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RESEARCH ARTICLE

Effects of Chitosan and Lactic Acid Immersion on the Mussels' Quality Changes During the Refrigerated Storage

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Summary

The objective of this study was to determine the effects of different pretreatment agents such as chitosan (0.5% (w/v), pH 2.9-3.2) and lactic acid (0.5% (v/v), pH 2.5-2.7) on the chemical and sensory qualities of mussels stored at 4ºC. Mussels were dipped in 100 mL of 0.5% solution of lactic acid (v/v) and chitosan (w/v) at room temperature (22ºC) for 15 min. Mussels from the control group were dipped in 100 mL of sterile distiled water (2% NaCl) without chitosan and lactic acid. Treatment of mussels with lactic acid and chitosan at the beginning of the experiment (day 0) for 15 min reduced bacterial counts of total aerobic mesophilic bacteria (0.53-1.07 log) psychrotrophic bacteria (0.11-0.13 log), *Lactobacillus* spp. (0.46-1.30 log), Enterobacteriaceae (0.43-0.48 log) and coliform bacteria (0.52-0.66 log). Total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), trimethylamine nitrogen (TMA-N) and histamine values of control group mussels were increased from (day 0) 13.1 mg N 100 g^{-1}, 1.37 mg MA kg^{-1}, 1.97 mg N 100 g^{-1} and 4 ppm to 39.9 mg N 100 g^{-1}, 3.02 mg MA kg^{-1}, 4.86 mg N 100 g^{-1} and 7.75 ppm at the end of the storage period (day 11), respectively (P<0.05). The results indicated that shelf-life of mussels stored at 4ºC were limited to 4 days in the control group. However, mussels pretreated with lactic acid and chitosan were stored for 6-7 days and the shelf-life of mussels was extended for ca. 2-3 days, as compared with the control group (P<0.05).

Keywords: *Chitosan, Lactic acid, Mussel, Quality, Shelf life*

Laktik Asit ve Kitosanın Soğukta Muhafaza Edilen Midyelerdeki Kalite Değişiklikleri Üzerine Etkileri

Özet

Bu çalışma +4ºC'de muhafaza edilen midyelerde kitosan (%0.5 (w/v), pH 2.9-3.2) ve laktik asit (%0.5 (v/v), pH 2.5-2.7) uygulamalarının midyenin kimyasal ve duyusal kalitesi üzerine etkisini belirlemek amacıyla yapıldı. Midyeler 100 mL, %0.5'lik laktik asit (v/v) ve kitosan (w/v) solusyonları içine daldırıldı oda ısısında 22ºC'de 15 dk. bekletildi. Kontrol grubu midyeler ise içinde kitosan ve laktik asit olmayan 100 mL steril distile su (%2 NaCl) içinde bekletildi. Midyeler başlangıç gününde (0. gün) 15 dk. süreyle laktik asit ve kitosanda muamele edilmesi sonucu toplam aerobik mezofilik bakteri (0.53-1.07 log), psikrotrofik bakteri (0.11-0.13 log), *Lactobacillus* spp. (0.46-1.30 log), Enterobacteriaceae (0.43-0.48 log) ve koliform bakteri (0.52-0.66 log) sayılarında düşüş sağlandı. Kontrol grup midyelerinde sıfırıncı günde total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), trimethylamine nitrogen (TMA-N) ve histamin değerleri sırasıyla 13.1 mg N 100 g^{-1}, 1.37 mg MA kg^{-1}, 1.97 mg N 100 g^{-1} ve 4 ppm iken depolamanın sonunda (11. günde) 39.9 mg N 100 g^{-1}, 3.02 mg MA kg^{-1}, 4.86 mg N 100 g^{-1} ve 7.75 ppm'e yükseldi (P<0.05). Elde edilen sonuçlara göre +4ºC de muhafaza edilen kontrol grup midyelerin raf ömrü 4 günle sınırlı kaldı. Buna karşın muhafaza öncesi laktik asit ve kitosan ile muamele edilen midyelerin raf ömrü 6-7 gün olarak belirlendi, kontrol grup ile karşılaştırıldığında raf ömrünün 2-3 gün daha fazla uzadığı görüldü (P<0.05).

