EFFECTS OF MARINATING PROCESS ON MUSSELS PHYSICOCHEMICAL AND MICROBIOLOGICAL QUALITY ATTRIBUTES DURING REFRIGERATED STORAGE

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RESUME

En Tunisie, la moule *Mytilus galloprovincialis* est un bivalve peu consommé par les tunisiens à cause du manque de conscience de sa valeur nutritive. Le but de cette étude est d'améliorer l'image de ce produit sur le marché en utilisant une nouvelle méthode de conservation à savoir le marinage. Durant cette étude, le procédé de marinage utilisant l'acide acétique, le sel et l'huile végétale a été adopté afin de prolonger la durée de vie du produit tout en conservant sa qualité nutritionnelle. L'effet synergique des différents composants de marinade (acide acétique, le sel et l'huile végétale) a été évalué sur la qualité physico-chimiques (pH et l'activité de l'eau), microbiologique, chimique, (ABVT, TBA) et biochimique des moules marinées emballées sous vide et conservées 15 jours dans la glace à 4 ° C. Les résultats obtenus pour les moules marinées ont été comparés avec les résultats d'un lot contrôle de moules. Les teneurs en Azote Basique Volatil totale (ABVT) et en acide thiobarbiturique (TBA) ont été en dessous des valeurs limites seuils d'acceptabilités (25mg / 100g et 3 mg MDA / kg) au cours de la période de stockage. Les teneurs en ABVT et en TBA étaient de 14,3 ± 0,006 mg/ 100g et 0,2 mg MDA / kg respectivement pour le lot de contrôle et 14,12 ± 0,02 mg / 100g et 0,3 mg MDA / Kg pour lot mariné. Le nombre de la flore mésophyle totale des moules marinées (3,21 log UFC / g) était significativement plus faible par rapport au moules témoins (4,14 log UFC / g). Les analyses effectuées ont déterminé une DLC de 15 jours pour les deux lots avec une détérioration de la qualité nutritionnelle nettement plus lente pour le lot mariné.

***Mots clés**:* Moules marinées, *Mytilus galloprovincialis*, Composition biochimique, Qualité microbiologique, Qualité physico-chimique

ABSTRACT

In Tunisia, mussels *Mytilus galloprovincialis* are not familiar seafood because of cultural influence and convenience aspects. The aim of the present study is to develop a ready to eat seafood product as a novel variety of mussel. Marinating process using acid, salt and vegetable oil was chosen in order to achieve a longer shelf life, to improve taste and to maintain nutritional quality of the product. Synergistic effect of different pickling agents (acetic acid, salt and vegetable oil) was evaluated on physicochemical (pH and water activity), microbiological, chemical (TVB-N and TBA) and biochemical (water, ash, proteins, lipids and carbohydrates) quality of marinated mussels *Mytilus galloprovincialis* during vacuum-packaged storage during 15 days at 4°C. Obtained results of marinated mussels samples were compared with results of a control mussels samples. Total Volatile Basic Nitrogen (TVB-N) and Thiobarbituric acid (TBA) values were determined below the thresholds of acceptability limits values (25mg/ 100g and 3 mg MDA /kg respectively) during the storage period. TVB-N and TBA values were found as 14.3 ± 0.006 mg / 100g and 0.2 mg MDA/ Kg respectively for control mussel and 14.12 ± 0.02 mg / 100g and 0.3 mg MDA / Kg for marinated mussel. Total mesophilic flora for marinated mussels (3.21 log CFU / g) had significantly lower level compared to control group (4.14 log CFU/ g). Analysis evaluated a shelf life of 15 days for both batches with a significantly slower deterioration of the quality for the marinated batch.

***Key words**:* Marinated Mussels, *Mytilus galloprovincialis*, Biochemical content, microbiological quality, physicochemical quality

INTRODUCTION

In Tunisia, shellfish farming is mostly limited to the lagoon of Bizerte, located in the North of the country. Presently, this production is valued at an average of 1635 tons (DGPA, 2015). Because of several aliases such as the increase of water temperature in summer, significant mortality of livestock is observed (ie, current year) especially for mussels *Mytilus galloprovincialis*, causing great economic losses for producers. Furthermore, in some periods of the year, alerts of biotoxins prohibit harvesting, marketing and consumption of the product. On the other hand, when mussels are normally produced, they are exclusively sold fresh for limited market such as superstores and restaurants (DGPA, 2015). As an alternative of a breakthrough, could be processing unsold mussels during high production turning it into convenient seafood with various applications and many new marketing opportunities. The development and application of innovative and efficient processing and preservation techniques are essential to produce high

added and nutritional value products (Aveiro et al., 2007).

