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Research Article

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Comparison of lipid content and fatty acid profile of fresh and frozen blue swimming crab *Portunus Segnis*

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Abstract

The blue swimming crab *Portunus segnis* has a great economic interest in local and foreign fishing markets and is in high demand by consumers. The aim of the present work was to evaluate the change in total lipid (TL), fatty acids composition and nutritional quality indices (NQI) of the meat of blue swimming crab *Portunus segnis* after freezing at -18°C for 15, 30, 60 and 90 days. The comparison of fresh and frozen crabs showed that freezing had significant effects on the nutritional quality of this marine product. Lipid peroxidation was enhanced during the freezing process. Total lipid content decreased significantly as a function of days of storage, especially from 30 days. A significant change was also observed in the fatty acid composition of frozen crab meat. During the freezing process, saturated fatty acids (SFA) increased significantly, while polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA) decreased. We can conclude that storage of blue crab *P. segnis* at -18°C was not efficient enough for long preservation, as it has a strong effect on the deterioration of the nutritional quality of the meat over time. Frozen crabs should preferably be consumed within 15 days of storage. Our research targets both domestic and international consumers of this crab, with a specific focus on restaurants and hotels that incorporate this item into their menus. We recommend that consumers of this product exercise increased caution regarding the advantages and drawbacks associated with the freezing techniques employed.

Keywords: *Portunus segnis*, frozen storage, lipid content, fatty acids profile, lipid peroxidation, nutritional quality

Introduction

In the Mediterranean Sea, crustaceans are the second most abundant taxon of confirmed non-native species [1]. In Tunisian waters, a total of 163 alien species have been reported, with crustaceans dominating with a 24% share of reported alien species [2]. Crabs are common in all regions of the world and are found in freshwater, brackish water, and marine habitats. However, other species are also found on land, where they can live hundreds of kilometers from aquatic habitats [3]. Crabs occupy a variety of trophic niches; they also play an important ecological role in aquatic ecosystems [4]. The blue swimming crab, formerly called *P. pelagicus* [5], is one of the first Lessepsian invaders. In Tunisian waters, the occurrence of *P. segnis* was first reported in 2014 by [6] in shallow, sandy areas with seagrass beds and algae. Since 2015, the number of *P. segnis* individuals in the Gulf of Gabes has greatly increased [7] and includes much larger areas on the southeastern coasts of Tunisia. *P. segnis* is in high demand by consumers and is regularly sold at all local fish markets.

Crustaceans are recommended for human consumption because of their beneficial and healthy properties [8]. They are rich in polyunsaturated fatty acids such as EPA (ecosapentaenoic fatty acid) and DHA (decosahexaenoic fatty acid). EPA and DHA are very useful for human health due to their anti-inflammatory, antithrombotic, and antiarrhythmic properties. In recent years, the increasing consumption of seafood has led to an improvement in preservation methods to obtain products of high nutritional quality.

Freezing is considered the most suitable and effective method to preserve food quality [9]. This method is most suitable for long-term preservation of seafood because it preserves the organoleptic properties of the food well [10]. However, the formation of ice crystals under freezing conditions could lead to cell cracking and tissue destruction [11]. Fishery products are indeed subject to natural postmortem degradation due to endogenous and exogenous reactions [12]. This phenomenon is largely dependent on storage conditions, which further promote oxidation reactions.

Due to the lack of information on the effects of freezing on blue swimming crab meat, lipid content, fatty acid composition, NQI, and lipid peroxidation markers in *P. segnis* after freezing at -18°C for 15, 30, 60, and 90 days were studied in this work.

Material and methods

Sampling Site

Although the Gulf of Gabes is considered one of the most productive fishing areas in Tunisia, it is subject to numerous anthropogenic impacts that affect its biodiversity [13]. In the present study, our samples were collected in the commercial port of "Ghannouch" in the Gulf of Gabes (34° 05' 37"N, 10° 26' 13"E) (Figure 1).