Anahtar sözcükler: *Kitosan, Laktik asit, Midye, Kalite, Raf Ömrü*

INTRODUCTION

In the Black Sea region of Turkey, fishing is an important industry. In Turkey, average consumption of fishery products per person is 6.9 kg in the year 2010. The Turkish Statistical Institute [1] has reported that the amount of fish and mussels caught in Turkey was 399. 656 and 27.968 tons, respectively in 2010.

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In Turkey, mussels are in general freshly consumed and often cooked with rice called "Stuffed Mussels" or they are vacuum packaged for marketing purposes. The high level of microorganisms in the mussels are probably due to their filter-feeding activities. Furthermore, heat treatment could not satisfactorily penetrate to the centre of mussels because of their thick shells. The consumption of raw or undercooked mussels containing pathogenic microorganisms such as *Vibrio parahaemolyticus*, Hepatitis A, *Shigella* spp. and Norwalk viruses results in serious public health problems [2].

When mussels stored under inappropriate conditions, trimethylamine nitrogen (TMA-N), ammonia and other basic nitrogenous compounds, and total volatile basic nitrogen (TVB-N) are produced in a shorter time. Many methods have been developed to prolong the shelf-life of fishery products to reduce bacteria counts and to maintain quality of products such as cold shock, freezing, ultraviolet irradiation, salt treatment, storing at low temperatures, modified atmosphere packaging, decontaminating by using chitosan, chlorine, organic acids, sodium lactate, sodium acetate, nisin, ozone and chloroform [3-5]. The most widely used chemical decontaminants in the meat industry are organic acids [6]. The antimicrobial activity of lactic acid has been demostrated in a number of foods including chicken, meat and seafood [7]. Lactic acid inhibits the growth of microorganisms such as gram negative Enterobactericeae and *Pseudomonadaceae* spp [8]. The other decontaminant that is used in the seafood to reduce bacterial count is chitosan. Chitosan is a $\beta(1-4)$ linked N- acetyl-D-glucosamine polymer. It is derived by deacetylation of chitin, a major component of the shells of crustaceans such as crab, shrimp and crawfish [9,10]. Chitosan has been shown to have antibacterial activities against gram-positive and gram-negative bacteria [10-12]. The present study was undertaken to investigate the effects of different pretreatment agents such as chitosan and lactic acid on the shelf-life and quality of mussels stored at 4°C.

MATERIALS and METHODS

Sample

A total of 400 fresh mussels *(Mytilus galloprovincialis)* (approximately 3 kg) were obtained from the Samsun region in the middle Black Sea coast of Turkey in May 2010. Mussels were transported in containers with ice bags to the Department of Food Hygiene and Technology, Ondokuz Mayis University, within 1-2 h after harvesting. The samples were washed in sterile distilled water, disinfected with alcohol (70%) and opened aseptically by using sterilised scalpel and pincers.

Chemicals and Reagents

Chitosan (catalogue number: 44887-7, medium molecular weight, 75-85% deacetylated, viscosity 200-800 cps) was purchased from the Aldrich (Milwaukee, WI, USA) and lactic acid (catalogue number: 100366.2500, 90%) purchased from Merck (Darmstadt, Germany). The media used for microbiological analysis were obtained from Oxoid (Basingstoke, UK).

Decontamination with Lactic Acid and Chitosan

Lactic acid solution (0.5% (v/v), pH 2.5-2.7) was prepared by diluting in sterile distilled water. Medium molecular weight chitosan solution (0.5% (w/v), pH 2.9-3.2) was prepared by dissolving in a 0.5% (v/v) lactic acid solution with magnetic stirring. All solutions were autoclaved for 15 min at 121°C. Each mussel was then dipped in 100 mL of 0.5% solution of lactic acid (v/v) and chitosan (w/v) at room temperature (22°C) for 15 min. Control group of mussels was dipped in 100 mL of sterile distiled water (2% NaCl) without chitosan and lactic acid. After dipping, mussel samples were stored at 4°C in aerobic condition and chemical and sensory analyses were performed in duplicate at 0, 2, 4, 7, 9 and 11 days of storage.

Chemical Analyses

pH measurements: Ten gram of mussel tissue was homogenised with 20 mL of distilled water. The pH of the homogenate was measured using a pH meter (Inolab pH730, Germany) [13].

