

Food safety and shelf life of cooked meat of brown mussel, *Perna perna* (Linnaeus, 1758) stored under different temperatures

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Abstract

Food safety of shucked meat of brown mussel (*Perna perna*) stored under three different temperature conditions, ambient (28°C), refrigerated (4°C) and frozen state (-5°C) was analyzed using sensory, microbiological and biochemical methods. The shelf life of the mussel was observed to be 14 hours at ambient temperature, 7 days at refrigeration temperature (4°C) and 6 weeks at frozen temperature (-5°C). Mussels stored at -5°C had the most acceptable quality. The total heterotrophic bacterial count of frozen mussel was 1.7 X 10⁴ CFU/g and Trimethylamine – Nitrogen and Total Volatile Base - Nitrogen values were 7.81 mg/100g and 17.59 mg/100g respectively. The pH stayed near neutral till the end of the 5th week. The limits of freshness stated here were mostly within the proposed limits of acceptability by some international quality standard organization for marine sea foods.

Keywords

Mussel
P. perna
Temperature
Shelf life assessment
Storage temperature

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Introduction

Seafood is being used as a main protein source from time immemorial. In developing countries, many people rely on sea foods for their animal protein requirements. Sea foods have received more attention from consumers due to their positive benefit on human health and nutrition (Jeena *et al.*, 2003). Seafood mainly comprises of fin fish, crustaceans and mollusks. Among the molluscan species, bivalves are considered a delicacy. Bivalves include mussels, clams, oysters and scallops. Mussels are low in fat and cholesterol (Holland *et al.*, 1994) and rich in polyunsaturated fatty acids (Misra *et al.*, 1985), mainly n-3 Poly unsaturated fatty acid (Orban *et al.*, 2002). Mussels have recently received more attention from consumers due to their benefit on human health and nutrition. Recent studies have clearly shown the importance of n-3 and n-6 fatty acids for human health (Miletic *et al.*, 1991). These fatty acids are biologically important and have been associated with a decreased risk of cardiovascular disease (Kromhout *et al.*, 1985). Mussels are very perishable seafood and keeping them in good condition is very essential for

In India brown and green mussels are available with the dominance of brown mussel. The brown mussels were named as *Perna indica* (Kuriakose and Nair, 1976) but recently it is identified as *Perna perna* (Jonathan *et al.*, 2016). Both mussels are available throughout the year in the southeast and south west

coast of India. Kanyakumari is a coastal district of Tamil Nadu situated along south east and south west coast of India dominated with rocky beaches thriving with abundant mussel population. Many consumers prefer the meat of brown mussel for its good nutritive value and delicacy. For the conservation of mussels of this region, mussel fishing is permitted only for five months from November to March. Normally consumers do not shuck out mussel meat by breaking the shell for cooking, instead they steam cook the mussel for proper opening of the shell to extract the meat. The steam cooked meat retains the shape for making delicious dishes. Little information is available on the quality changes of cooked mussel meat during storage. Slabjy and Carpenter (1977) have reported the processing effects on the proximate composition and mineral content of the meat of *Mytilus edulis*. The seasonal variation and frozen storage stability of the same species were assessed by Krzynowek and Wiggan (1979). Chemical decomposition indicators of *M. galloprovincialis* have been reported by Erkan (2005) and Goulas *et al.* (2005).

Quality assessment of sea foods has more to do with the determination of its shelf life or storage life which is the amount of time that sea foods remain palatable. Different species of sea foods have different shelf life which also varies depending on the oil levels, catch area, season and duration of rigor mortis, intrinsic conditions of the seafood and how it was captured and handled. The shelf life of most

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marine animals have been predicted to range between 2 - 24 days in ice, 5 days at 5°C and 3 days at 10°C (Huss, 1995). The shelf life of Croaker (*Pseudolithus elongatus*) found in Nigerian marine waters have been predicted to be 20 days in ice and 12 hours at ambient temperature (Ola *et al.*, 2004). Super chilling at -4°C extend the shelf life of frozen fish to several weeks because at such temperatures, microbial spoilage occurs after a considerable length of time (Huss, 1995; Adams and Moses, 2008). Changes in pH, microbial population, trimethylamine, volatile protein nitrogen and few other parameters have been used as indices of the freshness of iced aquatic species (Cheuk *et al.*, 1979). The chemical indicator for the decomposition is available about the shelf life of shrimps, lobster and crabs. For the fresh meat of mussels decomposition was reported by chemical indicators (Erkan, 2005).

