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Effect of Freezing Process on the Chemical Properties of Blue Swimming Crab *Portunus segnis*: Storage Stability and Acceptability

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ABSTRACT

The effect of frozen storage at a commercial temperature (−30°C) on the nutritional characteristics of the blue swimming crab *Portunus segnis* for a period of 120 days was investigated. Lipid and protein oxidation was investigated by analyses of peroxide value, thiobarbituric acid reactive substances, free fatty acids, and advanced oxidation protein products. Results showed significant lipid deterioration with extended storage time and changes in fatty acids composition and lipid classes. From a nutritional standpoint, it would be preferable for human consumption to eat frozen crabs for no more than 30 days as they retain a higher nutritional value.

KEYWORDS

Portunus segnis; freezing process; biochemical oxidation; fatty acids; health lipid indices

Introduction

Crabs play an important economic and bio-ecological role in most aquatic ecosystems since they occupy several trophic niches. These crustaceans are high-valued resources much appreciated worldwide as an important source of protein, essential elements, oil content, and healthy fatty acids (e.g. EPA and DHA) (Bejaoui et al. 2017; Marques et al. 2010). The family Portunidae is the most dominant in commercial crab fisheries worldwide (Deidun and Sciberras 2016) and is mainly represented in the Mediterranean by the lessepsian migrant *Portunus segnis* (Forskål 1775). This blue swimming crab, previously named *P. pelagicus* (Lai et al. 2010), is one of the first Lessepsian invaders, recorded in Egypt (Mediterranean Sea) in 1898, a few years after the opening of the Suez Canal. Its current distribution range extends along the western Indian subcontinent, Pakistan, the Persian Gulf, the Red Sea, the Mediterranean Sea, and the east coast of Africa (Lai et al. 2010). The presence of *P. segnis* in Tunisian waters was first reported in 2014, when crabs were collected in shallow sandy areas mainly covered with sea-grass and seaweed beds (Rifi et al. 2014). Then, they proliferated massively causing considerable damage to the coastal artisanal fishing sector, particularly in the Gulf of Gabes, where, in high season, blue crab represented more than 70% of catches off Gabes. After two years, this species was reported in the Gulf of Hammamet. In 2018, this invasive species emerged in the lagoons of Bizerte and Ghar El Melh (Mili et al. 2020; Shaiek et al. 2021). Given the overproduction of this resource in Tunisia, *P. segnis* has become common in all fishing markets and is in high demand by local and foreign consumers. However, coastal fishermen have found many difficulties related to the presence of this species in fisheries, such as the degradation of fishing nets, alteration of other caught species, time spent in its disentanglement, and the drop in stocks of many fish species of important commercial value (Mili et al. 2020). In view of these issues, the government has devised and supported a national plan to encourage fishing, development, and marketing of blue

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swimming crab in the Gulf of Gabes. Due to the high demand, Tunisian fishermen have succeeded with the support of the Food and Agriculture Organization (FAO) in transforming this invasive species into an export commodity, where the production of *P. segnis* increased sharply in May 2021 to reach 2090.9 tons compared to 796.1 tons for the same period in 2020 according to the FAO.

Generally, seafood, including crustaceans, is recommended for human consumption because of its favorable health characteristics. Over the past decades, seafood demand has steadily increased; therefore, efforts to improve the preservation procedure and the quality of these products increased too. Frozen storage processes of marine products have been described as an effective long-term preservation method in slowing the enzyme activity, inhibiting the microbial growth, and retaining the sensory and nutritional properties of frozen product (Nazemroaya et al. 2009). Many factors including freezing rate, storage temperature and duration, temperature fluctuations, and thawing rate may influence marine frozen food quality (Bejaoui et al. 2021). For seafood, loss of quality during frozen storage is predominately caused by lipid oxidation, protein denaturation, and ice recrystallization, which often leads to weight loss, rancidity, off-flavors, and loss of juiciness (Lorentzen et al. 2019).

Despite the high consumption of *P. segnis* blue crab in the Mediterranean countries, information about the effect of freezing on its nutritional quality is limited (Anacleto et al. 2011; Risso and Carelli 2017) and totally absent for blue swimming crab *P. segnis*. For this reason, the current work aimed to study the effect of frozen storage at −30°C for a period of 120 days on the nutritional characteristics of *P. segnis*. This freezing temperature is most used in conservation of seafood in the worldwide market. The recommended storage temperature for all fishery products is −30°C in the United Kingdom and Europe (FAO 1994). The proximate composition, neutral and polar lipids (such as triacylglycerols (TAG), cholesterol (CHL), free fatty acids (FFA), and phospholipids (PL)), fatty acids composition, and lipid and protein peroxidation indices (PV, TBARS, FFAs, and AOPP) were assessed in the fresh and frozen blue swimming crab breast meat. This paper provides valuable information that assesses the efficiency of freezing process on the quality of frozen crabs after a long-term storage period. The knowledge of the adequate duration of frozen storage is highly useful for regular users of this marine product in restaurants and hotels all over the Mediterranean countries. This study will be of practical value in promoting the consumption of frozen crab products in an ever-expanding global market.

Materials and methods

Sample preparation

Sixty mature blue swimming crabs of market size (weight: 150 g-200 g; length: 10–15 cm) were collected from the Gulf of Gabes (Southern Tunisian coasts) in January 2018. Crabs were immediately transported to the laboratory in a cold box, washed, and cleaned to remove the extraneous material. After cooling under cold water, samples of 10 individuals ($n = 10$) were packed into alimentary plastic bags, labeled, frozen at low (−30°C) temperature, respectively, and then stored in polyethylene boxes (Figure 1). Chemical analyses were examined at 30, 60, 90, and 120 days and compared with control (0 day). For each period, 10 individual breast meat tissues were extracted using the Tris-HCl buffer (20 mM; pH = 7.4) and prepared for protein quantification and oxidative damage markers analysis (Figure 1).

