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Effect of refrigerated conditions on lipid peroxidation and fatty acid composition of clams (*Venerupis decussata)* tissues

L'effet de la réfrigération sur la peroxydation lipidique et la composition en acide gras de la chaire de la palourde (*Venerupis decussata*)

S. BEJAOUI[1], F. GHRIBI[1], I. CHETOUI[1], FATMA AOUINI[1], NEJLA SOUDANI[1], M. EL CAFSI[1]

[1] Unit of Physiology and Aquatic Environment, Faculty of Science of Tunis, University of Tunis El Manar, 2092 Tunis, Tunisia.

*Corresponding author: safa.bejaoui@fst.utm.tn

Abstract – The effect of five refrigerating conditions (including fresh and stored lots at 15, 30, 90 and 180 days) on lipid peroxidation and fatty acid composition of clams (*Venerupis decussata*) tissues were investigated. All storage conditions reduced the moisture and lipid contents (p<0.05). Compared with fresh samples, the proportions of saturated (SFA) and monounsaturated (MUFA) storage increased (p<0.05), while polyunsaturated (PUFA) fatty acid content significantly decreased (p<0.05). This reduction was confirmed by a significant decrease in omega 3 content under all refrigerating conditions (p<0.01). Withal, Omega 6 increased after 90 and 180 days (p<0.05) but remained stable after 15 and 30 days of storage. Lipid peroxidation was observed for all stored samples when compared to the fresh ones. Results indicated that the effect of refrigeration on the fresh clams' has reduced their nutritional quality as revealed by decease in the EPA+DHA, atherogenicity, thrombogenicity indices and omega (3)/omega (6) ratio after storage process (p<0.05). Overall, it is recommended that clams should not be stored more than 15 days in the refrigerator (+4°C).

Keywords: Clams, Fatty acids, Nutritional quality indices, Thiobarbituric acid, Peroxide value

Résumé - Les effets de cinq conditions de réfrigération (incluant le lot frais et les lots conservés à 15, 30, 90 et 180 jours) sur la peroxydation lipidique et la composition en acide gras de la chaire de palourde (*Venerupis decussata*) ont été examinés. Toutes les conditions de stockage ont réduit le contenu lipidique et la teneur en eau (p<0.05). Les acides gras saturés (AGS) et monoinsaturés (AGMI) ont augmenté dans tous les échantillons conservés au réfrigérateur (p<0.05), tandis que le contenu des acides gras polyinsaturés (PUFA) a significativement diminué comparé aux échantillons frais (p<0.05). Cette réduction a été confirmée par une diminution significative des teneurs en oméga 3 dans toutes les conditions de réfrigération (p<0.01). En outre, les teneurs en oméga 6 ont augmenté après 90 et 180 jours (p<0.05), mais sont resté stable après 15 et 30 jours de conservation. La peroxydation lipidique a été observée pour tous les échantillons stockés par rapport aux échantillons frais (p<0.05). Les résultats indiquent que l'effet de la réfrigération sur les palourdes fraîches a réduit leur qualité nutritionnelle comme le révèle une diminution importante du rapport EPA + DHA, oméga (3) / oméga (6), d'athérogénicité et de thrombogénicité après le stockage. En général, il est recommandé de ne pas conserver les palourdes plus de 30 jours au réfrigérateur (+ 4 ° C).

Mots clés : Palourdes, Acides gras, Indices de la qualité nutritionnelle, acide thiobarbiturique, Indice de peroxyde

1. Introduction

Seafood, such as invertebrates, is good for human health because there are considered as healthy food, safe, nutritious and balanced diet (WHO 2003). Bivalves are low in saturated fats and rich in polyunsaturated n-3 fatty acids which are known to decrease the risks of coronary heart disease and cancer and to improve the response to inflammatory diseases, like eczema, psoriasis and rheumatoid arthritis (kristensen et al. 2016).

