± 0.09	20.29 ± 2.58	52.59 ± 2.39	51.93 ± 1.76	44.2 ± 4.33	27.82 ± 1.53	48.19 ± 1.88	52.93 ± 2.71
∑LC n-3 PUFA %	36.80 ± 0.60	45.87 ± 0.45	11.50 ± 0.05	16.30 ± 2.55	49.19 ± 2.27	48.18 ± 1.58	40.54 ± 4.32	23.36 ± 1.66	44.45 ± 1.54	49.22 ± 2.45

[A] Spring (2020–04-15), Fall (2020–10-21). Results are shown as mean ± SD (n = 2).
[B] Values are given as % of total fatty acids. Different small letters in each row show a significant difference ($p < 0.05$, Duncan's multiple range test).

respectively.

In the fall, the highest SFA-content was found in the head fraction (55 mg/g ww), followed by viscera + belly flap (52 mg/g ww) > tail > fillet > backbone (Table 2). Like SFA, the MUFA content was higher in head and viscera + belly flap compared with the other fractions. The PUFA and LC n-3 PUFA were highest in viscera + belly flap, followed by fillet > backbone > tail > head. Head lipids had a higher relative content of SFA (51% of total FA) and lower relative content of PUFA (15–20%) compared with the lipids of the other fractions, while viscera + belly flap had higher PUFA (51–52%) and lower SFA (28–30%). This means extraction of PUFA can be promising valorization approach for the viscera + belly flap.

3.3. Total Fe, hemoglobin, non-heme-Fe, and lipoxygenase

From reviewing the literature, heme proteins, non-heme-Fe, and lipoxygenases (LOX) are identified as the main endogenous prooxidants in fish muscle (Medina & Pazos, 2010; Undeland, 2016). Fig. 1A shows the total content of heme, expressed as hemoglobin (Hb) equivalents. The head had by far the highest values (95 and 100 μmol/kg mince), followed by, in decreasing order, viscera + belly flap, backbone, tail and fillet, the latter as low as 4.9 μmol/kg. In fillet and tail, there were slightly, but significantly ($p < 0.05$), lower Hb amounts in the fall compared with the spring samples. For total Fe, head had the highest amount (29 vs. 63 mg/kg) followed by viscera + belly flap > backbone > fillet ≈ tail (Fig. 1B). In spring compared with fall samples, a higher ($p < 0.05$) total Fe content was found in head, viscera + belly flap and fillet, but not in backbone and tail. This was confirmed also for samplings done in March and September (data not shown). The non-heme-Fe amount was higher in head compared with other fractions in spring, but in fall, the amount of non-heme-Fe in head was not significantly different from viscera + belly flap (Fig. 1C). The copper amount ranged from 0.23 to 0.79 mg/kg mince (Fig. 1D), which was lower than the total Fe amount (4.2–63 mg/kg). The viscera + belly flap and fillet contained the highest amount of copper, followed by tail, head and backbone. In head and viscera + belly flap, the fall samples had a higher ($p < 0.05$) amount of copper compared with spring. However, in tail, the spring showed a higher amount of copper. Fig. 2 shows that the head had at least 10 times higher LOX activity compared with other fractions and altogether both spring and fall samples were ranked as: viscera + belly flap > fillet ≈ backbone ≈ tail. However, in the head, spring samples had a higher LOX activity compared with the fall. Conversely, viscera + belly flap had higher LOX activity in fall. The backbone, fillet, and tail had low LOX activity in both spring and fall (Fig. 2).

