

## Quality Changes of Thermal Pasteurized Mussels (*Mytilus galloprovincialis*) During Refrigerated Storage at 4±1°C

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### ABSTRACT

This study investigated the changes in quality of pasteurized mussels during storage at 4°C±1°C. Mussels (*Mytilus galloprovincialis*) were harvested from the Marmara Sea in March. The mussel flesh was packed with lemon juice, apple vinegar, finely chopped onion, salt, and black pepper. Then, all the packets were pasteurized (at 70°C for 8 min) and stored for 21 days at 4°C±1°C. Results showed that the contents of protein, fat, and ash of pasteurized mussels were significantly higher ( $p<0.05$ ) than those of raw samples. The moisture content of mussels was significantly decreased ( $p<0.05$ ) after heat treatment. The meat yield of pasteurized mussels was 10.42%. Sensory evaluation results indicated that the acceptability of pasteurized mussels at refrigerated storage was limited to 9 days. The TVB-N value of pasteurized samples exceeded the acceptability limit of 22–25 mg/100 g on the 9<sup>th</sup> day. The TMA-N amount of pasteurized mussels remained lower than the acceptability limit during storage. The initial microbial load of the mussel samples reduced after the pasteurization process. This reduction was observed in the total mesophilic and psychrophilic bacterial count, with the yeast mold counts after pasteurization being 2.44, 2.07, and 2.37 log cfu/g, respectively.

**Keywords:** Pasteurization, mussel, quality, ready-to-eat

### INTRODUCTION

Nowadays, the trend towards ready-to-eat food at high quality makes thermal pasteurization applications important for food. Thermal pasteurization (usually below 100°C) is a classic food preservation method that provides food safety, which reduces undesirable pathogenic vegetative cells, and deteriorates microorganisms, inactivates enzymes, and prolongs the shelf life of foods. Pasteurization used in the food industry does not kill all microorganisms in food. It is used in the food industry targeting pathogens that are important for public health and microorganisms that cause food spoilage. Today, pasteurization is regarded as a technology in which the desired food quality is preserved with minimum loss and pathogenic microorganisms are killed (Skipnes, et al., 2002; Peng et al., 2017).

Mussels are rich in nutrient components; also their economic value is high. They are very popular in many countries in terms of hunting and processing compared to other aquatic products. They are relatively inexpensive, contain high quality protein source, and are usually consumed raw, fresh, frozen and canned. The mussels have low fat and cholesterol content. It is also very rich in vitamins including, A, B1, B2, B6, B12 and C, free amino acids, trace elements (selenium, calcium, iron, magnesium and phosphorous) and glycogen. At the same time they have high pH. This makes them a suitable substrate for the growth of microorganisms (Goulas, et al., 2005; Ovissipour, et al., 2013). On the other hand shellfish feed by filtering water. They accumulate various chemicals and microorganisms in their bodies (Vernocchi et al., 2007). Especially, raw and partially cooked

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molluscan shellfish (mussels, clams and oysters) are the most common tools of foodborne bacterial and viral diseases (Rippey, 1994).

Depending on all these properties, the mussel is a food that can deteriorate very quickly. Therefore, there is a need for appropriate storage technologies that prolong both shelf life and nutritional and sensory qualities. However, very little information and data are available on the quality changes that have taken place during the storage period, especially after heat treatment.

The purpose of this study, is to investigate the quality changes of mussel pasteurized with lemon juice, apple vinegar, onion, salt and black pepper during cold storage.

## MATERIAL AND METHOD

### Mussels Supply and Handling

Mussels (*Mytilus galloprovincialis*) were purchased from a wholesale fish market in Istanbul. The mussels were harvested from the Marmara Sea in March. Approximately 550 mussels were transferred to the laboratory in chilled polystyrene boxes within 60 minutes. Dead mussels were discarded after inspection. The average weight and length of the mussels were  $23.94 \pm 6.80$  g and  $6.99 \pm 0.78$  cm. The mussels were washed and shucked by hand using a sterile shucking knife. Raw mussel flesh analysis was done immediately.

