Changes in quality characteristics during cold storage of shucked mussels (*Mytilus galloprovincialis*) and selected chemical decomposition indicators

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Abstract: The following chemical changes were observed during the cold storage of mussels for 6 days at 4°C. Total volatile basic nitrogen (TVB-N, mg N per 100 g) and trimethylamine values (TMA-N, mg N per 100 g) were increased from 22.55 and 5.96 mg per 100 g at day 0 to 12.38 and 0.42 mg per 100 g, respectively, at the end of the storage period. The indole value and putrescine concentration were increased from 15.36 µg kg⁻¹ and 24.7 mg kg⁻¹ to 34.46 µg kg⁻¹ and 63.86 mg kg⁻¹, respectively, on the fourth day of storage. TVB-N, TMA-N and indole value could be selected as decomposition indicators for mussels during their cold storage. Acceptable limits of 15 mg per 100 g for TVB-N, 3 mg per 100 g for TMA-N, 35 µg kg⁻¹ for indole and 60 mg kg⁻¹ for putrescine are suggested. Sensory and chemical results indicated that the shelf-life of mussels at 4°C is limited to 4 days.

Keywords: mussel; freshness; indicator; quality assessment; spoilage

INTRODUCTION

Bivalves are valued seafood of maritime countries world-wide, mussels, scallops, oysters and clams being of the greatest commercial importance. Mussels are considered as a low-fat and low-cholesterol food with eicosapentaenoic (C20:5, n-3) and docosahexaenoic (C22:6, n-3) acids being the prevalent ones. Mussel is rich in protein, vitamins and minerals and has been used as one of the most popular items, especially in the production of frozen and cooked varieties. Mussels (*Mytilus galloprovincialis*) belong to the Mytilae family and are very popular mollusc species harvested in the Mediterranean, Marmara Sea and Bosphorus countries. Mussels are a very perishable seafood, and keeping seafood in good condition for as long as possible is a very important objective for producers. There are several reports of the general pattern of spoilage and storage stability of aquatic products.

Changes in pH, microbial numbers, trimethylamine, total nitrogen, non-volatile protein nitrogen, free amino acids, volatile acids and indole have been used and/or proposed as indices of the freshness of iced aquatic species. A number of compounds or groups of compounds have been suggested as chemical indicators of decomposition for seafoods. However, it is not known which chemical indicators are applicable to the detection of decomposition in these products. Total volatile bases and the trimethylamine (TMA-N) value are important parameters for determining the freshness of seafood products. Cadaverine, putrescine and histamine have also been suggested as chemical indicators of decomposition.

A review of the literature revealed some information about the shelf-life of shrimp, lobster and crab. Flores and Crawford evaluated the postmortem quality changes in iced Pacific shrimp (*Pandalus jordani*). Matches studied the effects of temperature on the decomposition of the same species. Chang et al. studied the effects of fresh storage temperature, freezing and boiling on indole levels of shrimp. Aman et al. examined the effects of ice and cold storage on the chemical and technological characteristics of Egyptian crab meat. Fatima and Qadri observed quality changes in lobster (*Panulirus polyphagus*) muscle during storage in ice. Shamshad et al. investigated the effects of different temperatures on the shelf-life of shrimp (*Penaeus merguiensis*). Hollingworth et al. studied the chemical indicators of decomposition for raw surimi and flaked artificial
crab. The same group investigated the chemical and microbiological analysis of vacuum-packed, pasteurised flaked imitation crab meat.

However, little information is available on quality changes in mussel. Slabjy and Carpenter studied processing effects on the proximate composition and mineral content of meat of blue mussels (*Mytilus edulis*). Krzynowek and Wiggins reported seasonal variations and frozen storage stability of the same species. Vasakou et al. studied the effect of sodium lactate and potassium sorbate on the quality characteristics and shelf-life of shucked mussels during chilled storage in pouches containing water. However, no chemical decomposition indicators have been established for mussels.

