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Preservative effect of jumbo squid (*Dosidicus gigas*) skin extract as glazing material during the frozen storage of Atlantic Chub mackerel (*Scomber colias*)

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The present research was focused on the quality loss of Atlantic Chub mackerel (*Scomber colias*) during the frozen storage. Its basic objective was to investigate the effect of including a lipophilic extract of jumbo squid (*Dosidicus gigas*) skin (JSS) in the glazing system applied previously to the frozen storage. For it, two different concentrations of skin extracts were tested and compared with two control treatments (water glazing and non-glazing conditions). Quality changes were monitored for a 8-month frozen storage by sensory (odour and taste) and chemical (lipid hydrolysis and oxidation development) evaluation. An inhibitory effect ($p<0.05$) of skin extracts on lipid hydrolysis (free fatty acid formation) evolution was observed; furthermore, lower average values for lipid oxidation indices (peroxide and fluorescent compounds formation) were observed in fish samples corresponding to the highest JSS presence in the glazing system. Some sensory quality enhancement was evident in mackerel as a result of including JSS extracts in the glazing medium, especially for raw-flesh and cooked-flesh odours; as for chemical indices, an increasing effect was implied by increasing the skin extract presence in the glazing medium. Under the conditions tested in the present study, the JSS extract has shown promising antioxidant properties that could be applied to enhance the seafood quality during the commercialisation in frozen conditions. Further research would be necessary to optimise its use in the glazing system.

Keywords: Frozen mackerel; Squid by-product; Glazing; Shelf life; Lipid damage

INTRODUCTION

Fatty fish are attracting considerable attention because of the positive role of marine lipids in human nutrition and health [1]. Nevertheless, during processing and storage, marine lipids are reported to undergo fast oxidation of highly unsaturated fatty acids, which is directly related to the production of off-flavours and odours and to decrease in the nutritional value [2, 3]. To extend the shelf life time, frozen storage has widely been employed to maintain fish properties before consumption or use in other technological processes. However, during frozen storage of fish, lipid hydrolysis and oxidation have been shown to occur and to influence fish acceptance [4, 5].

One particular established technology greatly used during freezing and frozen storage of seafood is the application of a layer of ice to the surface of a frozen product, referred as glazing [6, 7]. Thus, adequate glazing of fish fillets prior to frozen storage would protect the final product from dehydration, oxidation and quality loss. The amount of glaze applied would depend on various factors such as glazing time, seafood temperature, water temperature, product size and shape [8]. Previous research has shown profitable effects of glazing by inhibiting lipid hydrolysis [9] and oxidation [10-12] development and microbial activity [13,14].

To extend the shelf life time during the frozen storage of marine species, the employment of natural preservatives represents a relevant choice. In this sense, seafood by-products have been detected as a good source of antioxidant compounds. Among them, jumbo squid (*Dosidicus gigas*) skin (JSS) represents a promising possibility as being a rich source of pigments and other preserving molecules. Thus, marked antioxidant and antimicrobial effects have been proved on heated marine-oil systems [15] and chilled fish [16,17]. Moreover, no toxic effect was detected in such extracts [17].

The present research was focused on the quality loss of Atlantic Chub mackerel (*Scomber colias*) during the frozen storage. Its basic objective was to investigate the effect of including a lipophilic extract of JSS in the glazing system prior to the frozen storage. For it, two different concentrations of skin extracts were tested and compared with two control treatments (water glazing and non-glazing conditions). Quality changes were monitored for a 8-month frozen storage by sensory (odour and taste) and chemical (lipid hydrolysis and oxidation development) evaluation.

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MATERIAL AND METHODS

Preparation of squid skin extracts and glazing systems

Jumbo squid specimens were caught by local fishermen using the jigging fishing method at the Guaymas harbour (Sonora, Mexico; 8.75°N/112.25°W, 15–18 °C). The length and weight of the squid specimens ranged from 40 to 45 cm and from 2.0 to 3.0 kg, respectively. Squids were transported to the Seafood Laboratory at the University of Sonora 8 h after being captured. The skin was manually removed from the mantle and fins, cut into small pieces (about 15 cm length) and freeze-dried for two days (LABONCO Freeze Dry, Kansas City, MO, USA). The freeze-dried skin (100-mg portions) was placed in polyethylene-sealed bags, which were stored at –25 ± 2 °C.