3.4. Alpha, beta, delta, and gamma tocopherols

We analyzed four tocopherol isomers (α, β, γ, and δ) in all fractions from the two seasons before initiating the storage trials (Fig. 3). Only α-tocopherol was detected in the samples. In general, all fractions (except head) from spring showed a higher ($p < 0.05$) α-tocopherol amount compared with the fall samples. In the head samples, no

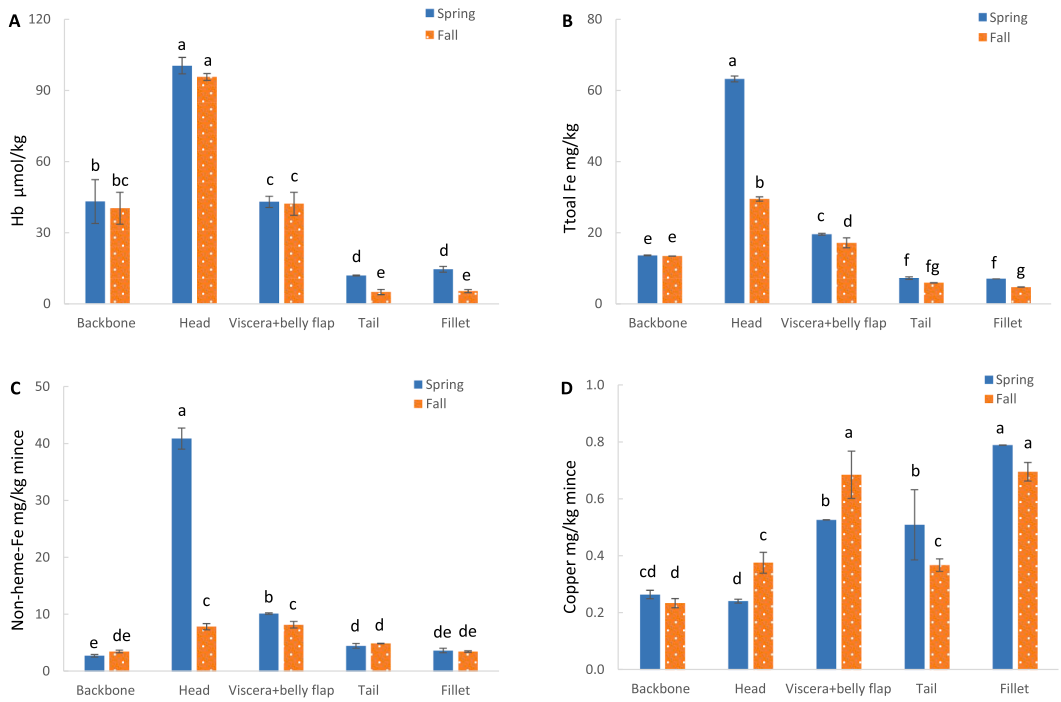

Fig. 1. Content of hemoglobin (A), total Fe (B), non-heme-Fe (C), and Cu (D) in wet tissue of sorted herring filleting co-products and fillets from two catches taking place April and October.