Bivalves are a potential source of nutrition compounds which undergrad major changes during refrigeration condition. In this context, refrigeration is the most usual system of fixing seafood for short storage period, permitting a good preservation of their nutritive value (Ojagh et al. 2010). Storage time is the major factors implicated in the deterioration of tissues life and has been known to affect the chemical composition and quality of clams' processed (Al-Bulushi et al. 2013). Among different mechanisms responsible for degradation during storage at +4°C, it is marked that polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have evidently altered more than linoleic acid and a-linolenic acid (Calder 2013). Several studies mentioned the reduction of the nutritional quality in seafood associate with the development of secondary products indicating lipid deterioration during refrigeration (Tenyang et al. 2017). In this context, lipid deterioration is one of the main preventive causes for the nutritional quality food products. During oxidation process, discoloration, drip loss, off- odor, off-flavor changes and the production of potentially harmful products were established (Alotaibi and Tahergorabi 2018). For storage of seafood, storage processes can stimulate lipid oxidation by modifying cell membranes and releasing pro-oxidants, thus lowering their nutritional quality (Benjakul et al. 2005).

The Mediterranean clam *Venerupis decussata* (*V. decussata*) is a bivalve of the veneridae family, which contains approximately 500 species and is extensively recorded in marine waters. This species is found along Mediterranean Sea, East Atlantic coasts, North and red seas and from Norwegians to Senegalese coasts. *V. decussata* is highly appreciated by local consumers and like many marine bivalves this species is characterized by an important nutritional value that is beneficial for human consumption (Ngo et al. 2012).

Indeed, previous work has shown that this species is a good source of omega-3 fatty acids, in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which play an important biological role in human health (Ojea et al. 2004). Fundamental knowledge about the nutritional quality of *V. decussata* under refrigerated condition is still lacking and these would be essential to facilitate the marketing and the consumption of this seafood.

This study describes for the first time the effect of refrigerated storage during 15, 30, 90 and 180 days at (+4 °C) on the amount of lipids and fatty acid composition of *V. decussata*. Our main objective is to study the variation of fatty acids after storage in the refrigerator and to evaluate the lipid degradation in clam tissues during this preservation process using thiobarbeturic acid (TBARS) and peroxide value (PV) as markers.

2. Materials and Methods

2.1. Sample preparation

One hundred specimens of similar market size of clams (Weight: 12.01 ± 1.02g; Length: 39.9 ± 2.35 mm) were purchased from the shellfish farmers of Bizerte (S.M.C.B) in the Bizerte lagoon (Northern Tunisia) during February 2016. Clams were immediately transported to the laboratory in ice box than washed and cleaned to remove the extraneous materiel. Twenty individuals were used fresh while the remaining samples were packed into alimentary plastic bags, labeled then refrigerated at +4°C. Chemical analyses were determined at 0 days and after 15, 30, 90 and 180 days for stored samples. For each storage condition, ten individuals whole tissues were extracted using Tris-HCl buffer (20mM; pH=7.4) then stored at -80°C until further analysis. Also, ten individuals were pooled and conserved at -80^{0}C until fatty acids analysis.

2.2. Chemical composition

Moisture and lipids contents of fresh and stored clam were measured in ten replicates for each condition. Moisture content (%) was measured by determination of weight difference before and after heating during 24h at 105°C as described by AOAC (2005). Lipid determination (mg. g^{-1} ww) was based on the method of Folch et al. (1957) using chloroform-methanol (2v/1v) as an extraction solvent.

2.3. Fatty acids analysis

Lipids were esterified according to the method mentioned by Cecchi et al (1985). Methyl nonadecanoate C19:0 (Sigma Aldrich) was added as an internal standard. Fatty acids separation was carried out using a HP6890 chromatograph (Agilent Technologies, Santa Clara, CA) coupled with INNOWax capillary column and a spilt/splitless injector equipped with a flame ionization detector at 275°C. Nitrogen was the carrier gas. Fatty acids peaks were integrated using HP chemstation software (CPG Agilent 7890A) and identified by comparison to a standard chromatogram (Menhaden Oil, Sigma, SUPELCO). The results were expressed as mg. g^{-1} ww.

2.4. Nutritional quality indices

Fatty acids ratios and sums were determined in our present study to evaluate the nutritional quality of fresh and storage tissues. The Atherogenicity (AI) and Thrombogenicity (TI) indices were giving by Ulbricht and Southgate (1991) and defined through equations (1) and (2):

(1) AI= ((4×myristic acid) + palmitic acid+ stearic acid) / (∑ monoinsaturated fatty acids + ∑ omega 6 + ∑ omega 3).

(2) TI= (myristic acid + palmitic acid+ stearic acid) / ((0.5× monoinsaturated fatty acids) + (0.5 × omega 6) + (3× omega 3) + (omega 3 / omega 6).

