

Post-mortem storage conditions and cooking methods affect long-chain omega-3 fatty acid content in Atlantic mackerel (Scomber scombrus)

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- *Post-mortem* storage conditions and cooking methods affect long-chain omega-3 fatty
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- 3

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9

10 Abstract

Long-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic 11 acid (DHA) are health beneficial lipids found in high concentration in pelagic fishes, 12 13 including Atlantic mackerel. While EPA and DHA are sensitive to oxidative degradation during fish storage and processing, *post-mortem* degradation in the first hours following fish 14 15 death is poorly documented. In this study, we stored fish at two temperatures (2-4°C and 16 18–20°C) and monitored EPA+DHA content in dorsal fillet 6, 12 and 24 hours after fish death and after cooking (grill or steam). Storage duration was the most influencing factor, and 17 EPA+DHA loss was faster at 18-20°C. Six hours after fish death, and regardless of storage 18 temperature EPA+DHA content decreased by $1.3\pm1.3 \text{ mg.g}^{-1}$ (9.6±9.5% of the initial content). 19 EPA+DHA losses were highly variable among individuals, probably because of other sources 20 of variability (e.g., sampling position). Handling between fishing operation and deep-frozen 21 22 storage should be as short and as cool as possible to preserve EPA+DHA and for food safety. Regarding cooking, both EPA+DHA and mono-unsaturated fatty acids increased in grilled 23 fillets. 24

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26 Key words: Oxidation, Fatty acids, Lipids, Small pelagic fish, Storage, Cooking method

Long-chain n-3 polyunsaturated fatty acids, and especially eicosapentaenoic acid (EPA; 28 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are essential lipids for several vital 29 30 functions in human (Siriwardhana et al., 2012; Swanson et al., 2012). Due to insufficient de novo synthesis capacities, a daily dietary EPA+DHA intake of about 300 mg is recommended 31 for healthy adults (FAO/WHO, 2010; Plourde & Cunnane, 2007). Several human populations 32 33 however, face an EPA+DHA deficiency, caused by malnutrition or undernutrition (Stark et al., 2016). EPA and DHA human supplies are mainly ensured by the consumption of marine 34 fish, which obtain these lipids through the food web, mainly from primary producers. Due to 35 36 climate changes that induces modifications in the physiology and community composition of primary producers (Galloway & Winder, 2015), the global EPA+DHA production is predicted 37 to decrease (Hixson & Arts, 2016). Such a decrease, combined with human population 38 growth, might cause a shortage in EPA+DHA availability that may become a challenge for 39 human food security in the next decades (Hicks et al., 2019). Preservation of EPA+DHA in 40 41 food is thus of high importance.

42 Small and medium pelagic fish, also called blue-backed fish, play an important role in food security, as they are cheaper than large fishes and rich in nutrients, including EPA+DHA 43 (Kawarazuka & Béné, 2011). Among them, the Atlantic mackerel Scomber scombrus is a 44 45 fatty fish occurring in cold and temperate regions of the northern Atlantic Ocean, where it forms large schools near the surface to feed on zooplankton and small fishes. About one 46 million tons of Atlantic mackerel are fished yearly, mainly by purse seine or pelagic trawl 47 (FAO Fisheries & Aquaculture, 2020). Mackerels are generally frozen or chilled whole at 48 collection, for further processing ashore (Sone et al., 2019). Storage method and duration, fish 49 50 processing, and cooking procedures are critical steps for fish EPA+DHA preservation, because EPA and DHA are highly sensitive to oxidation (Gladyshev & Sushchik, 2019; Secci 51

& Parisi, 2016). In the case of mackerel, whose lipid content can vary by a factor of three 52 among individuals, storage temperature is among the main factor determining a successful 53 storage (Romotowska et al., 2017). Studies have investigated lipid changes in long-term 54 storage of mackerel for a large panel of storage temperatures and duration, from -27°C to 55 +26°C and from a day to a year (Otero et al., 2019; Oucif et al., 2012; Romotowska et al., 56 2016, 2017; Standal et al., 2018). However, as for many other fish species, initial reference 57 58 values were based on fish obtained from commercial fishing and the exact time of death was not well documented. Consequently, having information on the EPA+DHA changes in the 59 first post-mortem hours could help detecting EPA+DHA losses during the first processing 60 61 steps of fish (e.g., between fishing and the processing plants).

