EFFECT OF BRINE FREEZING ON THE RANCIDITY DEVELOPMENT DURING THE FROZEN STORAGE OF SMALL PELAGIC FISH SPECIES

Santiago P. Aubourg* and José M. Gallardo

Instituto de Investigaciones Marinas (CSIC)

c/ Eduardo Cabello, 6

36208-Vigo (Spain)

* Correspondent: Fax: +34 986 292762; e-mail: saubourg@iim.csic.es

ABSTRACT

Brine freezing was applied to two small pelagic underutilised fish species (mackerel, *Scomber scombrus*; horse mackerel *Trachurus trachurus*). Rancidity development was studied during their frozen (-18ºC) storage up to 9 months and quality change results were compared to common freezing conditions (control treatment). Fish samples treated under brine freezing conditions showed a higher lipid oxidation development (peroxide value and thiobarbituric acid index) and worse marks on some sensory attributes (general aspect, odour and colour) than control fish. However, samples treated under brine freezing conditions provided a lower lipid hydrolysis development (free fatty acid formation) and better scores for consistency. Comparison between both fish species led to a higher secondary lipid oxidation formation (thiobarbituric acid index) for mackerel, while horse mackerel showed to be more prone to interaction compound formation (fluorescence detection); however, both fish species showed the same shelf-life times (3 and 5 months for brine and control freezing conditions, respectively). As a result of brine freezing conditions, an increase in NaCl content in white muscle of both species was observed. According to the results obtained in the present work, brine freezing treatment is not recommended for these two small pelagic fish species.

Running Title: Brine freezing of pelagic fish

Keywords: Brine freezing, mackerel, horse mackerel, underutilised fish, frozen storage, rancidity, shelf life

INTRODUCTION

Freezing followed by frozen storage constitute one of the best methods to retain marine species sensory and nutritional properties [1, 2]. Rapid freezing of fish has been recommended to prevent the formation of large ice crystals that can damage cells and allow loss of moisture during the frozen storage and upon thawing. In this sense, brine freezing has shown to provide a rapid convection freezing. The system consists of a saturated sodium chlorine (21%) brine immersion tank with a propeller maintained at –18ºC. The brine is then circulated around the fish until they are frozen [3, 4]. Brine freezing has been used in large fishing vessels that fish a considerable distance from home port and stay at sea for long periods and also by tuna canneries, that often have to store large quantities of starting fish material [5, 6].

A preservative effect of salt has been recognised according to a decrease in water activity, less availability to microbial attack, and enhancement of functional properties [7, 8], although lipid oxidation has been reported to be produced [9, 10]. Thus, previous research accounts for salt addition to the ice used to cool fish [11], immersion of fish material in a brine solution [12] or combination with other technological process such as drying [13] and smoking [14]. However, the effect of brine freezing on quality changes during the fish frozen storage has not been described.

The fish industry is actually suffering from dwindling stocks of traditional species as a result of drastic changes in their availability. Thus, fish technologists and fish trade have turned their attention to some unconventional sources of raw material [15, 16]. Small pelagic fish species can constitute food products of great economic importance in many European countries [17-19]. Some of these fish species are captured in high proportions when their demand is relatively low, so that a large portion

of their catches is underutilised and transformed into fish meals for animals. Thus, a great attention is being accorded by manufacturers in the search of appropriate technological treatments that may enlarge the shelf-life times and accordingly their trading value.

The present work concerns the commercialisation as whole fish frozen products of two small pelagic underutilised fish species (mackerel, *Scomber scombrus*; horse mackerel, *Trachurus trachurus*) [20]. The study is aimed to investigate the effect of brine freezing on the lipid stability of both fish species during the frozen storage. For it, different lipid damage indices are checked and complemented by the sensory assessment.

MATERIALS AND METHODS

Raw fish, sampling, processing and chemicals

Fresh mackerel and horse mackerel (39 individuals of each species) were obtained 10 h after being caught; during this interval, the fish were kept on ice. The weight of each individual was in the range 230-270g for both species. Upon arrival in our laboratory, one part of each fish species (18 individuals) was directly packaged in individual polyethylene bags and immediately placed in a common freezer at –18ºC (*control samples*). The other group (18 individuals of each species) was introduced in a brine (21%) immersion tank with a propeller maintained at –18ºC; after 3.5 hours, the fish were taken, packaged in individual polyethylene bags and placed in a common freezer at –18ºC (*brine samples*).