Proximate composition

Moisture (%) was determined according to AOAC (1989) method based on weight difference after heating the sample at 110°C for 24 h. Protein determination was performed using bovine serum albumin as standard according to the Lowry et al. (1951) method. Lipids were extracted according to the method of Folch et al. (1957) with the solvent mixture chloroform-methanol (2:1, v/v) containing 0.01% butylated hydroxyl toluene (BHT) as an antioxidant. Protein and lipid contents were expressed as mg/g wet weight (mg/g_{WW}).

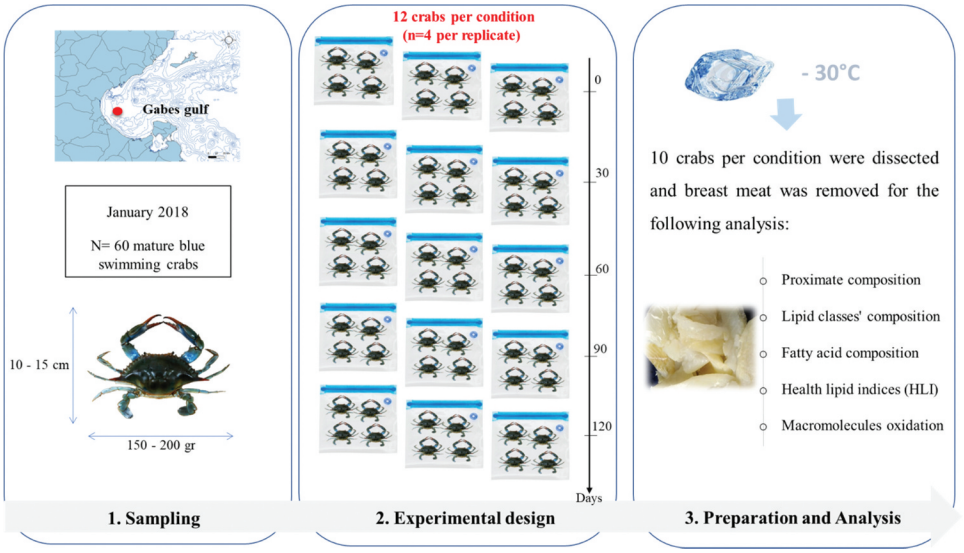

Figure 1. Experimental design.

Lipid class composition

Lipid classes were separated according to the method of Olsen and Henderson (1989), using thin-layer chromatography (TLC) with one-dimensional double development. Then, 500 µl of lipid extracts were separated on plates (20 × 20 cm, silica gel 60, Merck, Germany). The polar fraction was developed using a solvent containing methyl acetate, isopropanol, and chloroform/methanol: 0.25% KCl (25: 25: 25: 10, 9 v/v), and the neural lipids were developed using a solvent formed by hexane, diethyl ether, and glacial acetic acid (80: 20: 2, v/v) (Olsen and Henderson 1989). Once the migration was achieved, lipid classes were visualized under UV light after spraying with 0.1% 2′-7′dichloro-fluorescein in absolute methanol.

Fatty acid composition

Lipid extracts were trans-esterified according to the method of Cecchi et al. (1985). Nonadecanoic acid (C19:0) (Belefonte PA, USA, CRM47885) was added as an internal standard. Then, fatty acids methyl esters (FAMEs) were analyzed using nitrogen as transporter gas and an HP 6890 gas chromatograph equipped with a split/splitless injector, a flame ionization detector at 275°C, and a 30 m HP Innowax capillary column that was characterized by an internal diameter of 250 µm and a 0.25 µm film thickness. The injector temperature was unsubstantiated at 250°C. Briefly, the programmed oven was raised from 50 to 180°C at a degree of 40°C/min; then, from 180 to 220°C at 1.33°C/min and finally at 220°C for 7 min in order to stabilize. Identification of FAMEs was based on the comparison of their retention times with those of commercial standards methyl esters (SUPELCO PUFA-3 and menhaden oil by SUPELCO).

Health lipid indices (HLI)

The FA profile was used to determine several nutritional parameters in fresh and frozen crabs. They were calculated according to the following equations:

- Atherogenicity index (AI): According to Ulbricht and Southgate (1991), AI was determined following the below Equation (1):

$$AI = (12:0 + (4 \times 14:0) + 16:0)/(\Sigma MUFAs + PUFAn\text{-}6 + PUFAn\text{-}3) \quad (1)$$

- Thrombogenicity index (TI): TI index was evaluated according to Ulbricht and Southgate (1991). The following Equation (2) was applied:

$$TI = (C14:0 + C16:0 + C18:0)/(0.50 * MUFA) + (0.5 * PUFAn\text{-}6) + (3 * PUFAn\text{-}3) + (PUFAn\text{-}3/PUFAn\text{-}6) \quad (2)$$

- Polyene index (PI) was calculated according to Rodriguez et al. (2007) following the below Equation (3):

$$PI = (C20:5n\text{-}3 + C22:6n\text{-}3)(C16:0) \quad (3)$$

- Hypercholesterolemic index (h/H) is the ratio between hypocholesterolemic and hypercholesterolemic fatty acids. h/H ratio was calculated according to Fernandez et al. (2014) following Equation (4):

$$h/H = (C18:1n\text{-}9 + C18:2n\text{-}6 + C20:4n\text{-}6 + C18:3n\text{-}3 + C20:5n\text{-}3 + C22:5n\text{-}3 + C22:6n\text{-}3)(C14:0 + C16:0) \quad (4)$$

However, *n*-3/n-6 PUFA, PUFA/SFA, and EPA+DHA indices were determined according to Marques et al. (2010).