The objectives of this study were to quantify in Atlantic mackerel (i) EPA+DHA changes during the first *post-mortem* hours according to storage temperature; and (ii) the influence of the cooking method (grill and steam) on the EPA+DHA content. Fatty acids, including EPA+DHA, were quantified in the fillet of freshly euthanized mackerel, and at 6, 12 and 24 hours from death, on fish stored at two contrasted temperatures (2–4°C and 18–20°C).

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68 **2. Material & Method**

69 2.1. Sample collection

Fifteen mackerels *Scomber scombrus* (25.2 \pm 3.3 cm, min-max=20-30 cm in fork length) were collected by hand line in the Baie de Douarnenez, France on July 26th 2020. Seawater parameters were 16.2°C and 34.8 PSU, and air temperature was 18-20°C throughout the fishing operation. Fish were immediately euthanized by immersion in a 1 mL.L⁻¹ eugenol bath (Fili@vet Reseau Cristal, France) for 5-10 min, then immediately labelled for later identification, and dissected on board. Sampling was restricted to the dorsal fillet from the right side of the fish (i.e., white muscle under the first dorsal fin).This area is homogenous in

both lipid content and lipid oxidation in S. scombrus (Icekson et al., 1998), and in lipid and 77 fatty acids contents in the related species S. japonicus and S. australasicus (Bae et al., 2010). 78 In a previous estimate conducted on three Atlantic mackerels kept frozen for 8 months at -79 15°C, the EPA+DHA contents varied from ca. 7 % among the sampled positions (results not 80 shown). For each fish, about 0.5 g wet weight (ww) of dorsal white muscle was sampled 81 without skin, flash frozen in liquid nitrogen, and stored in a dry shipper frequently refuelled 82 with liquid nitrogen (T_0 sample). The total time between fish death and sample flash-freezing 83 was less than 10 min. Fishes were then kept whole (neither eviscerated, nor filleted), and 84 randomly stored either (i) at about 4°C in an insulated styrofoam box cooled with -20°C ice 85 86 packs (n=8 fish), or (ii) at ambient temperature in a styrofoam box (n=7 fish). To consider the inter-individual variability, measurements were repeated over time on the same individuals. 87 Six hours after the first tissue sampling, another 0.5 mg ww sample of dorsal muscle was 88 89 taken on each fish next to the previous one (T_6) (Fig.1). Samples were flash frozen and stored in a dry shipper, and fishes were stored back in their respective box (i.e., ice packs or 90 91 ambient). The same procedure was repeated 12 and 24 hours after the first tissue sampling (T_{12} and T_{24} , respectively) (Fig.1). Air temperature of the storage boxes (i.e., ice cold or 92 ambient temperature) was recorded before each tissue sampling and ranked 2-4°C and 93 94 18–20°C throughout the storage test, respectively.

95 After the last sampling, dorsal fillet from the left side was filleted and cooked on ethanol-96 cleaned equipment, with neither oil nor condiment. Fillets with skin were either (i) grilled on 97 a griddle at 80–90°C for five minutes, or (ii) steamed with 130 mL of tap water brought to a 98 boil for 15 minutes (for each cooking method, half individuals were from each storage 99 temperatures; Fig.1). European populations commonly used these two methods, steaming 100 being particularly preferred for babies. After cooking, about 0.5 mg ww of fillet without skin was collected, flash frozen, and stored in a dry shipper. All the samples were then stored at 80°C for 40 days before subsequent analyses.

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104 2.2.Moisture analysis

Moisture was determined by gravimetry. Samples were weighed before and after a 65-hours
freeze-drying (Christ Alpha 1-2 LD plus lyophilizer). The mean analytical variability was 1%.
Immediately after freeze-drying, samples were homogenized with ball mill (Restch MM400)
and stored back at -80°C for four days before lipid extraction. Moisture was expressed in
percentage of wet weight.