Acetic acid-ethanol pigment extracts from freeze-dried skin were prepared according to the method developed previously [15]. Briefly, 8 g of freeze-dried skin were blended in 80 mL of a 0.5% acetic acid-ethanol solution (v/v) at 0 °C for 1 min using an Ultra-Turrax equipment (IKA-UltraTurrax T-25, Staufen, Germany). The blended mixture was then submitted to an ultrasound bath at room temperature (18-20 ºC) (Ultrasons, Selecta, Barcelona, Spain) for 3 min. Afterwards, the homogeneous mixture was centrifuged at 3,500 × g at 4 °C for 10 min, then the supernatant being recovered. This process was repeated three times, so that the supernatant recovered after the last centrifugation process was colourless. Extracts were pooled together and carried out to a 200-mL volume solution with the acetic acid-ethanol mixture.

Three glazing systems were prepared. For it, 0, 24 and 144 mL of the above-prepared JSS extract were diluted, respectively, to 11 L with distilled water and led to water glazing (WG; water control), low-concentrated glazing (LCG) and high-concentrated glazing (HCG) systems, respectively. To maintain the same quantity of the solvent mixture in each glazing medium, 144 and 120 mL of 0.5% acetic acid-ethanol solution were added during the preparation of the WG and LCG systems, respectively.

Fish material, glazing and sampling

Fresh Atlantic Chub mackerel (102 specimens) were caught near the Galician Atlantic coast (North-Western Spain) and transported to the laboratory. Throughout this process (10 h), the fish were maintained in ice. The length and weight of the fish specimens ranged from 23.0 to 27.0 cm and from 104 to 121 g, respectively.

Upon arrival to the laboratory, six specimens were separated and analysed as initial fish. These fish specimens were divided into three different groups (two individuals per group) that were analysed independently to perform the statistical analysis; n=3). The remaining fish specimens were divided into four batches (24 individuals in each batch) that were immediately frozen at –40 ºC.

After 48 hours at –40 ºC, the first batch was packaged in polyethylene bags (two pieces per bag) and stored at –18 ºC (blank control; non-glazed batch; NG batch). At the same time, the remaining batches were immersed in the WG, LCG and HCG systems, respectively. In all cases, specimens were immersed for 30 s at 0 ºC, allowed to drain for 15 s, packaged in polyethylene bags (two pieces per bag) and stored at –18 ºC.

Sampling was undertaken at months 2, 4, 6 and 8 of frozen storage at –18 ºC. At each time and for each condition, six individuals were taken, that were divided into three groups (two individuals per group) and studied separately. Analysis of frozen material was undertaken after thawing; thawing was carried out by overnight storage in a cool room (4 ºC).

Chemical analyses related to quality loss

All solvents and chemical reagents used were of reagent grade (Merck, Darmstadt, Germany). Chemical analyses related to fish quality were carried out on the white muscle of mackerel.

Lipids were extracted from mackerel muscle by single-phase solubilisation with chloroform-methanol (1:1), as described by Bligh and Dyer [18]. Results are expressed as g lipid kg^{-1} muscle.

The free fatty acid (FFA) concentration of the mackerel lipid extract was determined by colorimetric reaction with cupric acetate-pyridine and absorbance was measured at 715 nm, according to Lowry and Tinsley [19]. Results are expressed as mg FFA kg^{-1} muscle.

The peroxide value (PV) of the mackerel lipid extract was determined by peroxide reduction with ferric thiocyanate and absorbance was measured at 500 nm (Beckman Coulter, DU 640; London, UK), as described by Chapman and McKay [20]. Results are expressed as meq active oxygen kg^{-1} lipids.

