Relationship between lipid and protein oxidation in fish

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1   Relationship between lipid and protein oxidation in fish: A Review

2   Running head: Relationship between lipid and protein oxidation

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23 **Abstract**

24 Reactive oxygen species (ROS) are generated in all aerobic organisms. Free radicals are highly

25 reactive ROS that cause damage to biological materials. Fish is rich in polyunsaturated fatty

26 acids, and hence, very prone to lipid peroxidation. Both lipid and protein oxidations are

27 important for quality loss during storage of fish, with high impact on taste and texture. Also,

28 there are interactions between protein and secondary lipid oxidation products (aldehydes) that

29 occur in foods because the oxidation products from one reaction can further react with both

30 lipids and proteins, respectively. This review focuses on the mechanisms and pathways of the

31 lipid and protein oxidation and their possible relationship. Additionally, the target amino acids

32 and final impacts of this relationship were considered. We propose that the products of lipid

33 oxidation promote protein oxidation in fish rather than the other way around specially, during

34 frozen storage, while during post-mortem changes protein oxidation dominate. Finally, it

35 seems that, secondary products of lipid oxidation might have more impact on the functionality

36 of proteins from both Michael addition and Schiff base reaction rather than lipid

37 hydroperoxides and lipid radical transfer.

38

39 Keywords: Lipid oxidation, protein oxidation, interaction, free radicals, fish

40   **1. Introduction**

41   Oxidation in food is a process that concerns both lipids and proteins by the effect of reactive

42   oxygen species (ROS) (Bernardini et al., 2011). Oxidative modifications can cause numerous

43   functional consequences and lead to changes in food texture, water holding capacity (WHC),

44   digestibility and juiciness in fish meat (Baron, Kjaersgard, Jessen, & Jacobsen, 2007). Also,

45   Sarma, Reddy, & Srikar (2000) found a negative correlation between lipid oxidation

46   parameters and functional properties of the protein in Indian oil sardine, indicating their

47   interdependence. However, still little is known about the kinetics of protein oxidation, the

48   interaction of protein and lipid oxidation and the subsequent impact on muscle food quality

49   (Baron et al., 2007; Kjaersgard & Jessen, 2004).

50   For many years, the focus in food was on lipid oxidation, mainly because of its effect on taste

51   and shelf life, but also because the lipid oxidation products can more easily be measured

52   (hydroperoxides, hexanal, and MDA) than protein oxidation product and therefore have been

53   detected and correlated to off-flavour in food. Especially in fish, which is rich in n-3

54   polyunsaturated fatty acids (PUFA), there is a high risk of quality loss due to oxidation

55   (Jeremiah, 2001; Medina, Gallardo, & Aubourg, 2009). Beside lipid oxidation, the oxidation

56   of proteins can also cause quality changes in the fish fillet. In general, the same factors causing

57   lipid oxidation will also cause protein oxidation. The kinetics of protein and lipid oxidation

58   with respect to the generation of hydroperoxide and carbonyls are quite similar but the

59   diversity of the protein oxidation products are more complex than those of lipid oxidation due

60   to more reactive targets in the proteins. The progress of initial oxidative reactions in foods is

61   enhanced by the interactions between the proteins and lipids due to the similarity of the

62   oxidation reactions. The development of lipid and protein oxidation can occur in parallel or

63   independently, but often there are interactions between them (Zhang, Xiao, & Ahn, 2013) .

64   Several authors reported a good correlation between oxidised products of lipid and protein in

65   fish (Soyer, & Hultin, 2000) beef (Estévez, & Cava, 2004) and chicken (Soyer, Özalp, Dalmış,

66   & Bilgin, 2010), which indicates that protein and lipid oxidation start together and can interact

67   with each other. In contrast to those conclusions, Aalhus & Dugan (2014) have suggested that

68   the products of lipid oxidation can promote protein oxidation since lipid oxidation may start

69   earlier.

70   In the initiation of lipid oxidation products can react with proteins and vice versa, but the most

71   well-described reaction is the reaction between secondary lipid oxidation products and

72   primary amino groups on proteins (Schauenstein, & Esterbauer, 1978; Burcham, & Kuhan,

73   1996).

74   As touched upon above, the reaction between secondary lipid oxidation products and amino

75   acids also takes place in fish. Saturated lipid aldehydes become covalently bound to

76   susceptible and functional groups of proteins like N-terminal groups of Tyr, Asp, Arg and Met

77   (Metz et al., 2004), the ϵ-NH2 group of Lys, and Cys-SH (Metz et al., 2004) through a Schiff

78   base formation while unsaturated aldehydes also can react via Michael addition (Cai,

79   Bhatnagar, & Pierce, 2009). By these interactions proteins, hydrophobicity and aggregation

80   are increased in fish flesh. In addition, the progress of lipid oxidation in fish muscle strongly

81   correlates to the formation of metHb/metMb and also leads to the reduction of extractability

82   haem proteins. For example, during frozen storage of herring fillets, peroxide value (PV) and

83   thiobarbituric acid reactive substances (TBARS) were increased while the amount of haem-

84   proteins decreased (Jonsson et al., 2007).

85   This review aims to give an overview of the lipid and protein oxidation, the known pathways

86   and correlations between lipid and protein oxidation in raw and post-mortem conditions and

87   also the effects of oxidation with a special focus on fish fillet quality.

