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Effects of frozen storage duration on biochemical composition, fatty acid profile, and oxidative damage markers of *Scomber scombrus* fillets over short period

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Abstract

The present work evaluated the effects of storage times (30, 45, and 90 days) at frozen temperature (-20°C) on biochemical composition and oxidative damage markers of *Scomber scombrus* fillets. The results showed a significant decrease in proteins and total lipids after the freezing process. We noted significant increases and decreases, respectively, in SFA and PUFA levels. Remarkable variations in oxidative damage markers were also observed. They were more significant after the first 30 days of storage. Therefore, it is highly recommended to consume the fish fillets, either fresh or frozen, within the period of not exceeding 45 days.

Introduction

Atlantic mackerel (*Scomber scombrus*) is a pelagic schooling fish found in the Mediterranean Sea along the North Atlantic coasts (FAO, 2007). This blue back species is among the most beneficial seafood products for human health. It has the best nutritional value due to its easy digestibility, high levels of protein and lipids, and richness in vitamins and different minerals (Dwyer *et al*., 2003). It is considered a valuable species as it is an excellent source of *n*-3 polyunsaturated fatty acids, mainly eicosapentaenoic and docosahexaenoic acids (Orban *et al.,* 2011; El Oudiani *et al.,* 2019).

The nutritional quality of seafood products is impacted by several storage methods including cooling, freezing, super-chilling, and chemical preservatives (Rathod *et al.,* 2022). These advanced food preservation techniques efficiently reduce microbial spoilage and oxidative degradation while prolonging the product's shelf life by limiting unfavourable changes in quality, nutritional value, texture, and flavour. The low-temperature storage technique is one of the most effective methods for fish preservation, successfully maintaining the nutritional and sensory properties of fish products (Erickson, 1997).

However, the product quality during freezing storage is primarily influenced by the presence of highly unsaturated fatty acids (UFA) and the high content of pro-oxidant molecules, which may lead to significant enzymatic and non-enzymatic rancidity (Richards and Hultin, 2002; Kolakowska, 2003). In this context, these last fatty acids are particularly susceptible to oxidation due to their high number of double bonds (unsaturation) on the carbon chain (Geret *et al.,* 2002). Additionally, proteins and vitamins are also prone to degradation during storage (Baron, 2014). Protein oxidation can be triggered by lipid radicals. This oxidation causes a biochemical

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and structural alteration, resulting in a loss and change in functional properties (Lund *et al.,* 2011). Moreover, several factors such as (i) temperature, (ii) duration, and (iii) whether the fish is stored whole or as fillets, significantly influence the preservation of food product quality. It has been demonstrated that during frozen storage, fillets from certain fish species tend to be more susceptible to lipid deterioration than whole fish due to increased exposure to air in the muscle (Aubourg *et al.,* 2004; 2005). Recent studies have shown that storing fish at lower frozen temperatures enhances protection against lipid oxidation and hydrolysis (Romotowska et *al.,* 2016; 2017). This suggestion was also supported by Aas *et al*. (2003) and Aubourg *et al*. (2005), who studied Atlantic mackerel fillets stored at -20°C (Zotos *et al*., 1994). Similarly, other studies on minced mackerel stored at -20°C and -30°C reported increased lipid and protein oxidation over time, with oxidation being more pronounced at -20°C than at -30°C (Saeed and Howell, 2002).

Several works have focused on the assessment of the effects of long-term freezing storage (from one to 12 months) of mackerel fillets. However, due to the lack of data on the effects of short-term freezing, the present work aimed to evaluate the impact of shorter freezing durations (30, 45, and 90 days) on the biochemical composition of *Scomber scombrus* fillets stored at -20°C. Specifically, the present work examined the fatty acid composition, macromolecule degradation, and nutritional quality indices.

Materials and methods

Sample preparation

Mackerel (*S. scombrus*) samples used in the present work were caught from fishing in Teboulba, located on the Middle East Coast of Tunisia from the Mediterranean Sea, during summer. The fresh samples with an average weight of 158.00 ± 17.04 g and an average length of 25.17 ± 2.65 cm were transported immediately from Teboulba port to the laboratory in polythene bags along with crushed ice. The fish samples were immediately washed and divided in two groups. The raw samples of fish were taken at day 0. During freezing, the mackerel fillets were packaged into commercial alimentary polyethylene bags (Stark®) and stored at -20°C (± 2) for 30, 45, and 90 d. The storage durations were selected based on several works (Nazemroaya *et al.,* 2011; Agustinelli and Yeannes, 2015; El-Dengawy *et al.,* 2017).

Mackerel fillets for each condition were homogenised in a Tris-HCl buffer (20 mM; pH = 7.4) in cold conditions, then centrifuged at 10,000 *g* for 20 min (4°C). Tissue supernatants were stored in Eppendorf Tubes at -80°C for biochemical analysis.

Proximate composition

Moisture content was determined by weight difference after heating the sample at 110°C for 24 h according to AOCS (1989). Protein determination was performed using bovine serum albumin as a standard according to Lowry *et al.* (1951). Total lipids (TL) were extracted using chloroform:methanol (2:1, v/v) solution containing 0.01% butylated hydroxyl toluene (BHT) as an antioxidant according to Folch *et al*. (1982). All proximate compounds were expressed as (g/100 g).

