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1 **Stabilization of herring (*Clupea harengus*) by-products against lipid oxidation by**
2 **rinsing and incubation with antioxidant solutions**

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9 **Abbreviated running title:** Stabilization of herring by-products against lipid oxidation

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Abstract

The content of hemoglobin (Hb) in different parts of herring (*Clupea harengus*) by-products, and three strategies for preventing lipid oxidation during their storage on ice were studied: (i) rinsing away Hb with water or 0.9% NaCl with/without antioxidants (Duralox-MANC, erythorbate and EDTA), (ii) incubation in water/0.9% NaCl with/without antioxidants, (iii) mincing and adding antioxidants into the mince. The by-product parts were ranked as follows regarding Hb-content: head>backbone>caudal fin>residuals. Only 10-18% Hb could be rinsed away with tap water or 0.9% NaCl, and the effect of such a rinsing on peroxide value (PV) or TBA-reactive substances (TBARS) development was limited. Rinsing or incubating by-products in antioxidant solutions however had remarkable effects; shelf life went from <1 day to >12 days. Duralox-MANC was particularly efficient. The presented strategies could be powerful tools to allow upgrading of herring by-products to food ingredients, which is currently hindered by their extreme sensitivity to lipid oxidation.

Keywords: Herring, Clupea harengus, by-products, lipid oxidation, hemoglobin, rinsing, incubation, antioxidants

34 **1. Introduction**

35 Fish makes up 17 % of the intake of animal proteins globally, in some coastal
36 countries more than 50 % (FAO, 2014). The fish demand is steadily increasing in
37 response to dietary recommendations and population growth. However, wild
38 fisheries cannot expand further based on endurance of ecosystems and climate
39 changes, and the expected aquaculture expansions will not cover the demand
40 (Alexandratos & Bruinsma, 2012). One way of increasing the supply of fish products
41 without increasing its environmental impacts is to use the landings of fish more
42 efficiently. This would involve better utilization of e.g. filleting by-products for food
43 production. However, the majority of all by-products from the fish industry are still
44 mainly targeted feed production or waste. Globally, it can be estimated that these
45 raw materials carry around 25 million tonnes of muscle rich in protein and long
46 chained (LC) n-3 polyunsaturated fatty acids (PUFA) (FAO, 2014), which instead could
47 be used in food production to meet the growing seafood demand. The largest
48 potential with fish filleting by-products in food production, lies in producing minces,
49 fish oil, functional protein isolates and protein hydrolysates. Innovative techniques
50 for such productions today exist. For example, Hultin and Kelleher (1999) first
51 developed the pH-shift process for muscle-based raw materials, and it has been
52 successfully recognized as a promising technique for lipid and protein recovery from
53 unconventional complex aquatic raw materials (Abdollahi & Undeland, 2019; P. K.
54 Vareltzis & Undeland, 2012). Other existing techniques are classic meat-bone
55 separation (Zhong, Liu, Cao, Chen, Wang, & Qin, 2016), or enzymatic hydrolysis

56 (Liaset, Lied, Espe, & Agriculture, 2000). However, one major problem currently
57 prevent a "take-off" for their implementation is fast oxidative destruction of the
58 valuable LC n-3 PUFA, causing rancidity in the by-products already within hours after
59 the filleting operation (Rustad, 2003), and thus, limiting their chances of staying
60 within the food chain.

61 Blood and its hemoglobin (Hb) have been revealed as the major components
62 responsible for lipid oxidation in fish (Richards & Hultin, 2002; Undeland, Kristinsson,
63 & Hultin, 2004). Fish filleting by-products are all very blood-rich, caused e.g. by gills,
64 caudial vein and the bleeding/injury caused by the actual cutting operation. We
65 therefore hereby state that blood and blood contamination is the main hurdle
66 preventing full utilization of fish by-products for food production. Richards *et al.*
67 (1998) and Undeland *et al.*(1998; 2003) rinsed mackerel fillets, herring mince, and
68 cod mince respectively, with water or 50 mM NaCl solution to remove Hb/blood prior
69 to storage on ice or in freezer. However, it was found that endogenous hydrophilic
70 antioxidants were removed in such washes which lead to a higher susceptibility of
71 the washed muscle tissue towards oxidation. One potential way to compensate for
72 such loss is by rinsing with an antioxidant solution. Previously, different naturally
73 occurring antioxidants have been used to improve the oxidative stability in minced
74 fish fillets. The addition of white grape dietary fiber (Sánchez-Alonso, Borderías,
75 Larsson, & Undeland, 2007), caffeic acid (Larsson & Undeland, 2010) and cranberry
76 extract (C. Lee, Krueger, Reed, & Richards, 2006) successfully inhibited lipid oxidation
77 in washed fish models or fish mince. However, to the best of our knowledge, there is

78 no literature on treating fish by-products with anti-oxidant containing solutions as
79 ways to prevent lipid oxidation, and thereby allowing for their further upgrading to
80 foods or food ingredients.

81 The aims of this study were to: (i) quantify Hb in different parts of herring
82 by-products, (ii) investigate whether Hb was located on the surface or interior in the
83 by-products, and (iii), investigate the effect of rinsing or incubating herring
84 by-products in different solutions on the removal of Hb and the development of lipid
85 oxidation during subsequent ice storage.

86 **2. Materials and Methods**

87 **2.1 Fish samples preparation**

88 Herring (*Clupea harengus*) was caught off the west coast of Sweden from September
89 to December of 2018. Fresh herring filleting by-products (head, backbone with
90 caudal fin and residuals (including intestines, skin and fish egg)) were provided by
91 Scandic Pelagic AB (Ellös, Sweden). The post mortem age of herring by-products
92 when arriving in our laboratory were between 1 and 2 days; however, the time
93 elapsing between filleting of the herring, and receival of the by-products was only
94 2-6 h. The by-products were kept below 5 °C during the transportation.