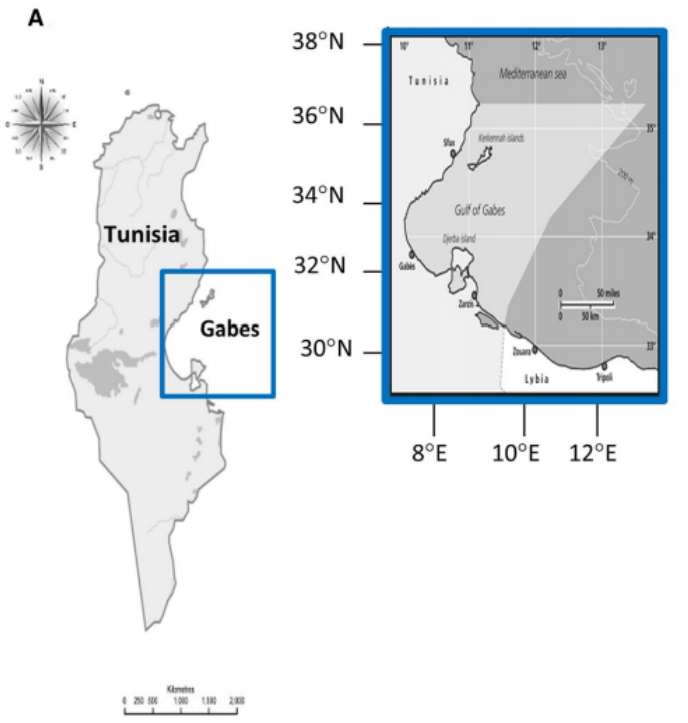

Figure 1. Sampling Site - Location of sample collection in the Gulf of Gabes.

Preparation of the Samples

Fifty adult specimens were collected in January 2018 to study the effects of freezing on the blue crab *Portunus segnis*. The crabs ranged in length from 41.75 to 62.9 mm, width from 85.8 to 130 mm, and total weight from 29.3 to 114.1 g. The samples were immediately brought to the laboratory in a cool box. Then, the crabs were well cleaned, packed in plastic bags, labeled, and frozen at -18°C for 15, 30, 60, and 90 days. For each period, the meat of ten whole animals was homogenized using a grinder (UltraTurrax®) and prepared for lipid and fatty acid content analysis. One batch of 10 animals was analyzed fresh.

Lipid Content

Total lipid content in fresh and frozen tissue samples was determined according to the method of Folch et al. (1957) using a mixture of chloroform-methanol (2v/1v) and 0.01% butylhydroxytoluene (BHT). The volume of chloroform-methanol extraction solvent is proportional to the weight of the organ: 30 ml of extraction solvent for 1 g of the tissue. Elimination of proteins was performed after the addition of a 15% NaCl solution. After centrifugation at 3000 rpm for 15 min, the organic phase was stored in previously weighed flasks.

Fatty Acid Profile

Fatty acid methyl esters (FAME) were determined by gas chromatography (HP 6890 GC). Qualitative identification of fatty acids (FA) was performed using a standard "PUFA3" chromatogram of menhaden oil from SUPELCO injected under the same chromatographic conditions. The determination of a fatty acid (X) is done by comparing its retention time with the time indicated in the "PUFA3" chromatogram. The areas of the peaks are proportional to the amounts of the corresponding fatty acids. In our study, the fatty acids in percent (%) were calculated from the areas of the peaks of the chromatogram and the internal standard (C19:0 Belefonte PA. USA. CRM47885), the amount of which is known in μg.

Nutritional Quality Indices (NQI)

The NQI of the fresh and frozen samples were determined using the indices mentioned by [14; 15; 16; 17; 18] and summarized in Table 1.

Lipid Peroxidation

Peroxide Value (PV)

PV was estimated based on the AOCS (1989) method. Results were expressed in ml g^{-1} and calculated according to equation (E1):

$$PV = \frac{(\text{sample titration} - \text{blank titration}) \times N_{\text{thiosulfate}} \times 1000}{\text{sample weight}} \quad \text{(E1)}$$

Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were determined according to the AOCS method (1989), and results were expressed in mg kg^{-1}.