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111 2.3. Fatty acid analysis

Lipids were extracted from ca. 60 mg of dry powder with 6 mL of solvent mixture 112 (CH₃Cl₃:MeOH, 2:1, v:v) directly added into glass vials (Sardenne et al., 2019). Extracts were 113 114 flushed with nitrogen gas, vortexed, sonicated for 15 min, and stored for 15 days at -20°C. Tricosanoic acid (23:0) was added as internal standard to lipid extract. Lipids were then 115 transesterified with H₂SO₄ (3.8% in MeOH) at 100°C for 10 min. Fatty acid methyl esters 116 117 (FAME) were separated and quantified on a Varian CP8400 gas chromatograph (GC) equipped in parallel with a Zebron ZB-WAX and a ZB-5HT column (both 30 m length, 0.25 118 119 mm internal diameter, 0.25 µm film thickness; Phenomenex) and flame ionisation detectors at the Lipidocean core facility, University of Brest, France. GC parameters were set as in 120 Sardenne et al. (2019). FAME were identified by comparing sample retention times to those 121 of commercial standard mixtures (Supelco 37-component FAME mix, BAME, and PUFA no. 122 1 and 3 mixes; Sigma-Aldrich) using Galaxie 1.9.3.2 software (Varian). FAME content was 123 converted into fatty acids (FA) content based on 23:0 recovery. Total FA content was 124

calculated as the sum of 41 identified FA (16 poly-unsaturated FA (PUFA), 15 monounsaturated FA (MUFA), and 10 saturated FA (SFA)). Data were expressed in mg.g⁻¹ dry
weight to avoid confounding lipid changing content with water changing content. The mean
analytical variability was 4.2% for the GC and 11.9% for the whole FA analysis.

129

130 2.4.Data analysis

131 Contents in moisture, total FA, EPA+DHA, and FA families (SFA, MUFA, PUFA) were compared among storage temperatures, durations and the interaction between both factors 132 tested using semi-parametric MANOVAs for repeated measures based on a central γ^2 -133 distribution, which does not assume multivariate normality nor covariance homogeneity (RM 134 function from the 'MANOVA.RM' package; Friedrich et al., 2018). Non-parametric paired 135 Wilcoxon tests (V statistic) were used to refine differences between duration modalities. 136 Unpaired Wilcoxon test (W statistic) were used to test for differences between storage 137 138 temperatures at each duration, and between cooking modes. Spearman correlation tests (S statistic) were used to test for correlation between EPA+DHA losses at T₆, T₁₂, and T₂₄, and 139 FA content at T₀, and linear regressions were used to test for fish length influence on FA 140 contents. Data were analysed using R software 3.5.0 (R Development Core Team et al., 141 142 2018), and 'stats' and 'MANOVA.RM' packages.

143

144 **3. Results**

Initial EPA+DHA content was $16.2\pm4.0 \text{ mg.g}^{-1} \text{ dw}$, representing about 30% and 80% of total FA and PUFA, respectively. The amount of FA varied as a function of fish length, with larger individuals having a higher content of total FA and EPA+DHA (i.e. from 12.2 ± 0.2 to $17.6\pm0.6 \text{ mg.g}^{-1}$ between 20 and 30 cm in fork length ; Fig 2). Biochemical contents of fish, including EPA+DHA (Table 1), changed with storage duration, but was not affected neither by storage temperature nor by the interaction between storage temperature and duration
(Table 2). Changes in EPA+DHA content between storage conditions were highly variable
among individuals (large SD; Table 1).

Regardless of the storage temperature, total FA content decreased between T_0 and T_{24} , from 60.9±21.8 to 51.0±24.1 mg.g⁻¹ (V=114, p<0.001; Table 1, Fig. 3b). Variability in total FA content (estimated through coefficients of variation) was lower at 2–4°C than at 18–20°C (T_6 : 32.2% and 42.8%, T_{12} : 44.0% and 49.9%, and T_{24} : 40.1% and 58.0% at 2–4°C and 18–20°C, respectively). Regarding cooking, total FA content did not change from T_{24} in either grilled or steamed fillets (W=42, p=0.12; Fig. 3b).