Such practice would not only ensure marketing and large distribution of the product during the periods of high and low mussel's production, but also encourage its wider aquaculture off the lagoon, in the open sea for a regular market supply. Mussels have high nutritional values making them ideal nutrients in human diet. Consumption of this bivalve helps to provide polyunsaturated fatty acids with known health beneficial effects, including essential vitamins, proteins with high biological value as well as minerals (Orban et al., 2002). This product is also known as a shellfish product with low fat and cholesterols contents (Erkan, 2005). Processed products, however must, fulfil the requirements of consumers, to be attracting, easy to prepare and good to be tasted.

Taking into account all cited considerations, and within SecurAqua project; the INSTM research group focused efforts to elaborate added-value product using mussels from the lagoon of Bizerte.

Numerous studies (Dalgiç & Erkoyuncu 2003; Kyriazi-Papadopoulou et al., 2003; Şengör et al., 2004; Goulas et Kontominas, 2005,; Ozgul & Balikci, 2013; Maktabi et al., 2015) have been done on various processing methods in order to preserve the nutritional quality of the seafood products while improving their shelf life.

Marinating is among technique used in food preserving. This technique is based on the treatment of the product with various solutions using salt, spices, lemon's juice, and numerous other compounds as pickling agents in order to ameliorate the organoleptic properties and tenderize the texture and structure of the product (Yashoda et al., 2005). Moreover, marinating reduces the bacterial and enzymatic activity, contributes to the improvement of sensory qualities of the product and ensures its extended but limited shelf life (Sallam et al., 2007).

Generally, the marinated seafood are treated with acids (acetic acid), sugar, salt, spices and oil in order to improve tenderness, juiciness and the flavour of the product's flesh (Hwang & Tamplin, 2005). Several studies were interested in the evaluation of the effect of the marinades on fishes' nutritional and microbiological quality (Goulas et Kontominas, 2005; Kilinc & Cakli, 2005; Cadun et al., 2008), however limited studies were carried out concerning marinated bivalves especially mussels.

In this work an interest was given to the evaluation of the effect of a mixture of salt, acetic acid and vegetable oil on the microbiological, physicochemical, and biochemical quality characteristics of the marinated mussels *Mytilus galloprovincialis* during vacuum-packaged storage during 15 days at 4°C

MATERIALS AND METHODS

Collection and preparation of the samples

Fresh mussels weighing about 8 kg were provided from shellfish company located in the lagoon of Bizerte, North Tunisia. On arrival to the laboratory, mussels were well cleaned, and byssi's thread was removed. All specimens were kept at 4°C until further processing.

Marinating process

Following shell cleaning, depurated mussels were cooked until all shells were open (5 to 10 minutes of cooking). Mussels which remained closed were eliminated (Turan et al., 2006). After cooking, marinating process were performed. The different steps of the marinating process are presented in table I

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Table I: Marinating process applied to the mussel *Mytilus galloprovincialis*

Steps	Marinating process	
Step 1	Cooking and collecting mussel's meat	
Step 2	Dividing mussels in 2 batches	
	Batch 1: control mussels	Batch 2: marinated mussels
Step 3	No application of the marinating process.	Application of the marinating process: - Adding salt - Rinsing with vinegar - Soaking in vegetable oil
Step 4	Sterilization: at 121°C for 20 min	
Step 5	Storage under vacuum packaging and conservation for 15 days in ice at 4°C	

During the storage period, samples were taken for analyses every three days (day 0, 3, 6, 9, 12 and 15) from control and marinated batch. For each sample, three bags of vacuum-packed mussels (n=30g/ bag) were taken and samples were well homogenised and preserved at -80°C for chemical analyses. Microbiological analyses, water activity and pH determination were performed the same day of the sampling.

Physical and chemical analysis

Water activity pH and measurement

During this study, pH value of mussel's meat was measured using pH indicator paper CARLO ERBA. The water activity (Aw) was measured using the LabSwift-aw system novasina (The art of Precision Measurement).