Fatty acid analysis

Total lipid was *trans*-esterified to methyl esters according to Cecchi *et al.* (1985). The methyl nonadecanoic acid C19:0 (Sigma), which was absent in our samples, was added as an internal standard. Methyl esters were analysed by gas chromatography using a chromatogram "Agilent Technologies" HP 6890 model equipped with a capillary column INNO-WAX (30 m × 0.25 μm), and supplied with a carrier gas (nitrogen). Identification of fatty acid methyl esters (FAMEs) was based on the comparison of their retention times with those of a mixture of methyl esters PUFA-3 (Menhaden oil by SUPELCO). Fatty acids were expressed in percentages.

Lipid quality indices

All lipid quality indices were evaluated using the fatty acid composition data. Fish Lipid Quality/Flesh Lipid Quality (FLQ) was established according to Senso *et al*. (2007) using Eq. 1:

$$FLQ = [100 *(C22:6n\text{-}3 + C20:5n\text{-}3) / SFA] \quad \text{(Eq. 1)}$$

Atherogenicity index (AI) and thrombogenicity index (TI) were used according to Ulbricht and Southgate (1991) using Eqs. 2 and 3, respectively:

$$\text{Atherogenicity index (AI)} = [(C12:0 + (4\times C14:0) + C16:0)] / [\Sigma MUFAs + \Sigma\ PUFA\ (n\text{-}6) + \Sigma\ PUFA\ (n\text{-}3)] \quad \text{(Eq. 2)}$$

Thrombogenicity index (TI = [(C14:0 + C16:0 + C18:0) / (0.50*ΣMUFA) + (0.5*ΣPUFA (*n*-6) + (3*ΣPUFA (*n*-3) + (ΣPUFA (*n*-3) / ΣPUFA (*n*-6)] (Eq. 3)

Polyene index (PI) was calculated according to Rodríguez *et al.* (2007) using Eq. 4:

PI= (C20:5*n*-3 + C22:6*n*-3) / (C16:0) (Eq. 4)

The Hypercholesterolaemic Index (h/H) ratio was estimated by the ratio between hypo- and hypercholesterolaemic fatty acids. According to Fernandes *et al.* (2014), the h/H ratio was calculated using Eq. 5:

Hypercholesterolaemic index (h/H = (C18:1*n*-9 + C18:2*n*-6 + C20:4*n*-6 + C18:3*n*-3 + C20:5*n*-3 + C22:5*n*-3 + C22:6*n*-3) / (C14:0 + C16:0) (Eq. 5)

The UFA/C16:0 ratio was calculated using Eq. 6:

Σ UFA/C16:0 = (MUFA + PUFA) / (C16:0) (Eq. 6)

Oxidative damage markers

Lipid peroxidation was assessed using 2-thiobarbituric acid (TBA) determined through the malondialdehyde levels (MDA) (AOCS, 1989). The quantity of MDA was determined at 532 nm. TBAR value was expressed as mg kg/WW.

According to the AOCS (1989), the peroxide value (PV) was carried out with pure chloroform and glacial acetic acid. Results were expressed as mL/g, and calculated using Eq. 7:

PV = [(titration of the sample – titration of the blank) × N thiosulfate × 1000] / weight of the sample (Eq. 7)

Free fatty acids (FFA) were assessed according to AOCS (1989). Results were expressed as a percentage, and calculated using Eq. 8:

FFA = [(sample titration – blank titration) × normality of mL of KOH solution × molar weight of KOH] / the weight of the sample (Eq. 8)

Advanced oxidation protein product (AOPP) was determined according to Kayali *et al*. (2006). The absorbance of this technique was determined at 340 nm, and expressed as µmol/mg of protein. The level of AOPP was quantified using the extinction coefficient of 261 cm^{-1} mM^{-1}.

Statistical analysis

The statistical analysis was carried out using STATISTICA Software Version 5.0. Results were expressed as means ± SD (standard deviation) for each analysis. The level of significance was determined at 0.05. The homogeneity and normality of variables were tested using the Shapiro-Wilcoxon test. For mackerel fillets, the differences in proximate composition, fatty acid composition, and oxidative damage markers between fresh tissues and from different freezing times (30, 45, and 90 days) at (-20°C) were assessed by one-way analysis of variance at 5%.

Principal components analysis (PCA) and Pearson's matrix correlation were established using «FactoMineR» R package in order to highlight the effects of frozen times (30, 45, and 90 days) at (-20°C) on biochemical composition, fatty acid composition, and nutritional quality of mackerel fillets.

Results

Proximate composition

A significant decrease in all biochemical compounds of *S. scombrus* fillets during (30, 45, and 90 days of freezing) at -20°C was observed (Table 1). Compared to the raw fillet, a significant decrease in the lipid quantity expressed by -30, -55, and -60% was observed in frozen fillets during 30, 45, and 90 days, respectively. The decrease in protein contents was recorded in all frozen fillets at -20°C during 30, 45, and 90 days (-8, -11, and -12%, respectively) compared to the raw fillet. Concerning moisture, significant decrease of -6, -11, and -15% were recorded in frozen fillet during 30, 45, and 90 days, respectively, compared to the raw fillet.

Fatty acid composition in raw and frozen S. scombrus fillets

Fatty acids composition of raw and frozen *S. scombrus* fillets at -20°C during 30, 45, and 90 days are shown in Table 2. Twenty-seven different fatty acids were identified and divided into three families as follows: saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA).

Table 1. Proximate composition of raw and frozen *Scomber scombrus* fillets at -20°C during 30, 45, and 90 days.