88

89   **2. Mechanisms of Lipid and Protein oxidation**

90   In order to investigate lipid-protein correlations in fish fillet, the mechanisms of lipid and

91   protein oxidation should be understood in depth. Particularly, the control of possible catalysts

92   has a key role because free radical chain reactions can be swiftly boosted by catalysts.

93   Lipid oxidation is a key factor, leading to a declined in food quality, predominantly of those

94   food products, which contain high amounts of unsaturated FAs (Secci, & Parisi, 2016).

95   Generally, the potential initiators of lipid oxidation can also initiate the protein oxidation

96   (Xiong, 2000). However, the mechanisms, pathways, and also the products of protein

97   oxidation are different (Stadtman, 2006). The functional groups, which are located in the

98   amino acid residues side chain and the peptide backbone are the targets for ROS.

99   The effect of lipid oxidation in muscle food (Estévez, & Cava, 2004) and seafood (Hultin,

100   1993; Secci, & Parisi, 2016; Mariutti, & Bragagnolo, 2017) and protein oxidation (Estévez,

101   2015; Soladoye, Juarez, Aalhus, Shand, & Estevez, 2015) has been reviewed extensively

102   whereas correlation between the lipid and protein oxidation has not really been touched upon

103   in a review.

104

105   **2.1. Lipid oxidation**

106   Unpleasant off-flavours and formation of volatiles can develop due to the oxidation of PUFA.

107   The most important factors which can affect rancidity in fish muscle are the high content of

108   PUFA and also the presence of pro-oxidants, especially the parts containing heme groups

109   (Richard, & Hultin, 2002).

110   Autoxidation enzymatic catalysed oxidation and photosensitized oxidation are three major

111   mechanisms in lipid oxidation.

112   Autoxidation in meat and fish can be initiated by light, heat, and the presence of metal ions

113   and radicals (Sampels, 2013). Autoxidation leads to the formation of the primary oxidation

114   products, hydroperoxides (ROOH). Once the oxidation process has started, a cascade of

115 reactions will occur with each new molecule increasing the reaction speed and variability

116 (Heinonen, Meyer, & Frankel, 1998). ROOH from lipid oxidation decompose easily at high

117 temperature or in the presence of metals to secondary products such aldehydes, short chain

118 hydrocarbons, alcohols, esters, acids, and ketones (Choe, & Min, 2006). Finally, termination

119 products can be crosslinking products e.g. where two radicals react with each other, thereby

120 terminating the chain reaction caused by radical reaction.

121 The atmospheric triplet oxygen is the most common oxygen species involved in the oxidation

122 of lipids but in the photosensitized reaction, both singlet and triple oxygen are two types of

123 oxygen have more interactions with lipids (Foote, 1976). Singlet oxygen has been suggested

124 to react 1,450 faster with linoleic acids than triplet oxygen (Rawls, & Santen, 1970). Singlet

125 oxygen is needed to start the so called type II photosensitized oxidation process, which can

126 react with unsaturated lipids (Foote, 1976) but compared to type I reaction the type II reaction

127 generate different types of hydroperoxide products. The greater importance is the energy of

128 oxygen, which has a significant impact on the initiation of the oxidation reaction.

129 Metal catalysis can be considered as an important lipid oxidation reaction in fish muscle, this

130 means that after post-slaughter processes, the released haem iron (ferrous (+2)) is converted

131 to the ferric (+3) and start the autoxidation progress. Both haem proteins (haemoglobin (Hb)

132 and myoglobin) can increase the lipid oxidation in fish fillet and other muscle foods (Kanner,

133 1994). Richards & Hultin (2002) reported that the blood residue in fish fillet, catalyse lipid

134 oxidation during storage of fatty fish and bleeding was also shown to retard lipid oxidation of

135 minced trout muscle during storage at $+2°C$. Maqsood & Benjakul (2011) showed that the

136 initiation and propagation of lipid oxidation in the un-bled samples compared with the bled

137 samples were more marked.

138 In addition, there are several enzymes in fish, which are capable to catalyse lipid oxidation

139 such as, lipoxygenases and myeloperoxidases. The first one exists in fish skin and gills and

140 can catalyse the incorporation O_2 into an unsaturated fatty acid and generate ROOH, and the

141 second one initiate lipid oxidation in the presence of halides and hydrogen peroxide. During

142 the processing, this can be critical because the lipid, oxygen and blood interaction will

143 increased (Mozuraityte, Kristinova, Rustad, & Storro, 2016).

144

145 **2.2. Protein oxidation**

146 Foods are constantly exposed to ROS and this will not only cause lipid oxidation but also

147 protein oxidation. However, for several decades proteins were ignored as a target for ROS, in

148 the opposed of lipid oxidation, which was investigated very deeply. In contrast to lipids,

149 proteins are complex macromolecules arranged in 3D-structures, and when oxidation occurs

150 it leads to various changes in the proteins, both chemical changes on individual amino acids

151 (Davies, Delsignore, & Lin, 1987) such as Met (Dalsgaard et al., 2010) Tyr (Dalsgaard,

152 Nielsen, Brown, Stadler, & Davies, 2011) His and Trp (Dalsgaard, Nielsen, & Larsen, 2007)

153 Lys and Arg (Dalsgaard et al., 2007; Lund, Heinonen, Baron, & Estevez, 2011) but also

154 structural changes may occur (Davies, & Delsignore, 1987). Because protein's functions are

155 very specific, oxidative modifications can cause numerous functional consequences and lead