The purpose of this research was to study the changes in quality characteristics of shucked mussels during their refrigerated storage and to select the most appropriate chemical indicators for their decomposition.

**MATERIALS AND METHODS**

Live mussels (*Mytilus galloprovincialis, LAMARCK, 1819*) were obtained from a seafood whole market in Istanbul. They had been harvested from the Bosphorus in May. The samples were immediately transported to the laboratory within 1 h of harvesting, in polystyrene foam boxes containing ice. They were subsequently inspected and dead animals were discarded. The remaining mussels (3 kg, ~1500) were rapidly washed and manually shucked by cutting the adductor muscle with a sterile knife. The mussels were divided into two lots. This study was performed in two series each being a pack of ~750 mussels giving about 100–120 g of mussel meat. The mussels were put into insulated sterile plastic boxes without ice or water. The plastic boxes were stored in a refrigerator (4 ± 1 °C) and samples were analysed every day for 6 days to determine the shelf-life. After sensory analysis, samples were homogenized and subjected to chemical analyses.

**Sensory analyses**

Five judges assessed the sensory properties of samples and a hedonic scale (Table 1) was used. General appearance, odour, colour and texture were used as criteria for acceptability. The mean values of these criteria from all panellists were calculated and the mussels were classified according to the following correspondence between points and quality bands: 9 ≥ E ≤ 8, 8 > A ≥ 6, 6 > B > 4, 4 ≥ C.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Extra category: 9 ≥ E ≥ 8</th>
<th>A category: 8 &gt; A ≥ 6</th>
<th>B category: 6 &gt; B &gt; 4</th>
<th>C category: 4 ≥ C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Glossy</td>
<td>Moist</td>
<td>Less moist</td>
<td>Dull</td>
</tr>
<tr>
<td>Odour</td>
<td>Characteristic sweet fresh</td>
<td>Non-specific slightly sweet</td>
<td>Slight to ammonia</td>
<td>Ammonia</td>
</tr>
<tr>
<td>Colour</td>
<td>Bright</td>
<td>Orange</td>
<td>Opaque</td>
<td>Grey discoloured</td>
</tr>
<tr>
<td>Texture</td>
<td>Very firm</td>
<td>Firm</td>
<td>Slightly firm</td>
<td>Soft</td>
</tr>
</tbody>
</table>

**Chemical analyses**

**pH**

pH was determined at room temperature on homogenates of mussel in distilled water (1:10, w/w). pH was measured using a microprocessor-controlled pH meter (Model WTW 537; WTW, Weilheim, Germany).

**Determination of total volatile basic nitrogen (TVB-N)**

TVB-N was determined using the method of Antonocopoulos and Vyncke. For TVB-N, a mussel sample (10 g) was homogenized with 6% perchloric acid (90 mL) for 1 min in an Ultra-Turrax homogeniser (IKA T 25 Basic, Staufen, Germany). The homogenates were filtered through a filter-paper (Whatman No. 1) and filtrates were distilled in duplicate in a Velp Marka (Model UDK 140, Milan, Italy) apparatus. After distillation, the contents of the conical flask were titrated with 0.01 mol L⁻¹ HCl. Results were expressed as mg TVB-N per 100 g of wet sample.

**Determination of trimethylamine nitrogen (TMA-N)**

TMA-N was determined by the AOAC method. The technique consisted in extracting the TMA from 20–25 mussel samples with trichloroacetic acid (7.5%) in a ratio of 1:9 (10 g:90 mL). The TMA was extracted with toluene and this extract was reacted with picric acid, which interacted with the primary and secondary amines to produce coloured reaction products (yellow picrates) with maximum absorption at 410 nm. Results were expressed as mg TMA-N per 100 g mussel.