95 **2.2 Preparation of treatment solutions**

96 The treatment solutions used in rinsing trials were 1) tap water, 2) 0.9% NaCl, 3) 5%
97 Duralox MANC-213 (Kalsec, Kalamazoo, Mich., UK), 4) isoascorbic acid (0.2%) + EDTA
98 (0.044%). The incubation solutions were: 1) tap water, 2) 0.9% NaCl, 3) 1% Duralox
99 MANC-213, 4) 0.5% Duralox MANC-213, 5) sodium phosphate buffer (50 mM, pH 7.5),

100 5) sodium phosphate buffer (50 mM, pH 6.5) 6) 2% isoascorbic acid. All treatment
101 solutions were freshly prepared from tap water and kept in a cold room (4 °C) at least
102 5 hours before use.

103 **2.3 Rinsing of herring by-products with different solutions**

104 One kilogram of fresh herring by-products were rinsed/shortly incubated in the
105 above treatment solutions (1:5 weight/volume) for 20 min in cold room (4 °C).
106 Thereafter, the herring by-products were removed from the solutions and drained
107 well in a stainless steel fine strainer. The control samples were not rinsed into any of
108 the solutions. Drained samples and controls were then immediately ground using a
109 table top meat grinder (C/E22 N, Minerva Omega group, Italy) equipped with a plate
110 with 4.5 mm holes, and thereafter stirred to complete homogeneity. The minces
111 were ice stored according to the method of Larson *et al.*(2007) after manually stirring
112 in 200 ppm streptomycin to prevent bacterial growth. Samples of the mince (25 g)
113 were flattened out on the bottom of a 250 mL screw-capped Erlenmeyer flask, giving
114 a thickness of ~5 mm, and stored on ice in darkness for up to 12 days. Samples for
115 chemical analysis (~1 g "plugs") were regularly taken out from the Erlenmeyer flask
116 using a hollow cylinder to obtain a constant surface-to-volume ratio between
117 different samplings. The plug was wrapped in aluminum foil and kept at - 80 °C until
118 analysis.

119 **2.4 Incubation of herring by-products in different solutions**

120 One kilogram herring by-products were incubated with the solutions described in 2.2
121 (1:1 weight/volume) up to X days. Two hundred ppm streptomycin was added into

122 the system to prevent bacterial growth. All treatments were stored in a cold room
123 (4 °C) in darkness for up to 7 days. Control by-products were stored at 4 °C without
124 solution for the same amount of time as the by-products were incubated in solution.
125 At each sampling point and for each treatment, 3 individual samples of each
126 by-product part (head, backbone with caudal fin and residuals) were ground together
127 using a table top meat grinder (C/E22 N, Minerva Omega group, Italy) equipped with
128 a plate with 4.5 mm holes and thereafter stirred to complete homogeneity. Samples
129 for chemical analysis (∼1 g "plugs") were taken out from the by-products mince. The
130 plug was wrapped in aluminum foil and kept at - 80 °C until analysis.

131 **2.5 Direct mixing of antioxidants into minced herring by-products**

132 One kilogram fresh herring by-products were ground as described in 2.3 and 2.4,
133 where after the following antioxidants were manually mixed into the by-products
134 mice: 1) isoascorbic acid (0.2%) + EDTA (0.044%), 2) 0.25 % Duralox MANC 3) 0.5%
135 Duralox MANC. Following manual stirring with 200 ppm streptomycin, all minces
136 were stored as described in 2.3-2.4.

137 **2.6 Total heme pigment measurement**

138 Herring by-product minces (50g in total) from the different treatments was carefully
139 placed one by one in a porcelain mortar containing liquid nitrogen. Additional liquid
140 nitrogen was slowly added alternating with sub-portions of the 50 g mince as needed
141 to completely freeze the mince. The frozen mince was pounded using a pestle to
142 break it into smaller pieces which were then transferred to a 1 L stainless steel
143 container for grinding into a fine powder. Milliliter amounts of liquid nitrogen were

144 added as needed to keep the mince frozen and to facilitate grinding as well as further

145 handling. Four grams of by-product powder was subsequently used to measure total

146 Hb using the acetone-based method of Hornsey (1956).

147 **2.7 Analyses of lipid oxidation**

148 Total lipids were extracted from 1-g samples of mince using chloroform:methanol

149 (2:1) (Cavonius & Undeland, 2017). The chloroform phase was recovered and

150 analyzed for peroxide value (PV) using the ferric thiocyanate method as described by

151 Undeland *et al* (2002). TBA-reactive substances (TBARS) was determined according to

152 the method of Larson *et al.*(2007), in the water–methanol phase recovered from the

153 extraction described above.

154 **2.8 Statistics**

155 All experiments were done in duplicates. Total Hb is reported as mean ± standard

156 deviation (SD) (n=2). An unpaired t-test was used to determine significant differences

157 between samples regarding their content of total Hb. To determine the effect of time,

158 treatment and their interaction on lipid oxidation, repeated measures mixed models

159 according to the method of Cavonius and Undeland (2017) were conducted. Data

160 were log-transformed when residuals from models were not normally distributed.

161 Tukey's post hoc test was used for pairwise comparisons when a significant effect

162 was observed. Differences are regarded as significant when $p < 0.05$.