156 to changes in e food texture, water holding capacity (WHC), digestibility and juiciness (Baron

157 et al., 2007; Sarma et al., 2000). Protein oxidation may be caused directly by ROS and reactive

158 nitrogen species (RNS) or indirectly as the result of reactions with products from lipid

159 oxidation with reducing sugars or carbohydrate (Lund et al., 2011). On the other hand,

160 nucleophilic reaction on the carbonyl groups of free sugars and aldehyde on the side chain of

161 amino acids can lead to production of Schiff base products (Dalsgaard, Nielsen, & Larsen,

162 2006, Dalsgaard et al., 2007). Generally, the pathways of protein carbonylation can be divided

163 to direct oxidation, metal-catalyzed oxidation, reaction with free sugars and also lipid

164 peroxidation products (Michael adducts) (Fedorova, Bollineni, & Hoffmann, 2014). Due to

165 side chain oxidation of some amino acids (Arg, Lys, His, and Pro) or backbone oxidation of

166 Asp, Pro and Glu residues carbonylation can be considered as a permanent and destructive

167 (Hawkins, & Davies, 2001) indicator of protein oxidation (Nystrom, 2005).

168 Protein oxidation is initiated when a hydrogen atom is abstracted from the protein to generate

169 a carbon-centered radical (C•) and in the presence of oxygen is converted to an alkylperoxy

170 radical (COO•). The following reaction of (COO•) with hydrogen atom abstraction from

171 another molecule leads to alkyl peroxide (COOH) formation. Subsequent reactions lead to the

172 formation of the alkoxy radical (CO•) and hydroxyl compounds (COH). In addition, two

173 carbon-centered radicals (alkyl-radical such as ethane, methane, and propane) can react with

174 each other in the absence of oxygen to generate carbon–carbon cross-linked derivatives

175 (Papuc, Goran, Predescu, & Nicorescu, 2017). The termination reaction, which in relation to

176 fish and meat quality may affect tenderness (Soladoye et al., 2015).

177 Additionally, aromatic amino acids like Tyr, Trp, His, and Phe are very susceptible to

178 oxidation (Hawkins, & Davies, 2001). During oxidation of aromatic amino acids, phenoxyl

179 radicals will be formed from tyrosine and their metabolites, dityrosine and other products, are

180 generated. This occurs especially when tyrosine is close to tyrosol radicals and also tyrosyl

181 radicals which cannot be repaired because they are not reductants (Aeschbach, Amado, &

182 Neukom, 1976).

183 On the other hand, the amino acids, which are aliphatic but do not contain sulfur like Pro or

184 Arg are oxidized via another way. In this group, oxidation takes place by hydrogen abstraction

185 at the α-carbon generating a carbon centered radical (Stadtman, 1993). This reaction occurs at

186 the terminal amine of the Lys side-chain and sites distance from deactivate α-amino group.

187 The generated product will be different depending on the presence or absence of oxygen

188 (Stadtman, 1993).

189 Protein oxidation via metal-catalysed cleavages main reason of oxidative damage *in vivo*

190 systems like fish (Moller, Rogowska-Wrzesinska, & Rao, 2011). Also, in the post mortem

191 fish muscle.

192 In some proteins, metal ion-catalysed oxidation systems can easily oxidize the side-chains of

193 amino acid residues. Lys, Pro, and Arg are the most important targets of metal catalysed

194 oxidation, (Amici, Levine, Tsai, & Stadtman, 1989). Requena, Chao, Levine, & Stadtman,

195 (2001) reported glutamic and then aminoadipic semialdehydes are respectively very important

196 carbonyl products in metal catalysed oxidation systems. For example, Lys can be a target for

197 Fe (II)-catalysed oxidation system. In the mentioned mechanism, the chelate form of Fe (II)

198 and amino group of Lys can generate hydroxyl radical by a reaction between Lys and hydrogen

199 peroxide. Hydroxyl radical preferentially attacks to the Lys moiety to convert Lys to a 2-

200 amino-adipic-semialdehyde residue. The similar reaction with other amino acids by Fe (II)

201 can produce carbonyl derivatives. The site-specific mechanism is supported by the

202 confirmation that the metal-catalysed reactions are prevented by catalase but not by $^\bullet$OH

203 scavengers, maybe because the scavengers are not be able to compete with the ''caged''

204 reaction at the metal binding site between amino acids and $^\bullet$OH.

205 Amadori products, which have been generated via Schiff base products rearrangement are

206 highly susceptible to the degradation and metal-catalysed oxidation reaction. Consequences

207 of the reactions lead to protein cross-linked adducts and Maillard reaction products (Lund et

208 al., 2011).