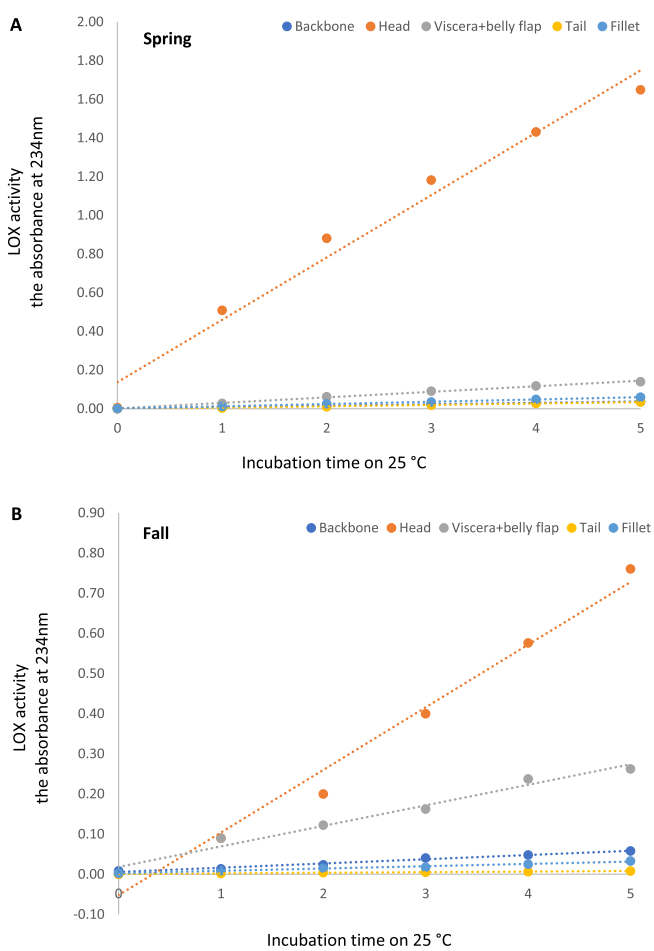

Fig. 2. Lipoxygenase (LOX) activity in wet tissue of sorted herring filleting co-products and fillet from spring (A) and fall (B).

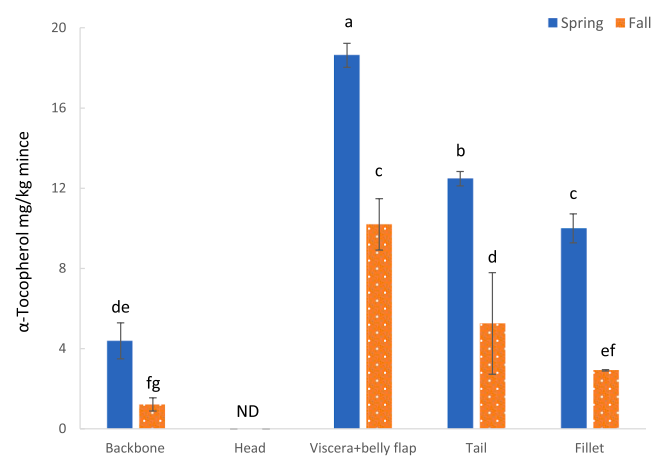

Fig. 3. α-Tocopherol contents in wet tissue of sorted herring filleting co-products and fillet from spring and fall.

α-tocopherol was detected in any of the seasons. In the spring samples, viscera + belly flap contained highest α-tocopherol (18.6 mg/kg), followed by tail (12.4 mg/kg), fillet (9.9 mg/kg), and backbone (4.3 mg/kg).

3.5. Lipid oxidation

The different fractions plus the skin-on fillets from the April and October samplings were minced and then stored on ice to investigate their susceptibility to lipid oxidation. Fig. 4A-D show the changes in PV and TBARS of all five fractions. Overall, all studied fractions showed a fast development of lipid oxidation, and combining all data, none of the samples had a longer lag phase than 1 day. For the spring samples, the fastest increase of PV was in head and the slowest increase was in viscera + belly flap (Fig. 4A); other fractions were ranked in between. The TBARS results showed the same trend as the PV for these samples