Proximate composition (g/100 g)	Raw	30 days	45 days	90 days
Protein	20.07 ± 0.51[a]	18.41 ± 0.40[b]	17.75 ± 0.32[b]	17.66 ± 0.20[b]
Lipid	12.5 ± 0.00[a]	8.1 ± 0.01[b]	5.06 ± 0.04[b]	5 ± 0.00[a]
Moisture	70.66 ± 1.52[a]	66 ± 1.28[b]	62.33 ± 2.08[b]	62 ± 20[b]

Data are mean ± SD (n = 10). Different lowercase superscripts in similar row indicate significantly different values (Duncan's $p < 0.05$) between raw and frozen fillets at different frozen durations.

Table 2. Fatty acids composition (%) of raw and frozen *Scomber scombrus* fillets at -20°C during 30, 45, and 90 days.

Fatty acid (%)	Raw	30 days	45 days	90 days
C14:0	0.56 ± 0.00[a]	1.02 ± 0.08[a]	2.50 ± 0.46[b]	2.67 ± 0.12[a]
C15:0	1.85 ± 0.02[a]	2.10 ± 0.12[b]	1.48 ± 0.05[b]	1.86 ± 0.07[a]
C16:0	23.40 ± 0.16[a]	31.20 ± 0.24[b]	34.40 ± 0.10[b]	37.00 ± 1.34[b]
C18:0	10.40 ± 0.05[a]	10.60 ± 1.1[a]	8.42 ± 0.03[a]	8.99 ± 1.04[a]
C20:0	0.41 ± 0.03[a]	0.23 ± 0.11[b]	0.20 ± 0.03[b]	0.13 ± 0.01[b]
C22:0	0.12 ± 0.03[a]	0.31 ± 0.11[a]	0.51 ± 0.12[b]	0.35 ± 0.02[b]
C14:1	0.40 ± 0.00[a]	0.62 ± 0.06[b]	0.63 ± 0.01[b]	0.28 ± 0.02[b]
C15:1	0.28 ± 0.00[a]	0.23 ± 0.02[a]	0.17 ± 0.06[b]	0.03 ± 0.01[b]
C16:1	4.02 ± 0.03[a]	4.57 ± 0.14[a]	6.32 ± 0.15[b]	7.36 ± 0.38[b]
C18:1	8.83 ± 0.01[a]	10.8 ± 0.96[b]	10.8 ± 0.69[b]	10.7 ± 0.01[b]
C20:1	0.35 ± 0.03[a]	0.19 ± 0.04[b]	0.19 ± 0.01[b]	0.29 ± 0.00[a]
C22:1	0.51 ± 0.03[a]	0.48 ± 0.01[a]	0.40 ± 0.02[b]	0.28 ± 0.04[b]
C18:3*n*-4	0.54 ± 0.03[a]	0.41 ± 0.02[a]	0.93 ± 0.18[b]	0.55 ± 0.21[a]
C18:3*n*-3	0.23 ± 0.05[a]	1.83 ± 0.12[b]	0.56 ± 0.03[b]	0.28 ± 0.02[a]
C18:4*n*-3	0.91 ± 0.03[a]	1.29 ± 0.65[a]	1.02 ± 0.00[a]	0.41 ± 0.05[a]
C20:3*n*-3	0.12 ± 0.03[a]	0.14 ± 0.12[a]	0.29 ± 0.07[a]	0.28 ± 0.02[a]
C20:4*n*-3	0.28 ± 0.03[a]	0.15 ± 0.12[a]	0.13 ± 0.06[a]	0.07 ± 0.01[b]
C20:5*n*-3 (EPA)	5.51 ± 0.01[a]	4.21 ± 1.15[a]	3.29 ± 0.66[b]	4.43 ± 0.19[a]
C22:5*n*-3	1.99 ± 0.02[a]	2.82 ± 0.87[a]	3.01 ± 0.27[a]	1.04 ± 0.19[a]
C22:6*n*-3 (DHA)	33.80 ± 0.25[a]	20.00 ± 1.36[b]	18.60 ± 0.34[b]	17.90 ± 0.59[b]
C18:2*n*-6	1.90 ± 0.02[a]	2.38 ± 0.09[a]	2.88 ± 0.44[b]	1.86 ± 0.23[a]
C18:3*n*-6	0.36 ± 0.03[a]	0.22 ± 0.18[a]	0.13 ± 0.02[a]	0.42 ± 0.06[a]
C20:2*n*-6	0.43 ± 0.03[a]	0.41 ± 0.20[a]	0.73 ± 0.46[a]	0.42 ± 0.15[a]
C20:4*n*-6 (ARA)	2.11 ± 0.02[a]	3.31 ± 0.33[b]	1.82 ± 0.14[a]	1.41 ± 0.06[b]
C22:2*n*-6	0.42 ± 0.03[a]	0.05 ± 0.01[b]	0.13 ± 0.04[b]	0.05 ± 0.00[b]
C22:5*n*-6	0.32 ± 0.03[a]	0.48 ± 0.09[a]	0.48 ± 0.14[a]	0.88 ± 0.63[a]
ΣSFA	36.70 ± 0.14[a]	45.39 ± 0.95[b]	47.50 ± 0.53[b]	50.90 ± 0.32[b]
ΣMUFA	14.40 ± 0.08[a]	16.90 ± 0.97[b]	18.60 ± 0.91[b]	19.00 ± 0.34[b]
ΣPUFA	48.90 ± 0.06[a]	37.70 ± 1.90[b]	33.80 ± 1.44[b]	30.00 ± 0.03[b]
ΣUFA	57.29 ± 0.07[a]	54.6 ± 1.43[a]	51.40 ± 1.02[b]	49.01 ± 0.18[b]
ΣPUFA (*n*-3)	42.89 ± 0.13[a]	30.4 ± 1.27[b]	26.90 ± 1.51[b]	24.40 ± 0.10[b]
ΣPUFA (*n*-6)	5.54 ± 0.15[a]	6.85 ± 0.64[b]	6.17 ± 0.24[a]	5.04 ± 0.13[a]