209 The major mechanisms in protein oxidation are still unclear because there are only a few

210 methods available to evaluate protein oxidation mechanisms, but the number of reaction

211 products is large. Detection of carbonyl groups, the formation of dityrosine and changes in

212 sulfhydryl groups are the most common methods to detect and quantify protein oxidation. In

213 addition, there are some advanced methods such as fluorescence spectroscopy, and electron

214 spin resonance, immune-spin trapping in combination with mass spectrometry used to

215 investigate the mechanism of protein oxidation (Dalsgaard et al., 2014)

216

217 **2.2.1 Function of Proteins as Antioxidants**

218 On the other hand, in some cases, proteins besides being the target for oxidation, are also

219 known to be able to function as antioxidants. The mechanisms of proteins and amino acids as

220 antioxidants in food have been related to their ability to chelate pro-oxidative metals;

221 sulfhydryl groups, which exist in the proteins and amino acids can inactivate free radicals

222 (Viljanen, Kylli, Hubbermann, Schwarz, & Heinonen, 2005). In some cases, the proteins may

223 act as a shield between ROS and the lipid (Dalsgaard et al., 2011). Carnosine can prevent lipid

224 oxidation in various model systems by free radical scavenging or metal ion chelating (Liu,

225 Xing, Fu, Zhou, & Zhang, 2016). His residues and carnosine can postpone oxidation by

226 removing metal ions from the surface of other macromolecules (Guiotto, Calderan, Ruzza, &

227 Borin, 2005). Furthermore, some peptides, which contain Trp or Tyr at the C-terminus have

228 shown to possess a high radical scavenging ability (Saito et al., 2003) and as mentioned above

229 e.g. dityrosine formation as a consequence of two tyrosine radicals reaction with each other

230 will terminate the radical (Dalsgaard et al., 2011). The amount of riboflavin, which is

231 responsible for photo-oxidation is variable in different fish species (Brjekkan, 1959), may play

232 an important role as well. It seems that fish with higher amount of proteins contain more

233 riboflavin. Therefore, Trp and Tyr can be considered as main target of photo-oxidation in fish

234 fillet, which are the only amino acids that can compete with oxygen or unsaturated fatty acids

235 in the quenching of triplet state riboflavin (Cardoso, Franco, Olsen, Andersen, & Skibsted,

236 2004; Dalsgaard et al., 2011). However, the sequence and categories of amino acid might have

237 a key role in the peptides antioxidant activity (Liu et al., 2016). Imidazole ring in the R group

238 of His has the ability of metal ion-chelating, lipid peroxyl radical trapping and hydrogen

239   donating (Chan, & Decker, 1994). In addition, Pro-His-His sequence showed higher

240   antioxidant ability in the linoleic acid system compare to other synthetic peptides (Liu et al.,

241   2016), while, the mechanism of hydrophobic amino acids such as Trp and Try might be differ.

242   The presence of peptides are enhanced at the water-lipid interface which can approach to the

243   lipid phase and scavenge free radicals (Ranathunga, Rajapakse, & Kim, 2006).

244   Hence lipid oxidation in fish could also be retarded due to the simultaneous presence of certain

245   proteins and amino acids. However, this connection is also still widely unexplored.

246

247   **3. Impact of lipid and protein oxidation on fish fillet traits**

248   **3.1 Lipid oxidation and quality parameters**

249   Sensory, nutritional value and colour are the major quality characteristics, which can be

250   affected in muscle foods by lipid oxidation. Several authors reported the negative effects of

251   lipid oxidation on the sensory aspect of fish fillet (Baron et al., 2007; Yin, Luo, Fan, Wu, &

252   Feng, 2014). Yin et al. (2014) studied effects of frozen storage on grass carp

253   (*Ctenopharyngodon idellus*) fillet and reported that the sensory parameters (colour, odour,

254   morphology and muscle elasticity) decreased significantly d by the effect of lipid oxidation.

255   Estévez, Ventanas, & Cava, (2005) reported a significant correlation between lipid oxidation

256   and fat content in liver of pâté. Fat content might have a key role in the development of lipid

257   and protein oxidation in the muscle foods (Estevez, Morcuende, & Cava, 2003; Stadtman,

258   1990). In order to find a correlation between fat content and the development of lipid oxidation

259   we compared PV results of two different kind of fish species (lean and fatty fish). Comparing

260   the studies by Saeed & Howell (2002) and Baron et al. (2007) on Atlantic mackerel (*Scomber*

261   *scombrus*) rainbow trout (*Oncorhynchus mykiss*), respectively, reveals that in fatty fish lipid

262   oxidation started earlier compared to lean fish and PV values were higher during storage at -

263   20 °C in fatty fish.

264 In most fish, the major contributor to the colour of muscle is myoglobin. During storage,

265 ferrous oxymyoglobin (Fe^{2+}) oxidized to ferric metmyoglobin (Fe^{3+}), which is responsible for

266 a discoloration reaction in fish fillets (Papuc et al., 2017). Secondary lipid oxidation products

267 (aldehydes) can, furthermore, alter the myoglobin structure via covalent bonds and change the

268 fillet colour during the storage time (Lynch, & Faustman, 2000). In addition, brown pigments

269 in fish can be generated via lipid-protein interaction. In this case, lipid peroxide can interact

270 with active types of proteins and leads to the transformation of the light coloured or colourless

271 precursor to brown pigments (Hidalgo, & Zamora, 2000).

272

273 **3.2 Consequences of protein oxidation**

274 The hydrophobicity, solubility, WHC tenderness and texture are the most important quality

275 parameters, which depend directly on protein oxidation in fish fillet (Lund et al., 2011).

276 Oxidation in a side chains of amino acids can produce carbonyl groups that eventually result

277 in a loss of solubility and protein aggregation (Rowe, Maddock, Lonergan, & Huff-Lonergan,

278 2004).

279

280 3.2.1 Hydrophobicity

281 Hydrophobic residues, in the native form of proteins, are hidden. In a semi oxidised protein,

282 the alteration of secondary and tertiary of protein structure expose the hydrophobic residues

283 to the proteases enzyme substrates follows by protein degradation (Jung, Hohn, & Grune,

284 2014), while heavily oxidation resulted in protein aggregation via cross-linked proteins (Hohn

285 et al., 2011). Aggregated proteins are stable against enzymatic degradation (Reeg, & Grune,

286 2015).