EPA + DHA and omega 3/ omega 6 were also determined according to Unusan (2007) and Marques et al (2010) respectively.

2.5. Lipid peroxidation measurement

2.5.1. Thiobarbituric acid (TBARS)

Thiobarbituric acid (TBARS) was determined according to Draper and Hadley (1990) by measuring the amount of malondialdehyde (MDA). Briefly, supernatant was mixed with 1 mL of trichloroacetic acid (30%) and centrifuged at 3500×g during10 min. Then, 1 ml of thiobarbituric acid solution (0.67 %; pH: 7,4) was added to 1 ml of supernatant and boiled at 100°C for 10 min. The absorbance was measured at 532 nm. A standard solution was used by 1,1,3,3-tetraethoxypropane and results were expressed as nmol. g^{-1} ww.

2.5.2. Peroxide value (PV)

Peroxide value was performed based on the AOCS (1989) method. Supernatants were mixed with chloroform and glacial acetic acid. Change of the coloration was obtained after addition of 200µl of potassium iodide solution. After agitation, the PV was measured through titration with sodium thiosulfate solution (0.1N) in presence of starch solution (1ml). Results are expressed as ml.g^{-1} and defined through equation (3) as follow:

PV= (((sample titration - blank titration) / thiosulfate molarity) × 1000) / samples weight.

2.6. Statistical analysis

All analyses were performed in ten replicates. Statistical analyses of variance and Tukey HSD tests by Statistica (8 version) were carried out to determine the degree of difference between fresh and the others storage conditions. *P* values at 0.05 was considered as statistically significant.

3. Results and discussion

3.1. Proximate composition

The moisture and lipids contents in *V. decussata* are shown in Fig.1. Initially, fresh tissues had higher moisture percent (85%) as compared to the stored samples. A significant decline in the moisture content was observed in stored clams after15 ($p<0.05$), 30 ($p<0.01$), 90 and 180 days ($p<0.001$).The amount of lipid varied considerably after storage and was higher in fresh samples (27.05 ± 5.74 mg.g^{-1}ww), but it was significantly decreased by 19%, 29%, 27% and 43% after 15, 30, 90 and 180 days of storage respectively (Figure 1).

The decrease in the moisture content has been described as the most prominent change that could occurred after storage and which is directly responsible of lipid reduction. Our results corroborate with

the finding of Gupta et al (2015) who related the low moisture and lipids contents of freshwater catfish *Wallago attu* to the storage period. Aberoumand (2013) reported that moisture and lipids content in the muscle tissues of four fish species (*lizadussmieri*, *sparidae*, *sciaenide* and *platycephalidae*) have characterized by a great decrease of the previous compounds during storage conditions.

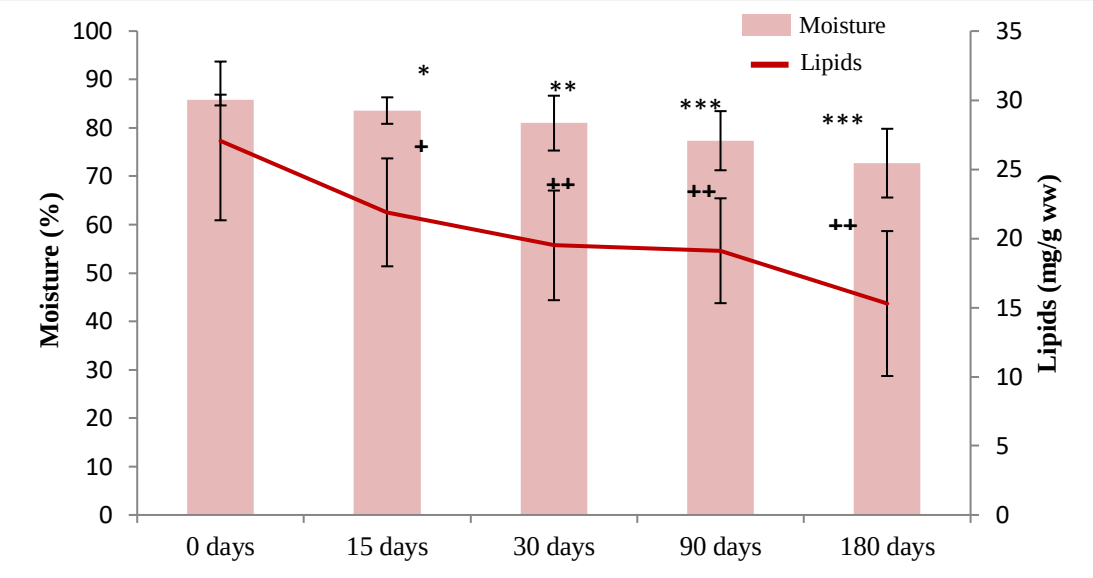

Figure 1. Proximate composition (moisture and lipids) in fresh and refrigerated clams

Significant differences between fresh and storage tissues were presented by asterisk (*<0.05; **<0.01; ***<0.001) in moisture and single crochet ($^{+}$<0.05; $^{++}$<0.01) in lipids.