Data are mean ± SD (n = 10). Different lowercase superscripts in similar row indicate significantly different values (Duncan's $p < 0.05$) between raw and frozen fillets at different frozen durations. ARA: arachidonic acid; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; and UFA: unsaturated fatty acid. C16:1 = (C16:1*n*-9 + C16:1*n*-7); and C18:1 = (C18:1*n*-9 + C18:1*n*-7).

Compared to the raw fillet, significant increases in SFA levels ($p < 0.001$) were recorded in frozen fillets at -20°C after 30, 45, and 90 days (with +23, +29, and +36%, respectively). Notably, a huge increase was recorded for C14:0 and C16:0 after 45 and 90 days of freezing. However, a significant decrease was observed for C18:0 during similar period. The MUFA levels increased significantly in frozen fillets during 30, 45, and 90 days (+17, +29, and +34%, respectively) compared to the raw fillet. Major changes were observed for MUFA, such as C16:1 and C18:1 levels after 45 and 90 freezing days.

The unsaturated fatty acid (UFA) considerably decreased after 30, 45, and 90 days of freezing (-23, -24, and -28%, respectively). The PUFA levels decreased significantly with time (-23, -30, and -37%, respectively). Decreases (-29, -37, and -41%) were observed in PUFA (*n*-3) in frozen fillets after 30, 45, and 90 days, respectively. PUFA (*n*-6) levels increased ($p < 0.001$) after 30 days (+11%) and 45 days (+29%) of freezing.

Two essential fatty acids such as DHA (-40, -91, and -47%) and EPA (-23, -45, and -19%) demonstrated some decline following 30, 45, and 90 freezing days, respectively.

The lipid quality indices are summarised in Table 3. The ΣPUFA/ΣSFA, *n*-3/*n*-6, h/H, ΣUFA/C16:0 ratios, EPA+DHA, FQL, and polyene index (PI) calculated in *S. scombrus* fillets decreased after 30, 45, and 90 freezing days ($p < 0.001$). However, AI and TI indices increased significantly in *S. scombrus* fillets after similar period compared to the raw fillet.

Table 3. Nutritional quality indices of raw and frozen *Scomber scombrus* fillets at -20°C during 30, 45, and 90 days.

Nutritional indices	Raw	30 days	45 days	90 days
EPA+DHA (%)	39.30 ± 0.27^{a}	24.20 ± 0.23^{b}	21.90 ± 1.00^{b}	22.30 ± 0.40^{b}
FLQ	39.30 ± 0.26^{a}	24.20 ± 0.23^{b}	21.90 ± 0.99^{b}	22.30 ± 0.39^{b}
PUFA/SFA	1.33 ± 0.01^{a}	0.83 ± 0.06^{b}	0.72 ± 0.04^{b}	0.59 ± 0.00^{b}
UFA/C16:0	2.51 ± 0.07^{a}	1.48 ± 0.12^{b}	1.81 ± 0.16^{b}	1.38 ± 0.09^{b}
n-3/*n*-6	7.74 ± 0.24^{a}	4.45 ± 0.24^{b}	4.38 ± 0.42^{b}	4.85 ± 0.11^{b}
PI	1.68 ± 0.00^{a}	0.78 ± 0.01^{b}	0.64 ± 0.03^{b}	0.6 ± 0.010^{b}
AI	0.41 ± 0.00^{a}	0.65 ± 0.02^{b}	0.86 ± 0.04^{b}	0.99 ± 0.04^{b}
TI	0.23 ± 0.00^{a}	0.43 ± 0.03^{b}	0.47 ± 0.02^{b}	0.54 ± 0.00^{b}
h/H	2.19 ± 0.01^{a}	1.32 ± 0.04^{b}	1.05 ± 0.02^{b}	0.89 ± 0.03^{b}

Data are mean ± SD ($n = 10$). Different lowercase superscripts in similar row indicate significantly different values (Duncan's $p < 0.05$) between raw and frozen fillets at different frozen durations. DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; UFA: unsaturated fatty acid; FLQ: fish lipid quality; PI: polyene index (PI), AI: atherogenicity index; TI: thrombogenicity index; and h/H: hypercholesterolaemic index.

Oxidative damage markers

The results showed a significant increase in lipid peroxidation in all frozen fillets as evidenced by the increase in malondialdehyde levels (MDA). Compared to the raw fillet, the MDA levels increased significantly after 30, 45, and 90 freezing days (+47, +119, and +175%, respectively) (Figure 1A).

A significant increase in peroxide value levels (PV) was observed (+16, +91, and +288%) during 30, 45, and 90 freezing days, respectively, as compared to the raw fillet (Figure 1B).

Free fatty acids (FFA) increased significantly in frozen fillets at -20°C during 30, 45, and 90 days of freezing (+26, +116, and +167%, respectively) compared to the raw fillet (Figure 1C).