Sampling was undertaken on the raw material and at months 0, 1, 3, 5, 7 and 9 of frozen storage at –18ºC for both fish species. Sampling at month 0 consisted of samples that had been frozen under brine or control conditions and analysed 24 hours after. For each freezing treatment and for each fish species, three different individual fishes were considered and studied separately for the statistical study (n = 3). Analysis of frozen material was carried out after thawing; thawing was carried out after overnight storage in a cool room (2-4ºC). Once the fish were analysed by sensory assessment, the white muscle was separated, homogenised and immediately employed for the biochemical analyses.

Chemicals employed along the present work (solvents, reagents) were reagent grade (E. Merck; Darmstadt, Germany).

Composition analyses

Water content was determined by weight difference between the homogenised fish muscle (1-2 g) and after 24 hr at 105 °C. Results are expressed as g water/100 g muscle.

Lipids were extracted from the fish muscle by the Bligh and Dyer [21] method. Quantification results are expressed as g lipids/100 g muscle.

NaCl content in fish muscle was calculated from the amount of chlorine by boiling in HNO_3 with excess of $AgNO_3$, followed by titration with NH_4SCN [22]. Results are expressed as g NaCl / 100 g muscle.

Lipid damage measurements

Free fatty acid (FFA) content was determined on the Bligh and Dyer [21] extract by the Lowry and Tinsley [23] method based on complex formation with cupric acetate-pyridine. Results are expressed as g FFA/100 g lipids.

Peroxide value (PV) expressed as meq oxygen/kg lipid was determined by the ferric thiocyanate method [24] on the Bligh and Dyer [21] extract.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke [25] on a 5% trichloracetic acid extract of the fish muscle. Results are expressed as mg malondialdehyde/kg fish sample.

Fluorescence formation (Perkin-Elmer LS 3B) at 327/415 nm and 393/463 nm was measured according to Aubourg et al. [26, 27]. The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission pair, and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 μg/ml in 0.05 M H_2SO_4) at the corresponding wavelength. The fluorescence ratio (FR) was obtained from the lipid extract [21] analysis, according to the following calculation: $FR = RF_{393/463nm} / RF_{327/415nm}$.

Sensory analysis

Sensory analysis was carried out by a taste panel consisting of five experienced judges in fish assessment, according to the guidelines presented in Table 1 [28]. Four categories were ranked: highest quality (E), good quality (A), fair quality (B) and rejectable quality (C). Sensory assessment of fish included the following parameters: General aspect, consistency, odour and colour.

Statistical analyses

Biochemical measurements corresponding to brine and control samples were subjected to one-way analysis of variance to assess significant ($p<0.05$) differences [29]; comparison of means was performed using a least-squares difference (LSD) method. The Spearman test for nonparametric correlations was applied [29].

RESULTS AND DISCUSSION

Composition analyses

Water content of white muscle ranged between 67% and 71% for mackerel, and between 74% and 78% for horse mackerel. Lipid matter was included in the range 6.5%-11.5% for mackerel and in the range 2.0%-4.5% for horse mackerel, according to the lipid content of fat and medium-fat fish species, respectively. In the present case, both species were captured in the period (Autumn) of the highest lipid content [30, 31]. Variations in both constituent (water and lipids) contents may be explained as a result of individual fish variation, and not arising from freezing conditions or frozen storage time.

Concerning the salt content in muscle, both species showed the same behaviour during the experiment (Figure 1). The brine freezing provided a significant ($p<0.05$) increase when comparing raw values with sample content at month 0. A significant increase ($p<0.05$) was again obtained at month 1 for both species. These content increases can be explained by salt diffusion into the white muscle and has already been studied in chilled fish where salt had been added to ice [12,18] and in frozen fish that had previously been soaked in brine solution [32]. After month 1, no significant

($p>0.05$) variations were observed till the end of the experiment. Comparison of both fish species only provided a significant difference ($p<0.05$) at the freezing step (month 0), in the sense that mackerel showed a bigger permeability to NaCl entrance than horse mackerel. Control samples only provided slight variations during the experiment, that can be explained as a result of differences from fish to fish samples, and not related to freezing conditions or frozen storage time.

Lipid hydrolysis

FFA content in white muscle increased with the frozen storage time for the four kinds of fish samples (Tables 2 and 3). Comparison of control and brine samples led to lower levels for brine frozen fish at months 1 and 7 for mackerel and at months 7 and 9 for horse mackerel. Accordingly, a partial inhibition effect of brine freezing on lipid hydrolysis development can be inferred. This inhibitory effect was also observed in previous research on salted sardine fillets stored at 5ºC, -3ºC, -20ºC and –35ºC [33] and on salted whole horse mackerel stored at –20ºC [32].