Microbiological analysis

Microbiological quality was evaluated according to the method of Harrigan and McCance (1976). For each sample, 10g were taken and homogenized in 90 ml peptone water. Decimals dilutions were prepared from 10^{-1} dilution. Total viable count was determined using Plate Count Agar as the medium. Plates were incubated at 26°C for 24-48 h. Analysis was made in triplicate in each sample.

Chemical analysis

The total Volatile Basic Nitrogen (TVB-N) content was determined according to the method of Ruiz-Capillas & Horner (1999) using the system of « Flow injection analysis » (FIA). Thiobarbituric acid (TBA) was evaluated using the method of Genot (1996). TBA values were expressed in units of mg/malonaldehydre /Kg sample.

Proximate composition

Evaluation of water and ash content was performed according to the method of AOAC (1990) Water content was determined at 105°C until a constant weight was obtained. Ashes were evaluated by ignition of the dry sample in an oven at 550°C during 6 hours. Total proteins content was determined according to the method of Lowry modified by Hartree (1972) and lipid content was determined by the method of Folch *et al.* (1957). All of these methods were submitted to harmonisation with the procedures used in CUPT (Consortium Universitaire de Trapani) laboratory within BIOVecQ project.

Statistical analysis

All obtained results were reported as Mean ± SD. One way ANOVA test followed by the least significant difference test (LSD) in the statistical software program SPSS were used to evaluate any significant difference ($p< 0.05$). The software used was SPSS version 17.0.

RESULTS AND DISCUSSION

Evaluation of pH and water activity in control and marinated mussels samples during the storage period at 4°C

High pH level in food is one of the main spoilage indicators in marinated products. The increase of pH levels, because of the nitrogenous compounds production by bacteria, indicates the loss of quality of the product and its spoilage (ICMSF, 1993).

The evolution of pH values in the control and marinated mussels samples during the refrigerated storage period is summarized in Figure 1**.** Initial pH value obtained for cooked mussels was 7.5; this result is compared to data reported by Turan *et al.* (2008) for boiled mussels.

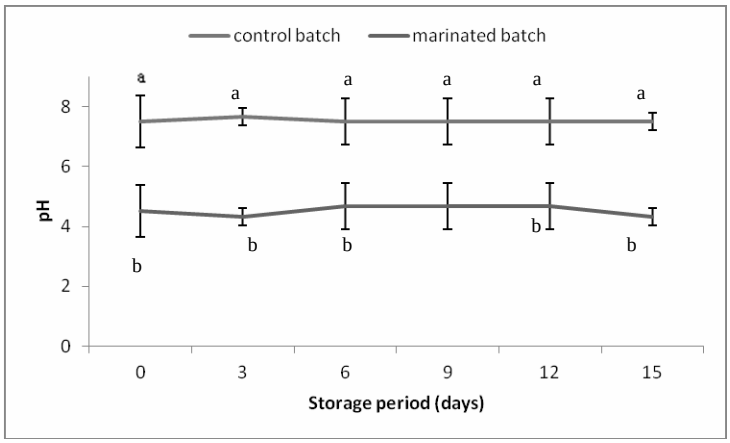

Figure 1: Evolution of pH value in control and marinated mussels samples stored under vacuum packaging during 15 days at (4°C). Bar = standard error; n= 6 in each case; different letters= mean values for each group are significantly different ($p<0.05$).

However, marinated mussels' flesh showed a significant lower pH values compared to those obtained for the control samples. This difference is due to the addition of the acetic acid during the marinating process (Gopal *et al*., 1985). Similar results were reported by Şengör et al (2004), Maktabi *et al* (2015), and Ozgul *et al* (2010) for smoked marinated mussels, marinated rainbow trout fillet and smoked marinated anchovy respectively. Marinating process performed to cooked mussels using acetic acid and salt aimed to ensure the stability and the extension of shelf life of the product. According to several studies, acetic acid has an effective antimicrobial activity (Aveiro *et al*., 2007). Throughout the 15 days of refrigerated storage, the pH values in control and marinated mussels' samples remained unchanged.

According to McLay (1972), food poisoning and growth of spoilage bacteria may occur when pH values exceed 4.8. In the present study, pH values in marinated mussels remained far below 4.6 throughout the period of storage. However in control mussels, pH values were > 7, which may favour the development of bacteria.