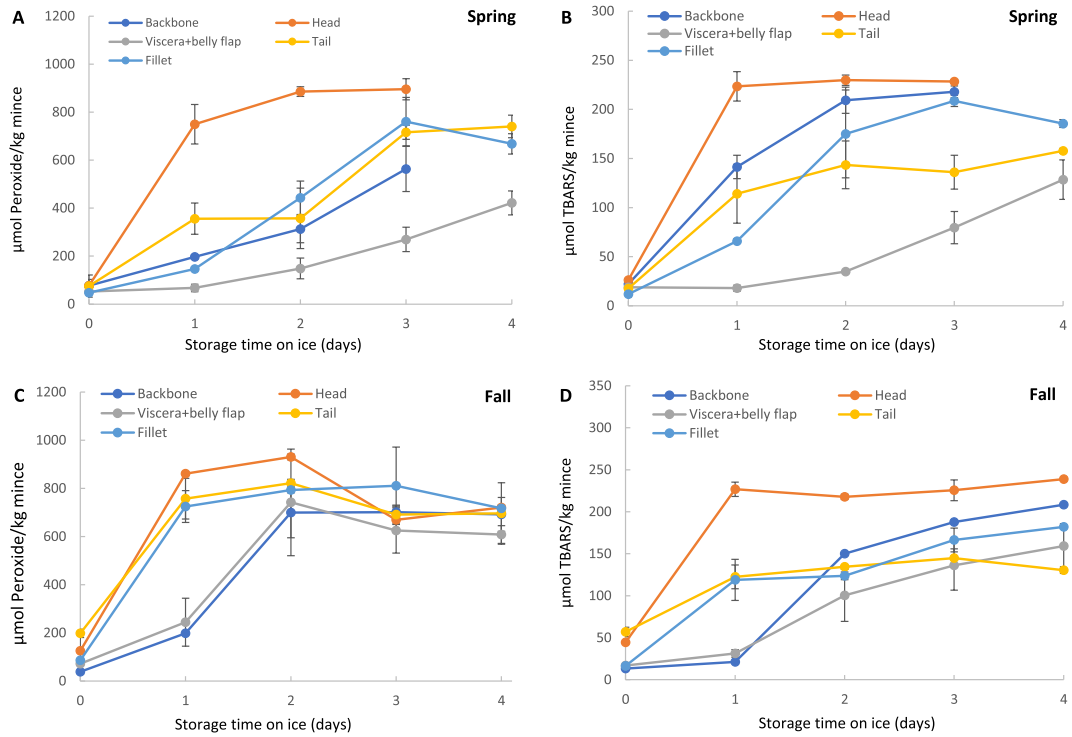

Fig. 4. Lipid oxidation TBARS and PV of sorted herring filleting co-products and fillet from spring (A, B) and fall (C, D) during ice storage.

(Fig. 4B). In fall samples, the PV development in the fillet and tail was equally fast as in the head; and the backbone as well as viscera + belly flap were more stable (Fig. 4C). However, the onset of TBARS for fillet and tail was slower ($p < 0.05$) than the onset for head. Moreover, the backbone and viscera + belly flap did not show a significant increase in TBARS until > day 1 (Fig. 4D).

4. Discussion

4.1. Variation in prooxidants, antioxidants, and lipid substrates in different fractions from the two seasons

We undertook this study to systematically investigate how lipid oxidation in different sub-fractions of herring caught during two seasons was affected by differences in prooxidants, antioxidants and lipid substrates. A very important compound to consider was hemoglobin (Hb), which in a long series of studies has been identified as a major catalyst for lipid oxidation in fish (Wu, Abdollahi, & Undeland, 2021). In this study, the fillet had a lower Hb amount compared with filleting co-products, except tail. These findings agreed with our previous studies in which Hb was 10.7 µmol/kg in herring fillet (Chaijan & Undeland, 2015), while mixed herring co-products contained 67.4 µmol/kg (Wu, Sajib, & Undeland, 2021). The result is attributed to the distribution of the main blood vessels of bony fish since Hb is located within erythrocytes (i.e., red blood cells). The main vessels in most cold-blooded fish run along the backbone and radiate outwards to the small vessels that supply oxygen to visceral organs and muscle. Moreover, fish acquire oxygen from the water through the gills (Brill & Bushnell, 2006), which explains why the head contains more blood vessels than other anatomical parts. That the content of total Fe showed an order similar to the total heme level was likely because heme iron is the main iron source in herring, which is in agreement with our previous studies (Abdollahi, Wu, & Undeland, 2021). The non-heme-Fe detected by subtracting heme–iron from total iron reflects iron bound to the Fe-storage protein ferritin as well as low molecular weight (LMW) iron chelated with organic phosphate esters (e.g NAD(P)H, AMP, ADP, and ATP), amino acids (e.g. histidine) or ascorbate since LMW-Fe is poorly soluble under physiological conditions (Hultin, 1994).