EPA+DHA content decreased between T_0 and T_6 (V=110, p<0.01): paired differences 159 indicated a loss of $1.3\pm1.3 \text{ mg.g}^{-1}$ (0.9±1.3 and $1.9\pm1.1 \text{ mg.g}^{-1}$ at 2-4°C and 18-20°C, 160 respectively), equating to a loss of 9.6±9.5% (5.5±8.1% and 14.2±9.3%, respectively) of the 161 initial EPA+DHA content. EPA+DHA content also decreased between T₆ and T₁₂ (V=103, 162 p<0.05), but not after (V=61, p=0.98; Fig. 3c). No correlations between total FA content at T_0 163 and EPA+DHA losses at T_6 , T_{12} , and T_{24} were detected (S=814, p=0.10; S=450, p=0.48; 164 S=438, p=0.43, respectively). Regarding cooking, grilled fillet gained 3.4 ± 3.5 mg.g⁻¹ and 165 steamed fillets lost $0.2\pm3.4 \text{ mg.g}^{-1}$ of EPA+DHA from T₂₄ (W=46, p<0.05; Table 1). As for 166 EPA+DHA, PUFA, MUFA and SFA contents decreased between T_0 and T_6 (all p<0.01; Fig. 167 3d to f), but only PUFA continued to decrease between T_6 and T_{12} (V=98, p<0.05). Cooking 168 methods affected both EPA+DHA and MUFA contents, which were already lowered by 169 storage (W=46, p<0.05 and W=47, p<0.05): EPA+DHA and MUFA gained 3.4±3.5 and 170 5.7 \pm 5.4 mg.g⁻¹ in grilled fillet and lost 0.2 \pm 3.3 and 2.3 \pm 6.3 mg.g⁻¹ in steamed fillet, 171 respectively (Fig. 3c and e). 172

173

174 **4. Discussion**

EPA and DHA are sensitive to oxidation, but their loss during fish processing in the first *postmortem* hours of the Atlantic mackerel is not well documented. Here, we find that i) storage duration was the only factor explaining FA losses in the first hours after fish death, and ii) grilled fillet had higher EPA+DHA content than steamed fillet. However, changes in EPA+DHA content were highly variable among individuals, probably in relation to other sources of variability (e.g. sampling position, analytical variability).

High inter-individual variability was observed both in FA contents and in FA losses. 181 Fish length explained most of the variability in the initial FA content, and storage duration 182 was the only significant factor explaining FA losses, despite faster FA losses at the high 183 184 storage temperature (18-20°C). Other sources of variability in FA losses might be the heterogeneity in FA compositions among tissue sampling positions, despite the fact that all 185 positions were close to each other, i.e., dorsal white muscle under the first dorsal spine. While 186 187 we tested the natural variability of EPA+DHA content in the dorsal fillet of frozen Atlantic mackerel, and that this remains relatively low, this variability remains to be assessed for fresh 188 individuals. Regarding changes due to storage duration, FA contents decreased only six hours 189 190 after fish death with: 9.6±9.5% of the initial EPA+DHA content been lost. Again, the large inter-individual differences observed can be explained by the sources of variability previously 191 192 mentioned (i.e., sampling position and analytical variability). However, only PUFA continued to decrease 12 hours after death. PUFA are generally considered as the FA the most sensitive 193 to degradation, especially to oxygen contact that causes peroxidation (Couturier et al., 2020). 194 195 While lipid hydrolysis tends to increase with fish fat content (Aubourg et al., 2005; Rudy et al., 2016), this relationship has not been reported for mackerel, even over several storage 196 months (Aubourg et al., 2005). Similarly, we did not observe any relationship between the 197 initial fish FA content and EPA+DHA losses in the first *post-mortem* hours of mackerel. 198 These results highlight the importance of fast handling, and to a lesser extent of cool handling, 199