Protein oxidation was determined using the advanced protein oxidation products (AOPP) levels. Significant increase in AOPP levels was recorded in the frozen fillets during 30, 45, and 90 days (+877, +987, and +1197%, respectively) compared to the raw fillet (Figure 1D).

Pearson correlation matrix and principal component analysis (PCA)

Results of PCA (Figure 2) allowed us to retain the first two factorial axes that explained 90.39% of

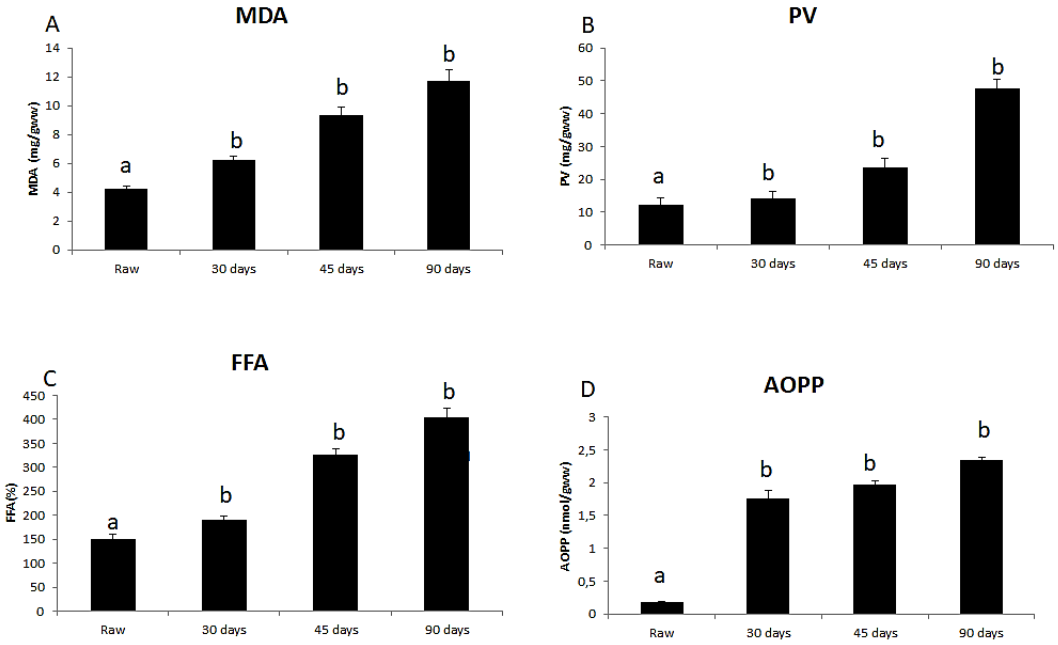

Figure 1. MDA **(A)**, PV **(B)**, FFA **(C)**, and AOPP **(D)** levels in raw and frozen *Scomber scombrus* fillets at -20°C during 30, 45, and 90 days. Data are mean ± SD (n = 10). Different lowercase letters indicate significantly different values (Duncan's $p < 0.05$) between raw and frozen fillets at different frozen durations.

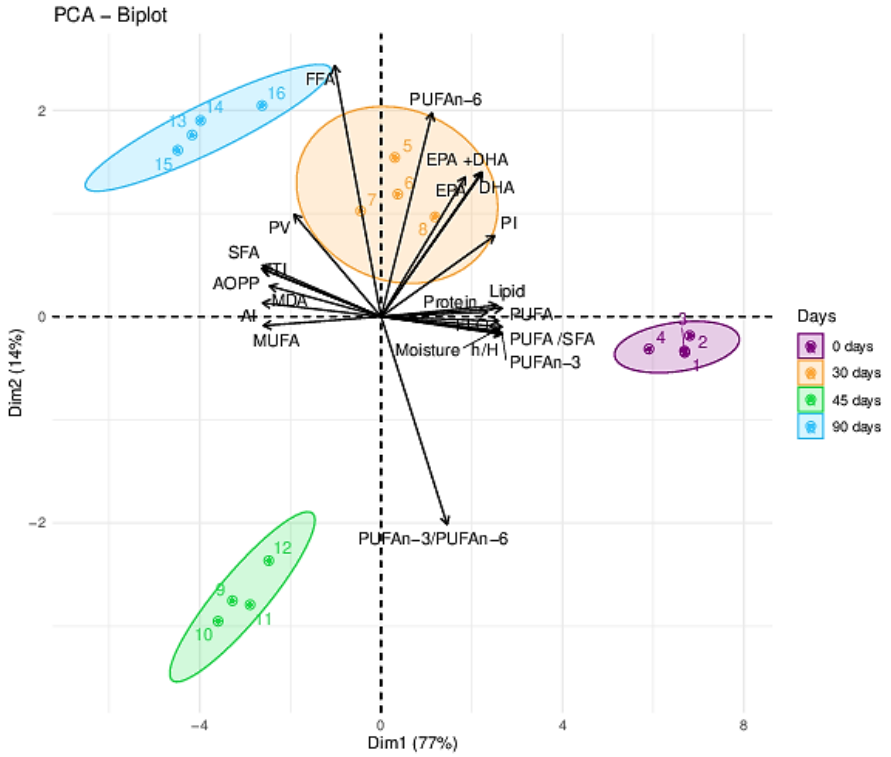

Figure 2. Principal analysis component (PCA) represented by two factors F1 and F2, and produced by biochemical parameters, fatty acid composition, nutritional quality indices, and oxidative damage markers in raw and frozen fillets of *Scomber scombrus* at -20°C for 30, 45, and 90 days. Projection of variables and cases on factor-plane (1 × 2).