The formation of fluorescent compounds (Fluorimeter LS 45; Perkin Elmer España, Tres Cantos, Madrid, Spain) was determined by measurements at 393/463 nm and 327/415 nm as described by Losada *et al*. [21]. The relative fluorescence (RF) was calculated as follows: $RF = F/F_{st}$, where F is the fluorescence measured at each excitation/emission maximum and F_{st} is the fluorescence intensity of a quinine sulphate solution (1 μg ml^{-1} in 0.05 M H_2SO_4) at the corresponding

wavelength. The fluorescence ratio (FR) was calculated as the ratio between the two RF values: $FR = RF_{393/463\ nm}/RF_{327/415\ nm}$. The FR value was determined using the aqueous phase that resulted from the lipid extraction of the fish muscle [18].

Determination of sensory acceptance

Sensory analysis was carried out by a sensory panel consisting of four to six experienced judges. Before carrying out the present experiment, the judges received special training on frozen mackerel, focused on the evaluation of specimens that exhibited different qualities. Special attention was paid to the evolution of the sensory descriptors that were found as limiting factors for the shelf life. Consequently, descriptors analysed were: external odour, raw flesh odour, cooked flesh odour and flesh taste. The different descriptors were evaluated on a scale from 7.0 (stage of highest quality) to 0.0 (stage of lowest quality) in agreement with Lehmann and Aubourg [22]. Four rang categories were considered [23]: 7.0-5.6 (excellent), 5.5-3.6 (good), 3.5-1.6 (fair) and 1.5-0.0 (rejectable).

At each sampling time, fish individuals from each batch were analysed. Evaluation began by the analysis of fish in the raw state and was followed by the analysis of samples in the cooked state. Cooking was accomplished at 95-100 ºC for 7 min in a pre-warmed oven with air circulation and then submitted to the panel. At each sampling time, whole fish specimens were coded with 3-digit random numbers and presented to the panellists in individual trays, which were scored individually. Each descriptor of each sample was scored a single time by each member of the panel. The panel members shared samples tested.

Statistical analysis

Data obtained (three replicates; n=3) from the different sensory and chemical analyses were subjected to the ANOVA method to explore differences resulting from the effect of the glazing system employed; the comparison of means was performed using the least-squares difference (LSD) method. Analyses were carried out using the PASW Statistics 18 software for Windows (SPSS Inc., Chicago, IL, USA); differences among batches were considered significant for a confidence interval at the 95% level ($p<0.05$) in all cases.

RESULTS AND DISCUSSION

Chemical analyses related to quality

Lipid content of mackerel fish ranged between 24.7 and 33.4 g kg^{-1} muscle. Results concerning the lipid hydrolysis development are depicted in Fig. 1. A marked FFA formation was obtained throughout the whole frozen storage period in all kinds of samples.

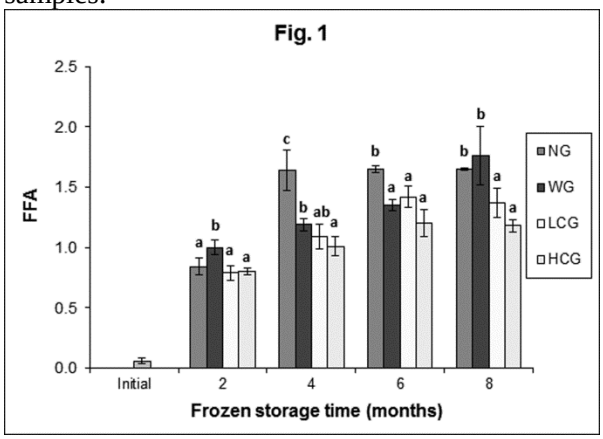

Figure 1. Assessment of free fatty acids (FFA; mg kg^{-1} muscle) formation* in frozen mackerel previously submitted to various glazing conditions**.

* Average values of three replicates (n=3). Standard deviations are indicated by bars. Average values accompanied by different letters (a, b, c) denote significant differences ($p<0.05$). No letters are included when no significant differences were found ($p>0.05$). ** Abbreviations of glazing conditions: NG (without glazing), WG (water glazing), LCG (low-concentrated squid skin glazing) and HCG (high-concentrated squid skin glazing) in agreement with the material and methods section.

Fish corresponding to the HCG system showed the lowest average values for the 4-8-month period; differences were found significant ($p<0.05$) at months 4 and 8 when compared with their counterparts from NG and WG conditions. Furthermore, an inhibitory effect ($p<0.05$) was also observed in mackerel corresponding to the LCG system at the end of the experiment. Consequently, an inhibitory effect on lipid hydrolysis development can be concluded by including the JSS extract in the glazing medium.