even before the rigor mortis that usually occurs at about 18-21 hours post-mortem in mackerel 200 201 (Anders et al., 2020). Although temperature did not play a crucial role for lipid oxidation on the short time scale of this study (24 hours), it is relevant for other degradation mechanisms 202 203 such as hydrolysis or histamine production (Couturier et al., 2020; Zou & Hou, 2017). Avoiding filleting, favoring fast frozen of fish, as well as the maintenance of low temperature 204 throughout long-term storage could save highly valuable FA in the Atlantic mackerel 205 206 (Aubourg et al., 2005; Romotowska et al., 2017), caveat that can probably be generalized to 207 other fatty and small pelagic fishes (e.g., Rudy et al., 2016). This procedure is also relevant for scientific samplings of fish obtained from professional fisheries or from remote areas, 208 209 which could rapidly loose FA before analysis, including EPA+DHA, due to a lack of proper storage. 210

Regarding cooking methods, grilled fish had higher FA contents, especially 211 212 EPA+DHA and MUFA, than steamed fish, as well as than T₀ fish (all in dry weight). A higher FA content measured after grilling might be related (i) to changes in the fillet texture that 213 214 might improve FA extraction from tissue, as high heating is commonly used by the industry 215 for fish oil extraction (Adeoti & Hawboldt, 2014); or (ii) to FA exchanges with the subcutaneous fat beneath the skin (fillets were cooked with the skin), favoured by the 216 217 increasing temperature. Studies have shown no exchanges of FA between fillet and skin of the Atlantic mackerel during iced and frozen storage (Xing et al., 1993), but extensive exchanges 218 can occur during frying (Sebedio et al., 1993). In addition, the skin of small pelagic fish is 219 relatively rich in EPA+DHA: about 19%, 26%, 25% and 13% of total FA for the Atlantic 220 mackerel, Rastrelliger kanagurta, Sardinella maderensis and Sardinella aurita, respectively 221 (Njinkoué et al., 2002; Sahena et al., 2010; Zuta et al., 2003). This suggests that the skin 222 should be kept to cook Atlantic mackerel fillets. Extended tests including other cooking 223 methods should however be conducted to determine the best cooking method to preserved the 224

valuable EPA+DHA from the Atlantic mackerel. Indeed, the summer Atlantic mackerel is an excellent food source of EPA+DHA: with about 4 to 5 mg.g⁻¹ in wet weight, a daily consumption of 75 g (raw or steamed) or 60 g (grilled) of dorsal fillet would be enough to cover the EPA+DHA daily requirements in adults.

229

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239

240 Author credits

Fany Sardenne: conceptualization, methodology, software, investigation, writing – original
draft, review & editing. Eleonora Puccinelli: investigation, writing – review & editing. Marie
Vagner: writing – review & editing, funding acquisition. Laure Pecquerie: writing – review &
editing, funding acquisition. Fabienne Le Grand: validation, resources. Antoine Bideau:
methodology. Philippe Soudant: validation, writing – review & editing, funding acquisition.

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Fig. 1 | Outline of the sampling design testing for the influence of storage temperature (2-4°C and 18-20°C), storage duration (from T_6 to T_{24} hours), and cooking method (grill and steam) on the fatty acid content of the Atlantic mackerel *Scomber scombrus*. Dark squares indicate the muscle sampling position at each step, and the grey ones the previously sampled positions. For cooking, we sampled the left side of the fish.



Fig. 2 | Total fatty acids (FA) and EPA+DHA contents (in $mg.g^{-1}$ of dry weight) in the dorsal fillet of Atlantic mackerel *Scomber scombrus* as a function of fish fork length. Fish (n=15) were collected from the Baie de Douarnenez, France, in July 2020. Grey areas are standard errors for the linear regressions.



Fig. 3 | Boxplots of differences from initial values (T_0 = at fish death) for dorsal fillet contents in (A) moisture, (B) total fatty acids (FA), (C)

EPA+DHA, (D) poly-unsaturated FA (PUFA), (E) mono-unsaturated FA (MUFA), and (F) saturated FA (SFA) during the first *post-mortem* hours (T_6 to T_{24}) and cooking of Atlantic mackerel, according to storage temperature and cooking methods (display by colour). In each boxplot, the thick black bar represents the median value, the box contains 50% of the data, and dots are outliers.