Marketing of sea foods is mostly carried out by local fish sellers at ambient temperatures. Therefore knowledge of spoilage patterns of shell fishes and their shelf life under ambient conditions is very important. Refrigeration temperature is also relevant because they are used by most households for temporary storage of seafood. Frozen state condition is also important since most of the imported seafood is in frozen condition. In recent times, modern biotechnology have introduced new techniques to detect early seafood contamination, improve the taste, modify the quality of seafood and prolong the shelf life and also impact disease resistance to the fish (William and Michael, 2009). There are two main methods of assessing seafood quality to determine its freshness and shelf life and they are the sensory and non sensory methods. Sensory methods rely mostly on appearance, odour, texture and taste of the seafood while non sensory methods use physical, biochemical, chemical and microbiological means (Huss, 1995).

In the present study, quality assessment was carried out on cooked meat of brown mussel, *Perna perna* under different storage temperatures such as ambient (28°C), refrigeration (4°C) and frozen state (-5°C) using sensory, microbiological and biochemical methods of evaluation. The limits of freshness proposed by some international standard organizations for sea foods were used as standard limits in this study.

Materials and Methods

Sample collection

Fresh 300 live brown mussels were collected from coastal villages of Kanyakumari district, India

and brought to the laboratory in an ice box. The shells were washed and steam cooked at 70°C for three minutes for sucking the meat from the shell. Twenty sucked mussel meat was aseptically transferred in to individual plastic bags and triplicate samples were stored at different temperatures for analysis.

Storage temperatures

The storage temperatures employed were -5°C, 4°C and 28°C. A refrigerator with a temperature control setting was used and it was adjusted to give a temperature of -5°C at the upper freezing chamber and 4°C at the lower refrigerator chamber. The temperature of the laboratory at the time of storage was 28°C and this was taken as the ambient temperature. The samples stored at ambient temperature were sampled every two hours; the samples stored at refrigerator temperature were sampled at one day intervals and samples stored at freezing temperature were sampled at 7 days intervals.

Sensory evaluation

The sensory evaluation was performed by 15 trained panelists. The assessment was conducted for the colour, odour and texture of the mussel samples using a 9-point hedonic scale (Mailgaard *et al.*, 1999). Evaluation of odour was carried out at the moment of opening the pack.

Determination of pH

pH of mussel meat was determined by the method of Goulas *et al.* (2005). 10 g of the mussel sample were homogenized with 50 ml of distilled water and the pH value of the homogenate was measured by means of a glass electrode pH meter (HANNA pH213) that was previously standardized.

Bio chemical analysis

Quality indicators, total volatile base nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents were determined by standard Conway micro diffusion method (Conway, 1950).

Microbiological analysis

Microbial analysis for the determination of Total Bacterial Count (TBC) and Total Fungal Count (TFC) was followed according to the procedure of International Commission on Microbiological Specification for Foods (1986).

Statistical analysis

Descriptive statistics such as means and standard deviations were computed. Two-way analysis of variance (ANOVA) was used to test the significance

difference in levels of quality indicators, sensory parameters and storage period from mussel species using Microsoft® Excel XP 2007 computer software.

Results

The steam cooked meat of brown mussel *Perna perna* was firm to the touch and retained its original shape even after cooking. The mussel meat stored at ambient temperature (28°C) was monitored for 14 hours, refrigerated meat (4°C) for 7 days and frozen meat (-5°C) for 6 weeks and the results are presented in Figure 1 - 3 and Table 1. The colour and texture of the flesh and any development of offensive odour were observed during storage and the mussel meat was acceptable for 12 hours at ambient temperature, 6 days at refrigeration temperature and 5 weeks at frozen temperature. The ANOVA results showed significant differences ($p < 0.05$) between the storage period and sensorial parameters.

The total bacterial count was > 30 CFU/g initially for all the samples subjected to different storage conditions. After two hours at ambient temperature, the bacterial population showed significant increase to 1.2×10^4 CFU/g and again showed a tenfold increase after 12 hours (4.0×10^5 CFU/g). Under refrigerated storage, significant growth was noticed on day 2 (0.2×10^4 CFU/g) and a tenfold increase was noticed on day 7 (2.05×10^5 CFU/g). Bacterial colonies were below 30 CFU/g throughout the storage period in the frozen mussel meat samples. Fungal colonies were not detected throughout the study period in all the samples.

Total volatile base nitrogen (TVB-N), Trimethylamine nitrogen (TMA-N) and pH were observed in mussel meat after storage at three different temperatures. The pH reached strong alkaline conditions after 10 hours at ambient temperature, after 7 days at refrigeration temperature and after 6 weeks at frozen temperatures. Evaluation of the quality was terminated when Total volatile base nitrogen (TVB-N) and Trimethylamine nitrogen (TMA-N) exceeded the international acceptable limits for fish and fishery products. Significant difference was noticed (ANOVA, $p < 0.05$) between the spoilage indicators and storage temperatures (28°C, 4°C and -5°C).