Lipid hydrolysis development is reported to be produced during the frozen storage as a result of lipase release from liposomes into the muscle, which then facilitates closer proximity between enzyme and substrate [4]. In a previous research [17], the JSS extract showed to inhibit the FFA formation during the chilled storage of hake; in this case, the JSS extract was included in the icing medium employed during the chilling process. In agreement with the current study, the employment of essential oils (sage, thyme and clove) extracts in the glazing system led to an inhibitory effect on the formation of FFA in frozen (6 months at –18 ºC) rainbow trout fillets [9]; additionally, an inhibitory effect was also observed in fish samples corresponding to water glazing when compared with samples not glazed.

Lipid oxidation development was determined by means of the peroxide and fluorescent compounds detection. Peroxide formation (Table 1) did not provide a definite trend throughout the storage for none of the fish batches; interestingly, the highest average value was obtained at month 4 in most kinds of samples.

Table 1. Peroxide value assessment* in frozen mackerel previously submitted to different glazing conditions**

Frozen storage time (months)	Glazing condition			
	NG	WG	LCG	HCG
Initial		2.09 (0.72)		
2	5.54 (2.25)	3.99 (0.61)	3.28 (1.73)	3.32 (2.38)
4	7.89 b (2.29)	4.69 ab (0.17)	5.14 ab (0.86)	3.61 a (1.28)
6	7.12 b (2.44)	4.12 ab (0.87)	2.30 a (0.49)	3.07 a (0.79)
8	1.57 a (0.34)	1.34 a (0.45)	7.93 b (1.55)	2.12 a (1.58)

* Average values of three replicates (n=3); standard deviations are indicated in brackets. Average values followed by different letters (a, b) denote significant differences ($p<0.05$). No letters are indicated when no differences are found ($p>0.05$). ** Abbreviations of glazing systems as expressed in Fig. 1.

Comparison among samples showed scarce significant differences; thus, significant differences could not be obtained ($p>0.05$) by comparing mackerel corresponding to any glazing system including the JSS extract with fish corresponding to NG and WG conditions. However, values of mackerel previously glazed under HCG system were included in a relatively low peroxide range (2.12-3.61).

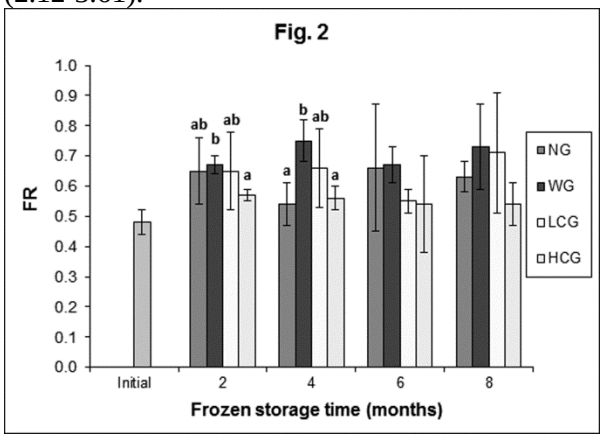

Figure 2. Assessment of the fluorescence ratio (FR)* in frozen mackerel previously submitted to various glazing conditions**.

* Average values of three replicates (*n*=3). Standard deviations are indicated by bars. Average values accompanied by different letters (a, b) denote significant differences ($p<0.05$). No letters are included when no significant differences are found ($p>0.05$). ** Abbreviations of glazing conditions as expressed in Fig. 1.

Scarce differences could be observed by assessing the tertiary lipid oxidation compounds (Fig. 2). When compared with initial values, a small increase in this quality parameter can be implied in all kinds of samples. Comparison among samples showed a lower ($p<0.05$) value in fish corresponding to the HCG batch than in their counterpart from the WG batch in the 2-4-month period. At most storage times, average values for fish from the HCG system were lower than from any other batch.