163 **3. Results and discussion**

164 **3.1 Hb level in different parts of the by-products before and after rinsing**

165 Hb in blood is an effective catalyst for the lipid oxidation reaction, often limiting shelf

166 life of fish (Richards & Hultin, 2002). In this study, we had the hypothesis that Hb is
167 particularly problematic for upgrading of fish by-products to food ingredients such as
168 protein and oil, since veins/ capillaries are concentrated in many of the non-fillet
169 parts of a fish (Brill & Bushnell, 2006). To identify the by-product parts which are
170 richest in Hb, and thus most challenging for upgrading, we measured levels of Hb in
171 different parts of herring by-products. Table 1 shows that the range of Hb in head,
172 backbone, caudal fin and residuals was from 39.1 to 70.9 µmol/kg. Previously, we
173 measured the concentration of Hb to be 10.7 and 28.6 µmol/kg in herring fillet light
174 and dark muscle, respectively (Chaijan & Undeland, 2015). Thus, the difference
175 between our previous and current studies confirmed that herring by-products
176 contained higher concentrations of Hb compared with the fillet. The head contained
177 the highest concentration of Hb (70.9 ± 1.1 µmol/kg) among the herring by-product
178 parts, followed by backbone, residuals and caudal fin. These results agree with the
179 distribution of the main blood vessels of bony fish. The main vessels in most
180 coldblooded fish run along the backbone and radiate outward to the small vessels
181 that supply visceral organs and muscle. Moreover, fish acquire oxygen from the water
182 through the gills, why gills of the head part contain more blood vessels than other
183 fish parts (Brill & Bushnell, 2006).

184 In smaller fish species like herring and mackerel, active bleeding is usually not
185 applied (Richards & Hultin, 2002). However, some blood passively leaches out, e.g.,
186 after the filleting operation and during the pre-processing storage. Lewis *et al.* (1966)
187 showed that Hb remained encapsulated in fish erythrocytes after rinsing blood with a

188 physiological saline (0.9% NaCl) washing solution. To reduce Hb contamination of the
189 herring by-products, we therefore used physiological saline for rinsing. Table 1 shows
190 that the percent of Hb that could be removed by rinsing with 5 volumes of 0.9% NaCl
191 was 10.3%, 17.9%, 6.6% and 18.0% in head, backbone, caudal fin, and residuals,
192 respectively. That the rinsing operation could remove more Hb/blood from backbone
193 and residuals compared with head and caudal fin, may be attributed to larger specific
194 surface areas of backbone and residuals compared with head and caudal fin.
195 Although the concentrations of Hb in the rinsed by-product group were significantly
196 lower ($P < 0.05$) compared to the non-rinsed group for head, backbone and residuals,
197 the amount of Hb removed by rinsing was relatively small. This indicated that most of
198 the residual blood was situated in the interior of the by-products rather than on the
199 surface, which in turn could be a results of the tap water rinsing that takes place
200 during the commercial herring filleting operation.

201 **3.2 Effect of rinsing mixed herring by-products on lipid oxidation**

202 It was investigated whether a rinsing treatment with 0.9% NaCl could stabilize herring
203 by-products against lipid oxidation, despite the limited Hb-removal. As a comparison,
204 rinsing with tap water, which has an extreme osmolarity (0% salt), was used. PV and
205 TBARS were used as indicators of primary and secondary lipid oxidation products,
206 respectively. Figure 1A and 1B show that the PV and TBARS for the control group
207 without rinsing were 244 ± 13 and 184 ± 8 µmol/kg, respectively, after 0.75 days,
208 which was significantly ($P < 0.05$) higher than at day 0, 71 ± 5 and 24 ± 4 µmol/kg.
209 These results indicated that there was almost no lag phase for lipid oxidation in

210 herring by-products, which confirmed hypothesis that herring by-products are even
211 more sensitive to lipid oxidation than herring fillets (Larsson, Almgren, & Undeland,
212 2007).
213 Although the PVs for all treatments were significantly influenced by storage time,
214 there was no significant effect of the rinsing treatment on PVs at all sampling times
215 during the five days of ice storage (Figure 1A). The TBARS development rates from
216 0.75 days to 2.75 days were ranked as follows: rinsing with 0.9% NaCl < rising with
217 tap water < no rinsing (Figure 1B). These results indicated that rinsing treatments to a
218 small extent could prevent the formation of secondary lipid oxidation products which
219 could be attributed to a partial Hb removal from the surface of the by-products (see
220 Table 1). This observation is in agreement with the capacity of Hb to break down lipid
221 hydroperoxides to e.g. carbonyls (Richards, 2010), and also agrees with previous
222 studies with mackerel fillets (Richards, Kelleher, & Hultin, 1998). Richards *et al.* (1998)
223 used distilled water to wash mackerel fillets from rigor fish and found that washing
224 improved the storage stability by preventing lipid oxidation, which was attributed in
225 part to washing-induced removal of blood. However, our present results contradict
226 our earlier study (Undeland, Ekstrand, & Lingnert, 1998), where we found that
227 washing of herring fillet mince with 0.05 M NaCl solution significantly promoted lipid
228 oxidation during the subsequent frozen storage at -20°C. This contradiction may be
229 explained by the difference in materials. In Undeland's study, it was a mince which
230 was subjected to the washing process, which provided a much large surface area
231 than that of whole fillets or by-products. This large surface area may have led to

232 removal of a substantial amount of antioxidants endogenous to the muscle, e.g.,
233 α-tocopherol, ascorbic acid, GSH-px, SOD, and catalase (Undeland, Ekstrand, &
234 Lingnert, 1998). Sannaveerappa *et al* (2007) clearly showed the strong antioxidative
235 power of the aqueous fraction ("press juice") of herring muscle, something we have
236 also shown for other fish species (Undeland, Hultin, & Richards, 2003). In addition,
237 rinsing or washing fish mince has been reported to increase the ratio between
238 pro-oxidants and phospholipids (Undeland, Ekstrand, & Lingnert, 1998), which may
239 be another reason why washing promoted lipid oxidation in herring mince, but not in
240 intact herring by-products parts.

