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Controlling hemoglobin-mediated lipid oxidation in herring (*Clupea harengus*) co-products via incubation or dipping in a recyclable antioxidant solution

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ABSTRACT

Applying value-adding techniques to fish co-products is rendered difficult due to their high susceptibility to hemoglobin (Hb)-mediated lipid oxidation. In this study, we investigated a dipping technology with a solution containing Duralox MANC 213- a mixture of rosemary extract, ascorbic acid, tocopherols and citric acid – to control lipid oxidation during storage at 0 °C and 20 °C. The possibilities to re-use the antioxidant solution was also analyzed, along with studies on the link between Duralox MANC and Hb-form. Dipping in Duralox MANC largely increased the oxidation lag phase; from <0.5 to >3.5 d at 20 °C, and from <1 d to >11 d at 0 °C. Even after re-use of the solution up to 10 times, lipid oxidation was completely inhibited at 0 °C. Duralox MANC could prevent auto-oxidation and hemin loss of herring Hb; which are suggested as the main mechanisms behind the observed stabilization of herring co-products against lipid oxidation.

1. Introduction

The demand for high value proteins is increasing every year due e.g. to population growth (FAO, 2014) and a recognition of the important role of protein for a healthy ageing (Beasley, Shikany, & Thomson, 2013). Co-products emerging in fish filleting operations offer large amounts of under-utilized muscle protein which could contribute to the growing food protein demand (Šližytė, Carvajal, Mozuraityte, Aursand, & Storrø, 2014). The residual fish muscle also contain high amounts of long chained (LC) n-3 polyunsaturated fatty acids (PUFAs), which gives this protein source an added value. Several innovative techniques, all applicable on fish co-products, are available today to separate muscle proteins from e.g. bones and skin. Examples are the pH-shift process, classic meat-bone separation, and enzymatic or non-enzymatic hydrolysis (Rustad, Storrø, & Slizyte, 2011; Sajib, Albers, Langeland, & Undeland, 2020). However, applying such techniques on fish co-products is rendered difficult by their high susceptibility to lipid oxidation (rancidity), strongly limiting the possible window of time from co-product generation to subsequent value-adding processes (Wu, Ghirmai, & Undeland, 2020). Stabilizing strategies for fish co-products are thus needed to allow for a certain holding time and/or transport time prior to processing, and, to allow the final quality of the

end-products to be as high as possible. Without stabilizing strategies, the chances of the co-products to stay within the food chain are limited, and their fates is rather animal feed or even waste.

Hb in blood is an effective lipid oxidation catalyst, often limiting the shelf life of fish (Harrysson, Swolin, Axelsson, & Undeland, 2020). Therefore, exsanguination is often applied within the fish industry to medium and large-sized species. Richards and Hultin (2002) found that bleeding significantly reduced lipid oxidation and rancid odour in minced trout muscle stored at 2 °C. The same group revealed that washed mackerel fillets oxidized and deteriorated more slowly compared to unwashed fillets, which was ascribed removal of blood from the fillet surface (Richards, Kelleher, & Hultin, 1998). However, for small species such as herring and sardines, active bleeding is not applied due to the large quantities that are handled at the same time and their low value (Sannaveerappa, Cai, Richards, & Undeland, 2014). Significant amounts of blood can therefore be released, e.g. during filleting of such species (Rustad et al., 2011), contaminating both fillets and co-products. Due to the rich capillarization of guts, backbone and head/gills, blood-contamination is even greater in the co-products than the fillets. In our recent study (Wu, Ghirmai, et al., 2020), we hypothesized that removing or diluting such residual blood from the surface would stabilize herring (*Clupea harengus)* co-products. However, we

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found that only 10-18% Hb was removed after rinsing in water or 0.9% NaCl, and such removal had none, or a very limited effect on the oxidation lag phase during subsequent ice storage. In agreement with some other previous studies (Sveinsdóttir et al., 2020; Tseng, Xiong, & Webster, 2005), we however observed that treating the surface with antioxidants was an effective method to stabilize fish muscle against lipid oxidation. A short incubation of herring co-products in 5 vol solution made from the commercial antioxidant Duralox MANC (a mixture of rosemary extract, citric acid, ascorbic acid and tocopherol) in fact extended the oxidation lag phase from <1 d to >12 d (Wu, Ghirmai, et al., 2020)! These very promising findings would however imply a relatively high cost; and a cheaper more scalable procedure is needed for industry to invest in stabilization technology for low value material as fish co-products. Further, it was not clarified how the antioxidant mixture specifically affected Hb as a pro-oxidant. It is well established that autoxidation and heme-loss are two crucial events to induce Hb-mediated oxidation of muscle (Aranda IV et al., 2009); and knowledge on how to inhibit these reactions would be of great relevance.

