1 # Effect of antioxidants on the sensory quality and physicochemical stability of
2 # Atlantic mackerel (*Scomber scombrus*) fillets during frozen storage

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14 **Abstract**

15 This study aimed to evaluate the shelf-life of mechanically filleted well-fed Atlantic mackerel during
16 frozen storage at -25 °C and effect of treatment with antioxidants (sodium erythorbate and a
17 polyphosphate mixture) and different antioxidant application methods (dipping, spraying and glazing).
18 Both physicochemical measurements and sensory analysis were applied. Antioxidant treatments
19 prolonged shelf-life of mackerel. Sensory analysis indicated that untreated fillets had a shelf-life of less
20 than 2.5 months, while all antioxidant treated fillets exceeded that. The most effective treatment,
21 dipping fillets into a sodium erythorbate solution, yielding a shelf-life of 15 months. Physicochemical
22 methods used to evaluate degradation of lipids in the fillets were free fatty acids (FFA), lipid
23 hydroperoxides (PV) and thiobarbituric acid reactive substances (TBARS). They did not correlate with
24 sensory results and might therefore be a questionable choice for evaluation of oxidation and
25 development of rancid flavour and odour in complex matrixes such as Atlantic mackerel.

26 **Keywords:** Atlantic mackerel (*Scomber scombrus*), fillets, sodium erythorbate, polyphosphate , lipid
27 oxidation

28 # 1 Introduction

29 Atlantic mackerel (*Scomber scombrus*) was for the first time caught in large quantities inside the
30 Icelandic fishing zone in 2007. It migrates into this zone in the summer months (June – September)
31 seeking feed to restore its energy reserves after spawning and travelling. The heavy feeding period
32 causes large changes in the muscle composition over a short period of time. The lipid content increases
33 from approximately 10-15% in June to 25-30% in September (Keay, 2011; Romotowska, Karlsdóttir,
34 Gudjónsdóttir, Kristinsson, & Arason, 2016a, 2016b). The high lipid content, in combination with the
35 constant abundance of heme proteins, makes mackerel caught during this period very susceptible to
36 lipid deterioration (Romotowska et al., 2016b; Richards et al., 1998). Initially mackerel caught around
37 Iceland was primarily processed into fish meal and oil. According to Statistics Iceland (2020) 93% of the
38 mackerel caught around Iceland in 2008 was processed into fishmeal and -oil compared to only 11% in
39 2018. This was accomplished through targeted research into the physical- and chemical properties of
40 Atlantic mackerel caught at this time of year. Specific catching and handling procedures were
41 introduced to improve quality making it possible to use larger portion of the catch for human
42 consumption, mainly freezing it whole or headed and gutted (Arason, Árnason, Helgason, Erlingsson,
43 Pétursdóttir, Þorgeirsson, Ottesen & Sveinbjörnsson, 2015; Romotowska et al., 2016a, 2016b). The
44 improved quality of mackerel caught in Iceland could create possibility for processing into even higher
45 valued products, such as frozen fillets. Rest raw materials from filleting contains high proportions of
46 valuable compounds like unsaturated fatty acids and can be utilized for production of other high value
47 products. Such production, however, is dependent on the fillets having a shelf-life that is adequate for
48 commercial distribution and a quality that is accepted by the market. But currently, knowledge
49 regarding stability and processability of the raw material caught during and directly following the heavy
50 feeding period is lacking.

51 Fillets from fatty fish species have been shown to be even more prone to lipid detoriation
52 during storage than whole fish, partly due to the increased access of air to the muscle (Aubourg et al.,
53 2004, 2005; Simeonidou et al., 1997). Therefore, the main challenge when processing this raw material

54 is to improve the shelf life by hindering lipid oxidation and hydrolysis. Traditionally shelf life is
55 prolonged by using e.g. additives with antioxidant properties or by choosing appropriate packaging
56 solutions (Aubourg et al., 2004; Karoui & Hassoun, 2017; Sone et al., 2020). Antioxidants are classified
57 depending on their mechanism. Primary antioxidants are radical quenchers, including e.g. sodium
58 erythorbate, ascorbic acid, and butylated hydroxytoluene (BHT). Secondary antioxidants are chelators,
59 including compounds such as citric acid and polyphosphates (Jacobsen, 2018). These different types of
60 antioxidants therefore have different inhibitory function within each food matrix (Jacobsen, 2018).
61 Reducing agents, e.g. sodium erythorbate, can also play an important role as antioxidants in heme-rich
62 systems such as mackerel since they prevent formation of the pro-oxidative met-form (Kelleher et al.,
63 1994). Kelleher et al. (1994) successfully used sodium ascorbate for this purpose in different mackerel-
64 based systems to retard lipid oxidation. Undeland, Hall, Wendin, Gangby and Rutgersson, (2005) also
65 saw the same strong antioxidative effect from using iso-ascorbic acid (i.e. erythorbate) during pH-shift
66 processing of herring. When applying phosphate and erythorbic acid as well as other antioxidants to
67 both mackerel mince and cubed mackerel prior to refrigerated storage, Weilmeier and Regenstein
68 (2004) observed that the amount of thiobarbituric reactive substances (TBARS) of the samples treated
69 with erythorbate was stable throughout 12 days of storage while the TBARS in untreated samples
70 increased ten fold. Phosphates did not inhibit lipid oxidation at a similar degree, although it was more
71 effective than other chelators, such as citric acid. Other studies have also been performed to evaluate
72 the effect of various antioxidants on the stability of lipids in Atlantic mackerel fillets or minced
73 mackerel muscle (Aubourg et al., 2005; Cropotova et al., 2019; Karoui & Hassoun, 2017; Özalp Özen &
74 Soyer, 2018; Richards et al., 1998; Weilmeier & Regenstein, 2004). To the best of our knowledge,
75 information about how antioxidant treatments affect sensory attributes of mechanically filleted well
76 fed frozen mackerel during long term frozen storage is lacking. This information is crucial to develop a
77 filleting process suitable for mackerel caught during the autumn.

78 Due to the complexity of lipid oxidation, there is no universal threshold value for when classic
79 chemical measures of these reactions, such as the perioxide value (PV) or thiobarbituric reactive

80 substances (TBARS), correspond to unpalatable levels of rancid flavour or odours. However, sensory
81 evaluation by a trained panel can provide valuable information regarding the intensitiy of these
82 attributes, which can in turn be used to define the shelf-life of the product.

83 The aim of this study was to evaluate the effect of two different antioxidants, sodium
84 erythorbate and a mixture of polyphosphates, on the sensory (flavour, odour, texture and appearance)
85 and physicochemical properties of filleted Atlantic mackerel stored at -25°C, compared to untreated
86 fillets and whole fish. The effectiveness of different antioxidant application methods was also
87 evaluated, including ***spraying*** the fresh fillets with the antioxidant solutions, ***dipping*** fillets into
88 solutions of antioxidants prior to freezing, or by adding the antioxidants to a ***glaze*** that was applied
89 after freezing of the fillets.