241 Furthermore, between 0.75 day and 2.75 days, rinsing with physiological saline
242 showed significantly lower ($P < 0.05$) TBARS values compared with tap water rinsing
243 (Figure 1B). This may be a result of the extreme osmolarity of tap water, which could
244 induce erythrocyte lysis, thereby releasing Hb allowing their contact with the lipids.
245 In contrast, physiological osmolarity (0.9% NaCl) would keep the Hb within the
246 erythrocytes (Lewis & Ferguson, 1966), thereby minimizing Hb interaction with
247 by-product lipids and preventing lipid oxidation.

248 To improve the lipid-stabilizing effect of rinsing during subsequent storage of
249 by-products, we used antioxidants in combination with tap water and 0.9% NaCl
250 solution as the rinsing media. In this case, the rinsing was regarded more as a dipping
251 instead of a wash, as it was meant to cover the surface and outer layer of the
252 by-products with antioxidants. Dipping with antioxidants was earlier used to stabilize
253 horse mackerel fillets against lipid oxidation (Pazos, Alonso, Fernández-Bolaños,

254 Torres, & Medina, 2006). Figure 2 shows that the lipid oxidation rate was lower when
255 dipping in isoascorbic acid + EDTA solution compared with no dipping, based on PV
256 and TBARS values from 0 to 2.75 days. However, there were no significant differences
257 for PV and TBARS values between isoascorbic acid + EDTA dipping and no dipping
258 after 4.75 days. These results indicated that isoascorbic acid + EDTA provided a lower
259 lipid oxidation rate in the initial storage period from 0 to 2.75 days, and then a higher
260 lipid oxidation rate from 2.75 days to 4.75 days. This phenomenon may be attributed
261 to the biphasic effect (anti- and pro-oxidant) of isoascorbic acid. Similarly, Richards *et*
262 *al.* (2004) reported that ascorbate (2.2 mM) presented both an antioxidant and a
263 pro-oxidant effect in a washed cod mince model enriched with trout Hb. The
264 inhibition provided by ascorbate could be attributed to the ability to scavenge free
265 radicals and reduce hypervalent forms of Hb (Kröger-Ohlsen & Skibsted, 1997).
266 Conversely, the shift of ascorbate from an antioxidant to a pro-oxidant could be due
267 to a break-down of accumulated lipid hydroperoxides to reactive lipid radicals
268 capable of propagating lipid oxidation (S. H. Lee, Oe, & Blair, 2001). The same could
269 be provided by the capacity of ascorbate to reduce low-molecular-weight iron to its
270 ferrous, and more pro-oxidant form (Yamamoto, Takahashi, & Niki, 1987). The shift
271 from anti- to pro-oxidant action is evident especially at low ascorbate concentrations;
272 according to Ramanathan and Das at <280 µmol/kg)(Ramanathan & Das, 1992). We
273 also added Duralox MANC, a commercial antioxidant mixture, to the dipping medium.
274 Figure 2A clearly shows that all PVs for MANC treated by-products were significantly
275 lower ($P < 0.05$) compared with isoascorbic acid + EDTA and control (no rinsing) from

276 0.75 to 4.75 days. Also with Duralox MANC treatment, the PVs did not change from
277 time zero in three sampling points 0.75, 2.75 and 4.75 days. There was a slight
278 increase in PV from 4.75 to 7.75 days and a rapid increase was observed after 7.75
279 days. These results indicated the Duralox MANC effectively inhibited production of
280 primary lipid oxidation products up to 7.75 days. Figure 2B shows that Duralox MANC
281 also completely inhibited formation of secondary lipid oxidation products (TBARS). All
282 observed TBARS were between 16 and 32 µmol/kg from 0 to 12 days, and there were
283 no significant differences between any of the sampling points. Duralox MANC is a
284 mixture of rosemary extract, ascorbic acid, tocopherols and citric acid. Rosemary
285 extract is the principal component on a weight basis and it may be key to inhibition
286 of lipid oxidation of the by-products. Vareltzis *et al.* (1997) performed dipping of
287 mackerel (*Trachurus trachurus*) fillets in a water solution containing 800 mg rosemary
288 extract/L where after the fillets were vacuum-packaged. The rosemary extract
289 significantly retarded oxidation of the fillets during subsequent frozen storage. The
290 mechanisms involved in retarding oxidation most likely include free radical
291 scavenging and possibly inactivation of low molecular weight metals by chelation
292 (Richards, 2010). Besides the rosemary extract, the other antioxidants in Duralox
293 MANC (ascorbic acid, tocopherols and citric acid) are believed to provide important
294 synergies with the polyphenols of the rosemary extract. Wada *et al* (1992) showed
295 that a mixture of rosemary extract and α-tocopherol (0.02% + 0.05%) had the highest
296 antioxidant activity in frozen fish muscle and delayed the onset of oxidation five days
297 longer than either rosemary extract or α-tocopherol alone. Similarly, Hraš *et al* (2000)