### Packaging and Pasteurization Process

Approximately, twenty five (approx. 150g) mussel flesh was placed in a thermostable polyethylene-polyamide pouch ( $O_2$  permeability of  $160 \text{ cm}^3/\text{m}^2$  per 24 h at 23C, 0% RH and water vapor permeability of  $8.5 \text{ g}/\text{m}^2$  per 24 h at 38C, 90% RH.) with lemon juice (10 mL), apple vinegar (10 mL), fine-chopped onion (10 g), salt (0.5 g) and black pepper (0.5 g). The packages were heat sealed under atmospheric air. Then, all packed samples were pasteurized (70°C for 8 min) by using autoclave. A total 18 packages were prepared. Pasteurized samples were stored refrigerated ( $4 \pm 1^\circ\text{C}$ ) conditions. After 0, 6, 9, 12, 15, 18 and 21 days randomly chosen two packages were removed for analysis.

### Yield Determination

After thermal pasteurization, mussel meat was subsequently drained by placing on filter paper and then covered with another filter paper for five minutes. Consequently the yield was compared with that from freshly unopened mussels. Mussels (25 pieces) yield are calculated by the following equation:

Yield (%):  $[\text{cooked mussel meat weight (g)}/\text{unopened mussel weight (g)}] \times 100$  (Cruz-Romero et al., 2007; Bongiorno et al., 2015).

### Proximate Analyses

The mussel samples were analyzed in triplicate for determination of the proximate composition: the lipid content of mussels was determined by the soxhlet method of the AOAC (1998a), the moisture content by the method of Mattissek et al. (1992), the ash content by the AOAC (1998b) method and total crude protein by the Kjeldhal method (AOAC, 1998c).

### Sensory Analysis

The sensory attributes of pasteurized mussels were evaluated on each sampling day by ten trained panelists. Sensory analysis was performed in individual booths under controlled conditions of air circulation, light, temperature and humidity. Pasteurized mussels were evaluated on the basis of appearance, odor, texture and taste characteristics. The scale points were: 10-9=excellent; 8.9-8=very good; 7.9-6=good; 5.9-4=sufficient; < 4=unacceptable (Karl et al., 2001). The overall quality score was calculated as the average value of the score of the each attributes evaluated.

### Microbiological Analyses

All microbiological analyses were performed in duplicate. Twenty five grams of mussel flesh for analysis was transferred aseptically to a sterile stomacher bag containing 225 mL of peptone from meat (Merck, 1.0214) (0.1%) and homogenized in a stomacher (IUL Instrument, Spain) for 60 second. Appropriate serial dilutions were prepared (1:10 diluent, 0.1% peptone water). Total aerobic mesophilic bacteria (TAMB) and total aerobic psychrophilic bacteria (TAPB) were determined using Plate Count Agar (PCA, Merck, 1.05463) after incubation for 24-48 hours at 37°C and for 10 days at 7°C, respectively (Baumgard, 1986). Violet Red Bile Agar (Merck, 1.01406) and Violet Red Bile-Mug Agar (Merck, 1.04030) were used for the enumeration of total coliform bacteria and *Escherichia coli* after incubation at 30°C 18-24 hours and 35°C 18-24 hours, respectively (Feng et al., 2002). Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466) was used for the enumeration of yeast and mold (YM) after incubation at 25°C for 5 days (Tournas et al., 2001). *Salmonella* spp. determination was performed according to Andrews et al., (2007). Twenty five grams of mussel flesh was added to the 225 mL Lactose broth (Merck, 1.07661), and incubated at 37°C for 24-48 hours. After the incubation, 0.1 mL homogenate was transferred into the 10 mL of Rappaport-Vassiliadis Broth (Merck 1.07700.500) and then incubated at 42°C for 24 hours for the selective enrichment. At the same time 1 mL homogenate was transferred in to 10 mL Tetrathionate Broth (Merck 1.05285) and then incubated for 24 hours at 43°C. After the incubation, a loopful of broth cultures were streaked onto XLT4 (Merck 1.13919) and Bismut Sulfite Agar Base (Merck 1.05418). Plates were incubated at 35°C for 48 h. After that, identification tests have been performed on suspected salmonella colonies. Mussel samples were analyzed for the presence of *Vibrio* spp. using FDA Bacteriological Analytical Manual (Kyasner and Depaola, 2004). Twenty five grams of mussel flesh was added to the 225 mL alkaline peptone water (1% NaCl+1% peptone from meat), after, a loopful of homogenate from alkaline peptone water was streaked on Thiosulphate Citrate Bile Salt Agar Base (TCBS, Merck 1.10263). Plates were incubated at 35°C for 18-24 h and identification tests have been performed on suspected colonies. Three agar plates per dilution were made in each medium.