**Indole analyses**

Indole was determined with the high performance liquid chromatographic (HPLC)–fluorescence detection method of Helle. The HPLC system consisted of a system controller, autoinjector, liquid chromatography pump A, liquid chromatography pump B, fluorescence detector and degasser, all from Shimadzu (Kyoto, Japan). The computer program used was Class-VP (Shimadzu). The chromatographic conditions were as follows: injection volume, 20 μL; eluent A, acetonitrile; eluent B, acetonitrile–water (1:1); flow rate, 0.7 mL min⁻¹; fluorescence detection, excitation at 275 nm, emission at 332 nm; column, M&N Nucleosil C18 HD-100, 3 μm HD precolumn, 250 × 4 mm i.d.; temperature, 35 °C; washing, methanol–water (4:1).
Biogenic amine analyses

Ten biogenic amines were measured by HPLC. The method involves an ion-pair chromatographic procedure on a Shimadzu Shim-Pack CLC-ODS (M) 250 reversed-phase column with a post-column reaction with o-phthalaldehyde (OPA).25

Preparation of standard amine solutions. Extraction of the samples and HPLC determination of biogenic amines were carried out according to a standard procedure.25 The detection limits were ~2 mg kg\(^{-1}\) for putrescine, histamine, tyramine and cadaverine. Standard solutions of putrescine, histamine, tyramine and cadaverine were prepared at concentrations of 20, 50, 100, 250 and 500 mg kg\(^{-1}\).

Preparation of sample extracts. To extract biogenic amines, 45 mL of 0.6 mol L\(^{-1}\) perchloric acid were added to 5 g of marinated mussel sample and the mixture was homogenized using an Ultra-Turrax homogeniser and the homogenate was filtered through a Schleicher & Schuell (Dassel, Germany) 595 filter-paper, φ 185 mm, and SMI Syringe Filters PTFE (SMI LabHut, Gloucester, UK), pore size 0.2 µm, filter φ 25 mm.

Chromatographic separation. The HPLC system consisted of a system controller, autoinjector, liquid chromatography pump A, liquid chromatography pump B, peristaltic pump, degasser and fluorescence detector (all from Shimadzu). The computer program used was Class-VP (Shimadzu). The chromatographic conditions were as follows, injection volume, 10 µL; eluent A, 8.03 g sodium acetate, 800 mL water, pH 4.5, and 2.16 g sodium octasulfonate (1000 mL), eluent B, 12.73 g sodium acetate, 600 mL water, pH 4.5, and 2.16 g sodium octasulfonate (230 mL) plus acetonitrile (1000 mL); flow rate, 1.0 mL min\(^{-1}\); derivative reagent (OPA), 0.7 mL min\(^{-1}\); fluorescence detection, excitation at 330 nm, emission at 465 nm; column, Shimadzu Shim-Pack CLC-ODS (M) 250.

Statistical analyses

Results are expressed as mean ± standard deviation. The significance (\(P < 0.05\)) of the variables studied was assessed by a one-way analysis of variance (ANOVA) test. A time-dependent linear regression analysis was performed on the results obtained for TVB-N, TMA-N, indole and putrescine values. Spearman’s correlation coefficients were calculated to study the relationship between amines and sensory evaluations. Statistical analyses of data were calculated using Microsoft Excel XP 2003.26

RESULTS AND DISCUSSION

Sensory evaluation data are presented in Fig. 1. The characteristic parameter of mussel gradually decreased (\(R^2 = 0.9633, y = -1.21x + 8.5\)) in intensity during storage. All sensory parameters were excellent or very good (grades E and A) scored during the first 2 days of storage and moderate grades (grade B) were obtained between days 2 and 4 of storage for mussel samples. Samples unfit for sale (grade C) were obtained after 4 days of cold storage. These results are in agreement with those of Kastanidou-Monousou et al., who kept shucked mussel meat at 3–4 °C. Pastoriza et al. reported a shelf-life of 3–4 days for live mussels stored in a refrigerator. Similar results were reported by Gökodlu for mussels stored in a refrigerator.