The physical, chemical and microbiological stability of food is strongly depends on the water content of the product. Therefore the concept of water activity (aw) was adopted as a reliable evaluation method of the microbial growth, non enzymatic and enzymatic activities and lipids oxidation (Rahman & Labuza, 1999). In this study, results related to the evolution of water activity in control and marinated mussels samples during the storage period at 4°C are illustrated in Figure 2.

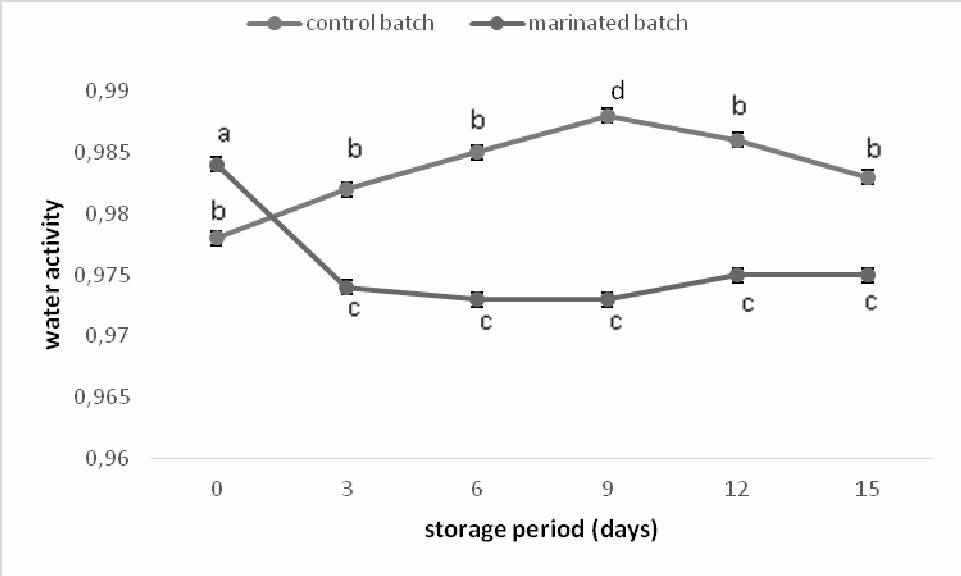

Figure 2: Evolution of water activity content in control and marinated mussels samples stored under vacuum packaging during 15 days at (4°C). Bar = standard error; n= 6 in each case; different letters= mean values for each group are significantly different ($p<0.05$).

According to conventional values (Ballesteros *et al*., 1993), both mussels' batches in this study, can be considered to be food products with high water activity limits as the recorded aw values were ≥ 0.86. However, water activity in marinated mussels showed a significant drop after 3 days of storage to remain significantly lower than values found in the control mussels' batch ($p< 0.05$) for the rest of the storage. Such aw decrease can be attributed to salt effect, acting as depressor agent of water activity during the maturation phase (Girard, 1988). Indeed, the water activity decreases as the sodium chloride concentration increases (Sainclivier, 1985). It is assumed that the measured values of water activity generally correlate well with metabolic activity and potential growth of microorganisms (Gould, 1985; Gould et Christian 1988). According to several studies, each microorganism has a critical water activity below which growth cannot occur (Leistner *et al*., 1981; Beuchat, 1983). For example pathogenic bacteria have a critical water activity varying from 0.85 to 0.86 (Silverman *et al*., 1983; Ballesteros *et al*., 1993). The obtained results showed that the addition of salt in marinating process ensure a significant lower water activity in marinated mussels' flesh compared to control batch and as a result a better conservation of the product from microorganisms activity. However, further investigation on salt/flesh ratio during marinating process is needed to assure lower water activities for better effects on the product quality with longer shelf life.

Evaluation of the microbiological quality in control and marinated mussels samples during the storage period at 4°C

Microorganism's proliferation is among the most important factors in the deterioration of food with special emphasis to seafood products as they are more alterable (Maktabi et al., 2015). Figure 3 presents the enumeration of the total mesophilic flora in control and marinated mussels samples during refrigerated storage.