A higher FFA content ($p<0.05$) was observed for raw horse mackerel than for raw mackerel according to an inverse ratio between lipid and FFA contents [34]. Lipid hydrolysis is deemed important because of its high incidence on lipid oxidation [35, 36] and on protein denaturation [37, 38].

Lipid oxidation

Different and complementary lipid oxidation indices were assessed to evaluate the rancidity development in the present experiment.

Peroxide value in white muscle increased with time in the four kinds of samples (Tables 2 and 3). For both fish species, control samples showed a slow peroxide

formation, so that low values (PV<5.0) for frozen fish were reached during the experiment. However, brine freezing samples showed a greater formation of peroxides attaining in the 7-9 month period mean values higher than 13, which correspond to an advanced rancidity stage for frozen fish. For both fish species, comparison between freezing conditions showed a higher PV in the 1-9 month period for brine samples than for control ones.

Assessment of secondary lipid oxidation compounds by the TBA-i afforded very similar results to the ones exposed for the PV (Tables 2 and 3). A general increasing trend with time was observed in all samples. For both fish species, the brine freezing samples showed higher mean values than the control ones, that were significantly different in the 7-9 month period. Comparison between both fish species showed a higher oxidation development ($p<0.05$) for mackerel than horse mackerel in both kinds of freezing samples at months 0, 1, 7 and 9.

Formation of interaction compounds between oxidised lipids and nucleophilic constituents (aminated mainly) was assessed by the fluorescence ratio. According to the mean values, a slight increasing trend was detected for all kinds of samples (Tables 2 and 3). However, no significant differences were observed by comparing both freezing condition samples in each fish species. Comparison between both fish species showed a higher ($p<0.05$) FR value for horse mackerel than for mackerel in the case of control (months 3-9) and brine (months 3, 5 and 9) freezing samples.

Sensory assessment

Progressive score decreases were observed with time for the four attributes considered in all kinds of samples (Tables 4 and 5). According to sensory acceptance scores, mackerel samples showed in control and brine condition samples a shelf-life

time of 5 and 3 months, respectively. In the case of horse mackerel, the same shelf-life times were obtained, so that a differential sensory acceptance was not observed between both frozen fish species.

In the case of the brine freezing fish, the limiting factors were the general aspect, odour and colour. In the case of control freezing samples, the four attributes provided rejectable scores at the same time (month 7). Consistency was the only attribute where brine freezing samples showed some better scores than control freezing ones (months 5 and 7 for mackerel and months 3, 5 and 7 for horse mackerel; Tables 4 and 5). This consistency enhancement agrees with the fish salting technology objectives [39] where in addition to a water loss, a firmness increase of fish is expected as a result of a higher NaCl presence.

Rancid odour development for mackerel showed a good correlation value with the time of storage for brine (r^2 = 0.92) and control (r^2 = 0.95) samples. Compared to biochemical lipid damage indices, the best correlation values were obtained with the FFA content (r^2 = 0.87 for brine samples; r^2 = 0.83 for control samples). In the case of horse mackerel, also good correlation values were obtained between the rancid odour development and the frozen storage time (r^2 = 0.92 for brine samples; r^2 = 0.89 for control samples). When compared to biochemical indices, the best correlation values were obtained with the TBA-i (r^2 = 0.88 for brine samples) and with the PV and FFA (r^2 = 0.83 and r^2 = 0.82, respectively, for control samples).

CONCLUDING REMARKS

According to sensory (general aspect, odour and colour) and biochemical (PV and TBA-i) analyses, the use of brine freezing has turned the fish muscle to be more prone to oxidation than their commonly frozen counterparts, so that a reduced shelf-life was obtained for the frozen storage. However, a partial reduction of lipid hydrolysis and better consistency scores were obtained when employing brine freezing conditions. Rancidity development comparison of both species showed a higher formation of secondary lipid oxidation compounds in mackerel, while horse mackerel showed to be more prone to the tertiary (interaction compounds) lipid oxidation compound formation; however, the sensory assessment led to the same shelf-life time for both fish species.

The brine freezing treatment has led to an increase in NaCl content in the white muscle. According to previous research, this increase can be responsible for the higher lipid oxidation development [9, 10, 12, 33] and the lower lipid hydrolysis development [12, 32, 33] observed. NaCl has been reported to act as prooxidant by enhancement of the prooxidant effect of chelatable iron ions widely present in fish muscle [40].

Since long shelf-life time would be required when commercialising both frozen pelagic fish species, the employment of other protective treatments such as glazing, vacuum packaging, modified atmosphere packaging and natural antioxidant application [11, 41, 42, 43] are encouraged to inhibit lipid rancidity development and accordingly, retain sensory and nutritional properties.