298 reported that, when combined with citric acid, rosemary extract showed an additive
299 antioxidative effect compared with rosemary extract alone in sunflower oil stored at
300 60 °C.
301 It was interesting to note that Duralox MANC treatment did not exert as strong
302 inhibitory effect on formation of primary lipid oxidation products (PV) from 7.75 to
303 12 days, as it did on generation of secondary lipid oxidation products (TBARS) in this
304 period. These results may be attributed to partial breakdown of the antioxidants that
305 inhibit formation of primary lipid oxidation products, while those antioxidants that
306 could inhibit secondary lipid oxidation products formation remained effective. On the
307 basis of the capacity to inhibit oxidation reactions, antioxidants can be classed into
308 primary and secondary antioxidants (Berdahl, Nahas, & Barren, 2010). Primary
309 antioxidants are substances that disrupt the free radical chain propagation process to
310 reduce the formation of hydroperoxides; secondary antioxidants are substances that
311 react with hydroperoxides, converting them to more stable, non-radical products
312 (Reische, Lillard, & Eitenmiller, 2002). This could be for instance tocopherols and
313 tocotrienols. In this sub-study, the primary antioxidants may begin to break down
314 after 7.75 days, which could result in a rapid increase of the PV. However, the
315 secondary antioxidants such as citric acid could still exert their antioxidative effects
316 to convert hydroperoxides to more stable, non-radical products, explaining why the
317 TBARS did not increase significantly during the 12 days of ice storage. Another
318 possibility is that Duralox MANC inactivated the ability of Hb to break down primary
319 oxidation products to carbonyls and new radicals, which is one of the major
320 pro-oxidative roles of heme-proteins (Richards, 2010).

321 **3.3 Incubation of herring by-products in water or physiological salt**

322 As a strategy to avoid direct contact between by-products and air during
323 pre-processing storage, we evaluated an incubation of the herring by-products in tap
324 water or 0.9% NaCl solution at 4 °C for up to 7 days. Figure 3 shows that there was no
325 significant difference in the lipid oxidation rates between tap water and 0.9% NaCl
326 treatments. However, a significantly lower lipid oxidation rate was observed in
327 incubated by-products compared with controls stored in air based on PV and TBARS
328 values. Similarly, Losada *et al.* (2006) reported that storage of sardine (*Sardina*
329 *pilchardus*) in an ice slurry (40% ice/60% water) resulted in lower oxidation values
330 (TBARS, peroxide value) compared with those stored in flake ice. Ortiz *et al.* (2008)
331 used flow ice (prepared from filtered sea water) to store rainbow trout *(On. mykiss)*
332 which increased shelf life from 8 days to 13 and 16 days compared with those stored
333 in normal ice.

334 To make the incubation solutions even more effective, different antioxidant additives
335 were used in one of our sub-studies. Figure 4 shows that all incubation treatments
336 had a lower lipid oxidation rate based on PV and TBARS values compared with
337 controls stored in air, which agrees with the results of Figure 3. According to the PV
338 and TBARS results, the effect of incubation with different additives was stronger than
339 just incubation with tap water or 0.9% NaCl. Besides the reduced contact between
340 oxygen and lipids (Richards, Kelleher, & Hultin, 1998), this could be attributed to one
341 or both of the following two reasons: i) the incubation solution could remove or
342 dilute a part of the Hb from the by-products and ii) the incubation could efficiently

343 cover the surface of the by-products with the tested antioxidants. Figure 4 shows
344 that the Duralox MANC treatment had the strongest inhibiting ability for lipid
345 oxidation, which confirmed this hypothesis. Similarly, Quitral *et al.*(2009) showed a
346 significant decrease in lipid oxidation when Chilean jack mackerel *(Trachurus murphyi)*
347 was stored in an ice-water system including rosemary extracts in refrigerated room
348 (4 °C) compared to the same system without rosemary extract. Figure 4 also shows
349 that there was no significant difference in lipid oxidation between pH 6.5 and pH 7.5
350 incubation PBS solution. However, our previous study showed that a higher pH (pH
351 7.2) prevented lipid oxidation in a washed cod mince model system compared to pH
352 6.0, as the latter stimulate formation of deoxyhemoglobin and methemoglobin
353 (Undeland, Kristinsson, & Hultin, 2004). This contradiction may be explained by the
354 difference in the actual systems studied. In this sub-study, the pH was adjusted only
355 in the solution which were incubated with the by-products, while in Undeland's study,
356 the entire washed cod mince model was pH-adjusted. Moreover, Figure 4 shows that
357 there was no significant difference in lipid oxidation between 1% and 0.5% of Duralox
358 MANC, which shows that a plateau was reached, which is indeed positive from an
359 economic perspective if the present antioxidant strategy would be implemented in
360 indystry.

361 **3.4 Direct add of antioxidants into minced herring by-products**

362 The above two strategies (rinsing and incubation) were proven promising ways to
363 stabilize by-products against oxidation if they are to be kept *intact* prior to further
364 processing. However, they never indicated which exact level of the added

365 antioxidants that reached into the herring tissue, and thus, which *in situ* levels there
366 were needed to prevent oxidation. In this sub-study, we therefore tried to confirm
367 which concentration of isoascorbic acid + EDTA and Duralox MANC, respectively,
368 which would be needed to efficiency inhibit lipid oxidation during ice storage
369 following direct addition into by-product minces, which could be a ready-to-process
370 material for further value adding operations. Figure 5A and 5B show that the PV and
371 TBARS values rapidly increased from zero-time and arrived at 737 ± 32 and 216 ± 25
372 µmol/kg, respectively, after 2 days without any addition of antioxidants. However, all
373 antioxidant treatments provided significantly lower PV and TBARS values after 2 days
374 compared to the control. Furthermore, the lag phases for PV were 1, 2 and > 8 days
375 for isoascorbic acid (0.2%) + EDTA (0.044%), 0.25% MANC and 0.5% MANC,
376 respectively (Figure 5A). The corresponding lag phases for TBARS were 2, 4 and > 8
377 days (Figure 5B). These results indicated the ability of the antioxidant ranked them as:
378 0.5% MANC > > 0.25% MANC > isoascorbic acid (0.2%) + EDTA (0.044%). These results
379 show that Duralox MANC is needed if by-product minces should be stored > 4 days
380 close to 0 °C.