90 2 Materials and methods

91 2.1 Raw material and handling

92 Atlantic mackerel (*Scomber scombrus*) was caught by a trawler off the East coast of Iceland (64°38,96'N
93 - 012°52,19'W) on the 31st of August 2016. It was caught in a mid-water pelagic trawl with a towing
94 time of 241 min and haul size of 211 tons. Most of the catch is killed in the trawl during hauling and
95 during pumping into cooling tanks. Post-mortem the catch was cooled rapidly, pre-rigor, in the tanks
96 to -1.5 °C with refrigerated seawater (RSW) to limit damage of the mackerel muscle caused by
97 proteolytic activity of the copepod *Calanus finmarchicus,* which is the main component of their feed
98 (Prokopchuk & Sentyabov ,2006). Grading based on the Stomach Fullness Index, evaluating the amount
99 of feed in the stomach resulted in a grade of 2 on a scale from 0 to 5, indicating some filling of the
100 stomach. Prior to landing the catch was kept in the cooled RSW storage tanks for 48 hours. After
101 landing and grading the mackerel (300-500 g) was mechanically filleted (VMK11-M120, Arenco VMK,
102 Sweden). The filleting protocol was decided in a pretrial evaluating the effects of superchilling before
103 filleting on gaping. According to the pretrial, the least gaping was seen in the fillets when the whole
104 fish was cooled to a core temperature of -1 to -1.5°C in a 15% brine with a fluid temperature of -10°C

105 prior to mechanical filleting. The brine cooling process made the mackerel slightly stiffer, and thus
106 easier to fillet, resulting in less gaping.

107 The fillets were treated with antioxidants either by dipping, spraying or glazing as described in
108 Table 1. The antioxidants used were sodium erythorbate (E316) (Bio-Engineering Co., Ltd, Zhengzhou,
109 China) and a mixture of sodium and potassium di- and tripolyphosphate (E450 and E451) (Carnal 2110,
110 CFB Bundheim, Bundheim, Germany). These antioxidants are both widely used in the fishing industry
111 and were chosen due to their different antioxidant mechanisms, i.e. the sodium erythorbate as a
112 primary antioxidant and reducing agent, and the polyphosphate mixture due to its secondary
113 antioxidant mechanism (Jacobsen, 2018; Weilmeier & Regenstein, 2004).

114 Untreated fillets and whole fish from the same haul were collected as reference samples.
115 Samples of whole mackerel were frozen using an industrial automatic box freezer (Skaginn, Akranes,
116 Iceland) and all fillets were individually quick frozen (IQF). After freezing the fillets were packed, 3 kg
117 per box, in low-density polyethylene (LDPE) plastic bags (Kivo, Volendam, Netherlands) and corrugated
118 cardboard boxes (Smurfit Kappa Narpapp AS, Dublin, Ireland). Samples were transported to the
119 research facility and stored at -25±1.8°C for up to 15 months. Samplings were performed after 2.5, 4,
120 8, 10, 12 and 15 months of frozen storage. Prior to analysis, the filleted samples were thawed at 0-2°C
121 for 16 hours while covered with plastic. The whole fish was thawed for 24 hours at 0-2°C prior to
122 analysis. Chemical and physical measurements, other than colour and cooking yield, were performed
123 on duplicate samples, each consisting of 3 minced fillets with skin. The whole fish were hand filleted
124 prior to analysis, and fillets with skin from three fish in each duplicate sample were used for analysis.
125 Sensory evaluation was performed on 10 fillets per group, and the cooking yield and colour was
126 evaluated on 5 fillets per group at each sampling occation.

127 2.2 Chemical composition of mackerel muscle

128 The water content of the samples was measured by determining the difference in weight of the minced
129 muscle samples before and after drying for 4 h at 102-104°C (ISO, 1999). The total lipid content of the

130 samples was obtained according to the method of (Bligh & Dyer, 1959). The protein content was
131 determined using the Kjeldahl method (ISO-5983-2, 2005) using a Tecator, with two deviations. Sulfuric
132 acid was used instead of hydrochloric acid, and a sample size of 1.5-2.0 g was used for samples with
133 protein content in the range from 3 to 30 g protein/100 g wet muscle instead of 1.0-1.2 g as described
134 in the original method. The salt content was determined using the Volhard titration method (AOAC,
135 2000). Content of water, total lipids, protein and salt were all presented as g per 100 g wet muscle.

136 2.3 Fatty acid profile of mackerel muscle

137 The fatty acid profile of the minced mackerel muscle was determined in the lipid extracts from each
138 group of minced fillets following 2.5 and 12 months of storage. It was done by gas chromatography
139 (Varian 3900 GC, Varian, Inc., Walnut Creek, CA, USA), of fatty acid methyl esters (FAMEs) according
140 to the AOCS method (AOCS, 1998), using methyl undecanoate (C23:0) as an internal standard. The GC
141 was equipped with a fused silica capillary column (HP-88, 100 m x 0.25 µm film), a split injector, and a
142 flame ionization detector, fitted with a Galaxie Chromatography Data System (Version 1.9.3.2
143 software, Varian Inc., Walnut Creek, CA, USA). The oven was set to 100 °C for 4 min, then increased to
144 240 °C at a rate of 3 °C/min and that temperature held for 15 min. The injector and detector
145 temperatures were 225 °C and 285 °C, respectively. Helium was used as a carrier gas at a column flow
146 rate of 0.8 mL/min, and a split ratio of 200:1. The program was based on the AOAC-996.06 (2001)
147 method.

148 The polyene index (PI) was calculated according to equation 1 (Rodríguez et al., 2007):

149 $$PI = \frac{(C22:6 + C20:5)}{C16:0} \quad (1)$$

150 where C22:6 represents docosahexanoic acid (DHA), C20:5 represents eicosapentaenoic acid (EPA) and
151 C16:0 palmatic acid.

152 To ease comparisons between treatments the total area of the peaks corresponding to the
153 evaluated fatty acids presented were used when calculating the ratio of each individual fatty acid.