287 3.2.2 Water Holding Capacity (WHC)

288   Protein oxidation has a negative affect on the WHC. As the accessibility of the polar groups

289   to pro-oxidants, which are present in fish muscle, is very high, they are more prone to oxidative

290   reactions (Standal et al., 2018). Protein carbonylation leads to the loss of amino groups, which

291   in turn results in the alteration of the distribution of the electrical charges and the overall

292   arrangement of myofibril protein. It appears that one result of intensive oxidative protein

293   modification is a change in the isoelectric points of proteins. Therefore, the oppositely charged

294   groups are more attracted to each other and thereby decrease the amount of water that is held

295   by the protein. Moreover, the repulsion of the myofibril protein structures is reduced due to

296   the isoelectric point therefore, the protein structures can be more compact and decrease WHC

297   (Huff-Lonergan, & Lonergan, 2005).

298

299   3.2.3 Tenderness and Firmness

300   The tenderness of fish fillets can change as a result of protein oxidation. There are two

301   hypotheses for a decrease in tenderness in relation to protein oxidation:

302   a) Amplification of the myofibrillar structure through the formation of MP cross-linking

303   (Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010) particularly, in the presence of

304   oxygen. Myosin proteins especially, MHC is susceptible to oxidation due to the

305   presence of Cys in the root tail of myosin, which is responsible for protein cross-link.

306   The results of this cross-link may decrease the fish muscle tenderness.

307   Glutathionylation of particular Cys in myosin binding protein C and troponin I might

308   be responsible to change the sensitivity of myofilament to calcium (Patel, Wilder, &

309   Solaro, 2013). In several studies, myosin has been reported as a target of

310   glutathionylation and also protein oxidation (Passarelli et al., 2010) but still there is a

311   lack of knowledge about the functional and structural alteration in myosin by the effect

312   of specific redox sites.

313   b) μ-Calpain inactivation (Rowe et al., 2004). For peptide bonds hydrolysis by calpains,

314   electron transfer is needed on the active side chain of His and Cys residues. In this

315   case, carbonyl derivatives of some amino acids, such as His, lead to the formation of

316   inter and/or intra-protein disulfide cross-links (Martinaud et al., 1997). Because SH-

317   containing Cys and His residues are present at the active sites of both μ-calpain and

318   m-calpain enzymes, these products might be inactivated by oxidation and lead to

319   reduce fish muscle tenderness (Carlin, Huff-Lonergan, Rowe, & Lonergan, 2006).

320 On the other hand, it has been proved that due to protein oxidation firmness is reduced during

321 frozen storage by the impact of ice crystal formation and cell disruption (Hematyar, Masilko,

322 Mraz, & Sampels, 2018; Subbaiah, 2015).

323 It seems that myoglobin and myofibril are the most important proteins to impact on fish

324 quality. Probably, the formation of covalent bonds and cross-links, by the impact of lipids and

325 proteins oxidation are responsible for quality changes in the fish fillet. Therefore, extensive

326 oxidation in fish muscle often leads to higher firmness while moderate protein oxidation can

327 decline the firmness due to more unfold protein structures. Additionally, degradation and

328 aggregation of proteins in the post mortem fish muscle lead to higher firmness and lower

329 tenderness. On the other hand, during frozen storage formation of unfolded proteins due to

330 oxidation or lipid-protein interaction probably are responsible for reduction of firmness.

331

332 4. Correlation and interaction of lipid and protein oxidation

333 During the storage of fish fillet, proteins are exposed to oxidised lipids or secondary

334 breakdown products that may cause some changes in protein functionality including

335 insolubilisation, polymerisation, loss of enzymatic activity and formation of lipid-protein

336 complexes (Howell, Herman, & Li-Chan, 2001).

337 4.1 Mechanisms of interaction

338　Two mechanisms can be considered for the interaction of lipids-proteins:

339　a) First, hydroperoxide decomposes to secondary lipid oxidation products e.g. malonaldehyde

340　that may react further with amino groups (Schaich, 1976) or for unsaturated lipid aldehydes

341　reacting with Cys, His, or Lys through Michael addition (Stadtman, & Levine, 2000).

342　b) Some products of lipid oxidation (hydroperoxides, lipid free radicals, and volatile secondary

343　oxidation products) can react with proteins to generate protein-centred free radicals (Saeed,

344　Fawthrop, & Howell, 1999).

345　Secondary lipid oxidation products can bind to proteins in two main ways by either binding to

346　an active site or cavity or at less well defined hydrophobic patches at positions close to the

347　surface of the protein (Fillery-Travis, Mills, & Wilde, 2000). Aldehydes as a product of metal

348　catalysed lipid oxidation cause, protein-lipid aggregation via crosslink bonds (Gardner, 1979).

349　The pathways for the interaction of lipid oxidation products (carbonyls) with proteins are

350　formation of Schiff base and Michael-type (Refsgaard, Tsai, & Stadtman, 2000). In the

351　carbonyl amine reaction, aldehydes can bind directly to amino groups in proteins through

352　covalent bonds. Interaction of dimethylamine and formaldehyde from trimethylamine-N-

353　oxide may result in decreased WHC as formaldehyde may form crosslinks between proteins,

354　which have been shown to decrease WHC and protein solubility in the Gadiform Fish fillet

355　(Nielsen, & Jorgensen, 2004).