Statistical Analysis

Data were presented as means ± standard deviations (SD) of 10 replicates for lipids, FA, and lipid peroxidation markers and

Table 1. Nutritional Quality Indices (NQI)

References	Index Calculation
Marques et al. 2010 [14]	$\frac{\text{n-3}}{\text{n-6}}$
Marques et al. 2010 [14]	$\frac{\text{PUFA}}{\text{SFA}}$
Unusan 2007 [15]	EPA + DHA
Ulbricht and Southgate 1991[16]	$AI = \frac{(4\times C14{:}0)+C16{:}0+C18{:}0}{\Sigma\text{MUFA}+\Sigma\ \text{PUFA}\ n-6+\Sigma\ \text{PUFA}\ n-3}$
Ulbricht and Southgate 1991 [16]	$TI = \frac{C14{:}0+C16{:}0+C18{:}0}{0.50\times\text{MUFA}+0.5\times\ \text{PUFA}\ n-6+3\times\ \text{PUFA}\ n-3+\frac{\text{PUFA}\ n-3}{\text{PUFA}\ n-6}}$
Rodriguez et al. 2007 [17]	$PI = \frac{C20{:}5n-3+C22{:}6n-3}{C16{:}0}$
Fernandez et al. 2014 [18]	$h/H = \frac{C18{:}1n-9+C18{:}2n-6+C20{:}4n-6+C18{:}3n-3+C20{:}5n-3+C22{:}5n-3+C22{:}6n-3}{C14{:}0+C16{:}0}$

analyzed with STATISTICA 8 software (Stat-Soft Inc). Homogeneity and normality of variables were tested with the Shapiro test. Significant differences were found at the 5% level. Results were checked with one-way analysis ANOVA and post-hoc Tukey and Kruskall-Wallis test. To show possible correlations between biochemical parameters of raw and frozen crabs, principal component analysis (PCA) was performed.

Results

Lipid content

Total lipid content in fresh blue crab was estimated to be 41.85 ± 4.33 mg/g (Figure 2). A significant decrease in lipid content was observed in the flesh of crabs frozen at −18°C after 30, 60, and 90 days of storage (27.28, 17.08, and 14.57 mg/g weight, respectively).

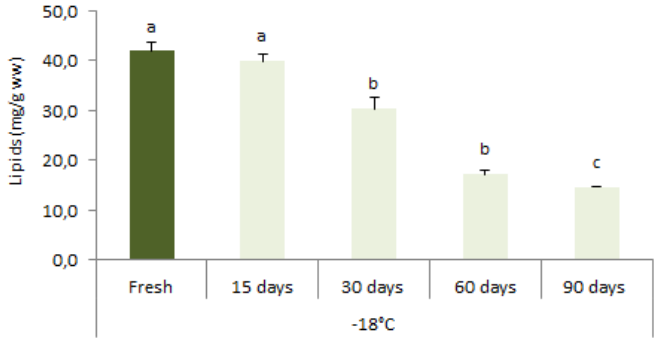

Figure 2. Lipid content of raw and frozen blue crab during freezing (−18°C)