### Chemical Analysis

Chemical analyses for performed triplicate. For the pH analysis mussel samples were homogenized and diluted with distilled water at 1:1 (w/v) ratio. After that, the pH value of the mussel samples was measured with pH meter (Hanna pH 211 Micro-processor pH meter, Ann Arbor, MI) (Olafsdottir et al., 1997).

Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) analyses were carried according to the methods described by Schormüller (1968). Mussel samples were boiled with MgO (Merck, 1.05862) and vapor components held with 0.1N HCl (Merck, 1.00317). The amount of TVB-N was calculated after the titration with 0.1N NaOH (Merck, 1.06498). The results were expressed as milligrams of TVB-N per 100 grams of mussel flesh. For the estimation of TMA-N content of mussel flesh, 10 g samples were homogenized with 10% trichloroacetic acid solution (90 mL)(Merck, 100807) and homogenized samples were filtrated by Whatman No: 1 filter paper. After filtration, 4 mL filtrate was shaken with 10 mL toluene (Balmumcu Ltd.), 1 mL 20% formaldehyde (Merck, 1.04002) and 3 mL 50% potassium hydroxide (Carlo Erba, 362257). Following this, upper layer (5 mL) was separated and mixed with 0.02% picric acid (5 mL). Absorbance was measured by a spectrophotometer (UV/VIS T80, PG Instruments Ltd) at 410 nm. The TMA values were calculated from the standard curve and expressed as mg/100g samples.

### Statistical Analysis

The average of the results from experimental studies was used for statistical analysis. The data were analyzed using IBM Statistical Package for the Social Sciences 21 software (SPSS Inc.; Chicago, IL, USA). Differences between means were analyzed by one way analysis of variance (ANOVA) followed by Tukey and Games-Howell tests. T-test was used to compare the results of the proximate composition. Differences at ( $p < 0.05$ ) were considered significant.

## RESULT AND DISCUSSION

In our study, pasteurized mussels had approximately 10.42% yield. Cruz-Romero et al., (2007), calculated the yield of traditional pasteurized oyster (at 75°C for 8 min) range 1.5-15.5%. Bongiorno et al., (2015), observed that, for *Mytilus galloprovincialis* maximum value of meat yield (cooked) in May (26.3%) and minimum value of yields in January (12.5%). In another study, Vernocchi et al., (2007), reported that meat yield (cooked) for *Mytilus galloprovincialis* ranged from 13.4% (July) and 25.2% (January). Cavalheiro et al., (2013) reported the global weight loss of the blue mussels as 17.20% after vapor cooking. Meat yield of bivalve species may vary depending on gametogenic cycle of animals, water temperature, salinity, pH, food availability and catching season (Orban et al., 2002; Yildiz et al., 2011).

**Table 1.** Proximate composition (%) of raw and pasteurized mussels.

	Raw Mussel	Pasteurized Mussel
Protein	10.61±0.09 <sup>a</sup>	20.00±0.41 <sup>b</sup>
Fat	2.11±0.10 <sup>a</sup>	3.82±0.09 <sup>b</sup>
Moisture	86.19±0.11 <sup>a</sup>	73.46±0.20 <sup>b</sup>
Ash	1.02±0.01 <sup>a</sup>	2.18±0.03 <sup>b</sup>

a-b: Different letters show significant differences ( $p < 0.05$ )