Quality changes that occur in iced seafood during storage are generally considered to result from the combined action of tissue enzymes and microbial contamination.

The pH was initially 5.96 and it decreased to 5.89 by the sixth day of storage. These values were not significant (\(P > 0.05\)) during storage. Pottinger proposed the following pH scale as a basis for determining the freshness of molluscs (oysters): pH 6.2–5.9, good; pH 5.8, ‘off’; pH 5.7–5.5, musty; pH ≤5.2, sour or putrid. An attempt to correlate changes in pH with the sensory quality of mussels was unsuccessful. The pH of the muscle of final live oysters ranged from 5.6 to 6.3. Dead oysters had a tissue pH of 5.2–5.4. Similar results have been reported for chilled scallops by Maxwell-Miller et al. Low pH levels (5.9) at the point of rejection have also been reported for mussels.33

As seafood spoils, proteins are broken down to peptides, free amino acids, amines and volatile ammonia. Trimethylamine oxide is broken down to the volatile amines di- and trimethylamine. The TVB-N of fish is an indicator of the freshness of the raw material. The TVB-N content of fresh mussel was initially 12.38 mg per 100 g. Thereafter, it increased (\(P < 0.05, R^2 = 0.6872, y = 1.69x + 9.95\)) with time, reaching 22.55 mg per 100 g after the sixth day (Fig. 2). At the borderline of acceptability (fourth day) the TVB-N level of chilled mussels was 15 mg per 100 g.

Cheuk et al. found constant TVB-N values during the first 11 days on ice for brown shrimp and during the first 15 days for pink shrimp. Spoilage set in
after 16 days for pink shrimp and 19 days for brown shrimp, when the TVN-N values rose to 30 mg per 100 g. A TVB-N level of 30 mg per 100 g of muscle has been considered as spoiled and unfit for human consumption.\textsuperscript{5} Lannelongue et al\textsuperscript{33} listed the following values (mg per 100 g TVB-N) for different degrees of freshness of fish: ≤12, fresh fish; 12–20, edible with only slight decomposition; 20–25 borderline; and >25, inedible and decomposed. Kim et al\textsuperscript{36} recorded TVB-N values between 19 and 35 mg per 100 g for oysters packaged in LDPE pouches after 12 days of refrigerated storage. Erkan and Gökoglu\textsuperscript{37} reported TVB-N values between 20–25 mg per 100 g for the acceptability limit of frozen fish and suggested that this parameter is a good indicator of freshness. Sikorski et al\textsuperscript{38} reported an acceptability level of TVB-N of 17 mg per 100 g in oysters. No limit of acceptability for mussel has been established by EC Decision 95/149/39.

The production of TMA-N and a pronounced increment in non-protein nitrogen in mussel during cold storage could be used as indicators of bacterial activity. TMA-N is considered a valuable tool in the evaluation of the quality of seafood products stored in ice because of its rapid accumulation in muscle under refrigerated conditions.\textsuperscript{40–42} The production of TMA-N followed a pattern similar to that of TVB. The TMA content of mussel samples increased slowly (5.96 mg per 100 g) during the storage period ($P < 0.05$, $R^2 = 0.9726$, $y = 0.94x - 0.04$). At the limit of acceptability the TMA-N value was 3 mg per 100 g (fourth day) in chilled mussel. The levels of TMA-N remained below the limit during the sensory shelf-life of mussels as determined by the test panel. A negative correlation was determined between the sensory and TVB-N values ($r_s = -0.7054$, $P < 0.05$) and TMA-N values ($r_s = -0.9732$, $P < 0.05$) during storage of mussels.