FA composition

The FA profile of fresh and frozen crabs, expressed as a percentage of the total identified FA, was presented in Table 2. For fresh crabs, PUFA accounted for 33.60% of the total FA, followed by SFA (20.49%) and MUFA (15.78%). Palmitic acid ($C_{16:0}$; 6.28%) and arachidic acid ($C_{20:0}$; 5.82%) dominated SFAs in fresh crab meat. PUFA were dominated by eicosenoic acid ($C_{20:1}$; 6.26%), oleic acid ($C_{18:1}$; 2.14%), and palmitoleic acid ($C_{16:1}$; 2.87%), while alpha-linolenic acid ($C_{18:3n-3}$; 7.82%), docosahexaenoic acid (DHA; 6.23%), eicosapentaenoic acid (EPA; 4.58%), and eicosatetraenoic acid ($C_{20:4n-3}$; 3.63%) were the most abundant FAs in PUFA (Table 2). In frozen crabs, PUFA and MUFA significantly decreased ($p < 0.05$), while SFA increased. In SFA, both $C_{16:0}$ and $C_{18:0}$ fatty acids significantly increased by 60.03% and 158.30%, respectively, after 90 days of freezing ($p < 0.05$). In contrast, PUFA significantly decreased after 90 days of storage (3.44%). This decrease was influenced by the decrease in major FA such as $C_{16:1}$ and $C_{18:1}$ (0.28% and 0.63%, respectively). Frozen crabs showed a significant decrease in n-3 PUFA after storage. The levels of omega-3 (PUFA n-3) and omega-6 fatty acids (PUFA n-6) in the fresh and frozen crabs are shown in Table 2. DHA and EPA reached their lowest levels of 0.61% and 0.56%, respectively, after 90 days of storage compared to the fresh samples (6.23% and 4.58%, respectively). In contrast, n-6 PUFAs in frozen crabs showed a different variation with a significant increase ($p < 0.05$) during the freezing process and reached their high values after 90 days of storage (16.19%) compared to fresh samples (2.92%).

Table 2. Fatty acid profile of fresh and frozen crabs for 15, 30, 60 and 90 days.

Fatty acids	Fresh	15 days	30 days	60 days	90 days
C14:0	1.67±0.28	2.38±0.36*	3.13±0.09***	4.50±0.22***	5.23±0.07***
C15:0	1.04±0.13	0.74±0.39***	2.12±0.05***	2.93±0.46***	4.17±0.06***
C16:0	6.28±0.25	7.74±0.61**	8.74±0.75***	9.18±0.51***	10.05±0.58**
C17:0	1.86±0.24	1.26±0.29	1.56±0.24***	4.05±0.50***	5.62±0.30***
C18:0	2.14±0.32	2.91±0.91	3.16±0.13*	5.02±0.50***	7.00±0.71***
C20:0	5.82±0.63	5.89±0.59	6.46±0.28	8.10±0.31***	9.65±0.45***
C22:0	1.41±0.06	1.62±0.48*	2.95±0.36***	5.54±0.97***	8.59±0.20***
C14:1	1.61±0.13	1.30±0.01**	0.87±0.06***	0.64±0.12***	0.16±0.03***
C15:1	0.57±0.38	0.66±0.22	0.23±0.03	0.18±0.01	0.14±0.05
C16:1	2.87±0.07	2.66±0.29	1.04±0.14***	0.85±0.06***	0.28±0.04***
C18:1	2.14±0.32	1.76±0.10	1.31±0.29*	0.87±0.09***	0.63±0.09***
C20:1	6.26±0.94	5.83±0.68	4.24±0.43***	2.78±1.05***	1.64±0.25***
C22:1	2.33±0.57	2.22±0.27**	1.89±0.54	1.30±0.17***	0.59±0.06***
C16:2n-4	3.17±1.58	0.33±0.20**	0.49±0.05***	0.25±0.02*	0.09±0.01***
C16:3n-4	1.39±0.31	1.54±0.40	0.47±0.15***	0.16±0.02***	0.11±0.01***
C18:2n-6	0.64±0.05	0.86±0.03**	0.91±0.08***	1.76±0.10*	2.68±0.29***
C18:3n-4	1.82±0.74	0.97±0.08*	0.54±0.15*	0.48±0.05***	0.14±0.02***
C18:3n-3	7.82±0.26	7.48±0.47**	5.95±0.71***	2.56±0.36***	0.27±0.06***
C20:3n-6	1.71±0.09	2.22±0.15**	3.96±0.29*	5.28±0.97***	7.05±0.06***
C20:4n-6	0.57±0.03	1.56±0.30**	2.52±0.61***	4.96±0.18***	6.46±0.33***
C20:3n-3	2.06±0.01	2.52±0.30*	2.77±0.42	1.22±0.09***	0.56±0.01***
C20:4n-3	3.63±0.45	3.61±0.36	2.26±0.12*	1.98±0.30***	0.61±0.06***
C20:5n-3	4.58±0.51	4.87±0.04	2.52±0.61***	1.52±0.74***	0.61±0.06***
C22:6n-3	6.23±0.38	5.51±0.48*	3.24±0.32***	1.98±0.12***	0.56±0.06***
PUFA n-3	24.31±0.07	23.99±1.09	16.57±0.11***	9.26±1.08***	3.40±0.16***
PUFA n-6	2.92±0.17	4.64±0.34***	8.47±0.33***	12.00±0.92***	16.19±0.28***