Table 1 | Mean \pm standard deviation for moisture in % per wet weight, total fatty acids (FA), EPA+DHA, poly-unsaturated FA (PUFA), monounsaturated FA (MUFA), and saturated FA (SFA) contents in mg.g⁻¹ dry weight (dw), from the dorsal fillet of Atlantic mackerel after three storage durations (T₆, T₁₂, and T₂₄ hours) at two storage temperatures (2-4°C and 18-20°C), and after two cooking methods (grill and steam). N is the number of individuals.

	Reference	Storage test						Cooking method	
		T ₆		T ₁₂		T ₂₄			
		[2-4°C]	[18-20°C]	[2-4°C]	[18-20°C]	[2-4°C]	[18-20°C]	Grill	Steam
Ν	15	8	7	8	7	8	7	8	7
Moisture (%)	75.4 ± 3.0	73.5 ± 2.9	74.3 ± 3.4	72.9 ± 4.3	74.3 ± 3.3	73.7 ± 3.2	74.0 ± 4.7	69.2 ± 2.7	68.3 ± 3.1
Total FA (mg.g ⁻¹ dw)	60.9 ± 21.8	60.6 ± 19.5	48.0 ± 20.5	55.6 ± 24.4	44.8 ± 22.3	56.7 ± 22.7	44.5 ± 25.8	66.5 ± 21.0	46.9 ± 19.7
EPA+DHA (mg. g ⁻¹ dw)	16.2 ± 4.0	15.9 ± 3.6	13.6 ± 3.8	13.8 ± 4.3	12.3 ± 4.4	14.0 ± 4.0	12.1 ± 5.2	16.5 ± 4.2	12.9 ± 4.1
PUFA (mg.g⁻¹ dw)	20.6 ± 5.6	20.3 ± 5.3	17.0 ± 5.2	17.7 ± 6.1	15.4 ± 5.9	17.9 ± 5.5	15.3 ± 7.0	21.6 ± 6.0	16.3 ± 5.6
MUFA (mg.g⁻¹ dw)	17.9 ± 10.1	18.0 ± 9.3	12.4 ± 9.3	17.1 ± 11.6	11.8 ± 9.9	17.6 ± 11.0	11.8 ± 11.2	20.8 ± 9.2	12.3 ± 9.2
SFA (mg.g⁻¹ dw)	16.2 ± 6.3	16.0 ± 5.5	12.3 ± 6.0	14.6 ± 7.0	11.4 ± 6.5	14.9 ± 6.4	11.3 ± 7.5	17.8 ± 6.0	12.1 ± 5.5

Table 2 | Summary of the results obtained from the repeated MANOVA to test the influence of storage temperature, duration and their interaction on biochemical contents of Atlantic mackerel dorsal fillet (moisture, total fatty acids (FA), EPA+DHA, poly-unsaturated FA (PUFA), mono-unsaturated FA (MUFA), and saturated FA (SFA)).

	χ^2	df	p value
Moisture			
Storage temperature	0.3	1	0.580
Storage duration	35.5	3	<0.001
Storage temperature * duration	2.4	3	0.530
Total FA			
Storage temperature	0.9	1	0.332
Storage duration	68.6	3	<0.001
Storage temperature * duration	2.9	3	0.400
EPA+DHA			
Storage temperature	0.7	1	0.409
Storage duration	127.4	3	< 0.001
Storage temperature * duration	3.6	3	0.305
PUFA			
Storage temperature	0.8	1	0.384
Storage duration	101.0	3	<0.001
Storage temperature * duration	3.3	3	0.342
MUFA			
Storage temperature	1.0	1	0.318
Storage duration	44.8	3	< 0.001
Storage temperature * duration	2.2	3	0.533
SFA			
Storage temperature	1.0	1	0.328
Storage duration	73.1	3	< 0.001
Storage temperature * duration	4.0	3	0.266