Initial number of total mesophilic bacteria was 3.04 log CFU/G and 2.96 log CFU/g for control and marinated samples respectively. These results are in agreements with those found for other marinated seafood products (Stamatis & Vafidis, 2009; Ozgul & Balikci, 2013).
In the present study, the initial microbial loads for control and marinated mussels samples were < the limits (10^6 CFU/g) established by International Regulation (ICMSF, 1986) and adopted by the DGSV (2011). Such low initial counts indicate the good quality of the raw product. During storage, an increase among total mesophilic bacteria was observed to reach 4.14 log CFU/g for the control samples and 3.21 log CFU/G for the marinated samples.
Compared to several other studies, both mussels' batches were qualified as "good" food product since they did not exceed the upper acceptability limit (Huss, 1988; Bilgin et al., 2006; Cakli *et al*., 2006; Bao *et al*., 2007; Duyar *et al*., 2012). Moreover, marinated mussels' batch presented lower total mesophilic flora than that of the control batch during the 15 days of storage. These results are in agreements with those found in the literature (Maktabi et al., 2015; Kilinc & Cakli, 2004). This result could be explained by the synergic effect of salt and vinegar added during marinating process, which inhibited the proliferation of micro-organisms (Jarvis et al., 1987). However, the increasing of total mesophilic bacteria during storage for marinated batch suggest that the acetic acid/salt ratio added during marinating process was not enough to protect the product, and further investigation on the adequate ratio should be done.

Evaluation of the chemical indices (TVB-N and TBA) in control and marinated mussels samples during the storage period at 4°C

The evaluation of Total Volatile Basic Nitrogen (TVB-N) and Thiobarbituric acid (TBA) are used for the determination of the spoilage level of seafood during storage period. As freshness indices, monitoring of TVB-N and TBA was carried out during 15 days of refrigerated storage.

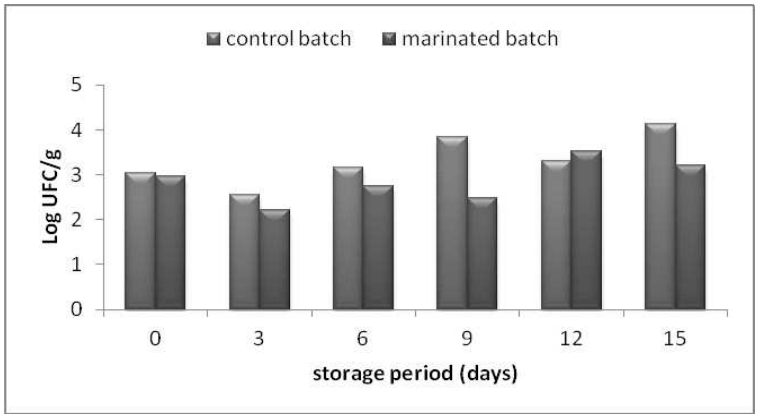

Figure 3: Enumeration of the total mesophilic flora (26°C) in control and marinated mussels samples stored under vacuum packaging during 15 days at 4°C (n= 3 in each case)

Evaluation of the Total Volatile Basic Nitrogen (TVB-N) content

The evaluation of TVB-N content is one of the most used methods of routine seafood quality determination. High TVB-N content is an indicator of deterioration of the product caused by both bacterial activities and endogenous enzymes (Ruiz-Capillas & Moral, 2005). In this study, the variation of TVB-N levels in control and marinated mussels samples under refrigerated storage are represented in figure 4.

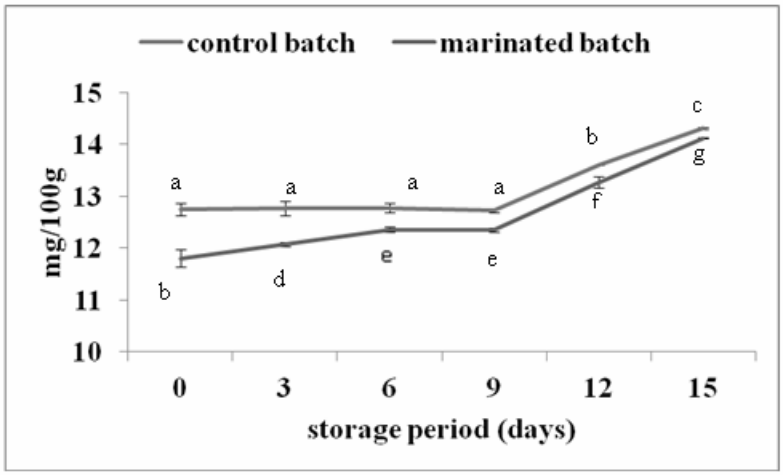

Figure 4: Evaluation of Total Volatil Basic Nitrogen (TVB-N) (mg/100g) content in vacuum packed control and marinated mussels samples stored during 15 days at 4°C. Vertical Bar= Standard Error, (n= 6 in each case); Different Letters= mean values for each group are significantly different ($p<0.05$).