Lipid peroxidation

Peroxide value (PV)

The peroxide value determines the concentration of hydroperoxide, which is the primary oxidation product. PV was estimated according to the AOCS (1989) method. Supernatants were vortexed with pure chloroform (5 ml) and glacial acetic acid (0.2 ml). Then, a saturated solution of potassium iodide was added, followed by the appearance of yellow color indicating activation of the reaction. Before titration with sodium thiosulfate solution (0.1N), starch solution (1 ml) was added to the previous mixture. Results were expressed as ml. g^{-1} and calculated according to the Equation (5):

$$PV = [(\text{titration of the sample} - \text{titration of the blank}) \times N\,\text{thiosulfate} \times 1000]/\text{weight of sample} \quad (5)$$

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances, which are secondary oxidation products, were determined according to AOCS (1989) using 2-thiobarbituric acid (TBA). A standard solution of 1,1,3,3-tetraethoxypropane (TEP) was used to obtain a calibration curve, and absorbance values were determined with this curve in order to calculate the quantity of MDA at 532 nm. Results were expressed as $mg.kg^{-1}$ WW.

Free fatty acid (FFAs)

Free fatty acids were examined according to the method described by AOCS (1989). Approximately 1 ml of supernatant was added to 5 ml of absolute ethanol and phenolphthalein. The obtained mixture was then vortexed for 10 min at rising temperature (60°C) with progressive addition of KOH solution (0.1N) until the apparition of pink color. Results were expressed as a percent and calculated using the following Equation (6):

$$\mathrm{FFA} = \frac{\left[\begin{array}{c}(\text{sample titration} - \text{blank titration}) \times \text{ normality of ml of KOH solution} \\ \times \text{molar weight of KOH}\end{array}\right]}{\text{the weight of the sample}} \quad (6)$$

Protein oxidation

Advanced oxidation protein product (AOPP)

AOPP levels were quantified according to Kayali et al. (2006). Briefly, 400 µl of crab meat tissue supernatant was mixed with 0.8 ml of phosphate buffer (0.1 M; pH 7.4). After 2 min, 0.1 ml of 1.16 M potassium iodide (KI) was treated with the previous solution followed by 0.2 ml of acetic acid. The absorbance of the reaction mixture was examined at 340 nm. The AOPP level for each sample was calculated using the extinction coefficient of 261, and results were expressed as nmol/mg protein.

Statistical analysis

Results were expressed as mean ± standard deviation (SD). Data were analyzed using the software STATISTICA 8 (Stat-Soft Inc.) The normality and homogeneity of variance were tested using Kolmogorov Smirnov test. Differences between fresh and frozen crab samples were deemed significant at $p < .05$. A one-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test was used. Principal component analysis (PCA) and a Spearman correlation matrix were used to display possible correlations between biochemical parameters of fresh and frozen crabs based on storage time and temperature.

Results

Proximate composition

Results showed that freezing process has no effect on the moisture percentage (%) during all the storage period when compared to fresh crabs (Table 1). During freezing, both lipid and protein contents were reduced over time. Protein decreased from 9.98 (mg/g ww) in raw crabs to reach 6.88 (mg/g ww) after 120 days. However, fresh crabs contained high lipid content (41.85 mg/g ww) when compared to frozen individuals during 30 (7.57%), 60 (28.02%), 90 (36.55%), and 120 (50.17%) days of storage (Table 1).

Neutral and polar lipid fractions

The results of triacylglycerols, cholesterol, free fatty acids, and phospholipid contents are presented in Table 2. TAG was the prominent fraction in raw samples followed by FFA, CHL, and PL. TAG decreased significantly from 9.43% in raw crabs to 3.90% after 120 days of frozen storage. Meanwhile, CHL, FFA, and PL contents increased considerably by 168.22%, 253.44%, and 408.69%, respectively, after 120 days of storage (Table 2).

Table 1. Proximate composition of raw and frozen crabs at – 30°C for 30, 60, 90, and 120 days.

		−30°C			
	Control	30 days	60 days	90 days	120 days
Moisture (%)	93.52 ± 0.41[a]	93.15 ± 0.66[a]	93.36 ± 0.92[a]	93.42 ± 2.13[a]	94.51 ± 1.12[a]
Lipid (mg/g ww)	41.85 ± 4.34[a]	38.68 ± 2.58[a]	30.12 ± 1.85[b]	26.55 ± 1.34[b]	20.85 ± 3.36[c]
Protein (mg/g ww)	9.98 ± 2.89[a]	8.00 ± 0.10[a]	7.69 ± 2.73[a]	7.28 ± 1.87[a]	6.88 ± 0.30[b]

Values are presented by mean ± SD of ten replicates.
Values in a row that do not share the same letter(s) are significantly different at $p < .05$.
WW: wet weight.

Table 2. Lipid classes of raw and frozen crabs at – 30°C for 30, 60, 90, and 120 days.

		−30°C			
	Control	30 days	60 days	90 days	120 days
Cholesterol (%)	5.35 ± 0.45[a]	8.90 ± 0.89[b]	10.10 ± 0.92[b]	12.22 ± 1.75[c]	14.35 ± 1.64[c]
Triacylglycerols (%)	9.34 ± 0.34[a]	7.04 ± 0.47[b]	5.92 ± 0.17[b]	4.21 ± 0.76[c]	3.90 ± 0.69[c]
Free fatty acids (%)	6.10 ± 0.55[a]	8.08 ± 0.17[b]	9.92 ± 0.17[b]	16.61 ± 2.50[c]	21.56 ± 1.62[d]
Phospholipids (%)	0.23 ± 0.01[a]	0.74 ± 0.05[b]	0.81 ± 0.02[b]	0.92 ± 0.02[b]	1.17 ± 0.02[c]

Values are presented by mean ± SD of ten replicates.
Values in a row that do not share the same letter(s) are significantly different at $p < .05$.

Table 3. Fatty acid composition and health lipid indices (HLI) of raw and frozen crabs at – 30°C for 30, 60, 90, and 120 days.