154 ## 2.4 Physical properties of mackerel muscle

155 Liquid holding capacity (LHC) of the mackerel mince samples was determined by a centrifugation
156 method (Eide, Børresen and Strøm, 1982). Since both water and lipids are separated out from the fatty
157 mackerel muscle during centrifugation the liquid holding capacity was evaluated, rather than water
158 holding capacity alone. Sample tubes made from cylindrical plexiglass (h 62 mm, inner Ø 19 mm, outer
159 Ø 25 mm) and a filtering membrane (100 µm mesh size) at the bottom, were used. Approximately 2 g
160 of minced sample was weighed into the tubes and centrifuged (Biofuges Stratas, Thermo electron
161 corporation, Germany) at 1350 g for 5 min at 4°C. The weight loss during centrifugation was then
162 recorded and the LHC calculated according to equation 2

163 $$\text{LHC}\ (\%) = \frac{((\%\ \text{water} + \%\ \text{lipid})\ \text{x g sample}) - (\text{g weight lost during centrifugation})}{(\%\ water + \%\ lipid)\ x\ g\ sample}\ x\ 100 \quad (2)$$

164 Cooking yield was determined from the weight loss recorded during cooking of a fillet (70 - 100
165 g) in a preheated steam oven (Convotherm, Elektrogeråte CmbH, Eglfing, Germany) at 100 °C for 6 min.
166 After cooking, the samples were drained and let to cool at room temperature (15 min) before being
167 weighed again. The cooking yield was calculated according to equation 3:

168 $$\text{Cooking yield}\ (\%) = \frac{g\ cooked\ sample}{g\ raw\ sample}\ x\ 100 \qquad (3)$$

169 ## 2.5 Colour of mackerel fillets

170 The colour of the samples was determined with a Minolta Chroma Meter CR-300 (Minolta, Osaka,
171 Japan) using the CIE Lab system. The instrument recorded the L^*-value, indicating lightness on the scale
172 from black to white, 0 to 100 repectively, the a^*-value, ranging from (+) red to (−) green, and the b^*-
173 value, ranging from (+) yellow to (−) blue. The colour was measured above the lateral line in three
174 positions, from the head to the tail of 5 raw fillets for each group, during each sampling.

175 ## 2.6 Lipid hydrolysis and oxidation

176 The free fatty acid (FFA) content was determined from the lipid extracts using the method described
177 by Lowry and Tinsley (1976) with a modification as described by Bernárdez, Pastoriza, Sampedro,

178 Herrera and Cabo (2005). The amount was determined using an oleic acid standard curve ranging 0-20

179 µmol and expressed as g FFA per 100 g lipids. Duplicates were analysed from each sample.

180 The peroxide value (PV) of the muscle was determined with the ferric thiocyanate method

181 (Shantha & Decker, 1994) with modifications as described by (Romotowska et al., 2016a) to determine

182 primary oxidation products. The results were expressed as µmol lipid hydroperoxides per g muscle.

183 Triplicates were extracted from each sample.

184 Thiobarbituric acid reactive substances (TBARS) were determined with the method described

185 by Lemon (1975) with modifications as described by (Romotowska et al., 2016a) to determine

186 secondary oxidation products. The results were expressed as malondialdehyde diethyl acetal (MDA)

187 equivalents per gram muscle. Triplicates were extracted from each sample.

188 2.7 Sensory analysis

189 A Generic descriptive analysis (GDA) (Stone and Sidel, 2004), was used to assess cooked mackerel fillet

190 samples, from all sample treatments. A panel of eight to eleven panellists, trained according to ISO

191 standard (1993), participated in the sensory evaluation during each session. The panel was trained

192 using Atlantic mackerel of different quliaty levels in three sessions. The panel training involved training

193 in the detection and recognition of flavour, odour, appearance and texture attributes, and describing

194 their intensity on an unstructured scale from 0 to 100. Twenty-seven attributes were evaluated, for

195 appearance (7), odour (8), flavour (8) and texture (4) (Table 2).

196 In preparation for the sensory evaluation, fillets or whole fish were thawed as described

197 earlier, and two equal portions (approximately 30 g per piece) were cut from the centre part of each

198 fillet, placed in aluminium boxes coded with random three-digit numbers. The samples were heated

199 at 100°C for 5 minutes in a pre-warmed oven (Convotherm Elektrogeräte GmbH, Eglfing, Germany)

200 with air-circulation and steam, and served to the panel at a temperature of 65-75°C. Duplicate samples

201 were evaluated by each panellist in a random order in all sessions. A maximum of four samples were

202 evaluated per sensory session and a maximum of two sensory sessions were held in one day.

203 The mackerel samples were defined to have reached its maximum shelf-life when a rancid
204 flavour or odour value >20 was obtained. At that value most panellists detected the attribute at an
205 intensity level that would deem the product unpalatable (Magnússon et al., 2006).

206 ## 2.8 Chemicals

207 All chemicals used for analysis in this study were of analytical grade and purchased from Sigma-
208 Aldrich (St. Louis, MO, USA), Sigma-Aldrich (Steinheim, Germany) and Fluka (Busch, Switzerland).

209 ## 2.9 Statistical analysis

210 Statistical analysis of data from evaluation of chemical and physical attributes was performed using
211 Microsoft Excel 2016 (Microsoft Inc. Redmond, Wash, USA), IBM SPSS Statistics v. 26 (International
212 Business Machines, Armonk, New York, USA) and SigmaPlot 12.0 (Dundas SoftwareLtd., GmbH,
213 Germany). Pearson's correlation, one-way and/or two-way analysis of variation (ANOVA) and Duncan's
214 post hoc test was applied on all samples for each group and the significance level was set to $p \leq 0.05$
215 for all statistical analyses.

216 Data collection during sensory analysis was performed using the FIZZ software (Version 2.50B,
217 Biosystémes, Couternon, France), and Panelcheck V1.4.0 (Nofima, Tromsø, Norway) was used to
218 monitor the panellists performance. To carry out the statistical analysis a General Linear Model (GLM)
219 corrected for panellists use of scale was performed using NCSS (NCSS 2000, Utah, USA). Duncan's post
220 hoc test was used to analyse statistical differences between the sample groups.

221 A principal component analysis (PCA) was performed using Unscrambler (Version 10.5.1,
222 CAMO ASA, Trondheim, Norway) to assess the connection between the evaluated variables and the
223 variation between samples. The data was centered and all variables weighed with the inverse of the
224 standard deviation to correct for different scales of variables. The model was fully cross-validated.

225 # 3 Results and discussion

226 ## 3.1 Chemical composition of mackerel muscle

227 The Atlantic mackerel used in this study had an average water content of 59.7±1.9 g / 100 g muscle, a
228 protein content of 16.4±0.7 g / 100 g muscle and a lipid content of 20.5±2.7 g / 100 g muscle. The
229 variation in the proximate composition (water, protein and lipid content) between sample groups or
230 over storage time was high, but no signifcant differences ($p>0.05$) were observed in these parameters
231 between the groups or during the storage period. A similar composition of Atlantic mackerel caught at
232 this time of year has been reported in earlier studies (Brix et al., 2009; Romotowska et al., 2016a).

233 The lipid content varied greatly among individual samples, ranging from 14.5 to 30.1 g / 100 g
234 muscle. Romotowska et al. (2016b) studied seasonal and geographical variation on the chemical
235 composition of Altantic mackerel caught in Icelandic waters, yielding similar results, but with larger
236 variation between samples (26.5±7.4 and 20.3±4.5 g lipid / 100 g muscle in 2012 and 2013,
237 respectively). This variation between the studies can be explained by individual variations, annual and
238 seasonal differences in environmental factors, as well as by different sampling techniques.
239 Romotowska et al. (2016b) performed analysis on three individual mackerels for each sampling in
240 order to show the variation between individuals specifically, while in the present study three
241 individuals were combined into each sample duplicate, lessening the effect of individual variation on
242 results.