Besides Hb, LOX is another important catalyst for fish lipid oxidation (German, Chen, & Kinsella, 1985), especially in gill/blood and skin (Hsu & Pan, 1996). LOX-derived volatiles are important for the fresh aroma of fish, but their contribution to off-flavor generation during storage is not fully clarified (Medina & Pazos, 2010). Fu et al. (2009) however identified 2,4-heptadienal (*E, E*) development in silver carp mince specifically ascribed to LOX-derived oxidation, while hexanal and nonanal were ascribed to Hb-mediated lipid oxidation. In the present study, the ranking of LOX activity (head > viscera + belly flap > fillet ≈ backbone ≈ tail) agreed with the findings of Wang et al. (2012), who reported that LOX activity of grass carp gradually increased in the order skin and muscle < gill < viscera < blood. Based on our results, the head contained the most blood, as indicated by the highest Hb. However, the LOX activity did not correlate with the Hb amount in the sense that backbone and viscera + belly flap had almost the same amount of Hb, but the latter had significantly ($p < 0.05$) higher LOX activity than backbone. This difference may be due to that LOX is mainly located in leucocytes instead of erythrocytes. The fact that LOX has Fe in its active site (Figure S3) could explain the high non-heme-Fe levels in spring heads; these had significantly higher LOX-activity than fall heads. The low LOX-activity in skin-on herring fillets reveals that for herring, skin was not a significant LOX-contributor, as was seen for gray mullet (Hsu & Pan, 1996). The copper detected in our samples; primarily in fillet and viscera are mainly derived from ceruloplasmin. The level of copper (0.23–0.79 mg/kg) in sorted herring fractions is in agreement with that reported by Aidos et al. (2001), who reported 0.45 and 0.66 mg/kg for herring fillet and mixed by-products (heads, frames, skin, viscera, etc), respectively.

Being membrane-bound antioxidants, tocopherols are important for protection of phospholipids from damage caused by free radicals formed e.g. during oxygen reduction in vivo and PUFA oxidation post mortem (Undeland, 2016). We detected only the α-isomer in the herring samples, which agreed with Syväoja et al. (1985) detecting only α-tocopherol in herring fillet and roe. Other investigators have made similar

observations in many other fish species, e.g., horse mackerel, flounder, sea bass, sea bream, and filefish (Matsushita, Inoue, & Tanaka, 2010). That viscera + belly flap had the highest α-tocopherol amount and head the lowest agreed with Cowey et al. (1981), who reported that the amounts of α-tocopherol in liver and kidney were 103 and 71 times greater, respectively, than in head of rainbow trout (*Salmo gairdneri*). The liver is the primary organ that accumulates α-tocopherol as a "delivery center" to maintain the α-tocopherol status of other organs and tissues (Hamre, 2011). In viscera + belly flap, fillet, backbone and tail, α-tocopherol levels were higher in spring samples than in fall samples. Similar findings were reported by Syväoja et al. (1985) who investigated α-tocopherol levels in 12 different marine fish (including herring) caught in spring and fall and found that, in almost all species, spring-caught fish had higher tocopherol than fall-caught fish. A reason outlined for this is that most fish spawn in the spring, and the tocopherol levels in fish reached a peak during the spawning season (Syväoja et al., 1985).

Regarding lipid substrates, the head had a higher total lipid content compared with the other four fractions in spring, most likely because brain contains a substantial amount of lipid. Hong et al. (2014) reported that fish brain contained 48–65% lipid (by wet weight), depending on species, habitat, and food consumed. The total lipid content of blue mackerel head (12.34%) was higher than in other fractions such as white muscle (1.3%), liver (9.4%), and viscera (8.1%) (Ahmmed et al., 2021). Similarly, it has been reported that the head has higher amount of lipid compared to the roe and skin in salmon (Ahmmed, Carne, Ahmmed, Stewart, Tian, & Bekhit, 2021) and in gurnard and snapper (Ahmmed, Carne, Bunga, Tian, & Bekhit, 2021). Samples from fall had a higher lipid content compared with spring samples, which was especially true for viscera + belly flap, rendering this fraction more lipid-rich than the head. Our previous study showed a similar finding that fall herring fillets without skin had a higher lipid content than those from spring (7.5% > 1.5% on wet weight basis) (Larsson, Almgren, & Undeland, 2007). The large change in lipid content of herring is attributed to the fact that herring has a higher rate of energy depletion during the spawning migration compared with the stationary fall period. In addition, herring do not feed during the spawning season, but, during the summer and fall, in order to store energy. The fatty acid analyses revealed that the fractions of herring having the highest PUFA content, both on an absolute and relative basis, were the viscera + belly flap and fillet fractions. Here, around 50% of all fatty acids were PUFA; the absolute values indeed peaking in fall along with the higher total lipid content. The most saturated lipids were found in the head (around 50% of total fatty acids). This is in agreement with Abiona et al. (2021), who reported the contents of saturated fatty acids from gills and head of cat fish (*Claris macrocephalus*) and Titus fish (*Scomber scombrus*) were 40–45% of total fatty acids.