Determination of histamine: The histamine analysis in mussel samples was carried out by using Ridascreen Histamine ELISA test kit (R-Biopharm A.G. Kit, Art. No. R 1604, Darmstad, Germany). According to the ELISA test kit procedure, one gram of sample was taken and 200 mL distilled water was added. Then it was homogenized for 15 min in a blender and the mixture was centrifuged for 5 min at 2500 *g* at room temperature (20-25°C). The supernatant was diluted appropriately, and analysis was carried out according to the method described by the manifacturer. The extracts were read at 405 nm in an ELISA reader (Digital. Analog Systems, DAS RS 232, Rome, Italy).

Determination of total volatile basic-nitrogen (TVB-N): Total volatile basic-nitrogen was determined using the TVB-N quantification kit of reagents (Kit 66662) (Fluka, Italy) according to the Official Method for the European Union [14]. Briefly, 10 g of mussel was homogenised with 90 mL of perchloric acid solution (0.6 M) in a stomacher (Interscience-BagMixer 400, St Nom, France) for 1 min. The homogenates were filtered through a filter paper (Whatman No. 1) and 50 mL of filtrate was made alkaline with sodium hydroxide 20%. The filtrates were distilled by steam distillation for 10 min and the volatile base components were absorbed by an acid receiver (boric acid solution). After distillation, filtrates were titrated with 0.01 mol L HCl. The results were expressed as mg TVB-N per 100 g of sample.

Determination of thiobarbituric acid (TBA): The thiobarbituric acid (TBA) was determined by the distillation method of Tarladgis et al.[15] Ten gram of the mussel sample was blended with 50 mL distilled water for 2 min. The mixture was transferred into a Kjeldahl flask, one drop of silicone anti-foaming agent

was added plus 2.5 mL HCl (4N) and the jar was rinsed with an additional 47.5 mL of distilled water. This sample was then distilled and 50 mL of distillate was collected. Five mL of distillate were added to 5 mL 0.02 M thiobarbituric acid (Merck, Germany) and heated in a boiling water bath for 35 min and then cooled in an ice bath. Absorbance of the solutions was determined at 538 nm on a UV spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, UK). The TBA values were expressed as milligrams of malonaldehyde per kilogram. The Standard curves were prepared by making appropriate dilutions of the $1x10^{-4}$ mol L 1,1,3,3-tetraethoxy-propane (TEP) (Sigma, USA) standard solutions.

Determination of trimethylamine nitrogen (TMA-N): Determination of TMA-N was carried out according to the method proposed by the AOAC [16] based on the colorimetric method. Briefly, ten gram of homogenised samples were weighed and blended with 90 mL of 7.5% trichloracetic acid solution. The blended solution was centrifuged at 2.000-3.000 rpm until the supernant was practically clear. Four mL of extract was transferred into test tubes and 1 mL formaldehyde (20%), 10 mL anhydrous toluene, and 3 mL potassium carbonate (K_2CO_3) solution (100 g%) were added. The tubes were shaken and the toluene phase was then transferred into a tube containing 0.2 g of anhydrous sodium sulfate and shaken to obtain a dehydrated extract. Five mL of the water-free toluene layer was pipetted and 5 mL of picric acid solution (0.02%) was added. The solution was mixed and transferred into a spectrophotometric cell. Absorbance was measured using a spectrophotometer (Helios Gamma, Thermo Spectronic, Cambridge, UK) at a wavelength of 410 nm. At the same time, blank and standards were prepared and measured. The amount of TMA-N in the samples was calculated from the optical densities by using the standard curve described. Results of TVB-N were expressed as mg N 100 g^{-1} sample.

Microbiological Analysis

For microbiological analysis 10 g of the mussel samples were transferred to a sterile bag with 90 mL of 0.1% sterile peptone water (Oxoid CM 0009, Hampshire, UK) and blended for 90 s in a stomacher (Interscience-BagMixer 400, St Nom, France). From this homogenate serial decimal dilutions were prepared in 0.1% peptone water according to standard method given by APHA [17]. Total aerobic mesophilic and psychrotrophic bacterial counts [18,19] were determined by Plate Count Agar (PCA, Oxoid CM 325) with the incubation at 37°C for 1-2 days and 7°C for 10 days, respectively. *Lactobacilli* counts [20] were enumerated on de Man Rogosa Sharpe agar (MRS, de Man Rogosa Sharpe Agar, Oxoid CM 0361) incubated at 30°C for 2 days. Enterobacteriaceae [21] were enumerated in Violet Red Bile Dextrose Agar (VRBG, Oxoid CM 0485) incubated at 37°C for 2 days. Coliforms [22] were enumerated on Violet Red Bile Agar (VRBA, Oxoid CM 0107) incubated at 37°C for 1-2 days.