The aims of the present study were to investigate: (i) the effect of incubating herring co-products with Duralox MANC on lipid oxidation at both ambient and cold temperature (i.e. 20 °C versus 0 °C), (ii) how different ratios of antioxidant solution to co-products, different incubation times, and re-use of the antioxidant solution for repeated dipping treatments would affect lipid oxidation, (iii) how iso-ascorbic acid, compared to the Duralox MANC mixture, works as an antioxidant and (iv), which effect Duralox MANC has on auto-oxidation and hemin loss of herring Hb.

2. Materials and methods

2.1. Fish sample preparation

Herring (*Clupea harengus*) was caught off from the west coast of Sweden in October and December of 2019. Fresh filleting co-products (head, backbone, caudal fin, skin, intestines and, at some occasions, roe) were provided by a company located at the Swedish West coast (Scandic Pelagic Ellös AB, Sweden). The time elapsing between filleting of the herring, and receival of the co-products was 2–6 h. During the transportation, the co-products were stored in a plastic bag covered by ice. The herring co-products used contained 72.3–74.5% moisture, 14.0–14.7% protein, 9.9–11.3% total lipid, and 2.3–2.4% ash (Supplementary Table 2)

2.2. Preparation of treatment solutions

The treatment solutions used in incubations/dipping trials were 1) 0.9% NaCl (Sigma Chemical Co., St. Louis, MO), 2) 5% Duralox MANC-213 (Kalsec, Kalamazoo, Mich., UK) in 0.9% NaCl solution, 3) 2% Duralox MANC-213 in 0.9% NaCl solution, and 4) 2% iso-ascorbic acid (Sigma Chemical Co., St. Louis, MO) in 0.9% NaCl solution. The 0.9% NaCl solution were freshly prepared with pre-cooled (4~6 °C) tap water.

2.3. Incubation or dipping of herring co-products with different solutions

Fig. 1. show the main steps involved in the whole procedure of incubating or dipping herring co-products in 0.9% NaCl or antioxidant solutions. In the incubation trials, the fresh co-products were incubated in 0.9% NaCl, 5% Duralox MANC-213 in 0.9% NaCl, and 2% Duralox MANC-213 in 0.9% NaCl (1:5 wt/volume) for 20 min in a cold room (4~6 °C.) In the dipping trials, herring co-products were dipped in 2% Duralox MANC-213 in 0.9% NaCl and 2% iso-ascorbic acid in 0.9% NaCl (1:3 wt/volume) for 10 s in a cold room (4~6 °C); the dipping solutions were re-used up to 10 times. In both trials, the herring co-products were thereafter drained well for 6-8 s in a fine stainless-steel strainer and then immediately ground using a meat grinder with a 4.5 mm hole plate (C/E22 N, Minerva Omega group, Italy). Control co-products were ground without any incubation or dipping. The pH of all minces was recorded using a pH-meter (PHM210 Radiometer Analytical S.A., Villeurbanne Cedex, France) as this factor strongly controls Hb-mediated lipid oxidation (Undeland, Kristinsson, & Hultin, 2004). After manually stirring in 200 ppm streptomycin, 25g of each mince were transferred into 250 mL screw-capped Erlenmeyer flask. The process of storage and
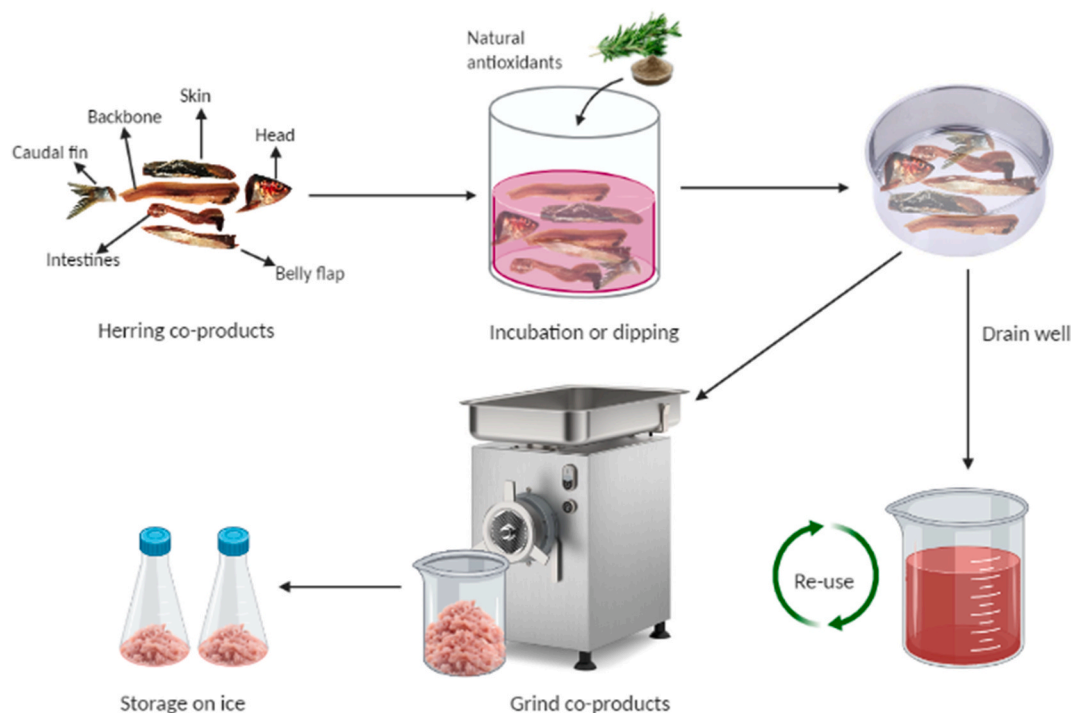