		−30°C			
	Control	30 days	60 days	90 days	120 days
C14:0	1.67 ± 0.28[a]	1.84 ± 1.00[b]	2.06 ± 0.73[b]	2.56 ± 0.27[b]	3.15 ± 0.45[b]
C15:0	1.04 ± 0.13[a]	0.74 ± 0.39[a]	0.64 ± 0.36[a]	0.39 ± 0.18[b]	0.26 ± 0.19[b]
C16:0	7.68 ± 0.25[a]	7.96 ± 1.66[b]	8.87 ± 3.12[b]	9.18 ± 3.10[b]	9.35 ± 2.38[c]
C17:0	1.86 ± 0.24[a]	1.26 ± 0.29[a]	1.28 ± 0.69[a]	1.34 ± 0.6[b]	0.86 ± 0.41[b]
C18:0	2.21 ± 0.28[a]	3.73 ± 0.93[b]	4.21 ± 0.07[b]	5.57 ± 0.26[b]	6.36 ± 1.40[b]
C20:0	5.82 ± 0.63[a]	7.46 ± 2.22[b]	9.46 ± 1.47[b]	10.08 ± 0.18[b]	11.40 ± 0.77[c]
C22:0	1.41 ± 0.06[a]	3.31 ± 1.13[b]	3.43 ± 0.04[b]	5.04 ± 1.37[c]	6.79 ± 2.40[c]
SFA	**21.69 ± 1.74**[a]	**26.30 ± 1.60**[b]	**29.95 ± 1.12**[b]	**34.56 ± 1.51**[c]	**38.17 ± 1.95**[d]
C14:1	1.61 ± 0.13	1.02 ± 0.55	0.97 ± 0.26	0.15 ± 0.07	0.23 ± 0.17
C15:1	0.57 ± 0.38	0.82 ± 0.79	0.72 ± 0.27	0.39 ± 0.27	0.33 ± 0.21
C16:1	2.87 ± 0.07[a]	2.52 ± 0.93[a]	2.15 ± 0.79[a]	1.76 ± 0.78[b]	0.60 ± 0.30[b]
C18:1	3.82 ± 0.37[a]	1.77 ± 0.48[b]	1.61 ± 0.22[b]	1.38 ± 0.07[b]	1.31 ± 0.59[b]
C20:1	15.76 ± 0.56[a]	14.31 ± 3.33[a]	12.08 ± 0.75[b]	7.82 ± 3.69[b]	7.39 ± 1.75[b]
C22:1	2.33 ± 0.57[a]	1.99 ± 1.36[a]	1.83 ± 0.11[a]	1.51 ± 0.71[b]	0.28 ± 0.13
MUFA	**26.96 ± 1.22**[a]	**22.43 ± 1.81**[b]	**19.37 ± 1.72**[b]	**13.00 ± 2.67**[b]	**10.13 ± 0.85**[c]
C16:2n-4	2.17 ± 1.58	1.94 ± 0.20	1.66 ± 0.09	1.54 ± 0.17	1.31 ± 0.16
C16:3n-4	2.89 ± 1.81	2.07 ± 0.11	1.42 ± 0.11	1.09 ± 0.43	0.86 ± 0.55
C18:2n-6	0.83 ± 0.24	1.64 ± 0.14	2.06 ± 0.10	2.50 ± 1.30	2.999 ± 1.22
C18:3n-4	1.82 ± 0.74	1.07 ± 0.19	0.84 ± 0.08	0.68 ± 0.78	0.55 ± 0.64
C18:3n-3	6.64 ± 1.56[a]	2.54 ± 0.21[b]	1.46 ± 0.40[b]	0.38 ± 0.10[c]	0.15 ± 0.06[c]
C20:3n-6	3.01 ± 0.79[a]	4.89 ± 0.75[b]	6.55 ± 1.14[b]	7.40 ± 0.39[b]	9.20 ± 1.95[c]
C20:4n-6	0.57 ± 0.03[a]	2.43 ± 1.04[b]	4.28 ± 0.31[b]	5.94 ± 0.43[c]	6.07 ± 1.09[c]
C20:3n-3	1.06 ± 0.01	0.63 ± 0.14	0.57 ± 0.09	0.44 ± 0.21	0.39 ± 0.21
C20:4n-3	3.63 ± 0.45	2.34 ± 1.32	2.26 ± 0.12	1.36 ± 0.54	0.86 ± 0.40
C20:5n-3	4.58 ± 0.51[a]	3.22 ± 0.72[b]	2.14 ± 1.32[b]	1.76 ± 1.97[b]	0.52 ± 0.69[c]
C22: 6n-3	4.73 ± 1.12[a]	3.86 ± 0.14[b]	2.51 ± 0.07[b]	1.82 ± 0.86[b]	1.10 ± 0.17[c]
PUFA	**31.91 ± 2.62**[a]	**26.63 ± 1.91**[b]	**25.76 ± 4.02**[b]	**24.89 ± 3.16**[b]	**23.19 ± 0.44**[b]
PUFA n-3	**20.64 ± 0.53**[a]	**12.60 ± 1.82**[b]	**8.94 ± 2.00**[b]	**5.75 ± 1.44**[b]	**3.01 ± 0.45**[c]
PUFA n-6	**4.41 ± 0.13**[a]	**8.96 ± 1.32**[b]	**12.89 ± 2.04**[b]	**15.84 ± 2.97**[c]	**18.26 ± 1.74**[d]
n-3/n-6	**4.68 ± 0.24**[a]	**1.41 ± 0.30**[b]	**0.69 ± 0.23**[b]	**0.36 ± 0.19**[c]	**0.16 ± 0.09**[d]
EPA+DHA	**9.31 ± 0.99**[a]	**7.09 ± 0.82**[b]	**4.56 ± 0.98**[b]	**3.58 ± 0.93**[c]	**1.61 ± 0.22**[d]
PUFA/SFA	**1.47 ± 0.31**[a]	**1.01 ± 0.12**[b]	**0.86 ± 0.34**[b]	**0.73 ± 0.08**[b]	**0.63 ± 0.12**[b]
PI	**1.21 ± 0.14**[a]	**0.89 ± 0.11**[b]	**0.53 ± 0.08**[b]	**0.39 ± 0.14**[b]	**0.17 ± 0.05**[c]
AI	**0.32 ± 0.03**[a]	**0.43 ± 0.03**[b]	**0.52 ± 0.05**[b]	**0.72 ± 0.05**[b]	**0.90 ± 0.06**[b]
TI	**0.14 ± 0.01**[a]	**0.25 ± 0.02**[b]	**0.35 ± 0.03**[b]	**0.54 ± 0.06**[b]	**0.81 ± 0.04**[b]
h/H	**2.58 ± 0.12**[a]	**2.08 ± 0.18**[b]	**1.89 ± 0.20**[b]	**1.80 ± 0.11**[b]	**1.71 ± 0.15**[b]