243 The whole fish had an average salt content of 0.3±0.1 g / 100 g muscle, while the fillets
244 contained an average of 0.6±0.1 g NaCl/ 100 g muscle ($p<0.001$) during the storage period. The slightly
245 higher salt content in the fillets was a result of the brine cooling performed prior to the mechanical
246 filleting.

247 ## 3.2 Fatty acid profiles of mackerel muscle

248 The fatty acid composition of the sample groups was analysed after 2.5 and 12 months of storage. The
249 fatty acid composition was not affected by the antioxidant treatments and/or mechanical filleting prior
250 to frozen storage ($p>0.05$). Therefore it is concluded that the antioxidant treatments did not effect the

251 fatty acid composition. Due to this the results shown in Table 3 are presented as mean values of the
252 fatty acid composition of all samples at 2.5 months and 12 months, respectively, regardless of other
253 treatments before storage. The Atlantic mackerel had a high ratio of monounsaturated fatty acids
254 (MUFA) and polyunsaturated fatty acids (PUFA). The most abundant PUFAs were docosahexaenoic acid
255 (DHA, C22:6n3) and eicosapentaenoic acid (EPA, C20:5n3). Romotowska et al. (2016a, 2016b) reported
256 similar fatty acid compositions in mackerel caught around Iceland. The most abundant
257 monounsaturated fatty acids (MUFA) were eicosenoic acid (C20:1) and cetoleic acid (C22:1n11), fatty
258 acids that are commonly found in the copepod *Calanus finmarchicus*, on which the mackerel feeds
259 around Iceland (Prokopchuk & Sentyabov, 2006).

260 Some minor changes in the relative fatty acid composition were observed during the frozen
261 storage in the present study. Samples analysed at 12 months of frozen storage contained on average
262 a 4.7 % higher PUFA concentration and 4.0 % lower concentration of MUFA than after 2.5 months of
263 frozen storage. The relative PUFA and MUFA concentrations thus significantly correlated with storage
264 time ($r=0.802$ and $r=-0.763$, respectively ($p<10^{-4}$)). This difference was caused by a small increase in
265 DHA (an average of 12.2 increasing to 15.0 %) and decrease in cetoleic acid (C22:1n11) (an average of
266 16.2 decreasing to 13.7 %) during the frozen storage. Therefore, samples analysed at 12 months of
267 storage had higher calculated PI than those measured at 2.5 months of storage ($p<0.05$). However, the
268 variation in composition of the raw material used in the study was large, e.g. lipid content varied 14.5
269 to 30.1 g / 100 g muscle. This slight difference in PI could e.g. be attributed to the large variation in the
270 mackerel.

271 3.3 Lipid hydrolysis and oxidation of mackerel muscle

272 The FFA content of the muscle was measured to evaluate lipid hydrolysis during frozen storage. The
273 amount of FFA in the mackerel samples increased significantly with storage time ($r=0.606$) (Figure 1).
274 Results from the two-way ANOVA and post hoc test showed that both the antioxidant treatments and
275 storage time affected the formation of FFA ($p<0.05$). All antioxidant treatments inhibited lipid
276 hydrolysis, especially when dipping was used. Dipping the fillets into sodium erythrobate (SED) had a

277 larger inhibitory effect on lipid hydrolysis of the fillets compared to dipping them into the
278 polyphosphate solution. A slight decrease in FFA was observed in the whole mackerel and SED fillets
279 at the end of the storage period. Due to large indiviudal variation in each group, however, this change
280 was not significant.

281 Lipid hydroperoxides (PV) were measured to evaluate the formation of primary oxidation
282 products. The PV in the samples increased with storage time, especially between the 2nd and 4th month
283 and the 8th and 12th month. Values peaked at the 12th month where after the PV then decreased
284 significantly between the 12th and 15th month of storage (Figure 1). The efficiency of the antioxidants
285 to inhibit primary oxidation products varied between treatments. The PV formation was significantly
286 slower in fillets treated with sodium erythorbate than in the polyphosphate treated fillets, as well as
287 compared to the untreated fillets and whole fish ($p<0.05$). However, the application method of the
288 erythorbate (dipping, spraying or glazing) did not significantly affect the rate of PV formation during
289 the storage period ($p<0.05$).

290 Many of the secondary oxidation products are volatile and therefore responsible for rancid
291 odour and flavour. Their formation can thus limit shelf-life due to quality loss and unpalatability.
292 Quantification of TBARS is commonly used to assess carbonylic secondary oxidation products in fish
293 products during frozen storage, and have in some systems such as washed cod mince correlated well
294 with rancid odour development (Larsson et al., 2007). In this study TBARS values first peaked at the 4th
295 month of storage and then decreased, most likey due to the high reactivity of carbonyls towards e.g.
296 proteins and phospholipids, causing e.g. the formation of Schiff bases and Michael adducts. Between
297 the 10th and 12th month, TBARS increased again (Figure 1). This trend can possibly be explained by
298 different oxidation reactions (autoxidation and photooxidation) with different speeds and initiation
299 times, e.g. depending on the level of saturation and position of the lipids within the muscle tissue
300 (Aidos et al., 2002). Overall lipid and muscle degradation during the early stages of the frozen storage
301 may also increase the access of radicals or other oxidizing agents to earlier inaccessible fatty acid

302 groups, triggering a second, delayed TBARS oxidation peak later during the frozen storage (Dang *et*
303 *al*.,2018; Romotowska, 2016) . Dang *et al*. (2018) noted a similar two peak TBARS trend in the dark
304 muscle of herring as did Romotowska,(2016) when assessing TBARS values of whole mackerel caught
305 in Icelandic waters in July 2012, both supporting this observation. Furthermore, (Aidos et al., 2002)
306 explained observed douple peaks in PV and anisidine in Maatjes herring oil by delayed oxidation
307 reactions as stated earlier. Small differences were present between the sample groups at the individual
308 sampling points, but these differences were neither consistent nor significant amongst groups through
309 the frozen storage according to the two way ANOVA.

310 In theory, a secondary oxiation product would be expected to peak after the decomposition of
311 the primary oxidation products, i.e. the lipid hydroperoxides (Jacobsen, 2018; Janero, 1990). However,
312 the TBARS assay measure carbonyl concentrations of the muscle. Some carbonyls, like MDA, can be
313 formed both during primary and secondary lipid oxidation processes (Janero, 1990), which can explain
314 why a strong peak in TBARS is observed before a peak in PV is observed. Furthermore, MDA is not the
315 only product of lipid peroxide decomposition (others include e.g. carbonyl compounds, hydrocarbons,
316 furans etc.) and may also react with other substances than TBA, which weakens the applicability of this
317 analytical method for a realistic assessment of the complex oxidation mechanisms occurring in
318 biological tissues (Janero, 1990).

319 These results therfore raise questions regarding the suitability of measuring TBARS to evaluate
320 secondary oxidation products as indication of possible changes in rancid flavour or odour in Atlantic
321 mackerel.