Fig. 1. Flowchart of the main steps involved in the procedure of incubation/dipping and storage of herring co-products. The picture of herring co-products was taken from Sajib et al. (2020).

sampling was conducted according to the method of Wu, Ghirmai, et al. (2020).

2.4. Total heme pigment measurement

The herring co-product minces were processed according to the method of Wu, Ghirmai, et al. (2020) using liquid nitrogen. Total heme content was then measured using the acetone-based method of Wu et al. (2020). Bovine Hb was used to construct a standard curve, and results were expressed as µmol Hb/kg mince.

2.5. Analyses of lipid oxidation

Total lipids were extracted from 1-g samples of co-product mince using chloroform:methanol (2:1) (Cavonius & Undeland, 2017). The lower phase (chloroform) was collected for peroxide value (PV) analysis as described by (Larsson, Almgren, & Undeland, 2007). The upper phase (water–methanol) was used to determine TBA-reactive substances (TBARS) according to the method of Wu, Xiao, Yin, Zhang, and Richards (2021). Results are expressed as µmol peroxides or µmol TBARS/kg mince.

2.6. Preparation of hemolysate

Herring blood was obtained as described by Ghirmai, Eriksson, Wu, Axelsson, and Undeland (2020), and hemolysate was prepared from the blood mixed with 1 vol of anticoagulant (sodium heparin) according to Fyhn et al. (1979) by washing the red blood cells four times in 1 mM Tris (pH 8) containing 0.9% NaCl and then lysing them in 1 mM Tris (pH 8). The method of quantify the Hb levels in hemolysate was described by Wu, Yin, Zhang, and Richards (2017).

2.7. Determination of auto-oxidation and hemin loss of herring Hb

Hb (5 µM) was incubated with or without Duralox MANC (0.5 g/L) in a sodium phosphate buffer (50 mM, pH 6.3) and stored at 4 °C. The pH was selected based on fish post-mortem pH (6.3–6.9). The percentage of methemoglobin was calculated according to the equations of Benesch, Benesch, and Yung (1973). The metHb was prepared as described previously (Wu et al., 2017). The hemin loss from metHb was measured according to the method described by Maestre, Pazos, and Medina (2009).

2.8. Statistics

In each experiment, minced samples from all treatments were stored in duplicate Erlenmeyer flask (n = 2). PV, TBARS and total Hb are reported as mean ± maximum-minimum value/2 from these duplicate samples (Larsson, Harrysson, Havenaar, Alminger, & Undeland, 2016). An unpaired *t*-test or ANOVA with Tukey's HSD test was used to determine significant differences between the different samples at a specific storage point, or, between different time points for a specific sample. Differences are regarded as significant when $p < 0.05$.

To investigate how different raw material batches influenced some of our main conclusions, key samples like the un-treated controls and the co-products incubated or dipped in 0.9% NaCl with or without 2% or 5% Duralox MANC, were included in 8 and 4 separate experiments, respectively. Data are shown in Supplementary information, Fig. 1, and illustrate that conclusions were the same, regardless of raw material batch.

3. Results and discussion

3.1. Effect of incubating herring co-products in antioxidant-solutions on moisture content, pH and Hb level

To reduce lipid oxidation induced by Hb in herring co-products, we incubated or dipped them in physiological saline, with or without Duralox MANC or isoascorbic acid, for removing blood and to cover surface tissue with antioxidants. The choice of saline was based on our recent findings showing that herring erythrocytes stayed intact for significantly longer time in 0.9% NaCl compared to in distilled water or 3% NaCl solution (Ghirmai et al., 2020). Table 1 shows that the moisture content of the herring co-products without incubation was 70.12%. This agreed with our previous study, which showed that the moisture content of mince from herring fillets obtained in the fall was 70.8% (Larsson et al., 2007). Although the incubation process slightly increased the moisture content of herring co-products up to 72.82–74.43%, we did not find a significant difference ($p > 0.05$) between incubated and non-incubated co-products. The pH was slightly reduced ($p < 0.05$) by the incubation in Duralox MANC, which, based on previous findings, could counteract the inhibitory effect from this antioxidant since acidification can accelerate Hb-mediated oxidation (Undeland et al., 2004).