356　Also, the products of lipid oxidation might have interaction with proteins on the hydrophobic

357　groups site, which lead to generate lipid-soluble fluorescence products. The consequence of

358　this interaction in the defatted samples can be resulted to decline in soluble protein

359　hydrophobicity (Liang, 1999) upon when the secondary and tertiary structure of proteins are

360　changed (Meng, Chan, Rousseau, & Li-Chan, 2005). This reaction mostly happens in the

361　presence of some prone amino acids such as His, Lys, Pro, Arg, Tyr, Trp, Cys, and Met via

362　side chain reaction with lipid oxidation products. As soon as protein unfolding occurs at the

363 water-lipid interface, the hydrophobic groups are absorbed and interact with the lipid phase.

364 While, the negatively charged groups of protein can remain in contact with water molecules

365 and hence increase the solubility or reduce the risk of protein-protein aggregation (Gitlin,

366 Carbeck, & Whitesides, 2006; Kramer, Shende, Motl, Pace, & Scholtz, 2012).

367 Thus, it appears that the covalent bonds are a very important type of bonds between free amino

368 groups of Cys, His, and Lys with MDA via side chain pathway (Pizzimenti et al., 2013). For

369 a better understanding of protein-lipid interaction, it is necessary to know more about the

370 water-lipid bonds and also the lipid-protein relationship, as it seems that there is a close

371 relationship between them (Alzagtat, & Alli, 2002). In the frozen fish muscle due to the

372 absence of water molecules, lipid-protein or protein-protein interactions are dominated.

373 Additionally, the interactions depend on the secondary products or radicals predominate that

374 can be specified by which proteins get involved in the lipid oxidation reaction chain (Ladikos,

375 & Lougovois, 1990). Furthermore, the stability of the proteins and volatiles may increase via

376 non-covalent bonds during the storage time of muscle foods.

377

378 **4.2 Role of haemoglobin and myoglobin on the progress of lipid oxidation**

379 It has also been demonstrated in model systems that lipid oxidation can increase in the

380 presence of protein radicals (Østdal, Davies, & Andersen, 2002). A good example for this kind

381 of interaction in fish muscle has been reported by Richard & Hultin (2002) that revealed

382 oxidised haemoglobin (deoxyhemoglobin) is a powerful catalyst of lipid oxidation.

383 Haemoglobin (Hb) autoxidation rate in different fish species, might be influenced by their

384 reside (Powers, 1972). Richards & Hultin (2003) revealed that Hb from mackerel (*Scomber*

385 *scombrus*) and herring (*Clupea harengus*) showed more pro-oxidative activity than from trout

386 (*Onchorhynchus mykiss*) Hb. This would be related to the frequent migration, which made Hb

387 more susceptible to autoxidation. Probably, mackerel blood contains more lipid oxidation

388 promoters or less powerful inhibitors (Richards, & Hultin, 2003). However, Maqsood &

389 Benjakul (2011); Maqsood et al. (2011) reported that active migratory fish like seabass (*Lates*

390 *calcarifer*) had less pro-oxidative Hb. In addition, Hb autoxidation in the fish from cold water

391 was 10-fold faster than warm water fish (Maqsood, & Benjakul, 2011). Might be His residue

392 is situated away from the centre and makes Hb more resistance to oxidise (Jensen, 2001).

393 Probably, when His and Phe residue are located on the distal part of the haem group the

394 accessibility of iron to the mentioned amino acids is decreased that leads to less interaction

395 between Hbs and lipid oxidation products. The rate of lipid oxidation can be affected by the

396 different Hbs formations in fish flesh (Maqsood, Benjakul, & Kamal-Eldin, 2012). Richards

397 & Hultin (2002) reported that deoxyHb is a stronger catalyst for lipid oxidation compare to

398 oxyHb. Formation of deoxyHb release the iron from the inside of the porphyrin group, which

399 is a catalyst for lipid oxidation and lipid-protein interaction. Immediate bleeding would keep

400 the freshness of muscle for a longer time and maintenance the fillet firmness during the storage

401 time. It has been demonstrated that inadequately bled or non-bled fish show the lower overall

402 quality in the fish fillet as bleeding decrease the total haemoglobin in the muscle (Richards, &

403 Hultin, 2002).

404 In fish, myoglobin oxidation and lipid oxidation are associated and influence each other

405 (Chaijan, 2008). Secondary lipid oxidation products (aldehydes) can modify the stability of

406 myoglobin and generate adducts through a covalent modification with myoglobin but in the

407 absence of lipid oxidation products metal catalyse via side chain reaction can unfold the

408 proteins. In line with this, Lynch & Faustman (2000) proposed that pro-oxidant activity of

409 metmyoglobin and oxymyoglobin oxidation will be increased by aldehydes. Furthermore,

410 metmyoglobin and H_2O_2, resulting from oxymyoglobin oxidation, can provoke lipid oxidation.

