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

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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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17   **Abstract**

18   The content of hemoglobin (Hb) in different parts of herring (*Clupea harengus*)

19   by-products, and three strategies for preventing lipid oxidation during their storage

20   on ice were studied: (i) rinsing away Hb with water or 0.9% NaCl with/without

21   antioxidants (Duralox-MANC, erythorbate and EDTA), (ii) incubation in water/0.9%

22   NaCl with/without antioxidants, (iii) mincing and adding antioxidants into the mince.

23   The   by-product   parts   were   ranked   as   follows   regarding   Hb-content:

24   head>backbone>caudal fin>residuals. Only 10-18% Hb could be rinsed away with tap

25   water or 0.9% NaCl, and the effect of such a rinsing on peroxide value (PV) or

26   TBA-reactive substances (TBARS) development was limited. Rinsing or incubating

27   by-products in antioxidant solutions however had remarkable effects; shelf life went

28   from <1 day to >12 days. Duralox-MANC was particularly efficient. The presented

29   strategies could be powerful tools to allow upgrading of herring by-products to food

30   ingredients, which is currently hindered by their extreme sensitivity to lipid

31   oxidation.

32   **Keywords:** Herring, Clupea harengus, by-products, lipid oxidation, hemoglobin,

33   rinsing, incubation, antioxidants

1. Introduction

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

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

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

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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).