Values are presented by mean ± SD of ten replicates.
Values in a row that does not share the same letter(s) are significantly different at $p < 0.05$.

Nutritional quality indices

The NQIs are listed in Table 3. After freezing, a significant decrease in the n-3/n-6 ratio was observed (Table 3). This ratio decreased by 97.48% after 90 days of storage. The PUFA/SFA ratio decreased significantly during freezing ($p < 0.05$). Similarly, EPA + DHA decreased significantly during storage and reached its lowest value after 90 days (1.17) compared to fresh crabs (10.81). The polyene index (PI), used as a marker of oxidative rancidity of crab meat, also decreased during freezing ($p < 0.05$) (Table 3). However, the indices of atherogenicity (AI) and thrombogenicity (TI) significantly increased by 385.33% and 818.66%, respectively, in frozen crab after 90 days of storage. Hypercholesterol ratio (h/H) decreased significantly ($p < 0.05$) by 59.73% in frozen crabs after 90 days compared to raw crabs (Table 3).

Lipid peroxidation

The results of lipid peroxidation are shown in Figure 3. This phenomenon increased significantly during the storage period, as TBARS and PV levels reached their highest values after 90 days of storage compared to fresh crabs (Figure 3).

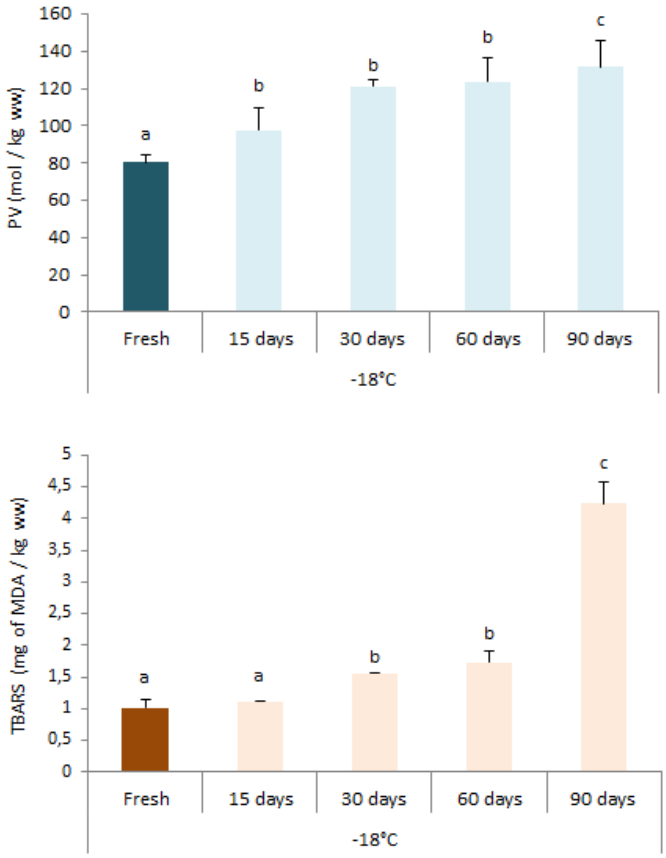

Figure 3. Lipid peroxydation indices in raw and frozen crabs.

Table 3. Nutritional quality indices of fresh and frozen crab's meat after 15, 30, 60 and 90 days of frozen storage.