3.2. Fatty acids profiles

The fatty acids profile of fresh and stored clams' is shown in Table 1.

The total fatty acid (TFA) profile of fresh clams was dominated by polyunsaturated fatty acid (PUFA) (35.24 ± 1.03 mg /g ww) followed by saturated (SFA) (19.85 ± 2.08 mg /g ww) and monounsaturated (MUFA) (14.05 ± 0.40 mg /g ww) fatty acids. These findings are in agreement with those obtained by Ojea et al. (2004) for the fatty acid composition of fresh *V. decussata* collected from Baldaio lagoon in Spain.

Significant changes in the fatty acid content occurred during 15, 30, 90 and 180 days of storage. Furthermore, n-3 and n-6 PUFA seemed to be the most influenced fractions by refrigerated storage especially the eicosapentaenoic (EPA), docosahexaenoic (DHA) and arachidonic (ARA) acids. For n-3 PUFA proportion, a significant decrease by 33%, 50%, 65% and 73% was observed in all refrigerated samples after storage during 15, 30, 90 and 180 days, respectively. The decrease in *V. decussata* stored samples seemed to be directly affected by the significant reduction of EPA and DHA contents (p<0.05). Whereas, n-6 PUFA showed an opposite variation characterized by an important increase during 90 (by 70%) and 180 (by 73%) days of storage when compared to the fresh tissues. This increase in n-6 PUFA proportion during 90 and 180 days of storage was followed by a significant increase of arachidonic (C20:4n-6) and linoleic (C18:2n-6) acids.

Tenyang et al (2017) have reported that n-3 PUFA content in the muscle of the catfish (*Arius maculatus*) reduced after several refrigeration times. Moreover, our results of n-6 PUFA were in agreement with the previous report of Simat et al (2014) who have found an increase of n-6 PUFA content in the conserved muscle of the fish *Boops boops* at 1°C for 18 days. Those authors have demonstrated that storage period induce an elevation of the ARA and C18:2n-6 contents.

In *V. decussata* fresh tissues, palmitic acid (C16:0; 12.22 mg/g ww) and stearic acid (C18:0; 5.90 mg/g ww) were the predominant among SFA. While, palmitoleic acid (C16:1; 2.30 mg/g ww), oleic acid (C18:1; 4.92 mg/g ww) and gadoleic acid (C20:1; 6.46 mg/g ww) were the main among MUFA. During storage significant increase was observed for C16:0, C18:0, C16:1, C18:1 and C20:1 especially after 30,

90 and 180 days ($p<0.05$). Similarly, Ozogul et al (2017) reported an increase of SFA and MUFA contents in the fish *Engraulis encrasicolus* under refrigerating conditions. Generally, previous studies dealing with the effect of refrigeration process on marine products concerned mainly fish and crab species (Pirini et al. 2000; Risso and Carelli 2017). Studies concerning the effect of refrigeration process on the fatty acid composition of stored bivalve's species are lacking.

It is well known that marine products with a high level of unsaturated fatty acids are considered as a healthy food in many dietary regimes (Ghribi et al. 2018). While, the higher saturated fatty acid content may increase the risks of cardiovascular diseases (Briggs et al. 2017). We suppose that the high level of SFA found on the stored tissues of *V. decussata* above 15 days may cause several health issues for clam consumers.

Table 1. Fatty acid profiles ($mg.g^{-1}$ ww) of fresh and refrigerated clams' tissues at 0; 15; 30; 90 and 180 days.