Initial values of the total volatile basic nitrogen content in control and marinated samples were 2.75 ± 0,001 and 1.80 ± 0,016 mg/100g respectively. These results are in agreement with those mentioned by Turan et al., (2006).

A significant increase was observed for the two batches from the 9th day of storage with significantly lower values for marinated mussels' samples compared to control samples. Obtained results are in agreement with those mentioned in the literature (Kilinc & Cakli, 2004; Ozyurt et al., 2012). Koutsoumanis & Nychas, 1999 and Ruiz-Capillas & Moral (2001) explain this increase by spoilage bacteria activity. Huss (1988) suggested different categories for seafood products quality according to its TVB-N content: samples with a content of 25 mg/ 100 g of TVB-N are considered as "very good", for a content of 30 mg/100 g of TVB-N product is regarded as "good", for a content of 35 mg/100g product is defined as "negotiable", and if the content is higher than 35 mg / 100 g TVB-N product is considered as "rotted".

During this study, reached values in the 15th day of storage were as followed: 4.3 ± 0,012 mg/ 100 g for the control samples and 4.12 ± 0,006 mg/ 100g for the marinated samples, however content of TVB-N in both batches remained largely lower than the higher limits of acceptability 25 mg/100g (CEC, 1995). Erkan (2005) and Goulas et Kontominas (2005) suggested values limits of acceptability for mussels of about 15 and 22-25 mg/100g respectively. Results of TVB-N content for both batches obtained during this work remained lower than the limits of acceptability mentioned by last cited works.

Evaluation of thiobarbituric acid (mg MDA/Kg) content

The content of thiobarbituric acid in seafood reflects the degree of lipid degradation corresponding to products of the secondary step of lipids oxidation. The oxidation of lipids involves considerable changes in the organoleptic properties of seafood as in the texture and the flavor (Aubourg et al., 2005, Brannan et Erickson, 1996; Pirini et al., 2000, Regost et al., 2004). This oxidation, is dependent on the presence of oxygen in the environment of storage, of the temperature of storage, the presence of pro-oxidants like the metal ions, and of the lack of natural antioxidants (like α-tocopherol) or of its deterioration in the flesh of the product (Huss, 1995). As a good indicator of food quality (Tarladgis et al., 1960; Vareltzis et al., 1993), the variation of the content of TBA recorded for control and marinated batches is represented on figure 5. Initially TBA content results obtained for control and marinated mussels samples were about 0.12 ± 0.01 mg MDA /Kg for control mussels and 0.17mg MDA/kg for the marinated mussels. Ozgul & Balikci (2013) and Cadun et al. (2005) also reported low contents of TBA for marinated fishes and shrimps respectively. During this study, a significant increase of the contents of TBA was observed from the 12th day of storage for control and marinated samples to reach 2 and 3 mg MDA/kg respectively. Similar results of increased TBA values during storage are mentioned by numerous studies done on the effect of marinating process on seafood during refrigerated storage (Ozgul et al., 2010; Maktabi et al., 2015). However, obtained results in the present work show significant higher lipid oxidation in marinated mussels' samples compared to control mussels. These results can be