The concentration of Hb in herring co-products without incubation was 67.44 µM/kg (Table 1). This Hb level was higher than the levels found in the light and dark muscle of a herring fillet, 10.7 and 28.6 µmol/kg, respectively (Chaijan & Undeland, 2015), and reflects the abundance of blood capillaries in the different co-products parts, e.g. along the back bone, in gills and along the gut/gonads (Bushnell & Brill, 1992). Moreover, Table 1 shows that the Hb concentrations in the incubated groups were from 57.28 to 59.99 µM/kg and were thus significantly lower ($p < 0.05$) compared with the non-incubated group. On a relative basis, the amount of Hb removed during incubation was however limited, from 11.05% to 15.07%. This indicated that a large proportion of the residual blood was situated in the interior rather than on the surface of the co-products, reflecting the nature of the capillarization described above. It should however also be stressed that during the actual filleting operation, there is a built-in rinsing with tap water, which will constitute an initial removal of surface blood from both fillets and co-products.

3.2. Effect of incubating herring co-products in antioxidant solutions on lipid oxidation as a function of storage temperature

Fig. 2 shows that there was no significant difference in lipid oxidation based on PV and TBARS values between non-incubated co-products and co-products incubated in 0.9% NaCl; both these samples oxidized within 1 d on ice. This result indicated that the incubation process *per se*

Table 1
The effect of incubation[a] on moisture, pH, total Hb level[b] and Hb-removal of herring co-products.

Treatment	Moisture %	pH	Total Hb µM/kg	Hb Removal (%)
No rinsing	70.12 ± 1.56[a]	6.72 ± 0.025[a]	67.44 ± 2.17[a]	-
0.9% NaCl	73.43 ± 0.53[a]	6.78 ± 0.020[a]	58.02 ± 1.50[b]	13.97
5% MANC in 0.9% NaCl	74.43 ± 1.28[a]	6.61 ± 0.015[b]	57.28 ± 3.44[b]	15.07
2% MANC in 0.9% NaCl	72.82 ± 0.94[a]	6.64 ± 0.010[b]	59.99 ± 2.50[b]	11.05

[a] The ratio was 5:1 for solution/co-products (volume/weight). The incubation lasted for 20 min in 4 °C under gentle stirring.