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FIGURE LEGENDS

Figure 1: Development of NaCl content* in white muscle of mackerel and horse mackerel from raw material (RM) till month 9 of frozen storage **

* Bars denote standard deviations of the mean (n = 3).

** Sample names: M-Brine and HM-Brine denote, respectively, mackerel and horse mackerel frozen under brine conditions; M-Control and HM-Control denote respectively, mackerel and horse mackerel frozen under common conditions.

TABLE 1

Scale used for evaluating quality of frozen mackerel and horse mackerel

Attribute	**E (Highest quality)**	**A (Good quality)**	**B (Fair quality)**	**C (Rejectable quality)**
General Aspect (dryness, myotomes breakdown, white spots)	Strongly hydrated; totally adhered myotomes; absence of white spots	Still hydrated; myotomes adhered; absence of white spots	Slightly dry; myotomes adhered in groups; small white spots	Dry; myotomes totally separated; big white spots
Consistency	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduce	Important shape changes due to mechanical factors
Odour	Shellfish	Weakly Shellfish	Slightly sour and incipient rancidity	Sharply sour and rancid
Colour	Strongly pinky	Still pinky	Slightly pale	Yellowish

TABLE 2

Lipid damage assessment* during mackerel frozen storage **

Storage Time (months)	Free Fatty Acids		Peroxide Value		Thiobarbituric Acid Index		Fluorescence Ratio	
	Control	Brine	Control	Brine	Control	Brine	Control	Brine
0	0.12 (0.03)	0.11 (0.02)	1.59 (0.76)	2.80 (0.66)	0.33 (0.05)	0.41 (0.12)	0.24 (0.05)	0.32 (0.07)
1	0.61 b (0.19)	0.22 a (0.09)	1.98 a (0.75)	5.60 b (1.63)	0.48 (0.17)	0.74 (0.17)	0.32 (0.07))	0.33 (0.06)
3	0.61 (0.18)	0.72 (0.09)	3.17 a (1.12)	5.74 b (0.23)	0.56 (0.19)	0.64 (0.13)	0.26 (0.04)	0.30 (0.04)
5	0.94 (0.16)	0.98 (0.26)	2.25 a (0.80)	7.20 b (1.04)	0.56 (0.13)	0.70 (0.18)	0.28 (0.10)	0.40 (0.06)
7	1.45 b (0.23)	0.93 a (0.17)	4.92 a (1.69)	13.98 b (1.20)	0.67 a (0.04)	1.05 b (0.24)	0.42 (0.03)	0.49 (0.05)
9	1.98 (0.29)	1.70 (0.30)	4.53 a (0.64)	17.87 b (3.23)	0.83 a (0.12)	1.25 b (0.19)	0.48 (0.09)	0.60 (0.11)

* Mean values of three independent determinations; standard deviations are indicated in brackets. For each index, values followed by different letters indicate significant ($p<0.05$) differences between fish frozen under common freezing at –18ºC (control) and under brine immersion at –18ºC (brine).

** Raw fish values: 0.12±0.03 (free fatty acids), 2.13±1.28 (peroxide value), 0.19±0.10 (thiobarbituric acid index) and 0.32±0.13 (fluorescence ratio).

TABLE 3

Lipid damage assessment* during horse mackerel frozen storage **

Storage Time (months)	Free Fatty Acids		Peroxide Value		Thiobarbituric Acid Index		Fluorescence Ratio	
	Control	Brine	Control	Brine	Control	Brine	Control	Brine
0	0.40 (0.15)	0.32 (0.12)	1.59 (0.60)	1.63 (0.98)	0.09 (0.06)	0.13 (0.03)	0.36 (0.10)	0.46 (0.20)
1	0.40 (0.15)	0.77 (0.16)	1.27 a (0.34)	4.54 b (0.16)	0.26 (0.12)	0.35 (0.06)	0.46 (0.14)	0.43 (0 .07)
3	0.50 (0.21)	0.63 (0.21)	2.71 a (0.10)	3.88 b (0.61)	0.21 a (0.06)	0.44 b (0.02)	0.66 (0.24)	0.59 (0.10)
5	1.05 (0.25)	0.93 (0.37)	2.78 a (1.31)	15.61 b (1.74)	0.50 (0.34)	0.72 (0.09)	0.73 (0.26)	0.63 (0.01)
7	1.64 b (0.23)	1.24 a (0.11)	2.54 a (0.99)	16.06 b (4.24)	0.42 a (0.14)	0.72 b (0.05)	0.73 (0.10)	0.58 (0.09)
9	2.03 b (0.11)	0.94 a (0.27)	4.23 a (0.66)	13.25 b (3.77)	0.49 a (0.15)	0.74 b (0.05)	0.93 (0.19)	0.87 (0.14)

* Mean values of three independent determinations; standard deviations are indicated in brackets. For each index, values followed by different letters indicate significant ($p<0.05$) differences between fish frozen under common freezing at –18ºC (control) and under brine immersion at –18ºC (brine).