381 **4. Conclusions**

382 Herring by-products have been successfully stabilized by three different antioxidant
383 strategies; rinsing and incubation of the intact by-products, or direct addition into the
384 by-products after mincing. The effects of the treatments were in some cases
385 remarkable; shelf life went from <1 day to >12 days, and Duralox MANC was in all
386 sub-studies the most effective antioxidant. Given it simplicity, the rinsing treatment
387 was particularly promising. Overall, our study paves the way for an extended window

388 of time during which it is possible to upgrade herring by-products to food ingredients,

389 despite the fact that they *per se* are a highly sensitive system when it comes to lipid

390 oxidation. This would be a more sound and holistic utilization of the valuable herring

391 biomass.

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501 **Figure Captions**

502 **Figure 1.** Lipid oxidation measured as (A) lipid hydroperoxides and (B) TBA-reactive
503 substances (TBARS) during ice storage of herring by-product mince that had been
504 pre-rinsed by rinsing the intact by-products with tap water, and 0.9% salt solutions.
505 The ratio of herring by-products to solution was 1:5 weight/volume.

506 **Figure 2.** Lipid oxidation measured as (A) lipid hydroperoxides and (B) TBA-reactive
507 substances (TBARS) during ice storage of herring by-product mince that had been
508 pre-treated by rinsing the intact by-products with antioxidant solutions. 1). 5%
509 Duralox MANC-213 in tap water; 2) Isoascorbic acid (0.2%) + EDTA (0.044%) in 0.9%
510 NaCl solution, 3) isoascorbic acid (0.2%) + EDTA (0.044%) in tap water. The ratio of
511 herring by-products to solution is 1:5 weight/volume.

512 **Figure 3.** Lipid oxidation measured as (A) lipid hydroperoxides and (B) TBA-reactive
513 substances (TBARS) during storage in a cold room (4 °C) of herring by-products that
514 were incubated with tap water, and 0.9% salt solutions. The ratio of herring
515 by-products to solution is 1:1 weight/volume.

516 **Figure 4.** Lipid oxidation measured as (A) lipid hydroperoxides and (B) TBA-reactive
517 substances (TBARS) during storage in cold room (4 °C) of herring by-products that
518 were incubated with different solutions. The ratio of herring by-products to solution
519 is 1:1 weight/volume.

520 **Figure 5.** Lipid oxidation measured as (A) lipid hydroperoxides and (B) TBA-reactive
521 substances (TBARS) during ice storage of minced herring by-products which were
522 fortified with antioxidants: 1) Isoascorbic acid (0.2%) + EDTA (0.044%), 2) 0.25 %
523 Duralox MANC 3) 0.5% Duralox MANC.

Table 1 The effect of 0.9% NaCl rinsing[A] on total Hb level[B] in different parts of herring by-products

Treatment	Head[C]	Backbone	Caudal fin	Residuals
No rinsing	70.9 ± 1.1[a]	42.8 ± 0.3[a]	39.1 ± 1.8[a]	40.6 ± 1.1[a]
Rinsing	63.6 ± 1.5[b]	35.1 ± 0.8[b]	36.47 ± 1.3[a]	33.3 ± 0.5[b]

[A]The ratio was 5:1 for 0.9% NaCl solution/by-products (volume/weight). The process of rinsing was 20 minutes in 4°C under gentle stirring. [B]The unit of total Hb is μmol/kg herring by-products. [C]Data are means ± standard deviations (n=2). Means bearing different designations (a, b) in a column differ significantly ($P < 0.05$).

Figure 1.