the total variance. Factor 1 displayed 77%, and factor 2 displayed 14% of the total variance. Analyses of proximate and fatty acid compositions, nutritional indices, and damage biomarkers of *S. scombrus* fillets, showed significant variations between raw and freezing times (30, 45, and 90 days) at -20°C. The results clustered into four separate groups. Group (1) consisted the raw fillets. The high levels of proximate compounds, fatty acids such as (DHA and EPA), and the healthy nutritional quality characterised the raw fillet. After 30 days of freezing, the fillets grouped as intermediate group (2), demonstrating an initiation of nutritional quality degradation. Groups (3) and (4) were characterised by fillets following 45 and 90 freezing days. These fillets presented low lipid, moisture, and protein quantities (i), the decrease in DHA levels, the *n*-3/*n*-6 ratio, FLQ, AI, and TI indices (ii), and the high expression of damage biomarkers (iii) such as lipid peroxidation (MDA, PV, and FFA) and protein oxidation (AOPP). These decreases in fillets' nutritional quality were more pronounced after 90 days of freezing. The decrease in PUFA (R = -0.90), PUFA (*n*-3) (R = -0.92), and DHA (R = -0.72) in these fillets demonstrated a negative correlation with the increase in MDA levels (Figure 3).

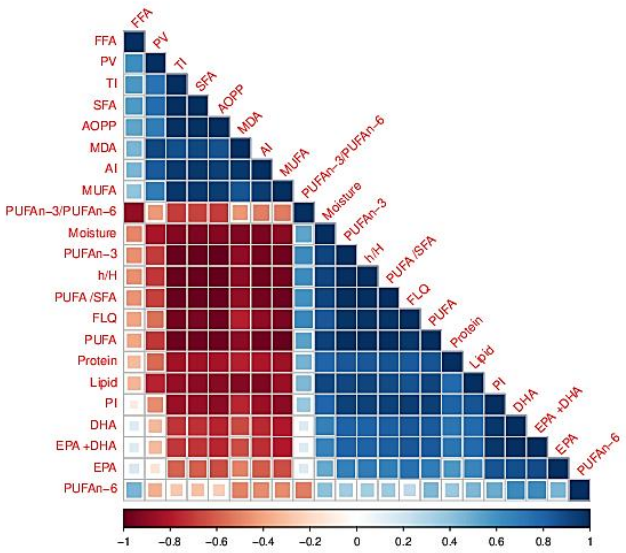

Figure 3. Pearson's matrix correlation between fatty acid composition (1); nutritional quality indices (2), and oxidative damage markers (3) in raw and frozen fillets of *Scomber scombrus* at -20°C for 30, 45, and 90 days. Each cell indicates the Pearson's correlation coefficient value, r, for a pair of parameters. Positive correlations (0 < r < 1.0) are displayed in blue colour, and negative correlations (1.0 < r < 0) are displayed in red colour. Colour intensity is proportional to the correlation coefficients.

Discussion

Mackerel has significant economic value. It is one of the most widely consumed blue fish, and highly recommended for human health. The present work aimed to evaluate the effects of short-term freezing (30, 45, and 90 days) on the biochemical composition and fatty acid profile of *S. scombrus* fillets stored at -20°C.

In the present work, the raw fillets of *S. scombrus* exhibited biochemical compound levels similar to those reported in previous studies (Agustinelli and Yeannes, 2015; Standal *et al.,* 2018; El Oudiani *et al.,* 2019). Also, the protein, moisture, and lipid levels decreased considerably over the freezing periods, with the most pronounced decrease observed after 90 days. These decreases may be attributed to the duration of freezing and storage. Consistent with our findings, previous studies have demonstrated a strong correlation between the decrease in protein and lipid contents in various fish species, and both storage duration and freezing temperature (Mazrouh, 2015; Mohamad and Saad, 2015; Agustinelli and Yeannes, 2015). The prolonged storage period and proteolysis in fish muscle may explain the decrease in protein content (Azam et *al.,* 1990) observed in the present work. According to Siddiqui and Ali (1979), such decrease could also be due explained by the leaching effect of amino acids and water-soluble proteins during the melting of ice.

Mackerel muscle consists of both dark and light muscles. It is considered as pelagic species characterised by abundance of sarcoplasmic protein in dark muscle, including enzymes and oxygen carriers, as well as myofibrillar proteins composed of myosin, actin, tropomyosin, and troponin which are responsible for muscle contraction (Ugwu *et al*., 2024). Despite this, fish proteins, especially myofibrillar proteins, are susceptible to freezing-induced denaturation during frozen storage. These denaturation leads to the formation of protein-protein bonds, and the development of high molecular-weight polymers (Benjakul *et al.,* 2003). In the present work, the significant increase in AOPP levels in frozen fillets indicated considerable effects of freezing times leading to the excessive oxidation of proteins, and likely resulting in the formation of denatured protein polymers, such as carbonyl proteins. This oxidation appeared to be more apparent after 90 freezing days.