322 3.4 Physical properties of mackerel muscle

323 The liquid holding capacity (LHC, 88.0±5.9 %) and cooking yield (CY, 91.7±1.0 %) did neither differ
324 amongst treatments nor with increased storage time partially due to high individual variation in
325 chemical composition within each treatment group. Therefore, this observation indicates that neither
326 the lipid oxidation, nor lipid hydrolysis affected the liquid holding characteristics of the muscle.

327 ## 3.5 Colour of mackerel fillets

328 The colour of the mackerel samples was measured and described according to the CIE Lab system. The
329 L^*-value (lightness) of the samples remained stable over time and no differences were observed
330 between the different groups. Higher a^*-values observed in whole fish than fillets indicated that the
331 whole fish was more red in colour than the mechanically filleted mackerel ($p<0.05$). It did, however,
332 not change with storage time. The higher a*-values in the whole fish may indicate a lower degree of
333 oxidation of heme-proteins in the muscle into the brownish-grey met-form. The b^*-value increased
334 with storage time in all groups, indicating an increase in yellow pigmentation associated with the
335 polymerization of tertiary lipid oxidation products during the frozen storage (Hamre et al., 2003). The
336 whole fish was in all cases less yellow and more red in colour than the fillets ($p<0.05$), supporting earlier
337 studies showing that fillets are more prone to lipid oxidation than whole fish (Aubourg et al., 2005;
338 Simeonidou et al., 1997). However, no differences were detected in the colour parameters between
339 filleted groups regardless of antioxidant treatment prior to storage.

340 ## 3.6 Sensory analysis

341 Sensory analysis was performed to evaluate 27 sensory attributes of the mackerel samples. During
342 panel training it was noted that the most indicative attributes of the shelf-life for this raw material
343 were rancid flavour and odour. Therefore, as mentioned in the materials and method section, samples
344 were defined as having reached the end of their shelf-life when an average value above 20 was reached
345 for rancid flavour and/or odour. The assessed changes in rancid odour and flavour of the mackerel
346 samples throughout storage are shown in Table 4.

347 Untreated fillets reached the shelf-life threshold for rancid flavour after 2.5 months of storage,
348 indicating that additional measures to increase the shelf-life of the mackerel fillets were required.
349 Although the untreated fillets obtained scores above the rancidity limit at the first sampling point it
350 was decided to continue evaluating them until other groups started showing signs of rancidity as well.
351 All antioxidant treatments resulted in an prolonged shelf-life, especially dipping the fillets into the SED.
352 After 15 months of storage, SED fillets had an average score of 15.2 for rancid flavour compared to

353 16.9 for whole fish, indicating that the SED fillets and the whole fish had not yet reached their
354 maximum shelf-life. This indicates that sodium erythorbate dipping is an effective method to prolong
355 the storage of mechanically filleted mackerel. Other treatments, i.e.all polyphosphate applications, as
356 well as sodium erythorbate spraying and glazing, resulted in a maximum shelf-life of 8 to 10 months at
357 -25°C, showing that both the antioxidant type and the methods of application (dipping, spraying or
358 glazing) affected the storage life. According to this study, sensory evaluation of rancid flavours and
359 odours are therefore clearly more appropriate methods to assess the shelf-life of Atlantic mackerel
360 products than the chemical measurements PV and TBARS. However, the sensory assessment does not
361 provide any details on the the chemical reactions leading to the rancidity formation. Lugasi et al. (2007)
362 reported when evaluating shelf-life of horse mackerel (*Trachurus trachurus*) that TBARS had no
363 correlation with the rancid flavour or odour as evaluated using sensory analysis.

364 The results from this study confirm conclusions of previous studies on pelagic fish showing that
365 antioxidant treatment leads to prolonged shelf-life as evaluated by sensory analysis (Kelleher et al.,
366 1992; Lugasi et al., 2007). The antioxidants application method clearly affected oxidation inhibition of
367 SED treated fillets. This could be explained by e.g. the different antioxidant mechanisms, as well as
368 how well the antioxidants were incorporated in to the muscle. Pazos, Alonso, Fernández-Bolaños,
369 Torres and Medina (2006) concluded that spraying antioxidant solutions on to fillets was more efficient
370 than glazing with them. Furthermore, the same study showed that washing fillets prior to spraying
371 could be preferable. Richards et al. (1998) also concluded that washing fresh fillets of Atlantic mackerel
372 could rinse away compounds with pro-oxidative activity, e.g. heme, to prolong shelf-life. In their study,
373 washing fillets with antioxidant solutions proved more efficient in prolonging shelf when compared to
374 fillets washed with water before being stored at -20°C. Thus, the dipping with SED probably allowed
375 removal of surface bound hemoglobin, and at the same time allowed deeper penetration of the
376 antioxidant into the fillet compared to spraying. The reason for the higher effectiveness of SED over
377 phosphates is thought to be a combination of radical scavenging, and prevention of heme-proteins
378 being oxidized into their met-form. The latter are very effective in cleaving lipid hydroperoxides into

379 e.g. volatile aldehydes and ketones (Richards & Hultin, 2002). Phosphates act mainly as chelators of
380 low molecular weight metals, and in fish, these appear to play a minor role as pro-oxidants compared
381 to e.g. haemoglobin and myoglobin (Undeland et al., 2002).

382 During sensory analysis panellists were asked to evaluate the colour (on a scale from pale to
383 dark) and to report discolouration of the samples. The sensory panel neither detected a change in
384 colour (from pale to dark) in the samples during the frozen storage nor between treatments, which is
385 in agreement with the CIE lab assessment for the lightness (L^*-value) of the samples. However, the
386 applied sensory scales were not able to assess yellow colour, which was the main discolouration
387 according to the CIE lab measurements. During further research, an adjustment of the sensory scale
388 might thus be in order, especially when evaluating fatty fish such as Atlantic mackerel, where both
389 lightness and yellow discolouration can be correlated to lipid oxidation processes (Hamre et al., 2003).

390 Sensory evaluation of the texture and flavour of the samples showed that from the 10th month
391 of storage, the fillets tasted slightly saltier than the whole fish, although within a palatable limit at all
392 sampling points. These results are supported by the differences in salt content of 0.3±0.1 and 0.6±0.1
393 g / 100 g muscle in the whole fish and fillets, respectively ($p<0.001$), caused by the brine treatment
394 prior to filleting. At the 10th month and throughout the rest of the storage, the texture of the samples
395 also differed slightly ($p<0.05$), but the mechanically filleted fish was softer, more tender and juicier
396 than the whole fish.