Proximate composition of raw and pasteurized mussel is shown in Table 1. The raw mussel meat had a moisture content of 86.19%, protein 10.61%, fat 2.11% and ash 1.02%. Our results are similar to the results of many studies with *Mytilus galloprovincialis* (Khan et al, 2005; Fuentes et al., 2009; Özden et al., 2010; Ulusoy and Özden, 2011; Bongiorno et al., 2015). It is known that catching season, size, age, food availability, sex and reproductive cycle may influence the meat yield and nutritional composition of mussels (Orban et al, 2002; Stratev et al., 2017; Merdzhanova et al., 2018). The moisture, protein fat and ash contents of pasteurized mussel meat were 73.46, 20.00, 3.82 and 2.18% respectively. The protein, fat, and ash contents of pasteurized mussels were significant higher ( $p < 0.05$ ) than raw samples. Shelf life of stuffed mussels at 4°C in modified atmosphere packaging was studied by Ulusoy and Özden (2011). In their study, the protein, fat, and ash contents of stuffed mussels was found statistically significant higher after cooking as observed in our study. In this study, the moisture content of mussels significantly decreased ( $p < 0.05$ ) after heat treatment, in agreement with data reported by Cruz-Romero et al. (2007), Ulusoy and Özden (2011) and Lekjing et al., (2017).

The pH value of pasteurized mussels during cold storage are shown in Table 2. Manousaridis et al., 2005, recommended the following pH scale points as a basis for evaluating the freshness of mollusks (oysters); 6.2-5.9=good; 5.8=off, 5.7-5.5= musty and 5.2 and below sour or putrid. In our study, pH value measured in raw mussel was 6.20±0.00. Approximate pH values (6.30, 6.20 and 6.20) were reported by Manousaridis et al., 2005, Masniyom and Benjama, (2007) and Bongiorno et al., (2018), respectively. In our study, initial pH of the sauce (lemon juice, apple vinegar, fine-chopped onion, salt and black pepper) was 2.55±0.03 which is acidic. The pH value of samples decreased after treatment with lemon juice and vinegar, similar to results reported by others (Sallam et al., 2007; Cosansu et al., 2011; Cosansu et al., 2013). Bhunia et al., (2017), observed that a decrease in initial pH of blue mussels after treatment with red sauce (red tomato sauce, salt, and paprika).

The scores of sensory evaluation of pasteurized mussels are presented in Table 2. During the first 6 days, mussel samples were rated at 8.9-6 scores. This range of points is defined as "very good" and "good". Acceptability scores for sensory quality of pasteurized mussel samples stored at 4±1°C decreased with time of refrigerated storage. The limit of acceptability of sensory quality was reached after 9<sup>th</sup> day of storage. Pasteurized mussel samples were determined as unacceptable (2.20±0.93) at the 12<sup>th</sup> day of storage. In a similar study, Ulusoy and Özden, 2011, reported that the shelf life of stuffed mussel with air packed was 11<sup>th</sup> day. In another study, sensory quality of cooked and chilled mussels without vacuum conditions unacceptable at 7<sup>th</sup> day (Bongiorno et al., 2018).

In this study, initial TVB-N content of raw mussels were 17.52±1.94 mg/100g. This value slightly higher than reported by previous studies (Masniyom and Benjama, 2007; Bongiorno et al., 2018). During refrigerated storage, TVB-N content increased in pasteurized mussels until end of storage (21 days), reached value of 29.24±0.38 mg/100 g (Table 2). The TVB-N content is often