Frozen storage is known to be associated with fish lipid oxidation processes where different kinds of endogenous enzymes may be involved [5]. Partial inhibition of lipid oxidation found in the current research can be explained on the basis of previous research [15]. In it, marked radical scavenging activity (ABTS assay) and oxygen radical absorbance capacity (ORAC assay) were detected; additionally, inhibition of lipid oxidation in a marine-oil heated model system was evident. In agreement with various spectroscopic and chemical analyses, JSS molecules responsible for this antioxidant behavior were identified as belonging to the ommochrome family [15].

In agreement with the present study, previous related research accounts for lipid oxidation inhibition (formation of thiobarbituric acid reactive substances in all cases) as a result of including various preservative compounds in the glazing system. This accounts for chitosan nano particles combined with sodium tripolyphosphate nano particles in frozen (30 days at –21 ºC) shrimp [12], green tea and grape seed extracts in frozen (5 months at –18 ºC) bonito [7], green tea extract in frozen (180 days at –21 ºC) shrimp [11] and Pollock skin hydrolysates in frozen (4 months at –35 ºC) pink salmon [10].

Additionally, Çoban [9] studied the lipid oxidation development by assessing primary and secondary lipid oxidation compounds formation during the frozen storage (6 months at –18 ºC) of rainbow trout fillets. By including essential oils

(sage, thyme or clove) extracts in the glazing system, an inhibitory effect on the formation of both kinds of lipid oxidation compounds was implied. Furthermore, an inhibitory effect was observed in fish samples corresponding to water glazing when compared with those samples not glazed.

Determination of the sensory acceptance

Sensory analysis showed a progressive quality decrease throughout the storage time for all descriptors under study (Table 2).

Table 2. Sensory analysis* of frozen mackerel previously submitted to different glazing conditions**

Descriptor/frozen time (months)	Glazing condition			
	NG	WG	LCG	HCG
External odour				
2	3.5 (0.7)	4.0 (1.4)	5.0 (1.4)	4.5 (0.7)
4	4.0 a (0.0)	4.0 ab (1.4)	4.0 a (0.0)	5.5 b (0.7)
6	0.7 a (0.6)	2.0 ab (1.0)	3.3 b (0.6)	2.7 ab (1.5)
8	1.3 a (0.5)	2.0 ab (0.7)	3.0 b (1.0)	3.2 b (1.0)
Raw flesh odour				
2	4.0 (1.4)	4.5 (0.7)	4.5 (0.7)	5.0 (1.4)
4	3.5 a (0.7)	4.5 a (0.7)	6.0 b (0.0)	4.5 a (0.7)
6	0.7 a (0.6)	2.3 b (0.6)	3.3 b (0.6)	2.0 ab (1.0)
8	1.4 a (0.6)	2.7 ab (0.6)	2.3 ab (1.2)	3.7 b (0.6)
Cooked flesh odour				
2	4.5 a (0.7)	4.5 a (0.7)	5.5 a (0.7)	6.0 b (0.0)
4	4.5 (0.7)	4.5 (0.7)	5.0 (1.4)	4.5 (0.7)
6	1.0 a (0.0)	2.7 b (0.6)	3.7 b (0.6)	2.3 ab (1.2)
8	1.3 a (0.6)	2.3 ab (1.2)	2.7 ab (1.5)	3.7 b (0.6)
Flesh taste				
2	4.0 (0.0)	4.0 (1.4)	4.5 (0.7)	5.0 (1.4)
4	4.5 (0.7)	4.5 (0.7)	5.5 (0.7)	5.5 (0.7)
6	1.3 a (0.6)	2.0 ab (1.0)	3.0 ab (1.0)	3.3 b (0.5)
8	1.0 a (0.0)	2.0 ab (1.0)	2.0 ab (1.0)	2.7 b (1.3)

* Descriptors were evaluated on a scale from 7.0 (highest stage of quality) to 0.0 (lowest stage of quality) in agreement with the material and methods section. Values followed by different letters (a, b) indicate significant differences ($p<0.05$). No letters are included when no significant differences are found ($p>0.05$). Initial fish was assigned score 7.0 in all descriptors. ** Abbreviations of glazing systems as expressed in Fig. 1.