	0 days	15 days	30 days	90 days	180 days
C14 :0	0.719 ± 0.191	1.325 ± 0.494	1.169 ± 0.215*	2.212 ± 0.258***	2.225 ± 0.577**
C15 :0	0.804 ± 0.253	0.622 ± 0.086	3.940 ± 0.375***	3.714 ± 0.331***	3.342 ± 0.667**
C16 :0	12.221 ± 1.524	16.836 ± 1.571*	19.293 ± 3.009*	20.924 ± 1.675**	22.965 ± 1.768***
C 18:0	5.903 ± 0.790	6.494 ± 0.533	8.162 ± 0.668**	12.245 ± 2.203**	15.307 ± 0.659***
C20 :0	0.163 ± 0.069	0.538 ± 0.091**	0.595 ± 0.061***	0.514 ± 0.027**	0.457 ± 0.193*
C22 :0	0.044 ± 0.009	0.026 ± 0.005*	0.033 ± 0.005	0.015 ± 0.007**	0.025 ± 0.007*
SFA	19.856 ± 2.081	25.834 ± 2.323*	33.195 ± 2.481**	39.457 ± 0.528***	44.172 ± 1.577***
C15 :1	0.214 ± 0.056	0.453 ± 0.006***	0.760 ± 0.032***	1.095 ± 0.092***	1.487 ± 0.038***
C16 :1	2.309 ± 0.972	3.259 ± 0.474	5.358 ± 0.412**	6.092 ± 0.681**	8.546 ± 0.744***
C18 :1	4.926 ± 0.552	7.964 ± 0.635**	9.106 ± 1.136**	15.413 ± 0.796***	16.671 ± 1.367***
C20 :1	6.467 ± 1.860	9.091 ± 0.312*	6.981 ± 0.568	9.786 ± 1.566*	2.103 ± 0.864*
C22 :1	0.136 ± 0.061	0.369 ± 0.058*	0.208 ± 0.039	0.541 ± 0.022**	1.225 ± 0.343
MUFA	14.054 ± 0.401	21.015 ± 0.298***	22.417 ± 1.732***	27.610 ± 6.766*	29.539 ± 1.647***
C16 :2	0.881 ± 0.195	0.591 ± 0.104*	1.094 ± 0.501	1.332 ± 0.639	1.082 ± 0.234
C16 :3	1.931 ± 0.405	0.612 ± 0.057*	0.331 ± 0.088**	0.806 ± 0.189**	0.425 ± 0.163**
C16 :4	2.529 ± 0.422	0.355 ± 0.077**	0.271 ± 0.099***	0.514 ± 0.001**	0.388 ± 0.037***
C18 :2n-6	0.746 ± 0.088	1.215 ± 0.076**	1.478 ± 0.991	2.051 ± 0.388**	5.688 ± 0.583***
C18 :3n-6	0.451 ± 0.082	0.576 ± 0.067	0.534 ± 0.053	0.883 ± 0.178**	1.115 ± 0.087***
C18 :3n-3	1.157 ± 0.141	0.961 ± 0.211	0.817 ± 0.153*	0.937 ± 0.217	0.245 ± 0.014***
C18 :4n-3	1.098 ± 0.069	1.342 ± 0.284	1.072 ± 0.533	0.922 ± 0.184	1.062 ± 0.507
C20 :2n-6	1.188 ± 0.405	1.114 ± 0.463	1.208 ± 0.378	1.560 ± 0.290	0.516 ± 0.100*
C20 :3n-6	1.207 ± 0.274	1.469 ± 0.904	2.473 ± 0.416*	2.899 ± 0.327**	0.633 ± 0.137*
C20 :4n-6	1.485 ± 0.739	2.196 ± 0.553	2.306 ± 0.240	3.136 ± 0.530*	3.515 ± 0.534**
C20 :3n-3	1.044 ± 0.091	0.086 ± 0.003***	0.080 ± 0.011***	0.029 ± 0.006***	0.062 ± 0.017***
C20 :5n-3	5.377 ± 1.179	3.603 ± 0.208*	2.097 ± 1.014*	1.462 ± 0.386**	1.483 ± 0.431**
C22 :2i/2j	2.540 ± 0.172	2.255 ± 0.464	2.829 ± 0.806	2.031 ± 0.797	1.709 ± 0.635*
C22 :2n-6	1.089 ± 0.233	1.174 ± 0.418	1.128±0.148	1.714 ± 0.109*	1.685 ± 0.409*
C22 :5n-6	1.835 ± 0.450	1.967 ± 0.140	1.171 ± 0.199*	2.991 ± 0.004*	1.163 ± 0.379
C22 :5n-3	2.111 ± 0.306	1.491 ± 0.115*	0.724 ± 0.046***	0.667 ± 0.116***	0.417 ± 0.157***
C22 :6n-3	8.567 ± 0.500	6.003 ± 0.584**	4.787 ± 0.460***	2.620 ± 0.363***	1.800 ± 0.446***
PUFA	35.243 ± 1.033	26.169 ± 4.359*	23.586 ± 5.023**	24.822 ± 2.764**	22.997 ± 0.534***
Omega (3)	19.356 ± 0.981	12.962 ± 1.227**	9.580 ± 1.822***	6.641 ± 1.126***	5.071 ± 1.172***
Omega (6)	8.004 ± 1.904	9.714 ± 2.247	9.478 ± 2.533	13.667 ± 3.476*	14.318 ± 0.609**