397 3.7 Multivariate data analysis

398 A principal component analysis (PCA) was performed to obtain an overview of the effects of each
399 treatment and the differences between samples. Despite the two PCs only describing 40% of the
400 variation of the samples (Figure 2) PC 1 and PC2 indicated that mechanically filleted mackerel was
401 more juicy, tender and soft compared to whole fish, as seen in the sensory evaluation. This was
402 associated with a higher salt content as well as salty flavour. More white precipitation was formed
403 during cooking of fillets in preparation for sensory analysis and they were also more yellow than the

404 whole fish. The PCA also indicated changes observed in the fillets and whole mackerel throughout the
405 frozen storage. At the beginning of storage, the mackerel had a sweeter, as well as more metal and
406 fresh oil flavour, and also higher MUFA. At the end of the frozen storage, higher concentrations of
407 PUFA, PV and rancid flavours were observed, in agreement with the fatty acid composition analysis
408 and the oxidation process evaluations. Mackerel fillets dipped in sodium erythorbat (SED) maintained
409 a sweet, metal and fresh oil flavour longer than other treatments, or up to 8 months, and experienced
410 less progressive lipid oxidation and rancid flavour than other mechanically filleted mackerel.

411 # 4 Conclusion

412 Atlantic mackerel caught around Iceland (July – September) can be mechanically filleted and maintain
413 a shelf life of 8 – 15 months at -25 °C if sodium erythrobate or polyphosphates are added to the
414 product. During the storage period the chemical composition of the mackerel did not change, with the
415 exception of a slight change in the fatty acid composition. All samples, regardless of treatment, became
416 more yellow in colour during the frozen storage, a change in colour associated with lipid oxidation. The
417 mechanically filleted mackerel were in all cases more yellow than the whole fish, indicating faster lipid
418 oxidation on the muscle surface of the fillets than in the whole fish. The limiting factor for the shelf-
419 life of frozen mackerel was the formation of rancid flavours and odours, and sensory analyses of these
420 parameters was used to determine shelf life of sample groups. PV and TBARS values did not correlate
421 with results of rancidity as determined by a sensory panel. The results of this study thus indicates that
422 alternative analytical methods should be pursued as evaluation of the shelf-life of mackerel, giving a
423 better insight in to the oxidative changes occurring in this complex raw material. The results of this
424 study thus indicate that in order to prolong shelf-life of mechanically filleted mackerel during frozen
425 storage, intervention is needed to slow down lipid oxidation and hydrolysis in the fillets. Both sodium
426 erythorbate and polyphosphate solution had an effect on the shelf-life as evaluated by sensory
427 analysis, but sodium erythorbate more so than polyphosphate. Overall dipping the fillets into the
428 sodium erythorbate solution proved to be more effective than spraying or glazing, resulting in a shelf-
429 life of at least 15 months, more than a year longer than the untreated fillets.

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436

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566

567 **Tables**

568 **Table 1:** Description of the different sample groups used in this study, including the antioxidant
569 treatments.

570 **Table 2:** Sensory attributes (n=27) evaluated in the mackerel samples using an unstructured scale.

571 **Tabel 3**: Fatty acid composition (g fatty acids/100 g total lipid, mean ± standard deviation) of Atlantic
572 mackerel after 2.5 and 12 months of frozen storage at -25 °C. Average values of all samples shown in
573 each sampling (n=19). Different superscript letters between sampling points indicate a significant
574 difference ($p<0.05$).

575 **Table 4**: Results (mean value) of sensory analysis of rancid odour and flavour of mackerel samples
576 during storage for up to 15 months at -25°C. Different superscript letters between groups within a
577 sampling point indicate a significant difference ($p<0.05$, Duncan's post-hoc test). Shelf life of samples
578 was defined as being exceeded when the average score for rancid flavour was greater than 20. (Who
579 = Whole fish, Con = Control fillets, PG = Phosphate glazed, PD = Phosphate dipped, PS = Phosphate
580 sprayed, SEG = Sodium erythorbate glazed, SED = Sodium erythrobate dipped, SES = Sodium
581 erythrobate sprayed).

582

583 **Figures**

584 **Figure 1**: Free fatty acids (FFAs; g FFA/100 g lipids)(A and B), lipid hydroperoxides (C and D) (µmol/g
585 muscle), thiobarbituric acid reactive substances (TBARS; MDA equivalents/g muscle) (E and F) of whole
586 hand-filleted or mechanically filleted mackerel. (Con = Control fillets, Who = Whole fish, PG =
587 Phosphate glazed fillets, PD = Phosphate dipped fillets, PS = Phosphate sprayed fillets, SEG = Sodium
588 erythorbate glazed fillets, SED = Sodium erythrobate dipped fillets, SES = Sodium erythrobate sprayed
589 fillets).

590

591 **Figure 2**: Scores and correlation loadings from PC1 and PC2 from the principal component analysis

592 (PCA) of frozen mackerel. Samples are named as to indicate the treatment they received as shown in

593 table 1 and storage time before sampling. SE showing treatment with sodium erythorbate and P a

594 treatment with a polyphosphate mixture. D indicating fillets were dipped, S that they were sprayed

595 and G glazed with antioxidant solutions. The months of storage prior to sampling indicated at the end

596 of the sample name with the number .

597

Effect of antioxidants on the sensory quality and physicochemical stability of Atlantic mackerel (*Scomber scombrus*) fillets during frozen storage

Highlights

- Mechanically filleted mackerel was susceptible to lipid oxidation during storage
- Dipping fillets into sodium erythorbate prolonged shelf-life from 2.5 to >15 months
- Results of TBARS did not correlate with sensory evaluation results
- TBARS a questionable method for 2. oxidation assessment in complex muscle systems

Table 1: Description of the different sample groups used in this study, including the antioxidant treatments.

Group	Raw material	Antioxidant	Antioxidant application method
Who	Whole mackerel	-	-
Con	Filleted mackerel	-	-
SED	Filleted mackerel	0.2% Sodium erythorbate	Dipped for 10 sec in solution before freezing
SEG	Filleted mackerel	0.2% Sodium erythorbate	Glazed with solution after freezing
SES	Filleted mackerel	0.2% Sodium erythorbate	Surface sprayed with solution before freezing
PD	Filleted mackerel	0.2% Polyphospate mixture	Dipped for 10 sec in solution before freezing
PG	Filleted mackerel	0.2% Polyphospate mixture	Glazed with solution after freezing
PS	Filleted mackerel	0.2% Polyphospate mixture	Surface sprayed with solution before freezing

Table 2: Sensory attributes (n=27) evaluated in the mackerel samples using an unstructured scale