Discussion

The criteria for rejection of sea foods are based on the development of strong ammoniacal and offensive odours, softening of the tissues, discoloration of the meat and very high microbial counts corroborated

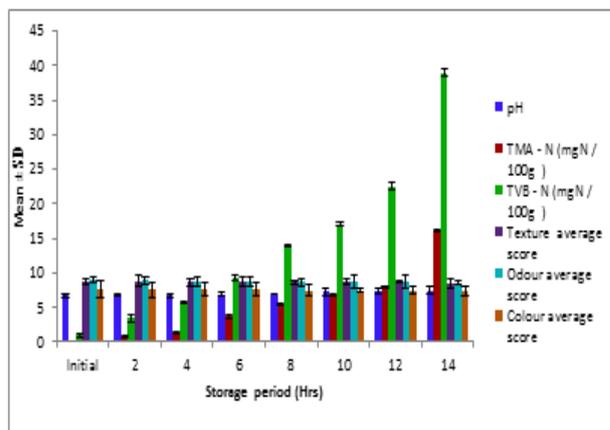


Figure 1. Values of spoilage indicators and sensory scores recorded at ambient temperature (28°C)

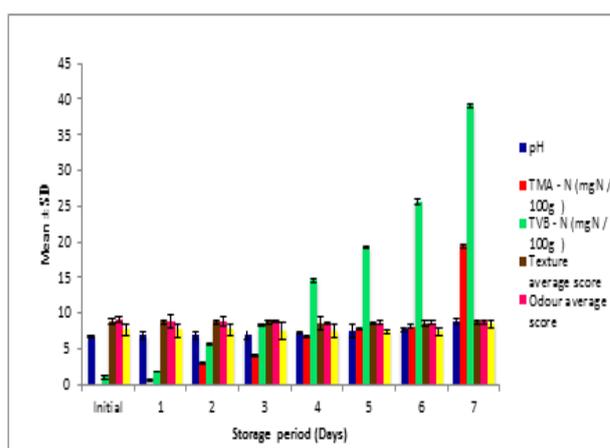


Figure 2. Values of spoilage indicators and sensory scores recorded at refrigeration temperature (4°C)

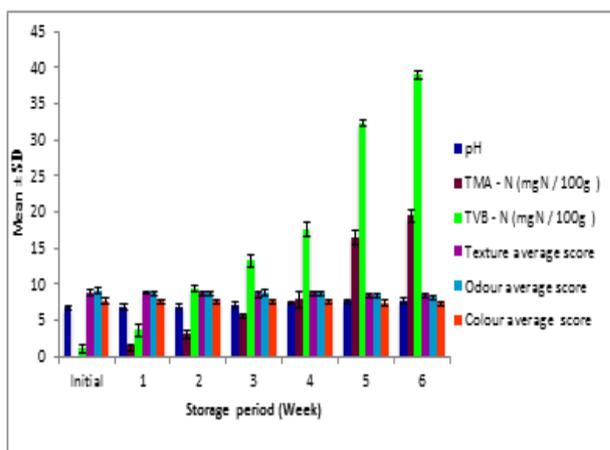


Figure 3. Values of spoilage indicators sensory score recorded at Freezing temperature (-5°C)

with high values of trimethylamine nitrogen (TMA-N), total volatile bases (TVB-N) and change in pH values. Pottinger (1948) reported pH value of from 6.2 to 6.9 as a good level for fresh oysters. The fresh brown mussel (*Perna perna*) meat had a value of pH 6.7. Hardey (1991) revealed low pH

Table 1. Microbial changes in brown mussel under different storage temperatures

Storage time (Hrs)	Ambient temperature (28°C)		Storage time (Days)	Refrigeration temperature (4°C)		Storage time (weeks)	Freezing temperature (-5°C)	
	TBC (CFU/g)	TFC (CFU/g)		TBC (CFU/g)	TFC (CFU/g)		TBC (CFU/g)	TFC (CFU/g)
Initial	TLTC	ABSENT	Initial	TLTC	ABSENT	initial	TLTC	ABSENT
2	1.2 X 10 ⁴	ABSENT	1	TLTC	ABSENT	1	TLTC	ABSENT
4	1.3 X 10 ⁴	ABSENT	2	0.2 X 10 ⁴	ABSENT	2	TLTC	ABSENT
6	1.5 X 10 ⁴	ABSENT	3	0.9 X 10 ⁴	ABSENT	3	TLTC	ABSENT
8	2.0 X 10 ⁴	ABSENT	4	1.2 X 10 ⁴	ABSENT	4	TLTC	ABSENT
10	2.4 X 10 ⁴	ABSENT	5	1.5 X 10 ⁴	ABSENT	5	TLTC	ABSENT
12	3.15 X 10 ⁴	TLTC	6	2.0 X 10 ⁴	ABSENT	6	TLTC	ABSENT
14	4.0 X 10 ⁵	TLTC	7	2.05 X 10 ⁵	TLTC	-	-	-