[b] The unit of total Hb is µmol/kg herring co-products. Results are expressed as mean ± maximum-minimum value/2 from duplicate samples. Means bearing different designations (a, b) in a column differ significantly ($p < 0.05$).
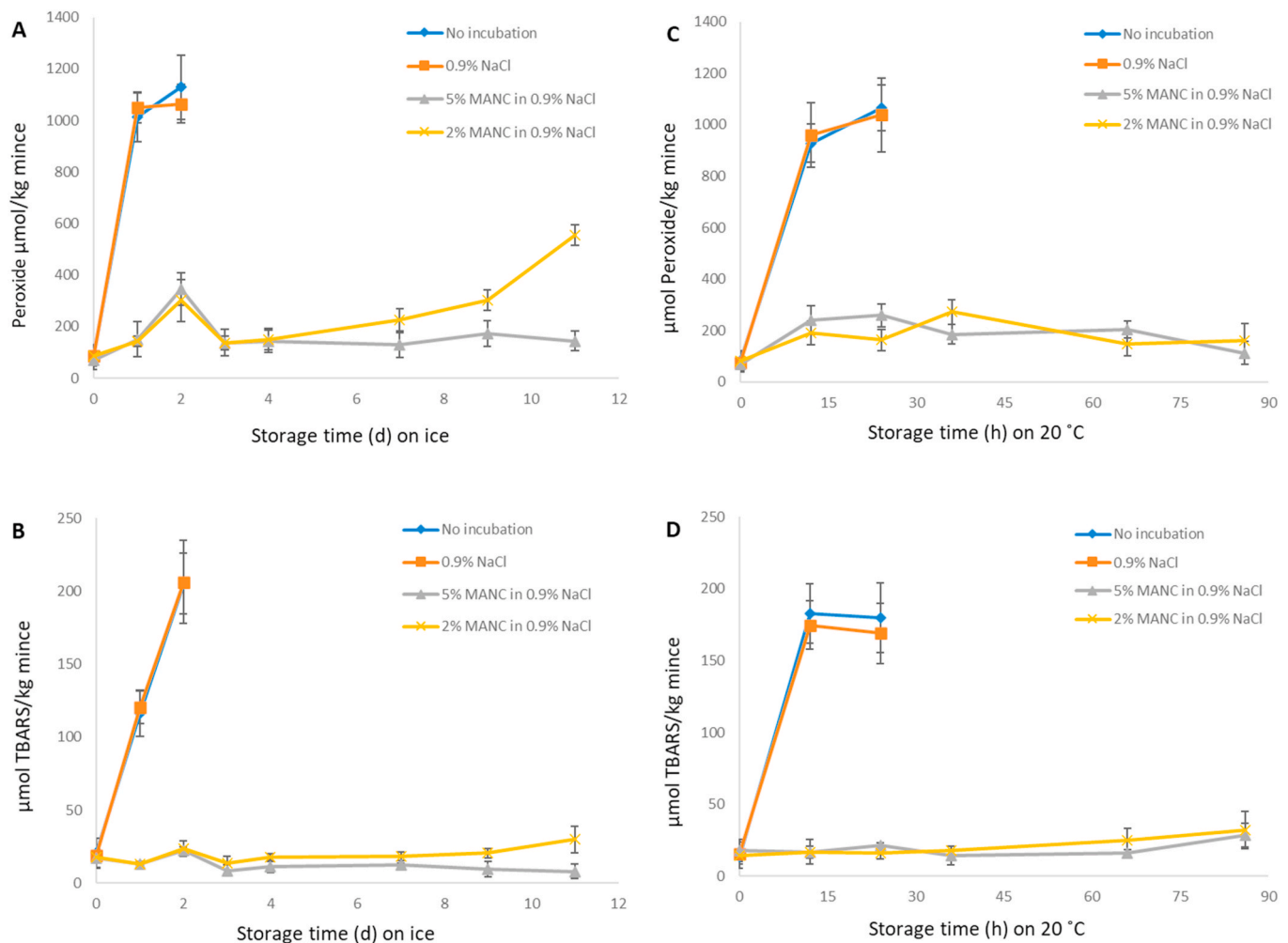
Fig. 2. Lipid oxidation measured as (A, C) lipid hydroperoxides (peroxide value, PV) and (B, D) TBA-reactive substances (TBARS) during storage of a mince made from herring co-products with or without pre-incubation in 0.9% salt solutions $\pm$ 2 or 5% Duralox-MANC. The ratio of herring co-products to solution was 1:5 wt/volume. The incubation time was 20 min.

did not affect lipid oxidation during storage, which may be attributed to the low Hb removal (Table 1) from the herring co-products. Richards et al. (1998) found that filleting very fresh mackerel under water vs. filleting it in air was effective in inhibiting lipid oxidation (PV and TBARS) during subsequent frozen storage. However, this study only addressed the surface tissue of the fillets, where as much as 90% heme was removed in the sub-merged filleting operation.

Fig. 2 also shows the difference in PV and TBARS kinetics depending on the storage temperature (20 °C versus 0 °C, i.e., on ice). At 20 °C, the rate of PV and TBARS development for the control group without rinsing levelled off after 12 h and then slightly increased (PV) or decreased (TBARS) (Fig. 2C and 2D). However, on ice, the PV rate for the control group without incubation levelled off after 24 h while TBARS continuously increased during 48 h of storage (Fig. 2A and 2B). These results indicated that the higher temperature greatly accelerated lipid oxidation of herring co-products, which is of industrial relevance as fish co-products are often stored without cooling, e.g., in boxes outdoors. The temperature effect could be due to a general increase in chemical re-action rates with elevated temperature (Richards, 2010), but also more specifically to the faster auto-oxidation rate and hemin loss of Hb during 20 °C storage compared with 0 °C on ice. Wilson et al. (1987) found that increasing the temperature in the range 14–40 °C causes rapid conversion of Hb from the ferrous to the met state, which accelerates hemin dissociation from Hbs; apoglobins normally have a 60-fold lower affinity for hemin (Fe^{3+}) than for heme (Fe^{2+}) (Aranda IV et al., 2009).

Previously, we postulated that hemin loss was a key step in the mechanism by which heme proteins oxidize a lipid substrates (Wu et al., 2017). Moreover, the rapid degradation of endogenous antioxidants in the herring co-products at 20 °C compared to 0 °C may be another reason for the faster lipid oxidation rate at this temperature. Passi, Cataudella, Tiano, and Littarru (2005) found that the degradation of endogenous ubiquinol and vitamin C was slower at −80 °C than −30 °C for ten species of Mediterranean fish.