Sensory attribute	Short name	Scale	Description
ODOUR			
fresh oil	O-oil	none | | much	Fresh fish oil odour
metallic	O-metallic	none | | much	Metallic odour
sweet	O-sweet	none | | much	Sweet characteristic odour of fresh mackerel
acidic	O-acidic	none | | much	Acidic odour, fresh
earthy	O-earthy	none | | much	Earthy odour, freshwater fish, arctic charr, salmon
butyric acid	O-butiric	none | | much	Butyric acid, smelly feet
frozen storage	O-frozen st.	none | | much	Cold storage, frozen storage odour
rancid	O-rancid	none | | much	Rancid odour
APPEARANCE			
colour	A-colour	pale | | dark	Outside of centre line; pale: pale grey, dark: dark grey / dark grey-brown
discoloured	A-discoloured	none | | much	Outside of centre line; discoloured, dark spots in flesh
yellow liquid	A-yellow l.	colourless | | yellow	How yellow is the liquid in the box
white precipitate	A-white p.	none | | much	White precipitate on sample surface
rusty precipitate	A-rusty p.	none | | much	Rusty (orange, yellow/brown) precipitate on sample surface
centre line	A-centre l.	distinct | | blurred	Distinct: dark and distinct. Blurred: pale and blurred
flakes	A-flakes	none | | much	Sample falls in flakes when pressed with a fork
FLAVOUR			
fresh oil	F-oil	none | | much	Fresh fish oil flavour
metallic	F-metallic	none | | much	Metallic flavour
sweet	F-sweet	none | | much	Sweet characteristic flavour of fresh mackerel
acidic	F-acidic	none | | much	Acidic flavour, fresh
salty	F-salty	none | | much	Salty flavour
earthy	F-earthy	none | | much	Earthy flavour freshwater fish, arctic charr, salmon
bitter	F-bitter	none | | much	Bitter flavour
frozen storage	F-frozen st.	none | | much	Cold storage, frozen storage flavour, cardboard, rancidity
rancid	F-rancid	none | | much	Rancid flavour
TEXTURE			
soft	T-soft	firm | | soft	Softness in first bite
juicy	T-juicy	dry | | juicy	Dry: draws liquid from mouth. Juicy: releases liquid when chewn
tender	T-tender	tough | | tender	Tenderness when chewn
mushy	T-mushy	none | | much	Mushy, porridge like texture

Table 3: Relative fatty acid composition (g fatty acids/100 g total lipid, mean ± standard deviation) of Atlantic mackerel after 2.5 and 12 months of frozen storage at -25 °C. Average values of all samples shown in each sampling (n=19). Different superscript letters between sampling points indicate a significant difference ($p<0.05$).

Storage	Average (%) 2.5 months	Average (%) 12 months
C14:0	6.84 ± 0.70^{a}	6.30 ± 0.56^{a}
C16:0	13.57 ± 0.56^{a}	13.07 ± 0.35^{a}
C16:1n7	4.42 ± 0.30^{a}	4.05 ± 0.41^{a}
C16:2n4	0.33 ± 0.06^{a}	0.28 ± 0.06^{a}
C17:0	0.28 ± 0.01^{a}	0.21 ± 0.06^{a}
C16:3n4	0.33 ± 0.03^{a}	0.27 ± 0.12^{a}
C18:0	2.04 ± 0.19^{a}	2.40 ± 0.18^{a}
C18:1n9	9.63 ± 1.25^{a}	9.92 ± 1.14^{a}
C18:1n7	1.99 ± 0.23^{a}	1.94 ± 0.25^{a}
C18:1n5	0.37 ± 0.02^{a}	0.34 ± 0.07^{a}
C18:2n6	1.54 ± 0.13^{a}	1.80 ± 0.15^{a}
C18:3n3	1.13 ± 0.11^{a}	1.32 ± 0.14^{a}
C18:4n3	4.29 ± 0.66^{a}	4.83 ± 0.82^{a}
C20:1	11.20 ± 0.49^{a}	10.23 ± 0.72^{a}
C20:4n6	0.35 ± 0.04^{a}	0.40 ± 0.09^{a}
C20:4n3	0.87 ± 0.04^{a}	0.97 ± 0.06^{a}
C20:5n3 (EPA)	9.13 ± 0.83^{a}	10.09 ± 1.08^{a}
C22:1n11	16.18 ± 1.77^{a}	13.70 ± 1.60^{a}
C22:1n9	0.90 ± 0.05^{a}	0.66 ± 0.14^{a}
C22:5n3	1.28 ± 0.13^{a}	1.30 ± 0.14^{a}
C22:6n3 (DHA)	12.22 ± 1.12^{a}	14.95 ± 1.00^{b}
C24:1n9	1.00 ± 0.09^{a}	0.80 ± 0.08^{a}
SFA	22.73 ± 0.48^{a}	21.97 ± 0.39^{a}
MUFA	45.78 ± 1.38^{a}	41.81 ± 1.94^{b}
PUFA	31.48 ± 1.56^{a}	36.19 ± 1.96^{b}
PI	1.58 ± 0.12^{a}	1.92 ± 0.13^{b}

Table 4: Results (mean value) of sensory analysis of rancid odour and flavour of mackerel samples during storage for up to 15 months at -25°C. Different superscript letters between groups within a sampling point indicate a significant difference ($p<0.05$, Duncan's post-hoc test). Shelf life of samples was defined as being exceeded when the average score for rancid flavour was greater than 20. (Who = Whole fish, Con = Control fillets, PG = Phosphate glazed, PD = Phosphate dipped, PS = Phosphate sprayed, SEG = Sodium erythorbate glazed, SED = Sodium erythrobate dipped, SES = Sodium erythrobate sprayed).

Rancid odour **Storage time (months)**	**Con**	**Who**	**SEG**	**SED**	**SES**	**PG**	**PD**	**PS**	**p-value**
2.5	19.2^{a}	12.4^{ab}	9.3^{ab}	19.8^{b}	19.3^{b}	6.6^{a}	6.7^{a}	24.0^{b}	0.012
4	13.6	15.3	14.3	11.1	10.7	9.6	7.2	7.5	0.898
8	15.1^{a}	3.9^{b}	18.6^{a}	7.0^{b}	15.4^{a}	15.6^{a}	19.5^{a}	18.5^{a}	0.047
10		11.0		18.8		23.4	18.6		0.061
12		6.6		8.9					0.469
15		10.6		18.5					0.222

Rancid flavour **Storage time (months)**	**Con**	**Who**	**SEG**	**SED**	**SES**	**PG**	**PD**	**PS**	**p-value**
2.5	25.6	19.6	11.3	19.7	16.2	6.9	12.9	19.8	0.091
4	28.3^{b}	8.1^{ab}	14.9^{ab}	6.5^{a}	9.4^{ab}	10.2^{ab}	12.1^{ab}	13.1^{ab}	0.048
8	20.5	6.1	25.7	7.9	22.5	13.5	13.3	22.1	0.095
10		10.6^{a}		13.8^{a}		27.8^{b}	27.6^{b}		0.001
12		19.4		9.4					0.088
15		16.9		15.3					0.791
Shelf life (months)	<2.5	>15	8	>15	8	10	10	8	