411 In one way the products of oxymyoglobin (metmyoglobin and H_2O_2) are necessary to start

412 lipid oxidation and on the other way aldehydes can change myoglobin stability and promote

413 oxymyoglobin oxidation

414

4.3 Myosin- lipid interaction

416 Myosin is another protein in fish that has a weak tendency to form a complex with lipids, but

417 the form of myosin can change during the storage due to unfolding. However, linoleic acid

418 hydroperoxides are highly destructive for myofibrillar structure that can precipitate and

419 denature the A-band (predominantly myosin). Chopin, Kone & Serot (2007) investigated the

420 interaction between fish myosin and secondary of lipid oxidation products and found a

421 correlation between myosin solution and aldehydes, which leads to lead to a decline of protein

422 solubility, due to aggregations, also supported by (Buttkus, 1966). It seems that the interaction

423 is taking place at some uncovered and new opened sites of unfolded myosin chain. During this

424 interaction myosin solubility, sulfhydryl groups and free amino groups significantly

425 decreased, thus supporting the idea of Schiff base and/or Michael addition reaction taking

426 place.

427

428 In order to make more visual, we showed some mechanisms and target proteins in the

429 relationship between lipid and protein oxidation products in a schematically graph (Figure 1

430 and 2).

431 During post mortem of fish muscle the lack of ATP and anaerobic conditions leading to the

432 antioxidants consumption which are resulted to the oxidation development. Probably during

433 post-mortem changes, first metal catalysed oxidation in proteins are dominated follow with

434 lipid oxidation development. We would say maybe protein oxidation is started earlier than

435 lipid oxidation or both are in parallel. Therefore, bleeding or washing the muscle after stunning

436 can be considered as a main factor to reduce this interaction. On the other hand, it appears that

437 during frozen storage formation of secondary products of lipid oxidation have key roll on

438 lipid-protein interaction via Schiff base reaction on the side chain of amino acids.

439 Secondary lipid oxidation products might have more impact on the functionality of proteins

440 from both Michael addition and Schiff base reaction rather than lipid hydroperoxides.

441

442 6. Conclusions

443 The present review summarizes the main actions of protein oxidation and the possible

444 connection to lipid oxidation with a focus on fish quality. Until now, both oxidation processes

445 have been investigated more or less separate from each other. However, both processes occur

446 in parallel in fish and fish products. The reaction products can then react further with each

447 other and form either volatile or non-volatile stable products. Formation of 'induced' protein–

448 lipid complexes is the result of this interaction.

449 We propose that the products of lipid oxidation promote protein oxidation in fish rather than

450 the other way around specially, during frozen storage. While during post-mortem changes,

451 protein oxidation is dominating. This hypothesis needs to be explored in model systems as

452 well as on real fish samples. In addition, a comparison of lean and fatty fish should be made.

453 There is the possibility, that retarding lipid oxidation could also slow down protein oxidation

454 and hence increase shelf life of fish. Furthermore, there is a strong need to investigate how

455 oxidation products influence the kinetics of the ongoing lipid and protein oxidation processes

456 to better prevent oxidation and food spoilage. Finally, it seems that, secondary products of

457 lipid oxidation might have more impact on the functionality of proteins from both Michael

458 addition and Schiff base reaction rather than lipid hydroperoxides.

459

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748 **X. Figure legends:**

749 **Figure. 1.** Main factors that can influence fish flesh quality before and after storage time and
750 prefer primary and secondary targets.

751 The efficiency of parameters which affect on fish flesh quality in the time period is indicated
752 by dotted lines.

753 **Figure. 2.** The main possible interaction pathways with the target amino acids and involved
754 products of lipid and protein oxidation with respect to the ideal condition. Main impact of the
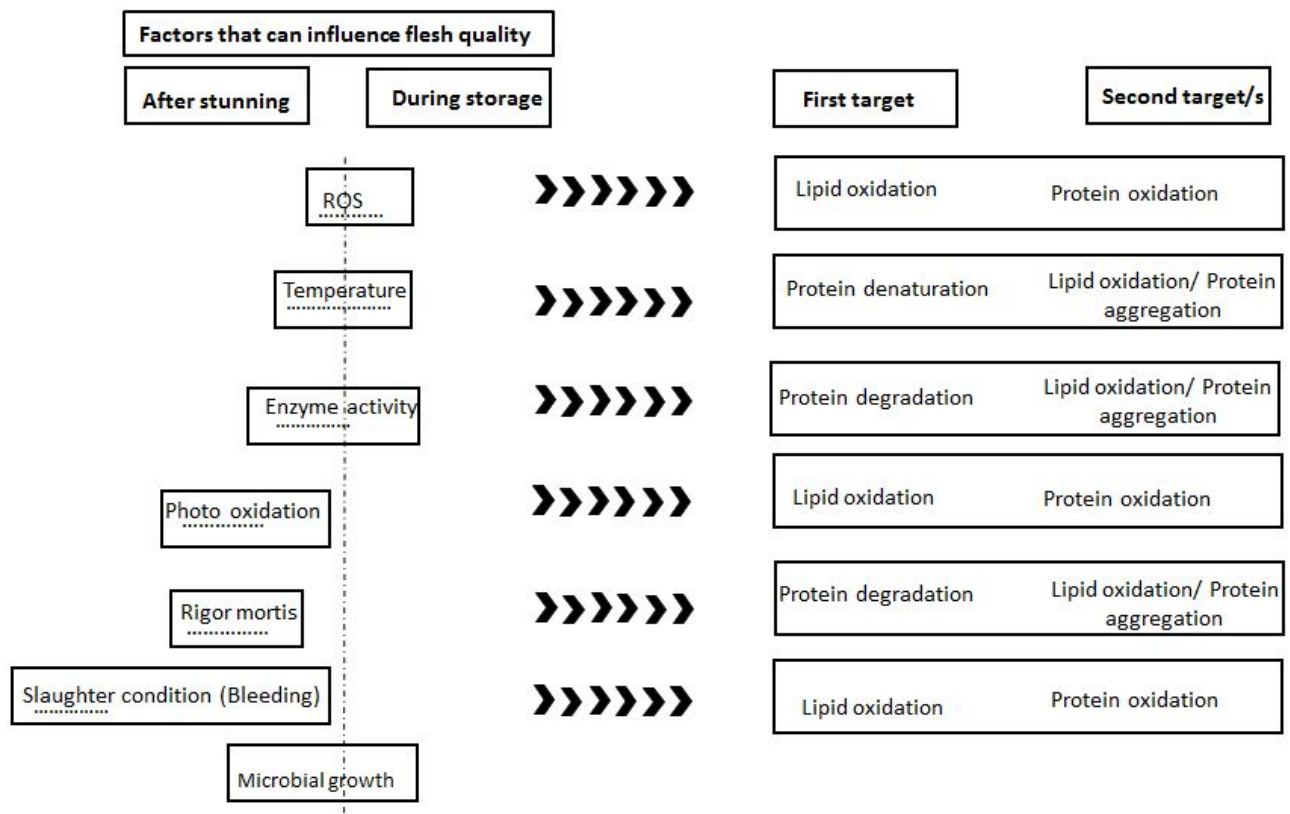755 mentioned interaction on the final flesh quality