Trimethylamine analysis is often used as an index in assessing the shelf-life and keeping quality of fishery products.\textsuperscript{9} Ruiz-Capillas et al\textsuperscript{43} cited 5 mg per 100 g as the acceptable limit of TMA-N in shrimp and lobster. Similarly, low TMA values have been reported for whole fresh Mediterranean fish stored in ice.\textsuperscript{44–47} The formation of TMA in fish muscle is mainly due to bacterial action on the TMAO content and the presence of specific spoilage organisms in the fish.\textsuperscript{48} A level of 10–15 mg per 100 g was suggested by Connell\textsuperscript{49} as the maximum limit of acceptability to indicate fish freshness. TMA is considered a good quality indicator for shrimp; it only reached 3 mg per 100 g TMA-N, which is below the suggested 5 mg per 100 g TMA-N limit for spoiled shrimp.\textsuperscript{44} No limit of TMA-N for the acceptability of mussels has been established in the literature.

The chemical changes taking place in fish and shellfish during spoilage are due primarily to bacterial action, although studies have shown a contribution by endogenous enzymes.\textsuperscript{8} In the studies reported here, the chemical changes measured were volatile base production calculated on nitrogen and indole.\textsuperscript{13} The changes in the indole level of the mussels are shown in Fig. 3. The indole level of very fresh mussel was initially $\sim 15.6 \mu g kg^{-1}$ and changed during chilled storage to 39.8 $\mu g kg^{-1}$ after 6 days ($P < 0.05$, $R^2 = 0.9511$, $y = 4.39x + 14.75$). Indole was an indicator of decomposition for shrimp and oysters. A good correlation between indole and sensory evaluation of fresh shrimp has been demonstrated.\textsuperscript{16,50} There was a significant correlation ($r_s = -0.9732$, $P < 0.05$) between the sensory and the indole values during storage. Indole is formed by bacterial degradation of the amino acid tryptophan.

Tryptophan, found in a large count of shrimp flesh, is broken down to indole by the enzyme tryptophanase found in some mesophilic microorganisms, frequently Gram-negative.\textsuperscript{51} A good correlation has been determined to exist between the levels of \textit{Escherichia coli} and the production of indole. The production of indole shows considerable development at relatively high temperatures.\textsuperscript{50,51} Indole is currently used by the US Food and Drug Administration (FDA) to validate the sensory evaluation of shrimp decomposition and a
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level of <25 µg per 100 g indole has been established for class 1 shrimp. If the pH of the shrimp tissue is <7.5, TVB <28.5 m per 100 g, TMA-N <5.0 mg per 100 g and indole <9.0 µg per 100 g, the sample will probably be of acceptable quality. Biogenic amines are generated by decarboxylase activity of both endogenous and microbial enzymes on free amino acids, so their presence could serve as an indicator of fish spoilage. In crustaceans, the biogenic amines act as neurotransmitters, neuromodulators or even neurohormones. Some bacterial groups such as Bacillus, Pseudomonas, Clostridium, Photobacterium, members of Enterobacteriaceae and lactic acid bacteria are capable of decarboxylating amino acids. Tyrosine is liberated from proteins and peptides during the process of enzymatic proteolysis in muscle and has been used as a measure of such activity. Several investigations of the use of tyrosine levels as an index of quality in fin fish have been reported. Putrescine is the decarboxylation product of ornithine and cadaverine arises from decarboxylation of lysine. Putrescine and cadaverine have been regarded as a freshness index in fish, as they were the only amines detected before the initial decomposition.

The putrescine level in fresh mussels was 24.7 mg kg⁻¹ and increased during the six days of cold storage to 62.56 mg kg⁻¹ (P < 0.05, R² = 0.7811, y = 7.32x + 25.89). A negative and significant correlation (r = -0.7232, P < 0.05) was observed between the sensory and putrescine values during storage. Other biogenic amines (tyramine and cadaverine) were not detected during the cold storage of mussels. Similar histamine, tyramine and cadaverine concentrations have been reported for crab, pasteurised crab and scallop.

In conclusion, chemical and sensory changes of mussels stored under refrigeration (4 ± 1 °C) were investigated by means of sensory assessments and chemical analyses. The sensory and chemical results indicated a shelf-life of 4 days for mussels stored at 4 °C.

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