Sensory Analyses

The sensory quality of raw mussels was evaluated by a sensory panel consisting of ten experienced judges. The sensory characteristics of mussels were evaluated using the 9-point hedonic scale according to the characteristics shown in *Table 1*. Each panelist evaluated approximately 20 g of mussel sample for appearance, odour, colour and texture. Four categories were ranked: scores between 9 and 8 indicated "excellent quality (E)"; scores between 8 and 6 indicated "good quality (A)"; scores between 6 and 4 indicated "fair quality (B)"; scores ≤ 4 indicated "unaccaptable (C)" samples [23,24].

Statistical Analyses

Statistical analysis was performed using SPSS [25] (Statistical Package for Social Sciences SPSS, Inc, Chicago) software package. All experiments were carried out in duplicate and the results were presented as means ± standard deviations. The results were evaluated by using ANOVA and any significant differences further evaluated using the Tukey Multiple Comparison Test. The level of significance was set at $P<0.05$.

RESULTS

The bacterial counts of total aerobic mesophilic bacteria, psychrotrophic bacteria, *Lactobacillus* spp., Enterobacteriaceae and coliform bacteria of control group mussels in initial day (0 day) were found $4.7x10^5$ cfu/g, $4.6x10^5$ cfu/g, $4.0x10^4$ cfu/g, $4.8x10^3$ cfu/g and $4.6x10^3$ cfu/g, respectively. Following treatment of mussels with lactic acid for 15 min, the bacterial counts of total aerobic mesophilic bacteria, psychrotrophic bacteria, *Lactobacillus* spp., Enterobacteriaceae and coliform bacteria were $1.4x10^5$ cfu/g, $3.6x10^5$ cfu/g, $1.4x10^4$ cfu/g, $1.8x10^3$ cfu/g and $1.4x10^3$ cfu/g, respectively. Following treatment of mussels with chitosan for 15 min, the bacterial counts of total aerobic mesophilic bacteria, psychrotrophic bacteria, *Lactobacillus* spp., enterobacteriaceae and coliform bacteria

Table 1. *Sensory analysis scale for mussels.*
Tablo 1. *Midyeler için duyusal analiz skalası*

Parameter	Excellent Quality (E) 9 - 8	Good Quality (A) 8 - 6	Fairly Good Quality (B) 6 - 4	Unaccaptable (C) ≤ 4
Appearance	Glossy	Moist	Less moist	Dull
Odour	Characteristically sweet and fresh	Non- specific/slightly sweet	Slightly ammonia- like	Ammonia
Colour	Bright	Orange	Opaque	Grey/discoloured
Texture	Very firm	Firm	Slightly firm	Soft

were $4.0x10^4$ cfu/g, $3.4x10^5$ cfu/g, $2.0x10^3$ cfu/g, $1.6x10^3$ cfu/g and $1.0x10^3$ cfu/g, respectively.

In the present study, following immersion of mussels to lactic acid for 15 min, the bacterial counts of total aerobic mesophilic bacteria, psychrotrophic bacteria, *Lactobacillus* spp., Enterobacteriaceae and coliform bacteria (0.53, 0.11, 0.46, 0.43 and 0.52 log cfu/g), decreased, respectively. Mussels treated with chitosan for 15 min, the bacterial counts of total aerobic mesophilic bacteria, psychrotrophic bacteria, *Lactobacillus* spp., Enterobacteriaceae and coliform bacteria (1.07, 0.13, 1.30, 0.48 and 0.66 log cfu/g) decreased, respectively.