In the present work, the analysis of fatty acid composition of *S. scombrus* raw fillet showed that the PUFAs, especially PUFA (*n-3*), were predominant, which characterise the mackerel species. Among PUFA (*n-3*), DHA and EPA have the highest proportions. SFA constitutes the second most important FA family; however, MUFA was the minor fraction in raw fillet assessed in the present work. In the SFA fraction, palmitic acid was predominant, while in the MUFA fraction, oleic acid was the major one. Similar results were observed in different mackerel species (Nazemroaya *et al.,* 2011; Agustinelli and Yeannes, 2015; El Oudiani *et al.,* 2019).

When mackerel fillets were stored at -20°C for 30, 45, and 90 freezing days, the FA quality declined after 30 days. Thus, the UFA and PUFA levels decreased considerably. In contrast, the MUFA and SFA levels increased greatly. These changes in FA composition were highly correlated with freezing times, and were more pronounced after 90 freezing days. Similar results were obtained by other authors for whole frozen mackerel at -19°C for 12 months (Nazemroaya *et al.,* 2011; Agustinelli and Yeannes, 2015), as well as for other fish species frozen at different temperatures during different times (Pirestani *et al.,* 2010).

In the present work, PUFA (*n-3*) was the most affected during freezing (30, 45, and 90 days), especially the two essential fatty acids such as EPA and DHA. Their decreases were more noticeable after 90 days of freezing. In this context, the decrease in DHA levels during the freezing process at -20°C reduced their beneficial roles. Other fatty acids such as C18:3*n*-3 and C20:4*n*-3 presented great changes after 30 and 45 days of freezing. Several studies focused on different fish species have demonstrated the same changes in these last fatty acids during the freezing process (Nazemroaya *et al.,* 2011; Agustinelli and Yeannes, 2015). Moreover, compared to the raw fillet, certain changes were recorded for PUFA (*n-6*) after 45 and 90 freezing days. Yet, significant declines were observed only in C18:4*n*-6 and C22:2*n*-6 after 90 freezing days. Our results agreed with other investigations determined after freezing the whole body of dark and light fillet of *S. japonicus* (Agustinelli and Yeannes, 2015).

The PUFAs are more susceptible to lipid peroxidation due to their high number of double bonds (Ke *et al*., 1977); the PUFA fraction acts as a substrate to initiate lipid peroxidation (Hultin, 1994). The lipid peroxidation in the present work was investigated through different oxidative damage markers. The malondialdehyde (MDA) level of frozen fillets increased considerably during freezing (30, 45, and 90 days) compared to the raw fillet. A negative correlation was recorded between the lipid peroxidation index (MDA), and the decrease in PUFA (*n-3*) and DHA levels during the freezing period, especially after 30 days, indicating the disruption of the nutritional quality of fillets. In this context, given the presence of highly unsaturated fatty acids, especially DHA, and the naturally pro-oxidant systems in fish tissue, the mackerel products seem to be very susceptible to loss of quality by the development of lipid peroxidation. Previous studies corroborated our results (Hultin, 1994, Chávez-Mendoza *et al.,* 2014; Agustinelli and Yeannes, 2015).

Furthermore, the frozen fillets of *S. scombrus* during 30, 45, and 90 days showed significant increases in MUFA levels, especially in C16:1 and C18:1. Similar results were observed by Chávez-Mendoza *et al*. (2014) in rainbow trout fillets (*Oncorhynchus mykiss*). Besides, compared to raw fillets, the significant increase in SFAs levels during freezing at -20°C for 30, 45, and 90 days was related to the enhancement of C16:0 and C22:0 levels, especially after 90 freezing days.

Moreover, the increase in SFA in the present work could be explained by the degradation of PUFAs during the freezing process that could generate low molecular weight compounds and

possibly a short chain FA (Chávez-Mendoza *et al.,* 2014). Besides, it has been demonstrated that EPA and DHA are considered as the crucial contributor to PUFA for the formation of low molecular weight compounds, including those in the SFA category (Chávez-Mendoza *et al.,* 2014).

Several nutritional quality indices were calculated in the present work in order to evaluate the healthy nutritive quality of fish following freezing process. Furthermore, the polyene index (PI), which represents the relationship between PUFA and palmitic acid (C16:0), was also calculated. PI decreased considerably during the frozen storage of *S. scombrus* fillets (R = -0.97). It could be explained by the decreases in EPA and DHA levels (R = 0.81 and 0.98, respectively), and the increase in C16:0 levels. Similar findings were reported by Pirestani *et al*. (2010) and Chávez-Mendoza *et al.* (2014). The ratio (PUFA(n-3)/PUFA(n-6) = 7.7) calculated in the raw fillet was similar to those determined by El Oudiani *et al.* (2019). However, it decreased remarkably during the frozen storage (30, 45, and 90 days, R = -0.87) at -20°C due to the significant decrease in PUFA levels (R = 0.95). These decreases were in concordance with those of EPA+DHA index. These results agreed with those determined in other fish fillets (Taheri *et al*., 2011; Chávez-Mendoza *et al.,* 2014). Fish lipid quality/flesh lipid quality (FLQ) was proposed by Senso *et al.* (2007) to access the quality of lipids in fish. The raw fillet of *S. scombrus* in the present work yielded FLQ value of 39%, which was similar to those determined for *Scomberomorus cavalla* (35.06%) (Fernandes *et al.,* 2014). Some decreases were recorded for FLQ during freezing storage, especially for 30, 45, and 90 days (R = -0.91), therefore substantiated the harmful effects of prolonged freezing on essential fatty acids.