Values are presented by mean ± SD of ten replicates. Values in a row that do not share the same letter(s) are significantly different at $p < .05$.

Fatty acid composition of total lipid (TL)

In Table 3, the fatty acid profile of raw and frozen crab meat was reported and expressed as a percentage of the total identified fatty acids. In fresh crabs, the FA profile shows that polyunsaturated fatty acid (PUFA) accounts for 31.91% of total FA, followed by monounsaturated fatty acid (MUFA: 26.96%) and saturated fatty acid (SFA: 21.69%). Fresh crabs contained high concentrations of *n*-3 PUFA, which included alpha-linolenic acid (C18:3n-3; 6.64%), docosahexaenoic acid (DHA; 4.73%), eicosapentaenoic acid (EPA; 4.58%), and eicosatetraenoic acid (C20:4n-3; 3.63%). In frozen crabs,

PUFA and MUFA decreased significantly ($p < .05$), while SFA increased considerably. A significant reduction of *n*-3 PUFA after 30 days of storage was noticed. At the end of the freezing process, DHA and EPA decreased by 76.74% and 88.64%, respectively. In contrast, *n*-6 PUFA in frozen crabs showed a different variation with a significant increase ($p < .05$) after 30 days of storage when compared to fresh samples (4.41%). The concentration of major MUFA in fresh crabs was as follows: oleic acid (C18:1; 3.82%); eicosenoic acid (C20:1; 15.76%), and palmitoleic acid (C16:1; 2.87%). Significant changes in MUFA levels were observed after 30 days storage ($p < .05$). SFA in raw crab meat was dominated by palmitic acid (C16:0; 7.68%) and stearic acid (C18:0; 2.21%). These two fatty acids (C16:0 and C18:0) increased after 120 days of frozen storage by 21.74% and 187.78%, respectively.

Health lipid indices

The *n*-3/n-6 ratio, known as an indicator of the nutritional value of marine organisms, was significantly reduced after frozen storage (Table 3). In the present study, this ratio decreased by 96.58% after 30 days of storage. PUFA/SFA and EPA+DHA ratios showed similar variations. PUFA/SFA decreased from 1.47 to 0.63 during 120 days of storage. Similarly, EPA+DHA decreased significantly during frozen storage and reached its lowest level after 120 days (1.61), as compared to raw crabs (9.31). Polyene index, used as a marker of oxidative rancidity of crab meat, declined during frozen storage ($p < .05$) (Table 3). However, atherogenic and thrombogenic indices increased in frozen crabs. AI and TI changed from 0.32 to 0.14, respectively, in non-frozen crabs to 0.90 and 0.81, respectively, in frozen crabs. The hypercholesterolemic ratio (h/H) decreased significantly from 2.58 to 1.71 ($p < .05$) (Table 3).

Lipid peroxidation

Results showed that lipid peroxidation occurred in crab samples after freezing (Figure 2). This phenomenon increased considerably during storage time. TBARS, PV, and FFAs levels increased significantly by 194.11%, 63.94%, and 86.20%, respectively, after 120 days of storage when compared to fresh crabs (1.02, 80.37, and 136.30, respectively) (Figure 2).

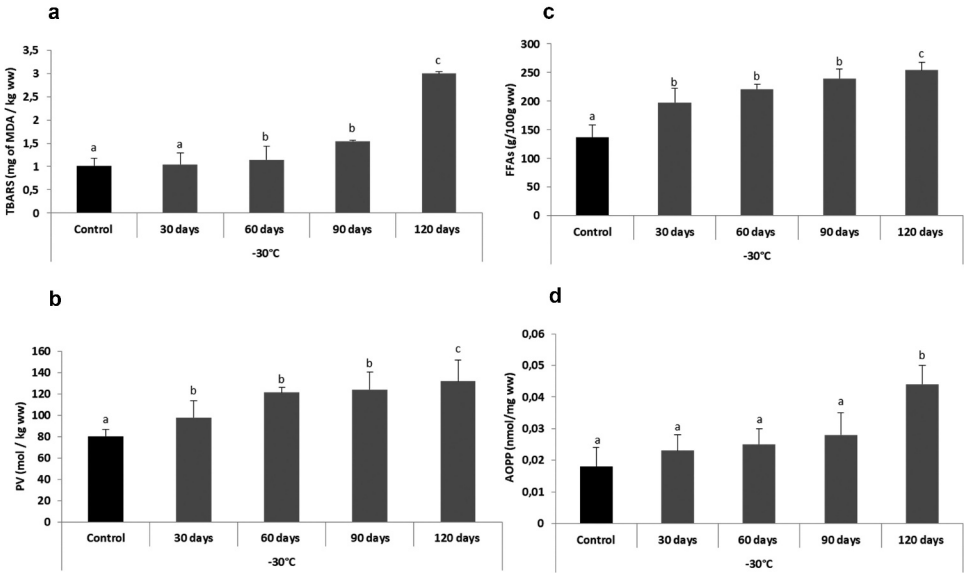

Figure 2. Lipid and protein oxidation indices in raw and frozen crabs (a. Thiobarbituric acid reactive substances; b. Peroxide value; c. Free fatty acids; d. Advanced oxidation protein product).