4.2. Lipid oxidation in the different herring fractions

The high speed by which all the five fractions from both batches oxidized during ice storage was in agreement with our previous results for mixed herring co-products which showed an oxidation lag phase of only 1 day, followed by a rapid raise in PV/TBARS (Wu, Ghirmai, & Undeland, 2020). From our present data, especially TBARS, it thus appears as if the high oxidation rate of minced mixed herring co-products is largely dominated by the presence of heads, which contributed to around 25% of the co-product weight. PV-data were however a little less conclusive, revealing equal oxidation rates for head, tail and fillet in the fall batch. That the viscera + belly flap fraction was most stable, a ranking it shared with backbone in the fall sampling, was somewhat surprising given the high Hb and PUFA-content, something which is further discussed below. It was also interesting to notify that the co-product fractions, despite their complex nature, in some cases were still more stable than the fillet, i.e. the current main product from the production. This reveals that at least from an oxidation perspective, these other parts are not more challenging to use for food production than the fillet. It is clear, however, that all the fractions from the herring would benefit from antioxidant protection to increase their oxidation lag phase beyond 1 day.

4.3. Relationship between endogenous factors and lipid oxidation

As stated initially, lipid oxidation in fish depends on many endogenous factors, e.g., the lipid substrates (lipid level and composition), prooxidants (Hb, LOX, non-heme-Fe), and antioxidants (e.g. tocopherols) (Undeland, 2016). To clarify which were the most crucial endogenous factors for oxidation of the different herring fractions, the zero-time compositional features and the storage-induced oxidation of April and October samples were subjected to Pearson correlation analysis.

In the Supplementary material, Table S1 shows that Hb levels had a significant ($p < 0.05$) positive correlation with the lipid oxidation susceptibility as measured by TBARS index. The high Hb content in head may thus partly explain the greater lipid oxidation rate compared with other fractions. Similarly, Hb levels in herring, cod and salmon backbones ranked them as: herring > cod > salmon, which was the same ranking as was found for lipid oxidation lag phase and rate (Wu, Abdollahi, & Undeland, 2021). This relationship also agreed with our previous study in which doubling the Hb concentration in a washed cod muscle mince system increased the rancid odor intensity by ~ 30% and approximately doubled the maximum intensity of TBARS (Undeland, Hultin, & Richards, 2002). Besides Hb, the LOX activity also displayed a significant ($p < 0.05$) positive correlation with lipid oxidation susceptibility, contributing to the explanation why head, with the highest LOX activity, had the fastest rate of lipid oxidation. However, it is still difficult based on our data to determine whether Hb or LOX is the dominant prooxidant in herring fractions during ice storage, and such distinction would require deeper analyses e.g. of specific oxidation products (Fu, Xu, & Wang, 2009).

The two factors promote oxidation by different mechanisms. Firstly, Hb or rather liberated heme, acts primarily as a pro-oxidant by cleaving pre-formed hydroperoxides (Grunwald & Richards, 2006), something which also explain the significant correlation between TBARS and zero-time PVs (Table S1). LOX on the other hand acts by aiding the addition of oxygen directly to the fatty acid via a cycloaddition mechanism, thus, without the formation of diene structures (German, Chen, & Kinsella, 1985). Thus, different hydroperoxides, and subsequently volatiles form via the two pathways. Also, LOX is an enzyme, which Hb strictly is not, although it has been ascribed pseudo-LOX activity. Thus, their content or activity cannot be compared in the same manner. Second, Hb can be degraded during lipid oxidation, which is not described for LOX. For example, Grunwald and Richards (2006) reported that Hb (Protoporphyrin IX) degradation was prevalent during Hb-mediated lipid oxidation in washed cod muscle while the LOX activities of Nile tilapia skin increased throughout 18 days of ice storage (Sae-Leaw et al., 2013).