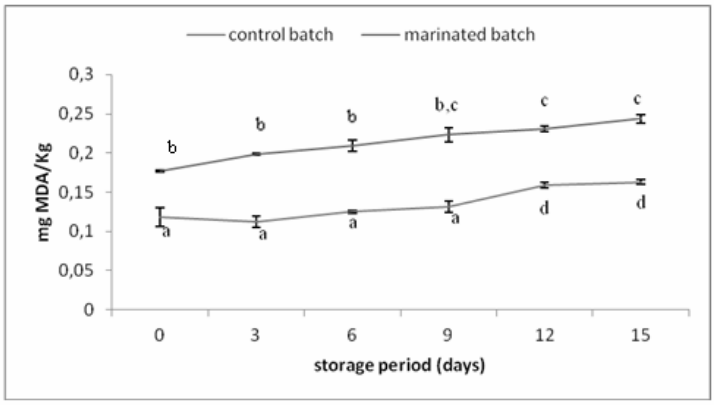

Figure 5: Evaluation of Thiobarbituric acid (TBA) (mg MDA/Kg) content in vacuum packed control and marinated mussels samples stored during 15 days at 4°C. Vertical Bar= Standard Error, (n= 6 in each case); Different Letters= mean values for each group are significantly different ($p<0.05$).

explained by higher rates of lipids in marinated mussels due to the addition of vegetable oil during marinating process and in consequence a greater susceptibility to their oxidation. Similar results were reported in other studies (Pakawatchai et al., 2009; Pezeshk et al., 2012, Maktabi et al., 2015) for marinated seafood products. According to several studies, the rate of TBA which is used for the determination of the rancidity of oil must be lower than 3 mg MDA/kg in a food defined as a very good quality product and should not exceed 5 mg MDA /Kg in food of good quality (Varlik et al., 1993). Other studies reported that rancidity starts in food products when TBA values exceeds 4 Mg MDA/Kg and that the limit beyond which the seafood products will develop an odour and/or unpleasant taste and become unsuitable for consumption is in the order of 7-8 mg MDA/Kg (Kaya & Basturk., 2015). Data in the present study suggest that the contents of TBA in both mussel's batches remained lower than the threshold of acceptability mentioned by literature (Kaya & Basturk., 2015).

Biochemical content in control and marinated mussels samples during the storage period at 4°C

Biochemical composition of bivalves is strongly related to water temperature, food availability and the gametogenic cycle of animals (Small & van Stralen, 1990). Processors have a direct interest in the biochemical composition of product, needing to know the nature of the raw material before different manufacturing techniques can be correctly applied (Murray & Burt, 1969). Monitoring of biochemical composition of raw material and marinated mussels was carried out during 15 days of refrigerated storage.

Characterization of the initial proximate composition

The quality and state of freshness of a finished product depend directly on the initial quality of the raw product. A characterization of the initial proximate composition of samples from the two batches of mussels was carried in order to evaluate occurring nutritional changes during vacuum-packaged storage during 15 days at 4°C (table II).

Tableau II: Comparison of initial proximate composition of control and marinated samples

Proximate composition	Control batch (day 0)	Marinated batch (day0)
Ash content (g/100g)	2.17 ± 0.2^{a}	2.96 ± 0.047^{b}
Moisture content (g/100g)	80.38 ± 0.09^{a}	77.21 ± 0.21^{b}
Total protein content (g/100g)	7.18 ± 0.12^{a}	7.43 ± 0.42^{a}
Total lipid content (g/100g)	2.40 ± 0.05^{a}	7.58 ± 0.06^{b}

Mean ± SD; different letters= mean values for each group are significantly different ($p<0.05$); n=6 in each case

Results in Table II show that the ash and total lipids content were significantly higher ($p <0.05$) in marinated mussels compared to control mussels. Turan et al (2006) showed that ash content increased in cooked salted mussels due to the effect of salt addition. These results are explained by a loss of

water from the flesh replaced in the marinated mussel's flesh by a salt penetration. The high lipid content in marinated mussels is explained by the addition of vegetable oil during the marinating process. Thus, this product is not recommended for people suffering from high cholesterol level in their blood.

Monitoring biochemical changes during the storage period at 4°C

The evaluation of the proximate composition: water content, total ash content, total protein content, total lipid content and total carbohydrates content in control and marinated mussels samples was carried out during storage period at 4°C. The results are represented in table III.

Literature data on biochemical composition of the marinated mussels are very limited (Aveiro et al., 2007; Turan et al., 2006; Guldas & Hecer, 2010). The majority of elaborated studies were related to fish marinating process (Ozden, 2005; Kilinc & Cakli, 2004; Cabrer et al., 2002; Ozgul & Balikci, 2013).

During storage, the water and ash contents in control and marinated samples did not present significant variation. Similar results were found by Turan et al., (2006) which studied the effect of various methods of salting on the Mediterranean quality of the mussels *Mytilus galloprovincialis*.