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

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

To make the incubation solutions even more effective, different antioxidant additives were used in one of our sub-studies. Figure 4 shows that all incubation treatments had a lower lipid oxidation rate based on PV and TBARS values compared with controls stored in air, which agrees with the results of Figure 3. According to the PV and TBARS results, the effect of incubation with different additives was stronger than just incubation with tap water or 0.9% NaCl. Besides the reduced contact between oxygen and lipids (Richards, Kelleher, & Hultin, 1998), this could be attributed to one or both of the following two reasons: i) the incubation solution could remove or 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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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 ($P < 0.05$).
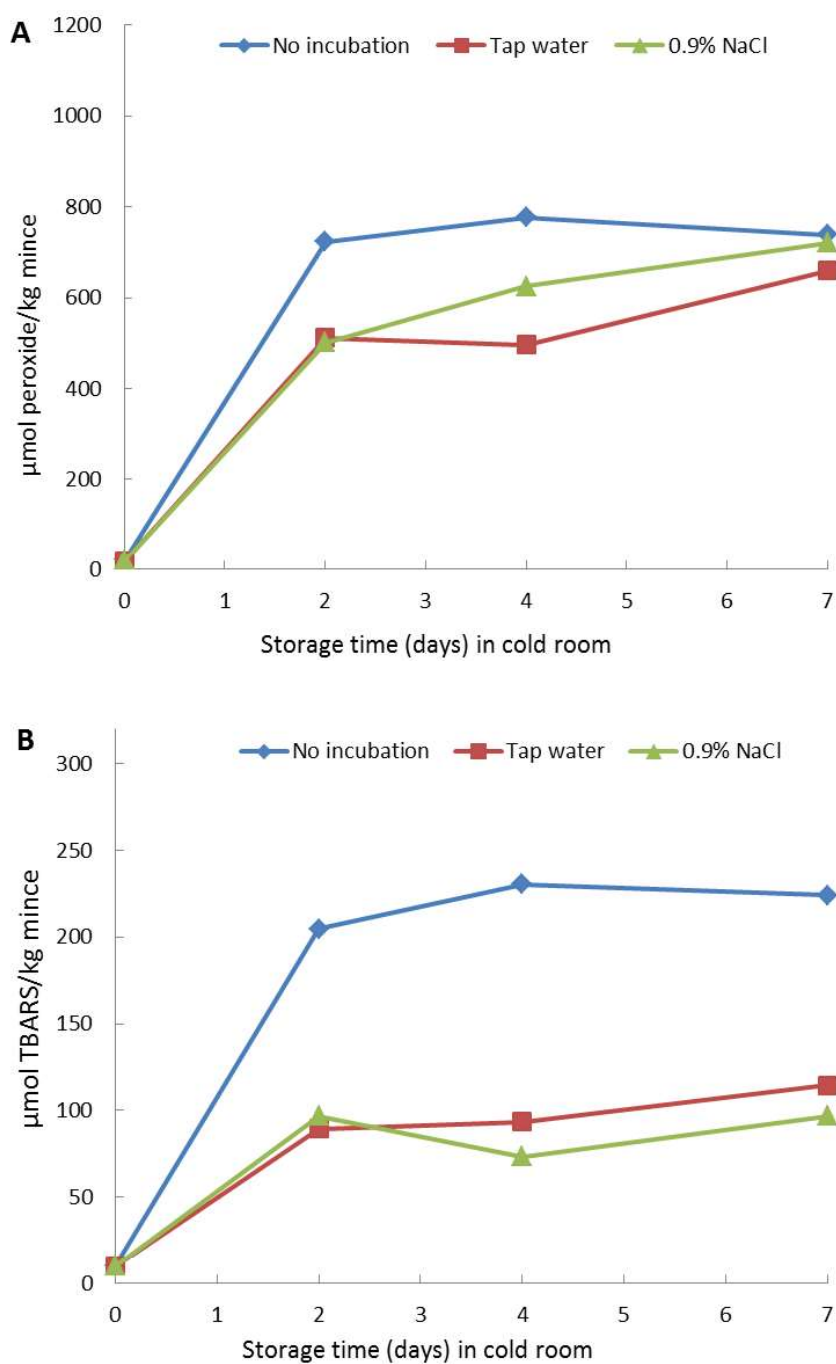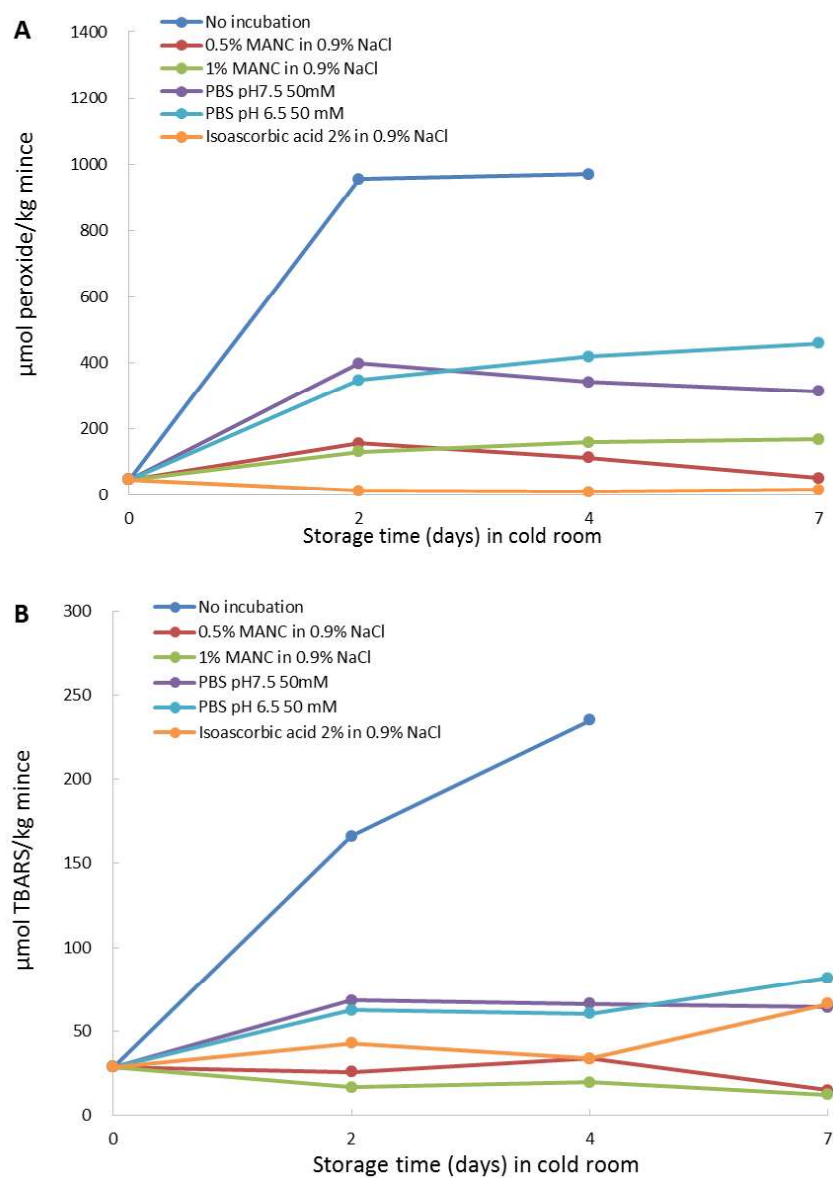
Figure 1.
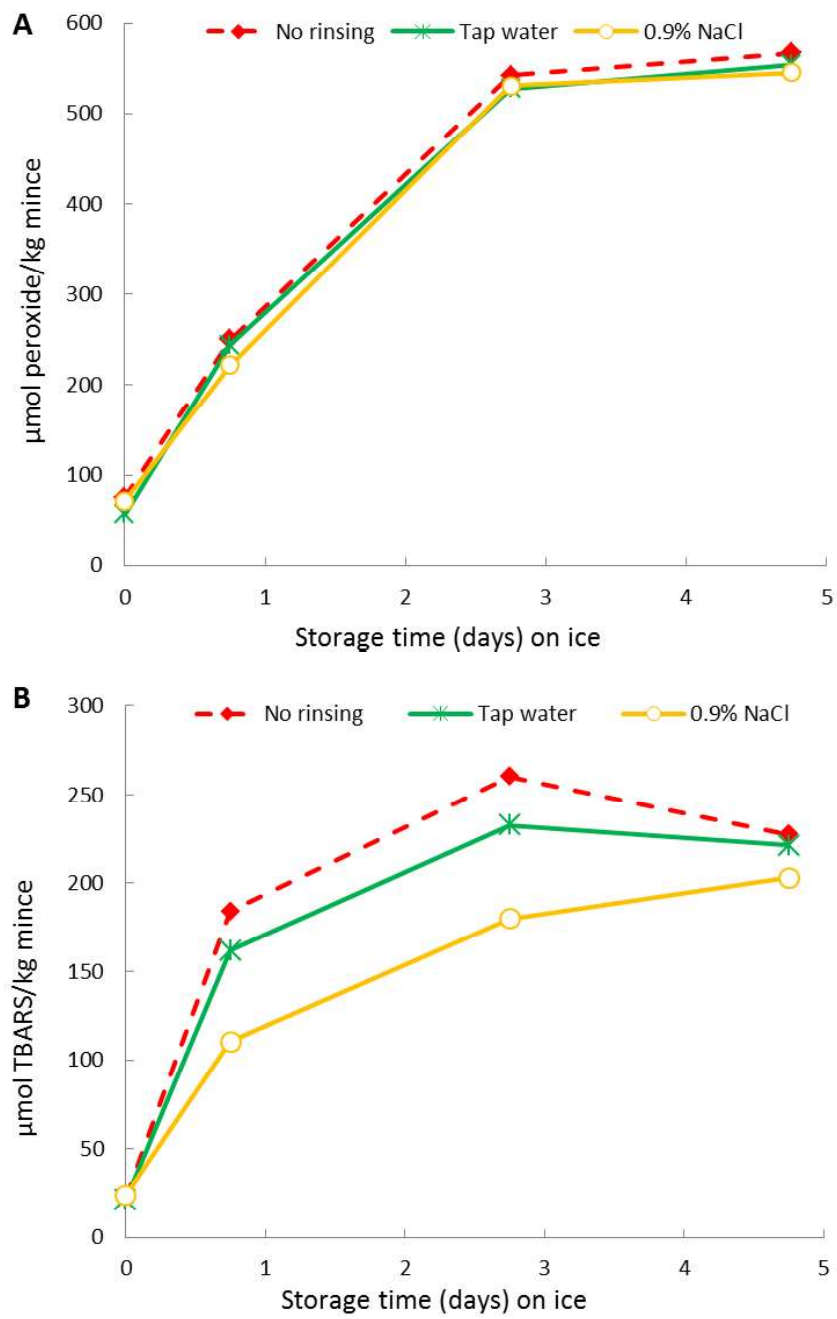
Figure 2.
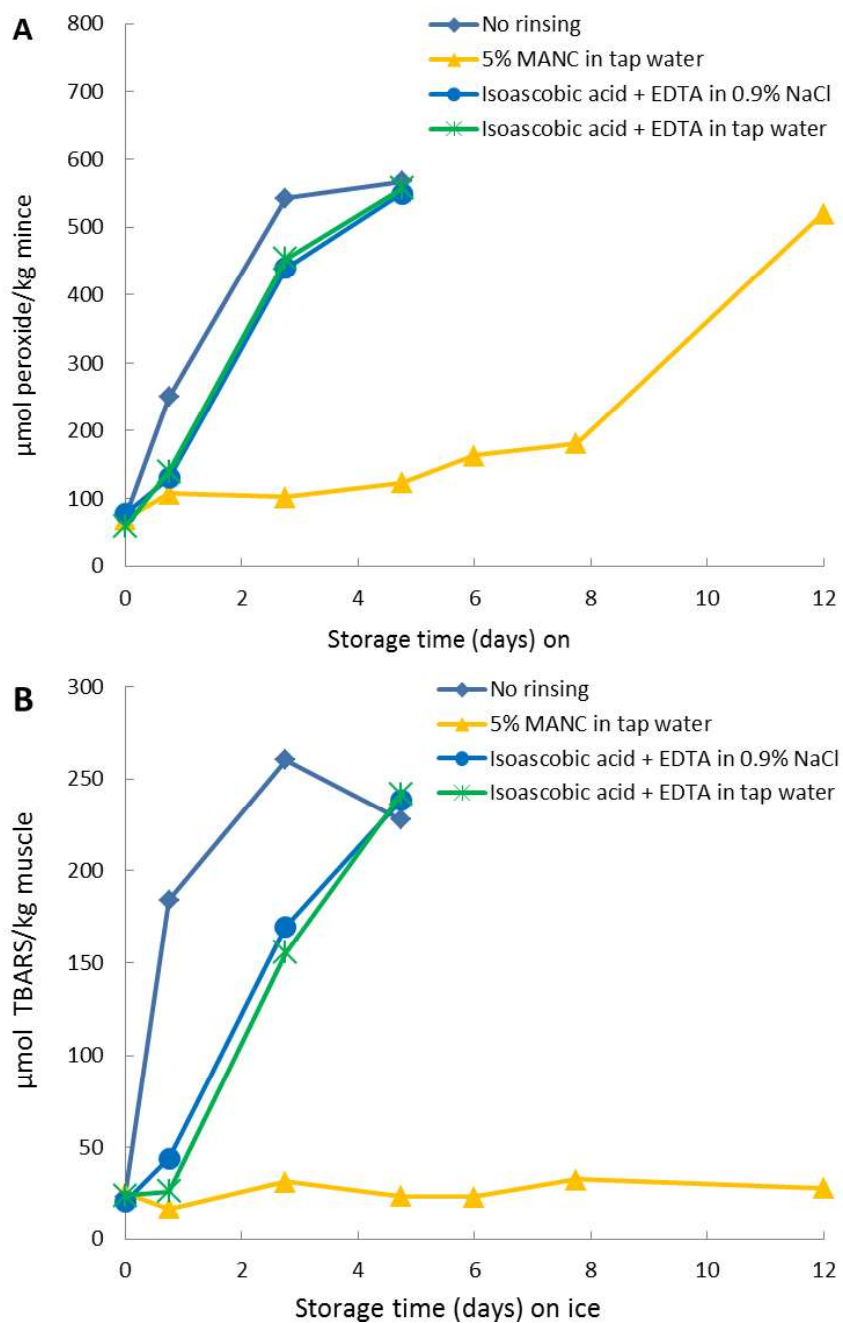
Figure 3.

Figure 4.

Figure 5.