Indices	Fresh	15 days	30 days	60 days	90 days
$\frac{n-3}{n-6}$	8.35±0.51	5.18±0.28***	0.95±0.05***	0.78±0.11***	0.21±0.01***
$\frac{PUFA}{SFA}$	1.64±0.02	1.33±0.14	0.95±0.05***	0.56±0.03***	0.40±0.01***
EPA+DHA	10.81±0.13	10.38±0.44***	5.76±0.64***	3.50±0.77***	1.17±0.03***
PI	1.72±0.09	1.35±0.16***	0.66±0.03***	0.38±0.10***	0.12±0.01***
AI	0.36±0.01	0.47±0.07***	0.71±0.03***	1.16±0.04***	1.65±0.06***
TI	0.12±0.01	0.15±0.02***	0.25±0.01***	0.50±0.03***	1.10±0.08***
$\frac{h}{H}$	2.98±0.10	2.42±0.31***	1.82±0.18***	1.39±0.15***	1.20±0.05***

Values are presented by mean ± SD of ten replicates.
Values in a row that does not share the same letter(s) are significantly different at $p < 0.05$.

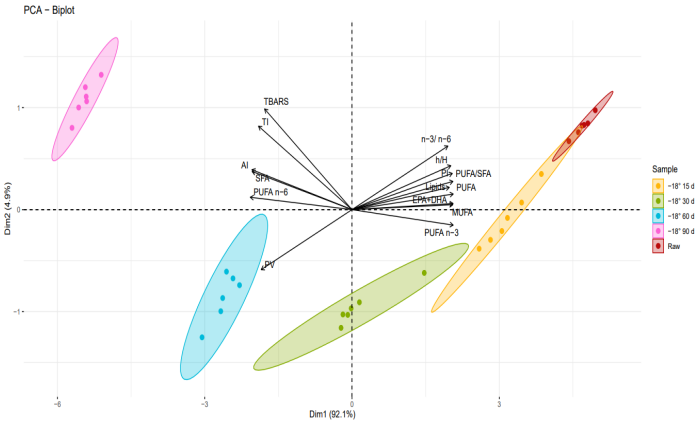

Figure 4. PCA Analysis - PCA analysis showing separation between fresh and frozen blue crab groups

Principal component analysis (PCA)

The PCA revealed a two-dimensional pattern that explained 85.3% of the total variance, including factor 1 (71.7%) and factor 2 (13.6%), and mainly showed the difference between raw and frozen individuals (Figure 4). The fresh crabs were characterized by higher contents and amounts of TL, MUFA, PUFA, PUFA/SFA, EPA + DHA, n-3/n-6, and h/H. The group of 15 days frozen crabs was the closest to the raw group. The 90-day frozen crab group had high values for SFA, PUFA n-6, TBARS, PV, AI, and TI (Figure 4). PCA analysis performed for all measured parameters highlighted the clear separation between the experimental groups during the freezing process.

Discussion

Crab species are considered a good source of lipids, proteins, essential elements, and vitamins beneficial for maintaining human health [19]. However, inadequate preservation of seafood could result in a significant loss of their nutritional quality. Freezing is the most popular form of food preservation (e.g., chicken, fish, beef, etc.). This process is known to preserve food quality by reducing the deterioration of color, flavor, and texture over time and limiting the degradation process caused by microorganism activity [20]. In the present study, total lipids (TL) in blue crab meat decreased significantly over time during storage at −18°C. This lipid degradation could be responsible for the shortening of the shelf life of the marine product due to the oxidation of unsaturated fatty acids (UFA). However, Benjakul *et al.,* [21] reported that catalysts released from denatured or destroyed muscle cells of marine products can accelerate lipid oxidation. The current results are consistent with the work of [22] on the mussel (*Perna canaliculus*) from New Zealand. The variations in lipid content in frozen samples could be related to lipid oxidation. Our results are in agreement with the work of [23], who confirmed that the decrease in TL content in the meat of frozen catfish was due to oxidation. [24] reported that the lipid content in the muscle of four fish species (Mugilidae, Sparidae, Sciaenidae, and Platycephalidae) decreased significantly during the freezing process.