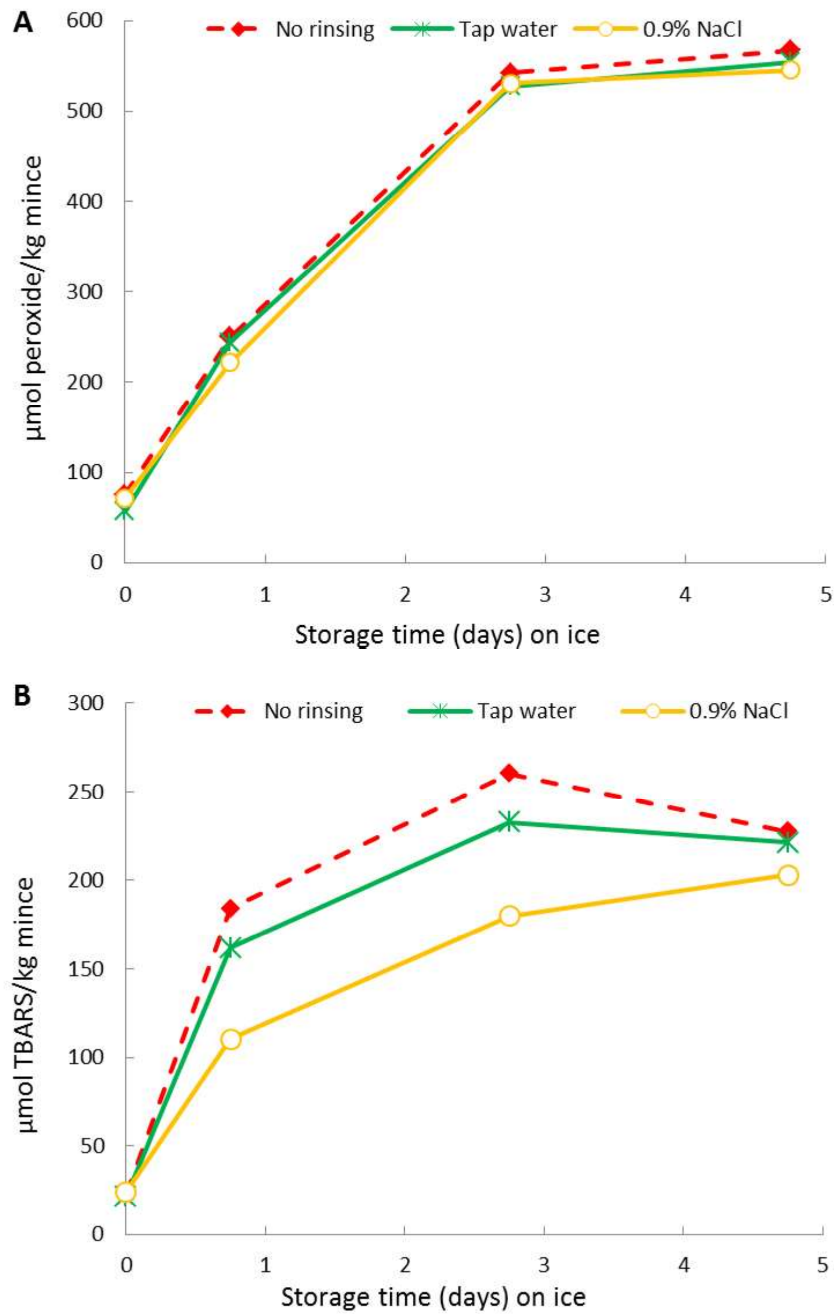
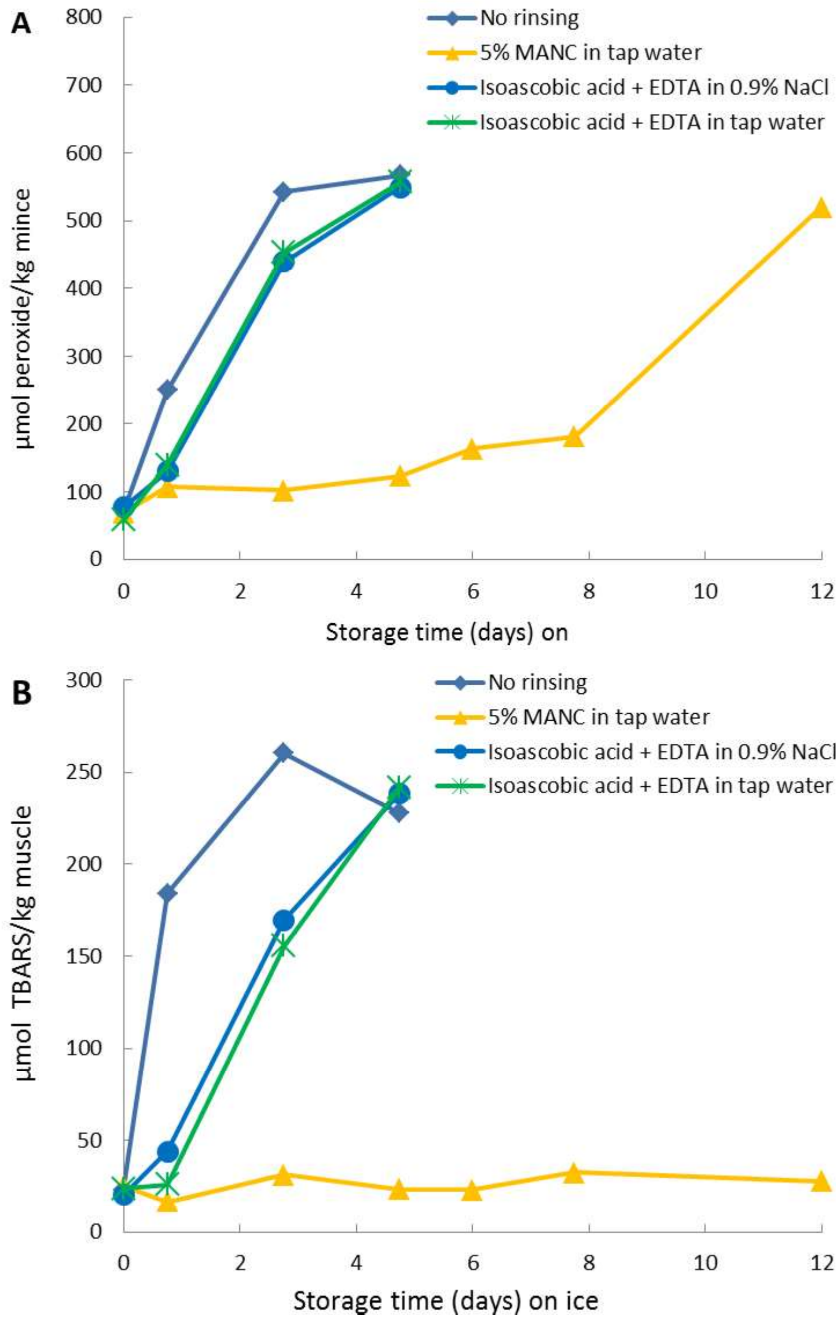


Figure 2.



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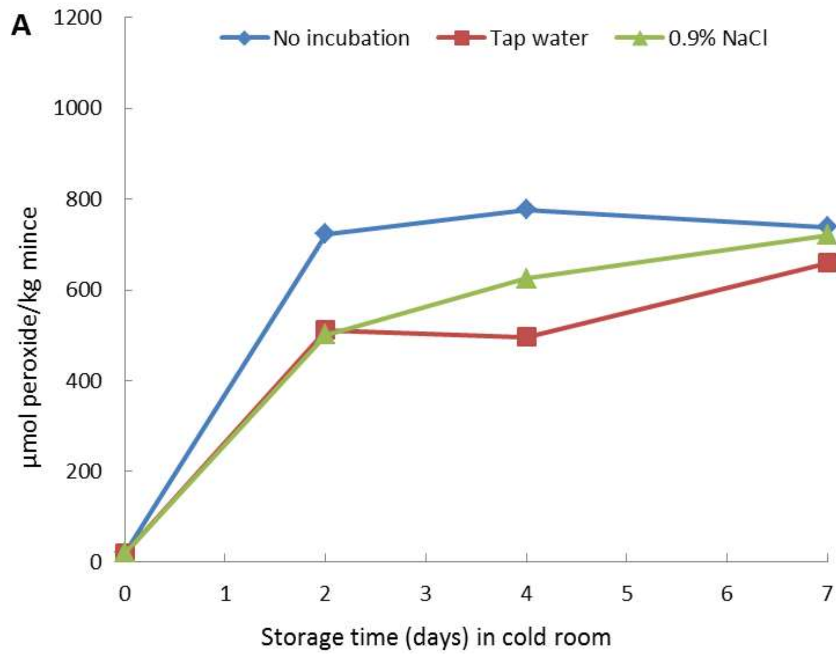