Table 2. *Changes in chemical constituents of mussels pretreated with chitosan and lactic acid during storage at 4°C (Mean+SD).*

Tablo 2. *Kitosan ve laktik asitle muamele edilen midyelerde +4°C'de muhafaza sürecinde şekillenen kimyasal değişiklikler*

Stroge Time (Day)	Control	Lactic Acid	Chitosan
TVB-N (mg N 100 g^{-1})			
0	13.1±0.2 ^{a1}	12.0±0.2^{a1}	11.9±0.2^{a1}
2	16.1±0.2 ^{a2}	14.0±0.2^{b2}	12.3±0.2^{c1}
4	30.1±0.3 ^{a3}	24.0±0.3^{b3}	15.0±0.4^{c2}
7	33.6±0.6 ^{a4}	28.0±0.4^{b4}	25.9±0.5^{c3}
9	38.5±0.5 ^{a5}	35.0±0.3^{b5}	33.6±0.2^{c4}
11	39.9±0.2 ^{a6}	38.0±0.2^{b6}	37.0±0.5^{b5}
TBA (mg MDA kg $^{-1}$)			
0	1.37±0.02^{a1}	1.21±0.02^{b1}	1.14±0.02^{c1}
2	1.72±0.02^{a2}	1.33±0.03^{b2}	1.23±0.03^{c2}
4	2.51±0.01^{a3}	1.34±0.01^{b2}	1.30±0.01^{c3}
7	2.72±0.01^{a4}	2.10±0.01^{b3}	1.96±0.01^{c4}
9	2.90±0.02^{a5}	2.47±0.02^{b4}	2.34±0.01^{c5}
11	3.02±0.01^{a6}	3.01±0.01^{a5}	2.87±0.02^{b6}
TMA-N (mg N 100 g^{-1})			
0	1.97±0.03^{a1}	1.27±0.02^{b1}	1.26±0.02^{b1}
2	3.25±0.02^{a2}	2.98±0.03^{b2}	2.79±0.04^{c2}
4	4.45±0.05^{a3}	2.79±0.09^{b3}	2.60±0.05^{c3}
7	4.60±0.04^{a4}	3.90±0.03^{b4}	3.50±0.05^{c4}
9	4.75±0.05^{a5}	4.16±0.06^{b5}	3.90±0.05^{c5}
11	4.86±0.06^{a5}	4.39±0.03^{b6}	4.35±0.02^{b6}
Histamine (ppm)			
0	4.00±0.02^{a1}	3.79±0.03^{b1}	3.80±0.03^{b1}
2	4.34±0.03^{a2}	4.10±0.02^{b2}	4.05±0.02^{c1}
4	4.48±0.02^{a3}	4.20±0.01^{b3}	4.18±0.01^{b1}
7	5.29±0.02^{a4}	4.78±0.02^{b4}	4.55±0.03^{c2}
9	5.69±0.03^{a5}	5.20±0.04^{b5}	5.12±0.03^{c2}
11	7.75±0.04^{a6}	5.60±0.02^{b6}	5.40±0.02^{c2}
pH			
0	5.99±0.02^{a1}	5.01±0.01^{b1}	5.48±0.02^{c1}
2	5.30±0.02^{a2}	5.04±0.02^{b1}	5.19±0.01^{c2}
4	5.02±0.01^{a3}	4.88±0.01^{b2}	5.00±0.01^{c2}
7	4.88±0.01^{a4}	4.60±0.02^{b3}	4.67±0.01^{c3}
9	4.66±0.02^{a5}	4.54±0.01^{b4}	4.65±0.02^{a4}
11	4.54±0.01^{a6}	4.52±0.01^{a4}	4.55±0.02^{a5}

^{a,b,c} Mean values within a column with different superscript letters differ significantly (P<0.05), $^{1,2,3....6}$ Mean values within a line with different superscript letters differ significantly (P<0.05)

Chemical and sensory analyses were used to evaluate the effect of these treatments during storage at 4°C. The changes that occured in pH, TVB-N, TBA, TMA-N and histamine of mussel meat during storage are presented in *Table 2*. The results of sensory evaluation are shown in *Fig. 1*. At the beginning of the storage, fresh mussel had a characteristic fresh odour (sealike, seaweed-like), bright orange colour, slightly firm, elastic, tight texture and moist appearance. Sensory scores significantly ($P<0.05$) changed by time. The acceptable shelf-