To compensate for the limited Hb removal from the co-products during the incubation in 5 vol of 0.9% NaCl, we fortified this solution with the commercial antioxidant Duralox MANC-213. Our previous study on herring co-products (Wu, Ghirmai, et al., 2020) showed excellent shelf life extension during ice storage after rinsing in a 5% solution of this antioxidant, which is a mixture of rosemary extract, ascorbic acid, tocopherols and citric acid. Fig. 2 A and B confirmed these results, and panels C and D also show that the PVs and TBARS of antioxidant-incubated samples were not significantly different (p < 0.05) between zero-time samples, and all subsequent sampling points even at 20 °C. Fig. 2 further shows that it was possible to reduce the level of Duralox MANC to 2% with maintained effectiveness, although associated with a slight but significant (p < 0.05) increase in lipid hydroperoxides after 11 d of ice storage (Fig. 2A). Therefore, the appropriate amount of Duralox MANC (5% or 2%) to use will depend on the expected storage time and temperature that the herring co-products will be subjected to prior to further value-adding. However, altogether the data

show that the residues of Duralox MANC penetrating the tissue or staying on the surface after the incubation treatment was an effective inhibitor of lipid oxidation.

3.3. Effect of dipping herring co-products in antioxidant solutions without and with recycling of solutions

As we found that the incubation process removed only a limited amount of Hb, and since it only inhibited lipid oxidation of herring co-products in the presence of antioxidants, the incubation treatment was from here and on regarded more as an *application* process than a washing process. Thus, its role was primarily to cover the surface of the co-products with antioxidants. To improve processing efficiency and thereby reduce costs, we evaluated a shortening of treatment time from 20 min to 10 s, a reduction in the ratio of herring co-products to treatment solution from 1:5 to 1:3 (weight/volume) and the possibilities to re-use the antioxidant solution for repeated dipping treatments. Further, we evaluated a replacement of the more expensive Duralox MANC with the cheaper isoascorbic acid.

Fig. 3A and 3B show that there was a nearly complete inhibition of lipid oxidation during ice storage, as measured by PVs and TBARS, also
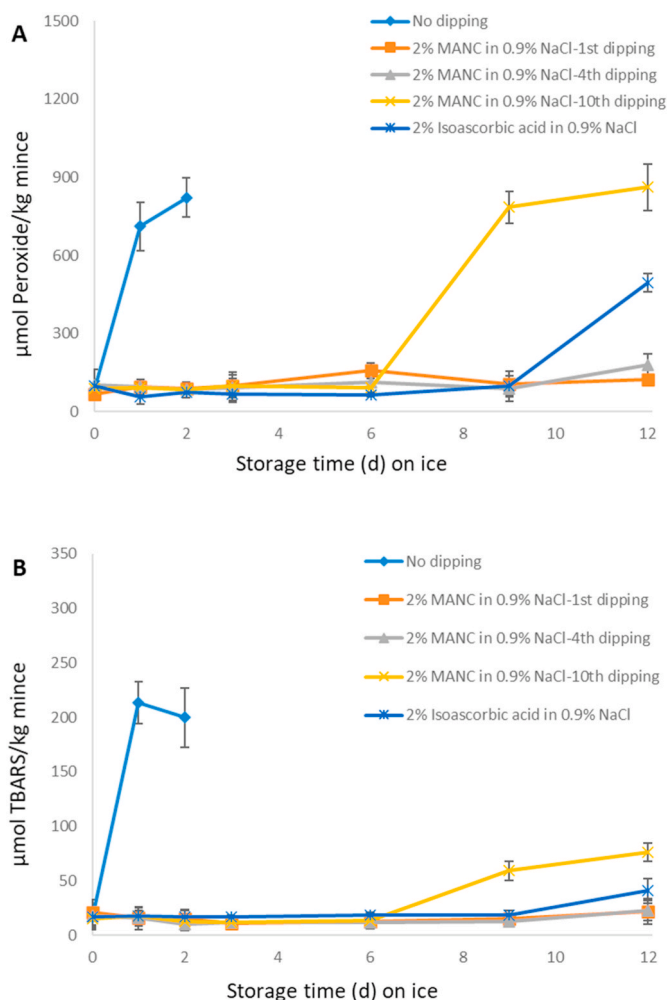
Fig. 3. Lipid oxidation measured as (A) lipid hydroperoxides (peroxide value, PV) and (B) TBA-reactive substances (TBARS) during ice storage of mince made from herring co-products that had been pre-dipped in 0.9% salt solutions ± 2% Duralox MANC or iso-ascorbic acid. The Duralox MANC solution was re-used up to 10 times and samples were taken after the 1st, 4th and 10th dipping cycle. The ratio of herring co-products to solution was 1:3 wt/volume. The dipping time was 10 s.