In the present work, the PUFA/SFA ratio was calculated and estimated to be 1.3 in the raw fillet. This value fell within the range determined by El Oudiani *et al.* (2019) in the fillets of *S. scombrus* for both sexes, harvested during the summer. On another side, our value was higher than those recommended for human health (0.45) (HMSO, 1994). During frozen storage at -20°C, the decrease in PUFA and the increase in SFA can explain the decrease in the PUFA/SFA index after different freezing times (30, 45, and 90 days). Same decreases were observed in surimi fish stored for 90 days at -22.3°C (Perez-Mateos and Byd-Lanier, 2004). In this line, other ratios were calculated in our study such as UFA/C16:0. It decreased considerably after 90 days of freezing, and demonstrated the alteration of fatty acid compositions of mackerel fillets during the freezing process, which accumulated more SFA, especially C16:0, following oxidation of UFA. Our findings showed that the raw fillet presented low AI and TI values. During the frozen storage of *Scomber* fillets for 30, 45, and 90 days (R > 0.93), these indices increased considerably. They could be interpreted by FA levels decreases, and the increase in atherogenic acids such as myristic (C14:0) and palmitic (C16:0) acids. Several authors have shown that the C14:0 and C16:0 fatty acids are among the most atherogenic, whereas C18:0 is believed to be neutral with respect to atherogenicity, but it is considered thrombogenic instead (Popa *et al.,* 2012). Our results agreed with those of Chávez-Mendoza *et al.* (2014).

Compared with the PUFA/SFA ratio, the h/H ratio is considered as a further index to reflect the impacts of the FA composition on cardiovascular diseases. For the raw fillet, h/H ratio was equal to 2.2 as reported on the other fish species (1.54 and 4.83) (Fernandes *et al.,* 2014). This ratio showed a significant decrease during the frozen storage days, becoming became more pronounced after 90 days of freezing, likely to the high level of SFA (C14:0 and C16:0). It was reported that a high h/H ratio, associated with low AI and TI, could help reduce the incidence of coronary heart disease (Ratusz *et al.,* 2018). However, the opposite results were observed in the present work for frozen fillets for 90 days, leading to the loss of the nutritional quality of the mackerel fillets, and supporting the potential for increased cardiovascular disease risk. UFA/SFA ratio is assumed that the higher it is, the more beneficial it is for the consumer's health (Attia *et al*., 2017). According to Thomsen *et al.* (1999) and Tholstrup *et al.* (2003), it is recommended to reduce the consumption of dietary rich in SFAs (mainly palmitic acid, C16:0), by replacing them with those rich in MUFAs (mainly oleic acid, C18:1n-9) in order to maintain the regulation of the haemostatic system. Due to the high level of C16:0 in the *S. scombrus* fillets obtained during the freezing process, the UFA/palmitic acid ratio has shown decreases respectively during all freezing days, suggesting a decrease in nutritional value and healthiness for human consumption.

The degradation of lipids expressed *via* damage markers such as MDA confirmed the fatty acid deterioration. Significant elevations were

recorded for peroxide value (PV) and free fatty acid (FFA) levels during the freezing times (30, 45, and 90 days). The lipid peroxidation was more pronounced after 90 days of freezing. High and positive correlations were recorded between the MDA, PV, FFA levels, and the freezing times *(R* > 0.93), as well as between the last oxidative damage markers and the decrease in lipid contents of *S. scombrus* fillets *(R* > 0.70), reflecting the harmful effects of prolonged freezing. Our results agreed with those of Agustinelli and Yeannes (2015).

The quality of frozen fish products depends on the preparation and packaging of fish, and the type of freezer. The increase in surface dehydration and mechanical air circulation during storage can lead to rapid myosin and lipid denaturation (Özogul *et al.,* 2004). To reduce oxidation, it is essential to minimise exposure to oxygen. Therefore, it is crucial to use effective packaging materials that are impermeable to moisture and oxygen, for example vacuum packaging. Such processes are more commonly used in industrial product settings to preserve fish quality. However, the present work was focused more on local and home consumption. For this reason, it is recommended to use high-quality freezer bags that are impermeable to humidity, and to ensure their emptiness of air before sealing. Another suggestion is to preserve fish in blocks, which appears to be more effective than individually freezing fish. Despite these recommendations, it is suggested consuming mackerel fillets stored at -20°C within 45 days, as they likely retain better and healthier nutritional quality compared to longer duration.

Conclusion

The present work evaluated the effects of short-frozen storage at -20°C for 30, 45, and 90 days on *S. scombrus* fillets' quality. Alterations in protein, lipids, and moisture contents were observed during the freezing process. The decrease in biochemical composition was associated with the increase in oxidative damage markers such as AOPP levels, reflecting the degradation of proteins and lipids, as evidenced by the significant increases in MDA, PV, and FFA. The changes observed in the proportions of PUFA, SFA, and MUFA revealed the high susceptibility of this marine product to the freezing process. The decrease in PUFA, EPA+DHA, FLQ, *n*3/*n*6 ratio, polyene index, and PUFA/SFA ratio, as well as the increase in SFA, atherogenic index, and thrombogenic index, indicated a substantial loss of nutritional quality of *S. scombrus* fillets. Also, the changes in the proportion of FAs of *S. scombrus* fillets that occurred during the first 30 days of storage was higher. Therefore, it is recommended for healthy diets to consume frozen fillets within 45 days, because this period preserves the biochemical quality of fish fillet.

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