Significant differences between fresh and storage tissues were presented by asterisk (*<0.05; **<0.01; ***<0.001).
SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid.

3.3. Nutritionnel quality indices

Fatty acids that are highly unsaturated abundantly found in refrigerated tissues were easily prone to oxidation and due to changed effects of fatty acids on health; it is essentially to describe the nutritional quality index (NQI). Table 2 shows the nutritional quality indices defined by omega (3)/ omega (6) ratio, EPA+DHA sum, AI and TI indices in fresh and stored clams.

The proportion of omega (3)/omega (6) decreased significantly ($p<0.05$) in all stored samples compared to the fresh ones. The UK Department of Health recommends a minimum omega 3/omega 6 ratio of 0.25. During our study, the obtained n-3/n-6 ratios in fresh and stored *V. decussata* agreed with the

recommendation set for this ratio and were even higher in fresh clams. This recommended value was lower than the values obtained in our study which can be a good index for combining the relative nutritional quality of clams as demonstrated in previous marine species (Senso et al. 2007). Similar to our outcomes, Risso and Carelli (2017) observed a significant reduction in this ratio in the refrigerated meat of crab (*Lithodes satolla)* after 10 days as compared to the raw one, suggesting that the higher ratio was a great importance in prevention of coronary hearth and cancer risks.

Refrigerated tissues showed a tendency towards a long storage period (Table 2) in the sum of EPA+DHA. A significant decrease in this sum, in stored samples during 15, 30, 90 and 180 days (9.60 $mg.g^{-1}$ ww; 6.88 $mg.g^{-1}$ ww; 4.08 $mg.g^{-1}$ ww and 3.28 $mg.g^{-1}$ ww respectively) was observed when compared to fresh clams (13.94 ± 0.90 $mg.g^{-1}$ ww). It has been suggested that consuming EPA and DHA may decrease the possibility of cardiovascular disease in persons who have previously qualified a cardiac event (Kris-Etherton et al. 2002). Our results corroborated with previous finding of Simat et al. (2014) performed on bogue fish storage during 18 days.

The nutritional quality of stored clams was also defined through AI and TI indices. Results showed significant increase of AI and TI indices after refrigerated storage to be even higher by 60% and 100% during 90 and 180 day. These values are in agreement with the previous reports of Pirini et al. (2000) carried on refrigerated *Dicentrarchus labrax.* It must be noted that the nutritional quality of *V. deccusata* investigated through this study has undergo significant changes during the kinetic refrigeration period. To sum up, all the studied indices showed an important variation during refrigerated storage indicating a considerable modification on the nutritional quality of clams during this process.

Table 2. Nutritional value of refrigerated and fresh clams at +4°C

	0 days	**15 days**	**30 days**	**90 days**	**180 days**
Omega (3) / Omega (6)	2.520 ± 0.650	1.360 ± 0.176*	1.043 ± 0.225*	0.525 ± 0.241**	0.356 ± 0.092**
EPA+DHA	13.944 ± 0.902	9.607 ± 0.791**	6.885 ± 1.131***	4.083 ± 0.746***	3.284 ± 0.837***
AI	0.015 ± 0.000	0.036 ± 0.006**	0.052 ± 0.016**	0.057 ± 0.020*	0.064 ± 0.006***
TI	0.255 ± 0.040	0.413 ± 0.008**	0.569 ± 0.028***	0.775 ± 0.109***	0.889 ± 0.112***

Significant differences between fresh and storage tissues were presented by asterisk (*<0.05; **<0.01; ***<0.001).

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; AI: atherogenicity; TI: thrombogenicity.