The predominant fatty acids (FA) in fresh crab meat were palmitic acid, oleic acid, EPA, and DHA fatty acids. When the crab meat was frozen at −18°C, the FA composition changed in their proportions; SFA increased, while MUFA and PUFA decreased significantly. Similar results on the changes of PUFA, MUFA, and SFA during freezing were observed in fish [10]. The reduction in PUFA content is likely due to oxidative reactions that occurred during the freezing process. It should be noted that the high degree of unsaturation favors the oxidation of PUFAs [25]. The n-3 PUFAs such as EPA and DHA were significantly reduced. In contrast, arachidonic acid (AA), which is known as a pro-inflammatory lipid compound, increased upon freezing. The results showed that the polyene index (PI) and the sum of DHA + EPA decreased due to the reduction of EPA and DHA, reflecting the degradation of PUFA, as previously reported by [26] in frozen hake. The n-3/n-6 index is considered a good standard for comparing fish oil quality [27]. A ratio of 1:1 or 1:5 could be beneficial for human health [28]. Our results showed that the n-3/n-6 ratio decreased after freezing, indicating a significant loss in the nutritional quality of the crab meat, but it was higher than the values recommended by the World Health Organization (WHO) up to 60 days of storage [29]. The same trend was observed for PUFA/SFA ratio. The reduction in PUFA resulted in a significant decrease in this index, confirming that frozen crabs are no longer a good source of PUFA. However, up to a storage period of 60 days, this ratio remained higher (0.45) than the minimum value recommended by HMSO (1994) for a healthy human diet. Similar results were observed in frozen fish at −18°C [30]. In this study, the h/H ratio decreased significantly, indicating a deterioration in meat quality, as higher values are considered healthier for humans [31]. Atherogenic (AI) and thrombogenic (TI) indices are commonly used to determine how healthy the fat content of the food in question is [32]. These indices increased, possibly due to the loss of beneficial FA and the enhancement of atherogenic FA. Others reported similar results in rainbow trout frozen at −15°C for 3 months [33].

The observed changes in the main FA showed that they were

involved in the oxidation process. The freezing temperature used was able to significantly increase PV and TBARS levels in frozen crab meat. The appearance of radicals and the formation of hydroperoxides can lead to oxidative changes [34]. The oxidation of lipids in seafood can produce a rancid taste and odor that compromises the nutritional value and safety of seafood through the formation of primary oxidation products that rapidly convert to secondary products [35]. The increase in PV, which is commonly used as an index to quantify the amount of hydroperoxide indicating oxidative deterioration [36], showed the development of rancidity, off-flavor, color, and nutritional deterioration in frozen crab meat. Our results confirm previous work on frozen fish and shrimp [37]. The higher increase in TBARS, known as secondary lipid oxidation products, during frozen storage suggests a high rate of lipid oxidation and decay. Similar results were observed in frozen silver catfish [38] and pink salmon [39].

From the above results, we can conclude that prolonged storage at −18°C leads to a loss of nutritional quality of crab meat due to the formation of oxidation products and contributes to the acceleration of lipid degradation. According to this study, shorter storage of no more than 15 days at −18°C is strongly recommended when crabs are frozen at this temperature.

Conclusion

The freeze had significant effects on the biochemical composition of the crabs. Major changes were observed in the FA profile and Nutritional Quality Indices (NQI) of frozen crabs. As with other seafood, the nutritional quality of crab species can be affected by frozen storage. The deterioration of flesh quality of frozen crabs increased with the duration of freezing, as shown by the elevated Peroxide Value (PV) and Thiobarbituric Acid Reactive Substances (TBARS) values. It can be concluded that blue crabs stored at −18°C are still a good source of fatty acids, especially omega-3, after a reasonable period of time. Our study concerns local consumers of this crab and, in particular, restaurants and hotels that use this product on their menus. We recommend that consumers of this product gain a deeper understanding of the potential hazards and advantages related to the freezing methods employed.

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