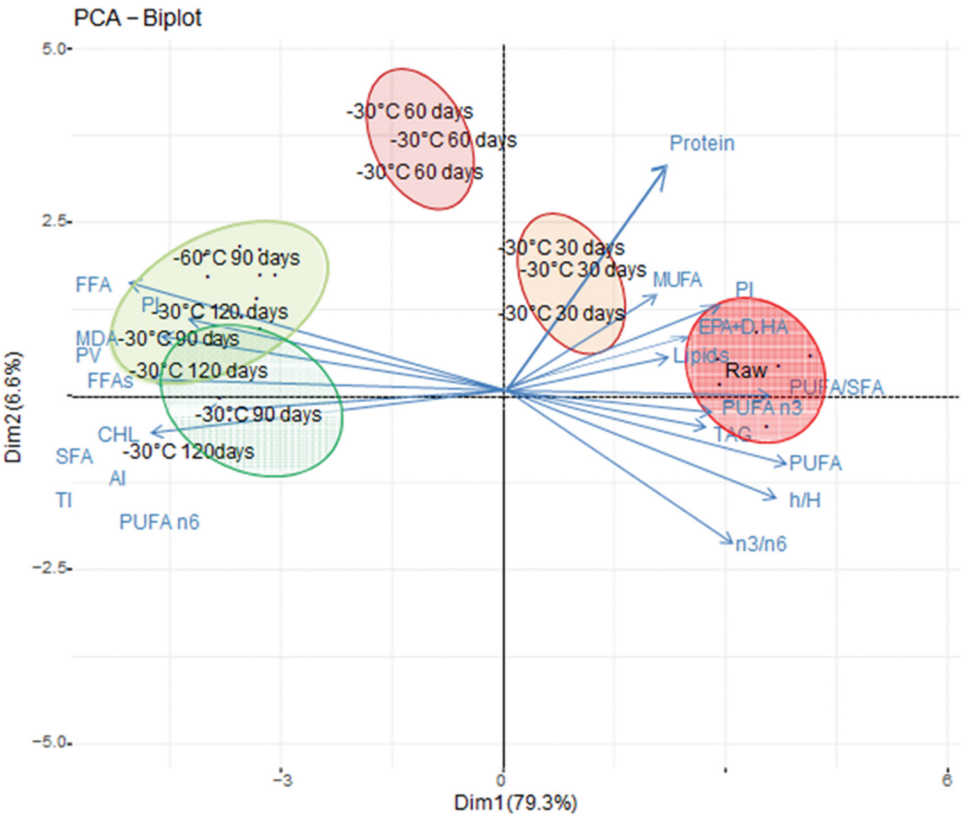

Figure 3. Principal component analysis (PCA) of raw and frozen crab biochemical parameters, lipid, and protein damage indices: Projection of the variables and the cases on the factor-plane (1×2).

Protein oxidation

The AOPP levels increased significantly by 51.72% in frozen crabs after 120 days when compared to raw samples (Figure 2).

Principal component analysis

The principal component analysis of the measured variables (moisture, lipids, proteins, lipid fractions, FA composition, and lipid deterioration markers) mainly revealed the difference between raw and frozen individuals, as well as the effects of storage time (Figure 3). The projection at PC1 allowed us to separate raw samples from the frozen (PC1 79.3%). The fresh crabs were characterized by a higher content of lipids, proteins, PUFA, PUFA *n*-3, EPA+DHA, TAG, *n*-3/n-6, and PI. Indeed, the variables that contributed mostly to the second component were SFA, PUFA *n*-6, FFA, PV, TBARS, CHL, AI, and TI, displaying a clear correlation with frozen samples (Figure 3).

Discussion

Blue swimming crab meat is known to be rich in lipids, mainly polyunsaturated fatty acids, which are very susceptible to oxidative deterioration (Bejaoui et al. 2017). Oxidative damage to lipid is a result of quality deterioration, texture/color modification, and nutritional value loss. Our results showed that blue swimming crab meat underwent chemical changes during frozen storage at −30°C. It has been observed that the freezing temperature was able to enhance significantly the levels of PV, TBARS, and

FFAs in frozen crab meat. Total lipids in blue crab meat decreased significantly over storage time. This lipid deterioration could be responsible for reduced shelf life of this marine product due to progressive oxidation and enzymatic hydrolysis of unsaturated fatty acids (UFA). For example, Benjakul and Sutthipan (2009) reported that catalysts released from the denatured or disrupted muscle cells of marine products could accelerate the lipid oxidation. Generally, frozen storage may trigger lipid oxidation since ice crystals formed during the freezing process could release pro-oxidants for lipid oxidation, especially free iron. This latter is able to take part in electron transfer reactions with molecular oxygen, generating a superoxide anion. The reaction of oxygen with UFA molecules could form hydroperoxides, which could be used as a measure of oxidation extent in the early stages. Our results showed significant differences in PV between raw and frozen, which have increased significantly over storage time. This rise revealed that the freezing temperature had significant effects on peroxide formation. Similar increases were reported for frozen fish and shrimp (Aydin and Gokoglu 2014). Lipid oxidation of seafood can produce rancid flavors and odors and, importantly, decrease nutritional quality and food safety through the formation of primary oxidation products that are rapidly transformed into secondary products. In the second stage, auto-oxidation peroxides are decomposed to aldehydes and ketones, which are known as secondary oxidation products (Choe and Min 2006). Usually, these products are detected by TBARS analysis, which has increased significantly in frozen meat of *P. segnis*. Our results indicated that lipid peroxidation increased with increasing storage time. A similar trend was observed for frozen silver catfish (Daniel et al. 2016) and pink salmon (Sathivel et al. 2007). This increase is a clear indication of oxidative deterioration that could be linked to the generation of reactive oxygen species. FFA levels in blue crab meat increased significantly and continuously over frozen storage time, indicating oxidative deterioration. This finding indicates that lipid oxidation is accelerated with prolonged storage up to 120 days. In line with our results, increases in FFAs levels in fish species have been observed following several freezing process (Tenyang et al. 2019). This was also confirmed by important negative correlations between the decrease in the total lipid and the increase in PV ($r = -0.695$), TBARs ($r = -0.700$), and FFAs ($r = -0.876$) (Table 4).