when the antioxidant treatment was reduced from 20 min incubation to 10 s dipping in 2% Duralox MANC. In addition, the dipping solution with 2% Duralox MANC at fourth re-use nearly completely inhibited lipid oxidation as measured by PVs and TBARS throughout 12 d storage (Fig. 3A and 3B). At a tenth re-use, this solution effectively inhibited lipid oxidation during 6 d of ice storage, after which there were slight but significant ($p < 0.05$) increases in TBARS and a large ($p < 0.05$) increase in PV. Thus, provided that 6 d of pre-processing storage would be enough for the co-products prior to processing into food ingredients, the antioxidant solution could be re-used at least 10 times. On a weight-basis, rosemary extract is the principal component of Duralox MANC, and such extracts are known to be powerful inhibitors of lipid oxidation based on their contents of e.g. carnosol, carnosic acid, rosmarinic acid or caffeic acid (Xie, VanAlstyne, Uhlir, & Yang, 2017). As an example, Tseng et al. (2005) described how Australian red claw crayfish tails dipped in a 0.06% (w/w) rosemary extract solution and stored at −20 °C prevented lipid oxidation better compared with dipping in water. According to the structure of the mentioned rosemary-derived compounds, i.e., phenolic rings with a high degree of methylation and hydroxylation, the main mechanisms by which rosemary extract inhibits lipid oxidation of herring co-products is expected to be free radical scavenging (Xie et al., 2017). However, it may also involve inactivation of low molecular weight (LMW) metals by chelation (Richards, 2010) and, tentatively, prevention of metHb/Mb-formation, the latter which needs further studies. In addition, the synergy between other components in Duralox MANC, e.g., the well-known one between ascorbic acid and tocopherols, and between these compounds and the rosemary extract are expected to contribute to the effectiveness of Duralox MANC to inhibit lipid oxidation (Xie et al., 2017). Wada et al. (1992) found that a mixture of rosemary extract and α-tocopherol (0.02% + 0.05%) had stronger antioxidant activity than either rosemary or α-tocopherol alone in a sardine oil model system and frozen fish mince.

Dipping the co-products in 2% isoascorbic acid, the PVs and TBARS did not significantly increase ($p < 0.05$) from time 0–9 d, indicating it was nearly as efficient as Duralox MANC. This was despite the fact that dipping in isoascorbic acid reduced the pH of the mince to 6.37 ± 0.021, i.e. 0.4 pH units under the un-dipped control (6.77 ± 0.028, see supplementary Table 1), which in itself could be expected to be pro-oxidative. Similarly, Sveinsdóttir et al. (2020) found that dipping mackerel fillets in 0.2% sodium isoascorbate for 10s prior to freezing to create a glaze was effective in inhibiting lipid oxidation of mackerel fillets during subsequent frozen storage. The isoascorbate effect could be attributed to scavenging of free radicals and reduction of hypervalent forms of Hb (Kröger-Ohlsen & Skibsted, 1997). That PVs started to increase after 9 d on ice for the isoascorbic acid treated samples could be attributed to storage-induced degradation of the isoascorbic acid. Petillo, Hultin, Krzynowek, and Autio (1998) reported that rancidity developed in mackerel dark muscle at the time that ascorbic acid was depleted during iced storage. Also Undeland, Hall, and Lingnert (1999) found a strong correlation between loss of endogenous ascorbic acid and lipid oxidation development in herring fillets during ice storage.

3.4. Effect of Duralox MANC on auto-oxidation and hemin release of herring Hb

We have shown that auto-oxidation and release of hemin were of primary importance in promoting lipid oxidation in fish tissue (Undeland et al., 2004; Wu et al., 2017). To explain Duralox MANC's effective inhibition of lipid oxidation in herring co-products, we investigated the effect of Duralox MANC on herring Hb auto-oxidation and hemin release. Fig. 4 shows that Duralox MANC significantly reduced ($p < 0.05$) metHb formation when 5 μM Hb was incubated with Duralox MANC (0.5 g/L) at pH 6.3 and 4 °C. The mechanism of fish Hb auto-oxidation involves deoxyHb formation, often as a result of low pH (pH < 7), which decreases the oxygen affinity of trout Hb (Aranda IV et al., 2009). Then deoxyHb reacts with copious amounts of O_2 to
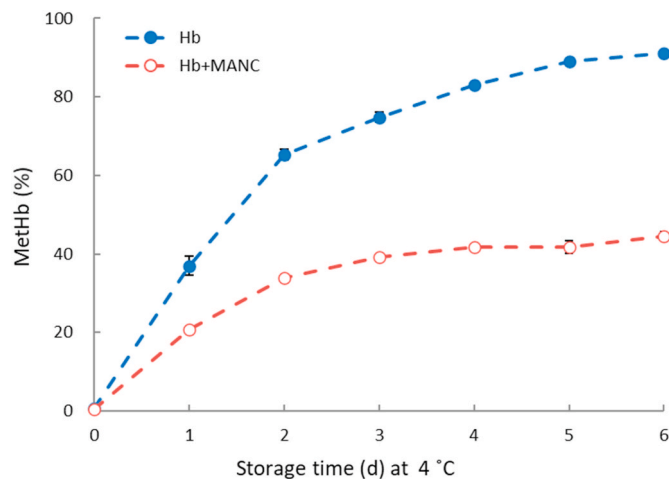
Fig. 4. Auto-oxidation of herring Hb during incubation with Duralox MANC. Hb was diluted to 5 μM in 50 mM phosphate buffer, pH 6.3 and incubated at 4 °C. The final concentration of Duralox MANC was 0.5 g/L.