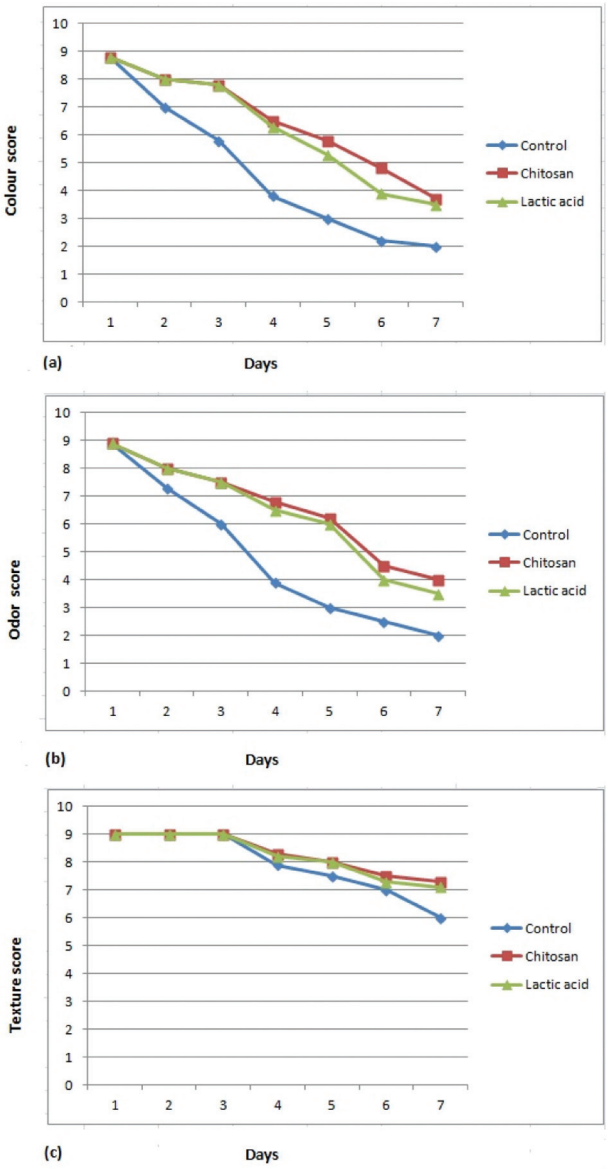

Fig 1. Sensory evaluation. Changes in (a) colour, (b) odor, (c) texture scores in mussels during storage at +4°C

Şekil 1. Duyusal değerlendirme. Midyelerde +4°C'de depolama süresince renk, koku ve tekstürdeki değişiklikler

life was considered from a sensory score of greater than 5.0. The results showed that sensory scores were greater than 5 for the non-treated mussels (control group) stored for 4 days at 4°C, while mussels pretreated with lactic acid and chitosan were stored for 6-7 days.

DISCUSSION

The initial pH of the mussels was 5.99 and gradually decreased in all treatments during storage ($P<0.05$). Similar results on the initial pH of mussels were reported as 5.96 by Erkan [24]. Mussels pretreated with lactic acid had lower pH than those pretreated with chitosan. The decrease in the pH of mussels may be due to the postmortem changes, degradation of muscle components and conversion of glycogen to lactic acid during the long term storage [26]. Pottinger [27] proposed the following pH scale as a basis for determining the freshness of mollusks: pH=6.2-5.9 good, pH=5.8 off, pH=5.7-5.5 musty, pH=5.2 and pH < 5.2 sour or putrid. The present study showed that pH value of control group mussels was unacceptable as 5.02 at the 4th day and was 4.54 at the end of the storage. On the other hand, Manousaridis et al.[4] reported that an attempt to correlate changes in pH to the sensory quality of mussels was unsuccessful and that pH is not useful as a quality index.

The TVB-N of seafood is an indicator of the freshness of the raw material. In this study the initial TVB-N value of mussel samples ranged from 11.9 to 13.1 mg N 100 g^{-1}. Thereafter it increased with time reaching 39.9 mg N 100 g^{-1} for the control group, whereas for mussels pretreated with lactic acid and chitosan it was 38 and 37 mg N 100 g^{-1}, respectively, on the eleventh day. The TVB-N value for the control group was found to be higher than the proposed acceptability limits of 35 mg N 100 g^{-1} of fish muscle set by the Commission Decision 95/149/EC [14] after 9 days of storage. On the other hand, when the mussels were pretreated with lactic acid and chitosan, the TVB-N value was found to be lower than the acceptability limits after 9 days of storage.