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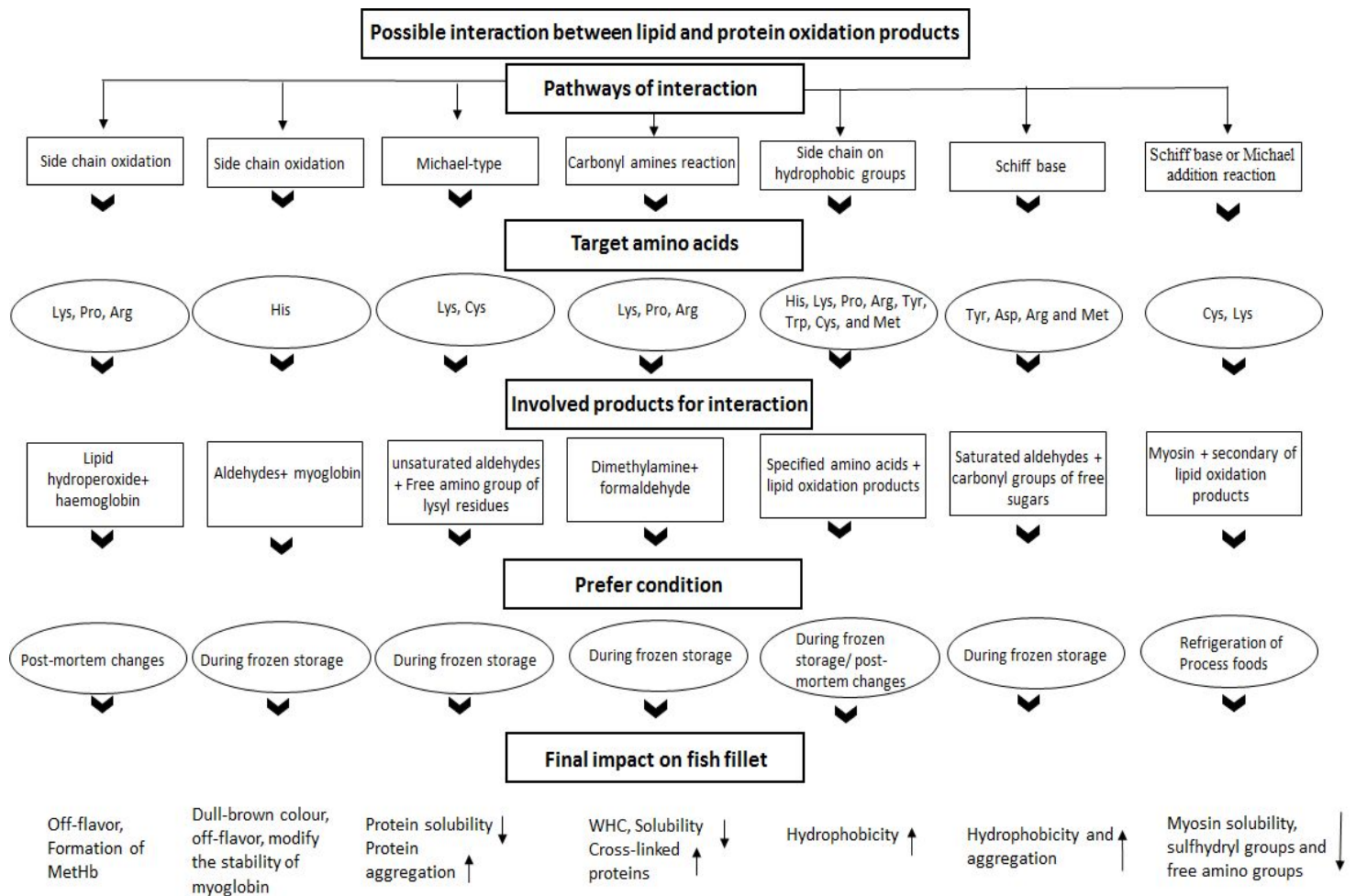
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