**Table 2.** Changes in sensory, pH, TVB-N and TMA-N values of raw and pasteurized mussels stored at 4±1°C.

Days	Sensory score	pH	TVB-N (mg/100g)	TMA-N (mg/100g)
0 (Raw Material)	7.66±0.27	6.20±0.00	17.52±1.94	1.65±0.13
0 (Pasteurized samples)	8.62±0.38 <sup>a</sup>	4.65±0.05 <sup>a</sup>	7.90±0.69 <sup>a</sup>	3.39±0.00 <sup>a</sup>
7	7.02±0.62 <sup>b</sup>	4.57±0.03 <sup>ac</sup>	21.92±0.98 <sup>b</sup>	3.34±0.19 <sup>ab</sup>
9	4.60±0.55 <sup>c</sup>	4.65±0.07 <sup>a</sup>	22.58±0.15 <sup>b</sup>	3.37±0.17 <sup>a</sup>
12	2.20±0.93 <sup>cd</sup>	4.84±0.07 <sup>b</sup>	22.24±0.67 <sup>b</sup>	3.54±0.28 <sup>ab</sup>
15	1.85±0.73 <sup>d</sup>	4.43±0.06 <sup>c</sup>	23.21±0.47 <sup>b</sup>	3.40±0.00 <sup>a</sup>
18	1.40±0.35 <sup>d</sup>	4.45±0.00 <sup>c</sup>	24.35±0.29 <sup>c</sup>	2.75±0.25 <sup>ab</sup>
21	1.17±0.20 <sup>d</sup>	4.54±0.04 <sup>ac</sup>	29.24±0.38 <sup>d</sup>	2.49±0.05 <sup>b</sup>

a-d: Different letters in the same column show significant differences (p<0.05)  
 TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen

**Table 3.** Changes in microbial counts of raw and pasteurized mussels stored at 4±1°C.

Days	TAMB (logcfu/g)	TAPB (logcfu/g)	YM (logcfu/g)
0 (Raw Material)	5.59±0.02	6.18±0.11	6.30±0.02
0 (Pasteurized mussel)	3.15±0.10 <sup>a</sup>	4.11±0.01 <sup>a</sup>	3.93±0.02 <sup>a</sup>
7	3.35±0.10 <sup>ab</sup>	2.20±0.17 <sup>b</sup>	3.35±0.31 <sup>ab</sup>
9	3.66±0.05 <sup>b</sup>	2.10±0.17 <sup>b</sup>	3.10±0.17 <sup>b</sup>
12	3.56±0.07 <sup>ab</sup>	2.00±0.00 <sup>b</sup>	3.10±0.17 <sup>b</sup>
15	3.20±0.17 <sup>ab</sup>	2.00±0.00 <sup>b</sup>	2.10±0.17 <sup>c</sup>
18	3.41±0.10 <sup>ab</sup>	1.20±0.17 <sup>bc</sup>	<100
21	3.47±0.00 <sup>ab</sup>	1.10±0.17 <sup>c</sup>	<100

a-c: Different letters in the same column show significant differences (p<0.05)  
 TAMB: total aerobic mesophilic bacteria; TAPB: total aerobic psychophilic bacteria; YM: yeast and mold

used as an index to evaluate the freshness and quality of seafood (Masniyom and Benjama, 2007). There are different views on acceptable limits in various literature. Kietzman et al., (1969) stated the TVB-N limit values in fish and fish products as follows; 25.00 mg/100g or lower TVB-N values; "very good", 30.00 mg/100g TVB-N values; "good", 35.00 mg/100g TVB-N values; "marketable" and over 35.00 mg/100g TVB-N values; "unacceptable". Sikorski et al., (1990) cited TVB-N acceptability limit value for fatty fish is 20 mg/100g and 17 mg/100g for oyster. The limit of acceptability is suggested as 22-25 mg/100g by Goulas et al., (2005), while it is 15 mg/100g according to Erkan (2005). Ulusoy and Özden (2011), reported the TVB-N value of cooked stuffed mussels reached to 21.90 mg/100g and exceeded the acceptable limit after nine days of storage. In our study, for pasteurized mussels the sensory score was approximately 4.60 on the 9<sup>th</sup> day when the TVB-N value reached 22.58±0.15 mg/100g. Panelists who performed sensory analysis evaluated mussel samples as "unacceptable" on the 12<sup>th</sup> day. At the 12<sup>th</sup> day of storage TVB-N content of mussel samples was 22.24±0.67 mg/100g. Our results were in accordance with that of Masniyom and Benjama, (2007) who reported a TVB-N value of 20 mg/100g for green mussel samples after 9 days of refrigerated storage. In our study, it would be more realistic to use the 22-25 mg/100g TVB-N unacceptable limit value recommended by Goulas et al., (2005) for the mussel

as compared with the value of 35.00 mg/100g proposed for fish (Kietzman et al., 1969).