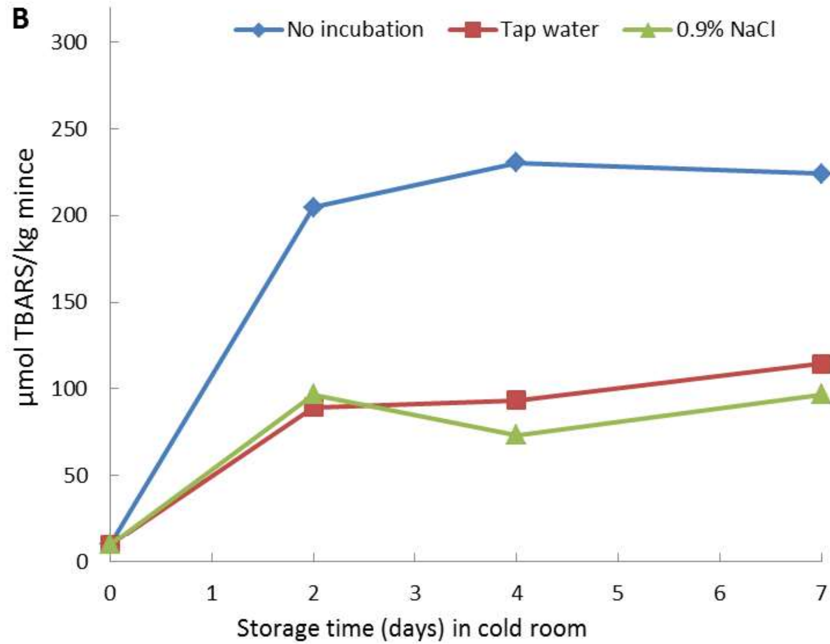

Figure 4.

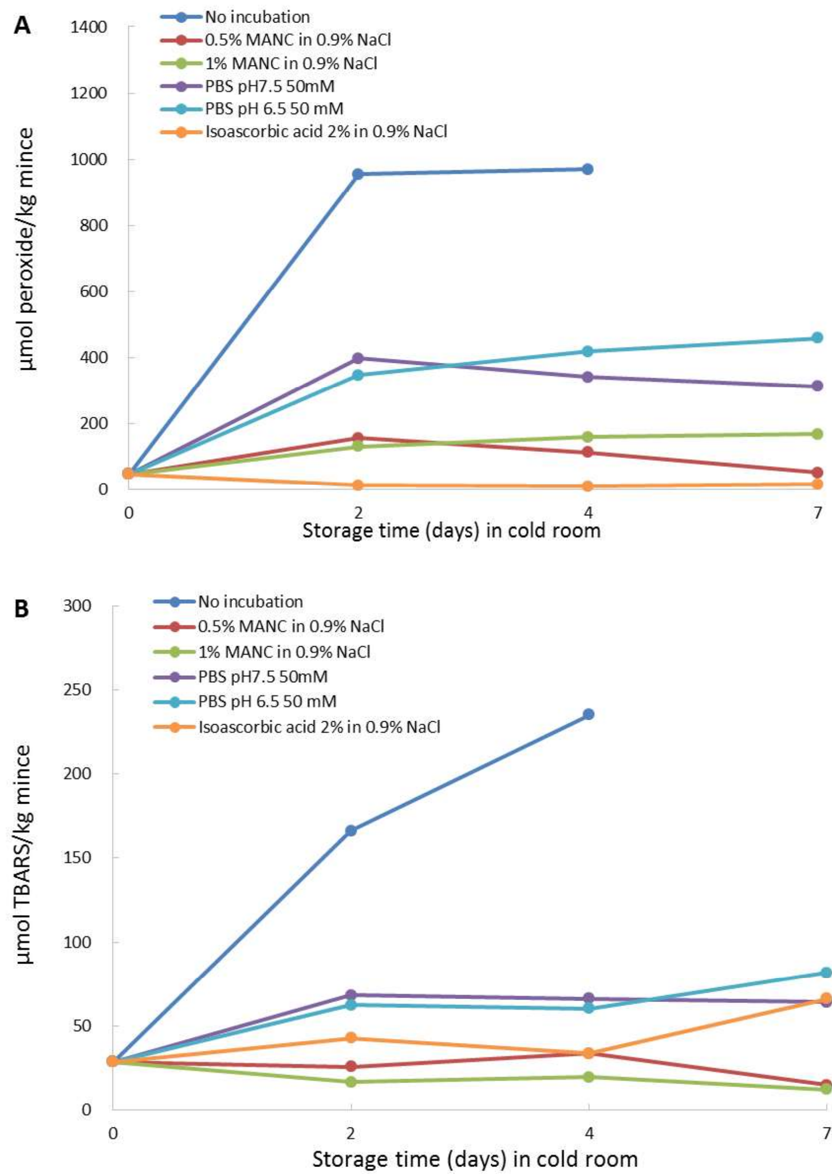

Figure 5.