As a result, fish corresponding to NG system (blank control) was found rejectable at month 6, while the remaining samples were still acceptable at the end of the experiment. Such rejection was observed in all descriptors under study. Scarce differences could be proved as a result of the presence of the JSS extract in the glazing system. Thus, higher scores ($p<0.05$) were observed in LCG samples at month 4 (raw flesh odour) and in HCG fish at month 2 (cooked flesh odour) when compared with their counterparts fish from NG and WG conditions. Interestingly, higher average values were obtained in all descriptors at the end of the study for mackerel corresponding to the highest

concentration of JSS extract in the glazing system. A profitable effect of JSS extracts on fish sensory quality has already been observed during the chilled storage of mackerel [16] and hake [17]; in both cases, the inclusion of this extract in the icing system employed for the chilling storage led to a marked enhancement of sensory acceptance. Furthermore, a previous phosphate dipping to freezing and frozen (15 days at –25 ºC) storage led to a sensory quality enhancement in red shrimp [13]. Contrary, Çoban [9] included essential oils (sage, thyme or clove) extracts in the glazing system prior to the frozen storage (6 months at –18 ºC) of rainbow trout fillets; however, no differences in sensory quality were observed when compared with fish corresponding to control-water glazing.

CONCLUSIONS

A novel glazing system based on the inclusion of a lipophilic extract of JSS was applied to frozen Atlantic Chub mackerel. For it, quality changes were monitored for a 8-month frozen storage by sensory and chemical evaluation. As a result, an inhibitory effect ($p<0.05$) of skin extracts presence on lipid hydrolysis (free fatty acid formation) evolution was observed; furthermore, lower average values for lipid oxidation indices (peroxide and fluorescent compounds formation) were observed in samples corresponding to the highest JSS presence in the glazing system. Some sensory quality enhancement was evident in mackerel as a result of including JSS extracts in the glazing medium, especially for raw-flesh and cooked-fresh odours. As for chemical indices, an increasing effect was implied by increasing the skin extract presence in the glazing medium. Under the conditions tested in the present study, the JSS extract has shown promising antioxidant properties that could be applied to enhance the seafood quality during the commercialisation in frozen conditions. Further research would be necessary to optimise its use in the glazing system.

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КОНСЕРВИРАЩ ЕФЕКТ НА ЕКСТРАКТ ОТ КОЖА НА ГИГАНТСКИ КАЛМАРИ (*Dosidicus gigas*) КАТО ГЛАЗИРАЩ МАТЕРИАЛ ПРИ СЪХРАНЕНИЕ НА АТЛАНТИЧЕСКА СКУМРИЯ (*Scomber colias*) В ЗАМРАЗЕНО СЪСТОЯНИЕ

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(Резюме)

В настоящата работа са изследвани промените в качеството на атлантическа скумрия (*Scomber colias*) по време на съхранението ѝ в замразено състояние. Проследено е влиянието на липофилен екстракт от кожа на гигантски калмари (*Dosidicus gigas*) (JSS) в глазиращата система, приложена преди замразяване. Тествани са две концентрации от кожни екстракти и са сравнени с две контролни обработки (глазиране с вода и без глазиране). Промените в качеството са проследени в продължение на 8-месечно съхранение в замразено състояние чрез сензорна (мирис и вкус) и химическа (хидролиза и окисление на липидите) оценка. Установен е инхибиторен ефект ($p<0.05$) на JSS екстрактите върху хидролизата на липидите (образуване на свободни мастни киселини), като по-ниски средни стойности на липидните окислителни индекси (образуване на пероксидни и флуоресцентни съединения) са наблюдавани при рибните проби с по-висока концентрация на JSS в глазиращата система. Установено е повишение на сензорното качество на скумрията в резултат на включване на JSS екстракти в глазиращата система., особено по отношение на мириса на суровото и свареното месо. Установено е и повишение на химическите индекси с увеличаване концентрацията на JSS екстракта в глазиращата система. При изследваните условия, JSS екстрактът е демонстрирал перспективни антиоксидационни свойства, които биха намерили приложение за повишаване на качеството на морската храна при търговията в замразено състояние. Предстоят изследвания за оптимизиране на употребата на JSS в глазиращата система.