Table III: Evaluation of the proximate composition changes in control and marinated mussels samples during the storage period at 4°C. Mean ± SD; different letters= mean values for each group are significantly different ($p<0.05$); n=6 in each case

Storage day / Analysis		0	3	6	9	12	15
moisture (g/100g)	**Control batch**	80.38^{a} ± 0,1	$79,2^{a}$ ± 0.16	79.18^{a} ± 0.09	79.31^{a} ± 0.17	80.41^{a} ± 0.01	81.13^{a} ± 0.23
	Marinated batch	76.13^{b} ± 0.15	$77,97^{b}$ ± 0.27	78.17^{b} ± 0.12	78.33^{b} ± 0.03	78.68^{b} ± 0.22	78.53^{b} ± 0.12
Ash content (g/100g)	**Control batch**	1.82^{a} ± 0.15	$1,85^{a}$ ± 0.01	1.86^{a} ± 0.03	1.84^{a} ± 0.002	1.57^{a} ± 0.06	1.11^{a} ± 0.08
	Marinated batch	2.68^{b} ± 0.15	$2,12^{b}$ ± 0.06	2.15^{b} ± 0.083	2.67^{b} ± 0.02	2.78^{b} ± 0.12	$1,121^{b}$ ± 0.04
Total protein content g/100g	**Control batch**	7.19^{a} ± 0.12	$3,6^{b}$ ± 0.10	3.32^{b} ± 0.02	2.2^{d} ± 0.06	2.53^{d} ± 0.04	2.2^{d} ± 0.04
	Marinated batch	7.43^{a} ± 0.04	4.95^{c} ± 0.22	4.9^{c} ± 0.09	3.2^{b} ± 0.2	2.84^{b} ± 0.06	3.06^{b} ± 0.003
Total lipid content (g/100g)	**Control batch**	$2,4^{a}$ ± 0.05	3^{b} ± 0.07	3.86^{c} ± 0.4	2.9^{b} ± 0.08	2.96^{b} ± 0.015	2.5^{a} ± 0.03
	Marinated batch	$7,58^{d}$ ± 0.06	$4,82^{e}$ ± 0.16	4.9^{e} ± 0.10	4.29^{e} ± 0.11	3.35^{b} ± 0.05	4.66^{e} ± 0.25
Total carbohydrate content (g/100g)	**Control batch**	$2,73^{a}$ ± 0.06	$2,35^{a}$ ± 0.09	2.25^{a} ± 0.03	2.52^{a} ± 0.04	2.52^{a} ±0.01	2.01^{a} ± 0.007
	Marinated batch	$2,58^{b}$ ±0.07	2.52^{b} ± 0.03	2.65^{b} ± 0.03	2.27^{b} ± 0.02	2.21^{b} ± 0.09	2^{b} ± 0.03

The contents of proteins presented a significant reduction for both batches of mussels during the first 3 days of storage then remained constant throughout the period of conservation. These results are comparable with those of Yeannes & Caslaes (2008) and Feeney (1977).

Lipid levels in marinated batches were significantly higher than levels observed in control batch. This difference can be explained by the addition of vegetable oil during marinating process.

The contents of carbohydrates did not show significant variation throughout the period of storage for both batches. Stamatis & Vafidis (2009) mentioned comparable results in marinated vacuum-packaged sea urchins during storage.

CONCLUSION

The present study showed that:

- Compared to control mussels (cooked mussels); marinating using vinegar and salt induces low pH and water activity, reducing thus, the quality degradation of vacum-packed mussels stored at 4°C during 15 days.
- Marinated mussels have a mesophilic flora below the upper limits of acceptability threshold (10^{6}UFC / g) during 15 days of storage
- Marinated mussels present quality indices; TVB-N and TBARs were below the upper limits of acceptability of respectively 25 mg / 100 mg and 1-2 MDA / kg for 15 days of storage.

On the basis of all these analyses, a shelf life of 15 days was determined for the conservation of marinated mussels under vacuum packaging at 4°C.
This work contribute to the development of a range of value added seafood products which may be available in markets, making mussel more popular locally and may thus, play a great role in this endeavour. Beside the development of the international seafood value chain may created new market opportunities for mussel that stimulated local economy.

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