Erkan [24] reported that the TVB-N value of fresh mussel was initially 12.38 mg N 100 g^{-1}, reaching 22.55 mg N 100 g^{-1} after the sixth day. Goulas et al.[28] found that the TVB-N value was initially 11.48 mg N 100 g^{-1} in mussel samples and it was 36.72 mg N 100 g^{-1} after 15 days of storage for the control group. They stated that when mixtures of (M1: 50%/50% (CO_2/N_2), M2: 80%/20% (CO_2/N_2) and M3: 40%/30%/30% (CO_2/N_2/O_2) were used, the TVB-N values remained significantly lower ($P<0.05$) than the acceptability limit after 15 days of storage. TVB-N content was found higher in the control mussels when compared with acid pretreated mussel samples throughout storage ($P<0.05$) by Masniyom and Benjama [29]. They indicated that TVB-N content increased rapidly and reached 20 mg N 100 g^{-1} after 9 days of storage for the control and all samples dipped in acids had TVB-N contents less than 20 mg N 100 g^{-1} within 27 days of storage.

TMA is produced by the reduction of trimethylamine oxide (TMA-O) by TMA-O reductase producing organisms and enzymatic activity [30]. TMA is considered as a good quality indicator for shrimp; it only reached 3 mg per 100 g^{-1} TMA-N, which is below the suggested 5 mg per 100 g^{-1} TMA-N limit for spoiled shrimp [31]. A level of 10-15 mg per 100 g^{-1} was suggested by Connell [30] as the maximum limit of acceptability to indicate fish freshness. Ruiz-Capillas et al.[32] cited 5 mg per 100 g^{-1} as the acceptable limit of TMA-N in shrimp and lobster. In the present study, TMA-N values of mussels were ranged from 1.26 to 1.97 mg N 100 g^{-1} on the first day of storage. At the end of the storage period of 11 days, TMA-N values increased to 4.86 mg N 100 g^{-1} for the control group and in the samples pretreated with lactic acid and chitosan TMA-N values were 4.36 and 4.35 mg N 100 g^{-1}, respectively. Masniyom and Benjama [29] found the highest concentration of TMA-N in the control mussel samples (0.06 mg/g), followed by those treated with citric, acetic and lactic acids (from 0.02 mg/g to 0.04 mg/g), respectively after 9 days of storage. They indicated that even after 27 days of storage, samples pretreated with acids had a TMA-N less than 5 mg N 100 g^{-1}. Similarly, in the present study control group samples and samples pretreated with lactic acid and chitosan had TMA-N less than 5 mg N 100 g^{-1} after 11 days of storage. Manousaridis et al.[4] reported that TMA-N values of all mussel samples remained relatively low throughout the entire storage period, attaining values of 7.5, 6.0 and 6.4 mg N/100 g for the control and samples ozonated for 60 and 90 min, respectively, on day 12 of storage.

The TBA assay is one of the most commonly used indicators for monitoring lipid oxidation. Lipid oxidation is responsible for undesirable effects such as the formation of off-odours and off-flavours in edible oils and fat-containing foods, called oxidative rancidity, and the loss of fat-soluble vitamins and palatability problems [33,34]. As can be seen in *Table 2*, the TBA values of the control and samples pretreated with lactic acid and chitosan were significantly ($P<0.05$) increased from 1.37, 1.21 and 1.14 (day 0) to 3.02, 3.01 and 2.87 mg MDA kg^{-1}, respectively, at the end of the storage period of 11 days. Similar results have been found by Masniyom and Benjama [29], who found that TBA value increased in all mussel samples when the storage time increased ($P<0.05$), indicating the lipid oxidation. TBA values found in the mussel samples by Caglak et al.[35] were between 2.16 to 3.7 mg MDA kg^{-1} at the end of the storage period of 12 days. In this study, TBA values of control mussels were 2.51 mg MDA kg^{-1} on the 4th day, exceeding the value of 1-2 mg MDA kg^{-1}, regarded as the limit beyond which fish will normally develop an objectionable odour/taste [30], whereas samples treated with lactic acid and chitosan did not exceed the limit after 4 days of storage. TBA values for samples pretreated with chitosan were lower than for those treated with lactic acid. On the other hand, Masniyom and Benjama [29] found that samples pretreated with citric acid showed lower TBARS than those treated with lactic and acetic acids.