In the Pearson correlation results, we did not observe any significant ($p > 0.05$) correlation between non-heme-Fe or Cu with the lipid oxidation susceptibility (Table S1), indicating that these compounds were less important in herring lipid oxidation. This conclusion agreed with our previous study showing that Fe^{2+}-ADP did not induce oxidation of washed minced cod (Undeland, Hultin, & Richards, 2002).

The Pearson correlation coefficients showed that α-tocopherol had a significant ($p < 0.05$) negative correlation with lipid oxidation (Table S1), a finding that further reinforces why head, lacking α-tocopherol, was most susceptible to lipid oxidation and why viscera + belly flap, with highest α-tocopherol, was more stable (Fig. 4). Similarly, our previous study of cod, herring and salmon backbones showed that high amounts of α-tocopherol correlated significantly with their stability towards oxidation (Wu, Abdollahi, & Undeland, 2021). That the head fraction was deficient in α-tocopherol was in line with the report by Aidos et al. (2002), who did not detect α-tocopherol in herring (*Clupea*

harengus) head. The mechanism of tocopherol prevention of lipid oxidation is likely based on its location in the interior of membranes and its capacity to donate two electrons to lipid radicals as a chain-breaking antioxidant before being recycled (Syväoja et al., 1985).

An important notification was that the lipid oxidation rate did not correlate significantly with the quantity of lipid substrate, i.e., total lipids, MUFA, PUFA or LC n-3 PUFA. For example, the viscera + belly flap from October showed the slowest lipid oxidation rate although this sample contained the highest amounts of total lipids and PUFA. The generally accepted view is that rapid oxidation in muscle food is attributed to the total lipid content, or the content of PUFA (Hultin, 1994). However, also our other recent study provided evidence contradicting this common view. For example, added phospholipids with polyenoic indexes of 282 and 24 activated myoglobin as an oxidant similarly in washed pig muscle during ice storage (Wu, Xiao, Yin, Zhang, & Richards, 2021). Also, Undeland et al. (2002) found the same rapid development of Hb-mediated TBARS and painty odor in a washed cod mince model system (0.7% lipids) without or with up to 15% menhaden oil added. Without Hb, development of TBARS and painty odor was slow in the washed cod mince, even with 15% added oil. These results thus indicated that type and quantity of pro-oxidants are far more critical for onset of lipid oxidation in fish muscle than lipid content and degree of substrate unsaturation.

5. Conclusion

This study revealed a high susceptibility of all the five sorted herring fractions to lipid oxidation during ice storage, but interestingly, some of the co-product fractions emerging from herring filleting were more stable than the actual fillets. Overall, the greatest lipid oxidation rate was found in heads, and the slowest rate in viscera + belly flap. Large variations in Hb, LOX, α-tocopherol and zero-time PV as a function of fraction and season were found, and based on correlation analyses, these four features largely explained the different lipid oxidation rates. On the other hand, the amount of lipid substrate (e.g., total lipid, MUFA, PUFA and LC n-3 PUFA) did not dictate the lipid oxidation rates in the different fractions and from spring and fall herring. It is thus clear that sorting herring filleting co-products to reach a broadened food production from this species, going beyond just the classic fillet, is advantageous to avoid that the most pro-oxidant-rich parts "contaminate" the more stable ones. For all parts, or combinations of them, it is however our recommendation to apply cost-effective antioxidant strategies tailor-made to inhibit Hb- and LOX-mediated oxidation, to reach a more robust food production chain with minimized losses.

CRediT authorship contribution statement

Haizhou Wu: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Bita Forghani:** Conceptualization, Writing – review & editing. **Mehdi Abdollahi:** Conceptualization, 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.2021.131523.

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