Trimethylamine is a non-protein nitrogenous compound, and is responsible for further fish degradation. TMA is formed by the reduction of Trimethylamine oxide (TMAO) caused by microbial action and possibly through the activity of endogenous enzymes. At the same time, it contributes to the characteristic ammonia-like off-odor in fish spoilage (Sikorski et al., 1990; Gram and Huss, 1996; Goulas et al., 2005). TMA-N limit values for fish and other seafood were determined as follows. 4.00 mg/100g TMA-N content; "good", 10.00 mg/100g TMA-N content; "marketable" and 12.00 mg/100g TMA-N content; "unacceptable" (Connell, 1980). Sikorski et al., (1990), suggested 5 to 10 mg TMA-N per 100g as the rejection limit. In our study, initial value of TMA-N in raw mussels was found 1.65±0.13 mg/100g. Approximate TMA-N values were reported by Kaba and Erkoyuncu, (2005) and Erkan, (2005). TMA-N value of pasteurized mussels did not exceed the limit value during storage period (Table 2).

Total viable count is an important criterion in assessing the quality of fresh and refrigerated seafood (Chouhan, et al., 2015). Changes in the microbiological count during cold storage were shown in Table 3. In our study, the initial total aer-



obic mesophilic bacteria count in raw mussel was  $5.59 \pm 0.02$  log cfu/g. Linton et al., 2003 also reported that total aerobic count for mussel samples was 5.00 log cfu/g. Total aerobic mesophilic bacteria count decreased ( $3.15 \pm 0.10$  log cfu/g) after pasteurization. Total bacterial count reached maximum load ( $3.66 \pm 0.05$  log cfu/g) at the 9<sup>th</sup> day of storage. In this study, total aerobic psychrophilic count in raw mussel was  $6.18 \pm 0.11$  log cfu/g. Similar, psychrotrophic count ( $>6$  log cfu/g) was found by Linton et al., (2003) for mussel. After pasteurization, the total aerobic psychrophilic load was reduced ( $4.11 \pm 0.01$  log cfu/g). Total psychrophilic count of pasteurized mussels decreased steadily throughout the storage period (Table 3). Many studies have shown that various heat treatment application in aquatic products are effective in reducing total bacterial load (González-Fandos et al., 2004; Martínez-Alvarez, et al., 2009; Mol et al., 2012; Cosansu et al., 2011, Cosansu et al., 2013, De Lima et al., 2017 ; Doğruyol and Mol, 2017 ; Bongiorno et al., 2018). The results of our study are consistent with the findings of other researchers. The raw material had initial yeast-mold counts of  $6.30 \pm 0.02$  log cfu/g. Yeast-mold load of the raw material decreased ( $3.93 \pm 0.02$  log cfu/g) after pasteurization process (Table 3). The counts of yeast-mold decreased throughout storage. The yeast and mold were not detected in the later days of storage. Velammal et al., (2017) did not detect fungal colonies throughout the storage period in cooked meat of brown mussel. Kilinc and Cakli (2005a,b) did not detect mold and yeast in pasteurized sardines during the cold storage. *Vibrio* spp. and *Salmonella* spp. were not detected in raw and pasteurized mussels in our study. Total coliform bacteria and *Escherichia coli* were found  $5.28 \pm 0.07$  and  $2.23 \pm 0.33$  log cfu/g in raw mussel, respectively. In our study, the pasteurization process eliminated total coliform bacteria and *Escherichia coli* in mussels. Thermal processing and refrigeration are important means of controlling these bacteria. Cosansu et al., (2011) and (2013) reported no growth of these bacteria in sous-vide fish.

## CONCLUSION

In our study, the pasteurization process reduced the microbial load of the mussels and stabilized during the storage. Pasteurization was very effective on microbial flora. According to the TVB-N and sensory analysis results obtained in the study, the pasteurized mussel can be safely consumed for nine days storage at 4°C.

**Conflict of Interest:** The authors have no conflicts of interest to declare.

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