The formation of lipid-oxidized products during frozen storage is able to influence the protein solubility of fish (Hematyar et al. 2019). Actually, high AOPP levels were observed after 120 days of frozen storage, revealing protein deterioration at this stage. The modification of seafood muscle protein structure and/or integrity during freezing process has previously been associated with water content (Hematyar et al. 2019). In the current work, moisture (%) was relatively constant during freezing. This is probably due to the richness of crab meat on fat. Lipid may act as an insulator to prevent water from escaping from the intracellular space and consequently prevent water loss from the surface of the tissue. Usually, during freezing, water molecules migrate to form ice crystals; the organized H-bonding system that stabilizes the protein structure, especially the myofibrillar protein structure, would be disrupted, causing protein denaturation (Standal et al. 2018). Thus, the stability of protein content in *P. segnis* meat during frozen storage could be explained by the preservation of water content as a sign of slow formation of ice crystals.

Saturated fatty acids in blue crab meat increased significantly, while monounsaturated and polyunsaturated fatty acids decreased over the storage period. In general, the decrease in PUFA levels during freezing could be due to oxidative and hydrolytic reactions that occurred during frozen storage. The high unsaturation degree and long hydrocarbon chains of PUFA could make them more susceptible to hydrolytic reaction than SFA. In fact, the degradation of PUFA could generate low molecular weight compounds that may be included in the SFA. This could explain the opposite trend observed between SFA and PUFA variation over storage, which has been confirmed through the Spearman correlation matrix (Table 4). Indeed, it is important to note that PUFA play a significant role in oxidation processes, and their vulnerability to these reactions is greatly influenced by the stability of certain fatty acids, namely DHA and EPA, which are diminished during frozen storage. On the contrary, frozen crabs exhibited an increase in pro-inflammatory lipid compounds such as arachidonic acid (AA). These FAs have been reported to be involved in cellular inflammatory, cardiovascular, and metabolic diseases (Bejaoui et al. 2021). Overall, the observed changes in FA

Table 4. Spearman correlations between *P. segnis* biochemical parameters, lipid fractions, fatty acids, health lipid indices and oxidative markers at −30°C. Numbers in bold indicate significant correlations. Asterisks denote the significance level of the correlations: *$p < .05$; **$p < .01$; ***$p < .001$.

	Protein	Lipid	CHL	TAG	FFA	PL	TBARS	PV	FFAs	AOPP	SFA	MUFA	PUFA	*n*-3	*n*-6	*n*-3/n-6	PI	AI	TI	h/H
Protein																				
Lipid	.302																			
CHL	−.171	−.782***																		
TAG	.359	.851***	**−0.931*** **																	
FFA	−.342	−.901***	**0.872*** **	**−0.884*** **																
PL	−.223	−.950***	**0.844*** **	**−0.891*** **	**0.950*** **															
TBARS	−.351	−.695***	**0.630*** **	**−0.640** **	**0.808*** **	.778***														
PV	−.198	−.700***	**0.582** **	**−0.698** **	**0.612** **	.732***	**0.430*** **													
FFAs	−.271	−.876***	**0.767*** **	**−0.752*** **	**0.752*** **	.836***	**0.619*** **	.730***												
AOPP	−.153	−.140	0.025	−0.244	0.125	.173	0.208	.127	0.097											
SFA	−.270	−.959***	**0.844*** **	**−0.896*** **	**0.901*** **	.949***	**0.746*** **	.748***	**0.889*** **	.021										
MUFA	.349	.828***	**−0.829*** **	**0.814*** **	**−0.891*** **	−.849***	**−0.798*** **	−.646**	**−0.724*** **	−.154	**−0.904*** **									
PUFA	.392	.627***	**−0.412***	**0.483** **	**−0.616** **	−.622***	**−0.573*** **	−.710***	**−0.688** **	−.281	**−0.502** **	**0.540** **								
n-3	.284	.880***	**−0.962*** **	**0.924*** **	**−0.922*** **	−.901***	**−0.652*** **	−.629**	**−0.816*** **	−.325	**−0.919*** **	**0.886*** **	.460**							
n-6	−.182	−.926***	**0.796*** **	**−0.905*** **	**0.940*** **	.921***	**0.739*** **	.639***	**0.720*** **	.135	**0.891*** **	**−0.819*** **	−.690**	**−0.843*** **						
n-3/n-6	.161	.899***	**−0.866*** **	**0.889*** **	**−0.999*** **	−.949***	**−0.808*** **	−.613**	**−0.740*** **	−.033	**−0.901*** **	**0.892*** **	.620**	**0.921*** **	**−0.943*** **					
PI	.300	.906***	**−0.888*** **	**0.867*** **	**−0.923*** **	−.915***	**−0.651*** **	−.691**	**−0.779*** **	−.171	**−0.905*** **	**0.912*** **	.618**	**0.957*** **	**−0.857*** **	**0.923*** **				
AI	−.199	−.819***	**0.753*** **	**−0.770*** **	**0.847*** **	.757***	**0.785*** **	.540**	**0.722*** **	.254	**0.834*** **	**−0.903*** **	−.561**	**−0.803*** **	**0.851*** **	**−0.846*** **	−.813***			
TI	−.123	−.899***	**0.744*** **	**−0.858*** **	**0.894*** **	.862***	**0.792*** **	.645**	**0.754*** **	.281	**0.885*** **	**−0.826*** **	−.622**	**−0.800*** **	**0.959*** **	**−0.897*** **	−.800***	**0.925*** **		
h/H	−.100	.600***	**−0.921*** **	**0.820*** **	**−0.694** **	−.670***	**−0.380*** **	−.451*	**−0.609** **	−.104	**−0.638** **	**0.585** **	.537**	**0.841*** **	**−0.625** **	**0.686** **	.743***	**−0.532** **	**−0.552** **	

composition during frozen storage seem to be an evident outcome of the lipid peroxidation occurrence in *P. segnis* frozen meat.