Fig. 5. Spontaneous hemin loss from herring metHb. MetHb was diluted to 5 μM in 50 mM phosphate buffer, pH 6.3 and incubated at 4 °C. The final concentration of Duralox MANC was 0.5 g/L.

produce metHb and a superoxide anion radical (Brantley, Smerdon, Wilkinson, Singleton, & Olson, 1993), the latter causing rapid production of hydrogen peroxide, which can convert residual oxyHb to metHb (Weiss, 1982). Rosemary extracts, ascorbic acid, and tocopherols can scavenge superoxide anion radicals or the hydrogen peroxide (Richards, 2010), thereby reducing metHb formation.

Hemin released from Hb is more hydrophobic than the hemin-globin complex, that is why it dissolves better into cellular membranes where it readily decomposes preformed lipid hydroperoxides, producing alkoxyl and peroxyl radicals that propagate lipid oxidation in muscle tissue (Richards, 2010). To study the effect of Duralox MANC on hemin release of herring Hb, we used metHb instead of oxyHb, as the porphyrin moiety in oxidized Hb is 60-fold less anchored in the globin compared with reduced Hb (Tang, Kalsbeck, Olson, & Bocian, 1998). As Fig. 5 shows, Duralox MANC resulted in a significantly lower rate of hemin loss at pH 6.3 and 4 °C. This inhibition could be explained by reducing agents, e.g., ascorbic acid in the Duralox MANC, which could reduce back metHb to oxyHb (Tomoda, Takeshita, & Yoneyama, 1978) and, consequently, reduce the rate of hemin loss. Further, the herring Hb structure could become more stable when it binds to phenols or flavonoids, e.g., rosmarinic acid, and caffeic acid (Xi & Guo, 2007). For example, Yin, Bingman, Tatiyaborworntham, Zhang, and Richards (2016) found that caffeic acid formed a covalent adduct with Cys 130 in the α-chain of turkey Hb. The adduct (Hb-caffeic acid) had a lower rate of hemin loss compared with the Hb monomer, and this prevented Hb-mediated lipid oxidation in washed cod muscle.

4. Conclusions

Lipid oxidation of herring co-products was largely inhibited at both 20 °C and 0 °C storage after incubation in 2% or 5% Duralox MANC; shelf life extended from <1 d to >12 d on ice and from 0.5 to >3.5 d at 20 °C. The high stabilizing effect from 2% Duralox MANC on ice stored herring co-products remained the same even after reducing the treatment time and the ratio of antioxidant solution to herring co-products. Promising results were also obtained when the antioxidant solution was re-used, i.e. no effect was lost after 4 times of re-using the solution, while the oxidation lag phase decreased from 12 d to 6 d after 10 times of re-using the solution. Dipping the co-products in 2% isoascorbic acid also increased the oxidation lag phase up to 9 d. Duralox MANC prevented auto-oxidation and hemin loss of herring Hb, which could partly explain its effective inhibition of lipid oxidation in herring co-products.
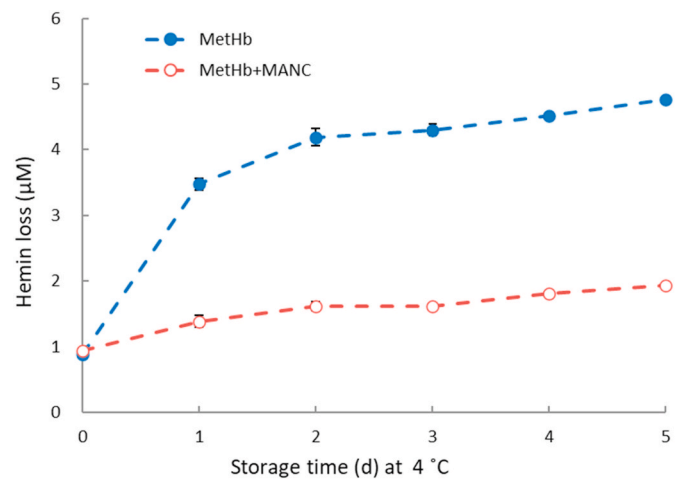
CRediT authorship contribution statement

Haizhou Wu: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft, preparation. **Mursalin Sajib:** Investigation. **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.foodcont.2021.107963.

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