Figures

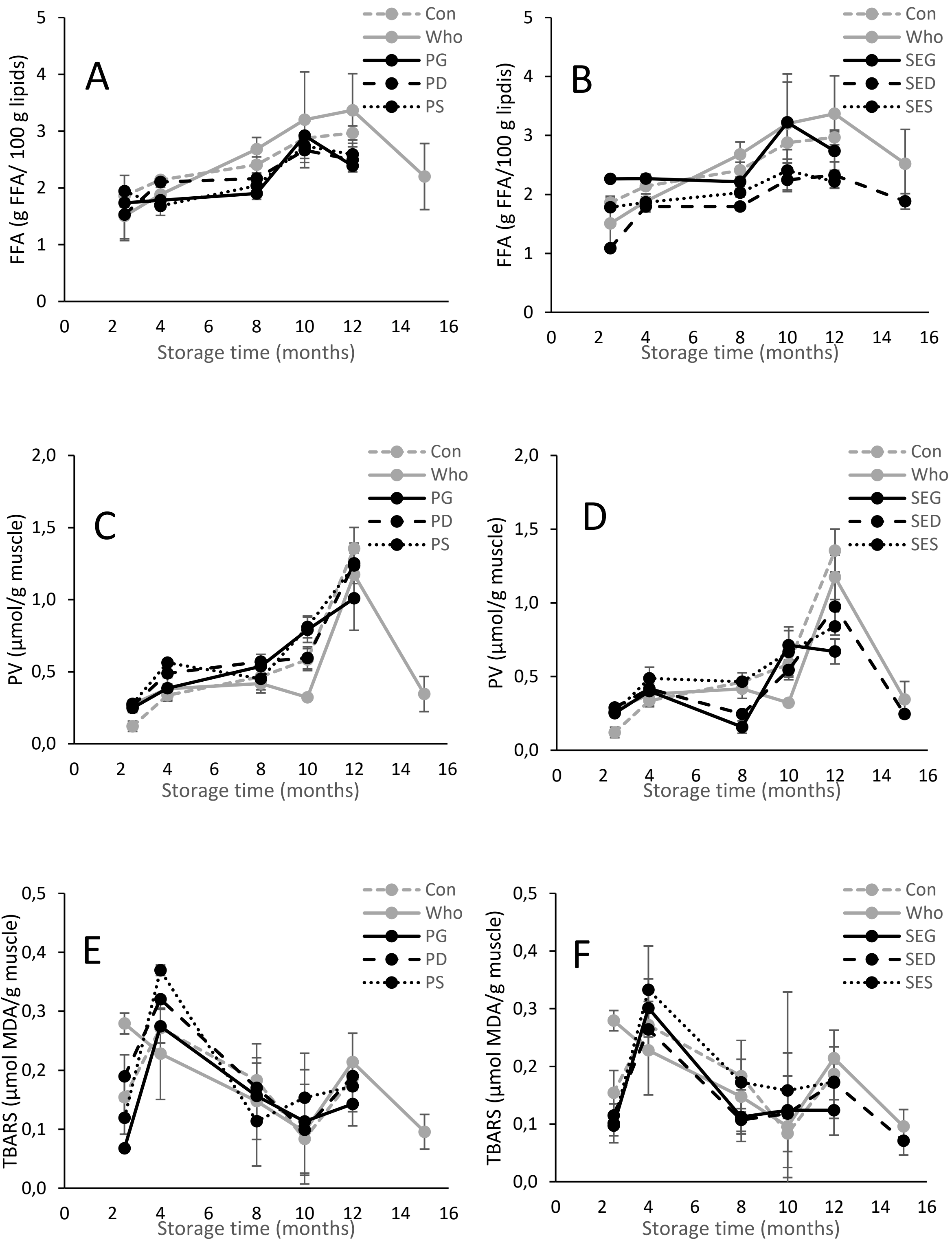

Figure 1

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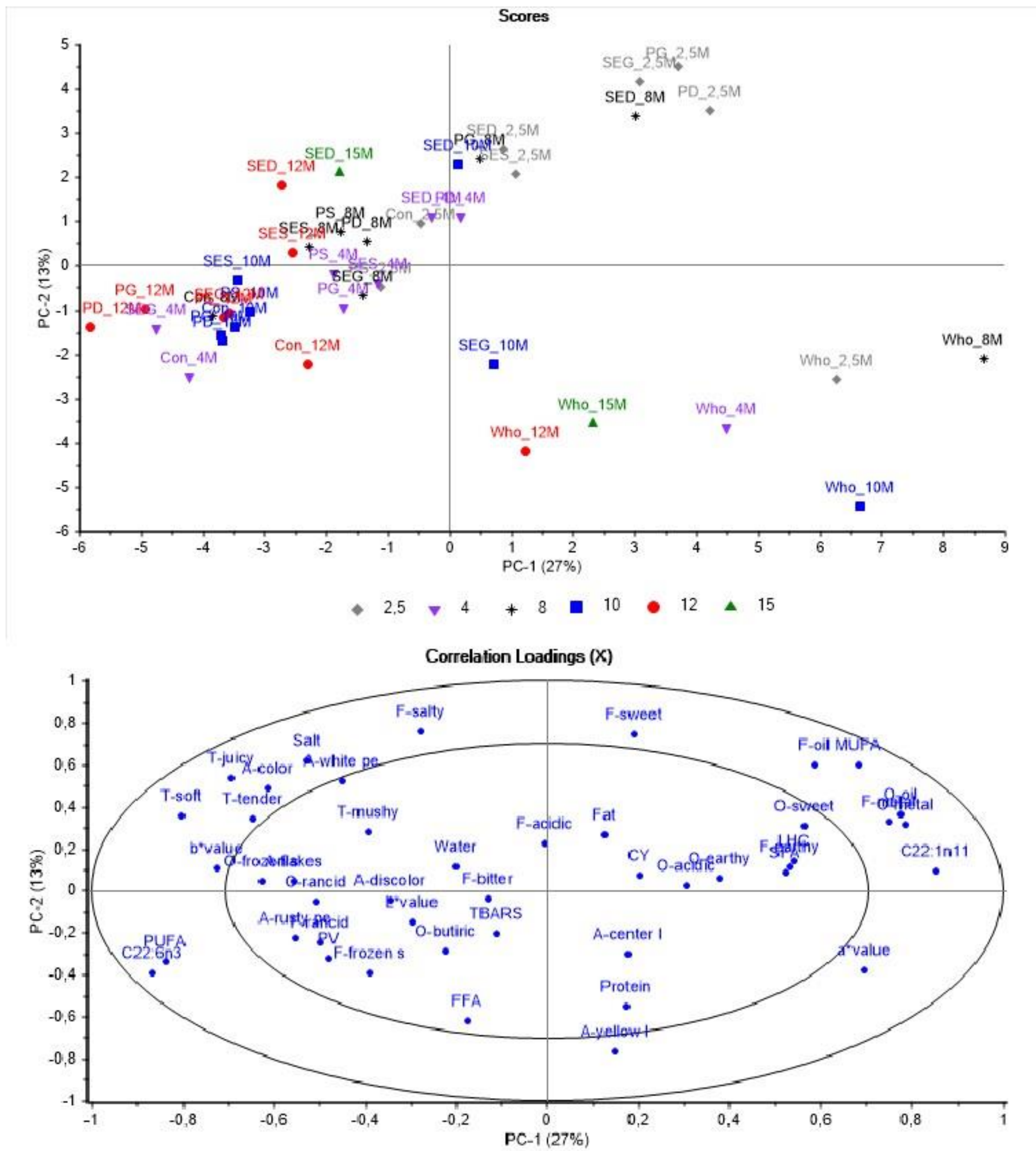

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601 Figure 2