Histamine is formed through the decarboxylation of

specific free amino acids by exogenous decarboxylases released from microbial populations associated with seafood [36]. Fish species belonging to the family Scombridae (e.g. tuna, bonito and mackerel), which contain high levels of free histidine in their muscle, are often implicated in scombroid poisoning incidents when not properly processed and stored [37]. Microbial contamination, temperature, proteolytic enzymes, pH and oxygen are the factors affecting the formation of histamine in foods [38]. In the present study, histamine level in the mussel samples was between 3.80-4.00 ppm on day 0. Afterwards, the histamine level was found to be 7.74, 5.60 and 5.40 ppm for the control group and samples treated with lactic acid and chitosan, respectively, at the end of the storage period of 11 days. The histamine level was increased gradually during the storage period at 4°C. However, these concentrations were lower than 50 ppm of histamine, the allowed limit of the US Food and Drug Administration (FDA) for scombroid fish and/or products [39] Similarly, very low or negligible histamine concentrations were found in calamari, prawn and mussel samples by Auerswald et al.[40]. They found a histamine value of 5.1 ppm in black mussels *(Mytilus galloprovincialis*) purchased from various outlets.

Crapo and Himelbloom [41] stated that "histamine value was 6 ppm in pacific herring fish after two days storage at 10°C. In fish between six and ten days, histamine levels were slightly less than 50 ppm. Only the fourteen day sample exceeded the regulatory action level." Olley and Baranowski [42] pointed out that low-temperature enzyme activity by microorganisms that grow at warm temperatures is important in histamine formation, provided sufficient bacterial numbers have been reached before cold storage. It is estimated that the difference between these histamine levels may be caused by the differences in storage methods used, bacterial load in sample, storage temperature and type of fishes.

The results showed that non-treated mussels (control group) could be stored for 4 days at 4°C, whereas mussels pre-treated with lactic acid and chitosan could be stored for 6-7days. Mussels treated with lactic acid and chitosan had an extended shelf-life of ca. 2-3 days, as compared with the control samples. The highest sensory scores were observed for mussel samples treated with chitosan. These results are in agreement with other researchers who reported a shelf-life of 3-4 days for mussels stored in a refrigerator [24,43,44]. However Goulas et al.[28] reported a shelf-life of 8-9 days for mussels at +4°C. On the other hand, Gokoglu [45] states that the samples were not safe to consume after 5 days.

In the present study, treatment of mussels with lactic acid and chitosan for 15 min reduced bacterial counts of total aerobic mesophilic bacteria (0.53-1.07 log) psychrotrophic bacteria (0.11-0.13 log), *Lactobacillus* spp. (0.46-1.30 log), Enterobacteriaceae (0.43-0.48 log) and coliform bacteria (0.52-0.66 log). Chitosan was more effective for reducing bacterial loads of mussels than that of lactic acid. The superiority of the chitosan versus lactic acid was originated from that of chitosan was not only effective against both gram (+) ve gram (-) bacteria but the use of lactic acid and acetic acid for the dissolving of chitosan further contributed to the antimicrobial effectiveness of the chitosan.

Chemical and sensory analyses revealed that mussel samples (control group) were still "acceptable" on day 4; however, on day 5, they were no longer acceptable. On the other hand, mussels treated with lactic acid and chitosan had an extended shelf-life of ca 2-3 days, as compared with the control group ($P<0.05$). The TVB-N value exceeded the acceptable limit of 35 mg N 100 g^{-1} on the 9th day. The TBA value exceeded the limit of 2 mg MDA kg^{-1} on the 4th day. Sensory quality limits were exceeded on day 4. However, TMA-N and histamine values did not exceed the acceptable limits at the end of the storage (day 11) in the control group mussels stored at 4°C. The highest sensory scores were obtained for mussel samples treated with chitosan. In conclusion, the results of this study suggest that the lactic acid and chitosan can be used to extend the shelf-life and maintain the quality of mussels.

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