3.4. Lipid peroxidation

Figure 2 illustrates the changes in lipid peroxidation in clams' tissues during refrigeration process (+4°C). The level of TBARS increased significantly from 12.70 ± 1.95 nmol. g^{-1} ww to 14.41 ± 1.16 nmol. g^{-1} ww (after 15 days); 16.61± 2.25 nmol. g^{-1} ww (after 30 days); 19.12 ± 2.27 nmol. g^{-1} ww (after 90 days) and 21.94 ± 3.51 nmol. g^{-1} ww (after 180 days). However, PV increased considerably by 24%, 42%, 130% and 144% during 15; 30; 90 and 180 days of storage respectively. The preservation of clam tissues in the refrigerator at (+ 4 ° C) can lead to the oxidation of PUFA which is most often the cause of the deterioration of its nutritional value (change of color and smell etc.) (Tenyang et al. 201). High PV and TBARS levels in stored samples can be attributed to decreases in PUFA content since these fatty acids are sensitive to oxidation and therefore reflect a higher degradation of peroxides as secondary oxidation products, resulting in a decrease of their nutritional quality. Such increases during shorted refrigerated condition for 9 days at +4°C have been reported by Erkan and Ozkan (2006). Duration of storage had a significant effect on several chemical indices (TBARS, PV, TMA…) of *Sardine pilchardus* muscle. Similar increases in PV and MDA during refrigeration at + 4 ° C after 150 days have been reported by Boran et al. (2006) in several fish species (garfish, golden mullet, shad and horse mackerel). Similarly, Erkan and Ozkan (2006) reported similar changes in *Sardina pilchardus* muscle during 9 days storage at + 4 ° C. It can be concluded that the conservation of fresh clams in the refrigerator has favored the degradation of lipids in their tissues over time.

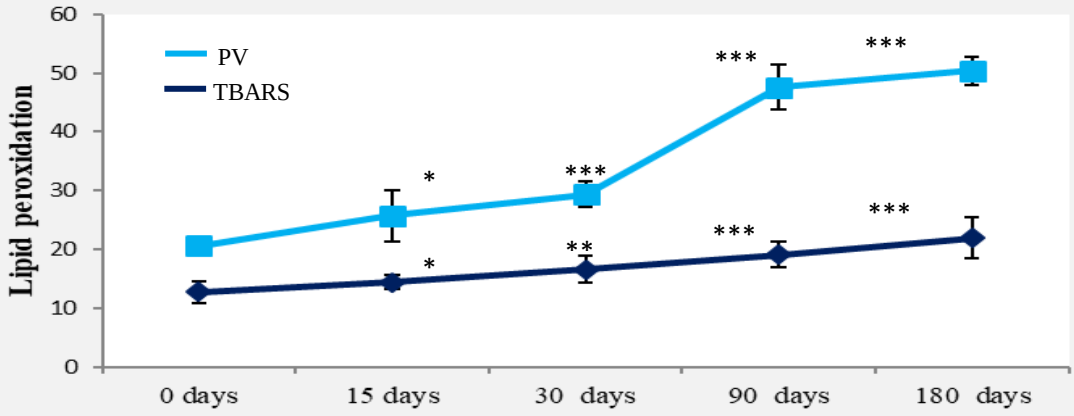

Figure 2. Changes in lipid peroxidation indices during kinetic refrigerated condition at +4°C

Significant differences between fresh and storage tissues were presented by asterisk (*<0.05; **<0.01; ***<0.001).

4. Conclusion

The effect of refrigerated storage on moisture, lipids, fatty acids profile and the nutritional quality of the commercial clam (*V. decussata*) were investigated during the current study. It was found that storage conditions and different duration had considerable effects on the nutritional value of this species. Clam tissues stored at +4°C showed lower moisture content, PUFA proportion, in particularly EPA and DHA and higher SFA content when compared to fresh clams. The analysis of the oxidation degree by the use of TBARS and PV showed that lipid damages occurred during all refrigerated storage periods. Generally, changes that occurred in the composition of fatty acids in stored clam tissues for more than 15 days were consistent with the mechanism of lipid peroxidation. Overall, it is recommended that clams should not be stored more than 15 days in the refrigerator (+4°C). Indeed, further investigations on the effect of refrigerated storage on other biochemical constituents (protein, vitamins, minerals etc.) in clam tissues are strongly recommended.

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