Freezing process could also affect the lipid fractions of the frozen meat that are commonly known as phospholipids and reserve lipids. To the best of our knowledge, our current research is the first attempt elucidating the impact of freezing process on the lipid classes of crab species. TAG fraction was significantly reduced while CHL, FFA, and PL increased over storage time. The decrease in TAG levels in frozen crabs could be related to the hydrolysis process. The same trend was found in hake (*Merluccius merluccius*) and squid (*Illex argentinus*) during frozen storage for 120 days and 10 months, respectively (Paredi et al. 2006; Roldan et al. 2005). The hydrolysis of triglycerides by lipases is a well-known process that can result in the accumulation of FFA in the muscle tissue of fish (Sista et al. 1997). Similarly, CHL content showed a significant increase during the frozen storage process. This increase could be related to the alteration that may occur in the protein-cholesterol complexes of the sample tissue after freezing. According to Cornelius et al. (2015), protein-cholesterol complexes are considered as a major component of the unit membrane structure that are involved in the maintenance and stabilization of membrane permeability. The complexing of structural elements of the cell is affected by freezing process (Hunter and Madin 1978) and is easily prone to alteration due to the denaturation of proteins (Krishnamoorthy et al. 1979). Frozen storage may be responsible for protein-lipid complexes dissociation, where cholesterol is freed and could explain the observed significant increases in the CHL levels. Our findings corroborate with previous studies of Krishnamoorthy et al. (1979), who reported that cholesterol content increased in oyster and shrimp meat after the freezing process. Meanwhile, the increase of PL in crab meat could probably be attributed to the effect of storage conditions (time, T°C) on membrane lipid metabolism, which is responsible for phospholipid extractability enhancement (Saoussem 2000). Similar results were reported by Romotowska et al. (2016) for frozen Atlantic mackerel *Scomber scombrus* after 9 months of storage. Overall, the mechanism of lipid fractions prone to hydrolysis during frozen storage remains very complex.

Seafood, with a high *n*-3 PUFA content, well-balanced *n*-3/n-6, and PUFA/SFA ratios is important for human health since it supports protection against cardiovascular and inflammatory diseases (Dalle Zotte et al. 2013). The *n*-3/n-6 ratio was considered as a useful indicator for comparing relative nutritional values of marine product oils. Meanwhile, PUFA/SFA ratio has been suggested as a good indicator of marine fish oil with dietary PUFA (Nazemroaya et al. 2009). In the current study, *n*-3/n-6 ratio reduction during freezing indicated an important loss of beneficial PUFA. These results are similar to the findings of Risso and Amalia (2016) for the frozen crab *Lithodes santolla*. On the other hand, PUFA/SFA reduction during frozen storage is probably an indication of PUFA depletion and SFA enhancement. Although these latter ratios decreased during frozen storage, they remained at acceptable levels. According to the World Health Organization (WHO) recommendations, *n*-3/n-6 and PUFA/SFA ratios should be above 0.25 and 0.40, respectively (WHO/FAO 2003). EPA+DHA sum was higher in fresh crab meat and decreased during frozen storage. AI and TI take into account the different effects of particular fatty acids on human health perturbation (e.g. the incidence of pathogenic phenomena, such as atheroma and/or thrombus formation, etc.) (Rossano et al. 2005). In the present study, AI and TI levels in frozen crabs were higher than those reported for fresh crabs. It can be suggested that AI and TI increases during frozen storage are due to the loss of FA considered beneficial to human health and the increase of atherogenic FAs (e.g. myristic acid). This phenomenon was also observed in rainbow trout that was frozen at −15°C for 3 months (Chávez-Mendoza et al. 2014). Results showed that during storage, polyene index decreased significantly, which confirms lipid oxidation. This decrease could be explained by the reduction of DHA and EPA and an increase in palmitic acid concentration during frozen storage. The PI index represents the relationship between the FA composition of the tissue, the stability of PUFA, and their susceptibility to oxidation. A decrease in PI values during storage reflected the decomposition of PUFAs, which is responsible for primary (PV) and secondary (TBARs) oxidation product enhancement. Thus, the higher the PI value, the greater the protective potential for coronary artery disease (Wołoszyn

et al. 2020). The h/H index indicated the effects of specific fatty acids on cholesterol metabolism. In the current study, h/H was reduced significantly during frozen storage. From a nutritional stand point, this could be a sign of meat quality degradation since higher h/H values are considered more beneficial for human health.

Conclusions

This study highlights the possible impacts of frozen storage on the nutritional value of swimming crab *P. segnis* meat. Results showed that lipid deterioration occurred mainly after 30 days of storage and was closely related to progressive oxidation and enzymatic hydrolysis of UFA. It can be concluded that although freezing had an impact on the nutritional characteristics of *P. segnis*, this marine product remains an acceptable source of omega-3 fatty acids for up to 90 days of frozen storage. However, it would be better for human health to consume frozen crabs for up to 30 days as they retain a higher nutritional value and showed HLI values above standard limits recommended by the WHO. It would be of further interest to investigate the effect of other storage conditions (packaging, application of vacuum, antioxidants, oxygen absorber, glaze layer, etc.) on the nutritional quality of crabs. Based on the current findings, it is important to identify a suitable storage temperature/time for individual types of crab species, at which the quality and nutritional value is the most retained. Therefore, further research and technical developments are needed to elucidate the appropriate storage conditions specific to the characteristics of each seafood product.

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