** Raw fish values: 0.22±0.06 (free fatty acids), 1.44±0.42 (peroxide value), 0.11±0.01 (thiobarbituric acid index) and 0.31±0.08 (fluorescence ratio).

TABLE 4

Sensory acceptance* of frozen mackerel that was treated under different freezing conditions**

Frozen Storage Time (months)	General Aspect		Consistency		Odour		Colour	
	Control	Brine	Control	Brine	Control	Brine	Control	Brine
0	A	A	A	A	A	A	A	A
1	A	A	A	A	A	A	A	A
3	B	B	A	A	B	B	B	B
5	B	C	B	A	B	C	B	C
7	C	C	C	B	C	C	C	C
9	C	C	C	C	C	C	C	C

* Category marks according to Table 1. Initial fish material was E category in the four attributes.

** Freezing conditions: Common freezing at –18ºC (Control) and brine immersion at –18ºC (Brine).

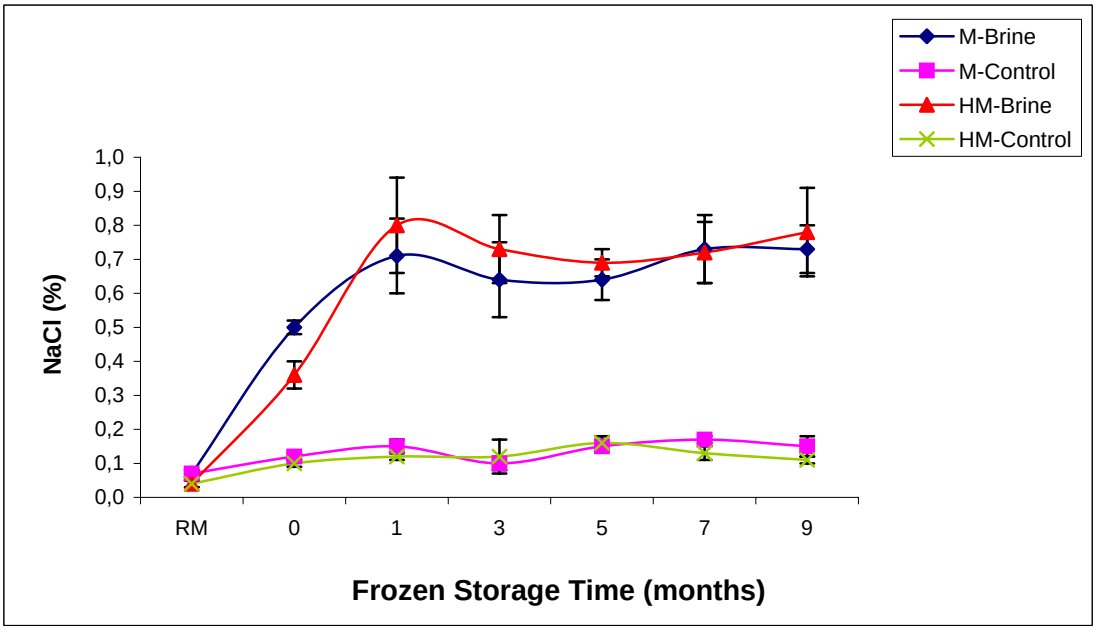
TABLE 5

Sensory acceptance* of frozen horse mackerel that was treated under different freezing conditions**

Frozen Storage Time (months)	General Aspect		Consistency		Odour		Colour	
	Control	Brine	Control	Brine	Control	Brine	Control	Brine
0	A	A	A	A	A	A	A	A
1	A	A	A	A	A	B	A	A
3	A	B	B	A	A	B	A	B
5	B	C	B	B	B	C	B	C
7	C	C	C	B	C	C	C	C
9	C	C	C	C	C	C	C	C

* Category marks according to Table 1. Initial fish material was E category in the four attributes.

** Freezing conditions: Common freezing at –18ºC (Control) and brine immersion at –18ºC (Brine).


M-Brine
M-Control
HM-Brine
HM-Control
NaCl (%)
1,0
0,9
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
RM
0
1
3
5
7
9
Frozen Storage Time (months)