level of 5.9 in mussels at the point of rejection. In the present study, the pH values stayed near neutral to slight alkaline conditions in all the samples stored at different temperatures except for samples stored under refrigerated conditions when the pH values reached strong alkaline conditions on day 7. When seafood spoils, proteins are broken down to free amino acids, amines and volatile ammonia. Trimethylamine oxide is broken down to volatile amines and trimethylamine. Veciana (1991) suggested TVB-N as an indicator of the freshness of fish. Goulas *et al.* (2005) suggested TVB-N limit for mussels as 22 - 25 mg N 100g⁻¹ as compared with the value of 35 mg N 100 g⁻¹ proposed for fish (Connell, 1990). This value is lower (25 - 30 mg N 100 g⁻¹) than that of spoilage of oyster (Lopez- Caballero *et al.*, 2000). Fatiman and Qadri (1985) revealed that the exceeded level of acceptability has been considered as spoiled and unfit for human consumption. Erkan (2005) reported the TVB - N values of fresh mussel were 12.38 mg/100g and it increased to 22.55 mg/100g and exceeded the acceptable limit after six days of storage in ice. In the present study, the evaluation of the quality of mussel meat stored at three different temperatures was terminated when the TMA-N and TVB-N values exceeded the international acceptable limits for fish and fishery products (International Commission on Microbiological Specification for Foods, 1978; Martin *et al.*, 1978; Gorczyca and Pohlen, 1985; Connell, 1990). Some of the definitive attempts that were made to study sea food spoilage at ambient temperature include the work of Reilly *et al.* (1985) where it was observed that brackish water prawns spoil within 16 hours at ambient temperature. Gorczyca and Pohlen (1985) observed that trout, Beam and mullet spoil within 13 hours at ambient

temperature. Ola and Oladipo (2004) and Chuma *et al.* (2010) have also predicted the shelf life of a Nigerian marine fish, Croaker (*Pseudolithus senegaliensis*) to be 12 hours, at ambient temperature using sensory, microbiological and chemical approach.

Cheuk *et al.* (1979) reported constant TVB - N values during first 11 and 15 days of ice stored brown and pink shrimp respectively but spoilage set after 16 and 19 days of storage of brown and pink shrimps when the Total volatile base nitrogen (TVB-N) values rose to 30 mg/ 100 g. The same authors revealed that the chemical changes took place in sea foods due to bacterial action and by endogenous enzymes. Hülya and Rabiya, 2015 reported that the mussels (*Mytilus galloprovincialis*) packed with vacuum packing and modified atmosphere had higher total volatile basic-nitrogen (TVB-N), trimethylamine-nitrogen (TMA-N), thiobarbituric acid (TBA) and the mussels became inconsumable at 21 and 15 days respectively. Payap, Ommee and Jaruan, 2011 reported that mussels packed with 80% MAP and 10% CO₂ are optimum condition for extending the shelf-life of green mussel *Perna viridis*.

It is interesting that the results of the bacterial population in mussel meat showed a sudden increase from the second hour for samples stored at ambient temperature and from second day for samples stored at refrigerated temperature. Fungal colonies were absent in all the samples. All these values were within the recommended international standard limits of acceptability (International Commission on Microbiological Specification for Foods, 1978; Martin *et al.*, 1978; Gorczyca and Pohlen, 1985; Connell, 1990). The sudden increase suggests that all the resident flora and the contaminants may not be have been eliminated during the steaming process

and those bacteria which had survived grew well in the absence of the competing flora. The handling procedure of fish in tropical countries like India stimulated the storage trial at ambient temperature. The common handling practice involves partial icing during fishing followed by marketing at ambient temperature. It is a matter of concern because even though bacteria were >30 CFU/g in frozen samples, thawing at room temperature encourages the growth of bacteria.

Some authors like Matches (1982) have also observed that shrimps can keep up to 6 days at refrigeration temperature (5°C). Huss (1995) have equally observed that the shelf life of most sea foods at refrigeration temperature (5°C) can last up to 5 days depending on the species, oil level of the species, catch area and intrinsic conditions of the species.

At freezing temperature (-5°C), the predicted shelf life was 5 weeks. At this stage, only very slight changes on the external appearance of mussel were observed. There was no ammoniacal odour because bacterial spoilage was drastically reduced and tissue softening was minimal but the trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) values were 16.47 and 32.31 mg/100g respectively and within the stated international limits of acceptability. Huss (1995) stated that super chilling at (-4°C) and below can effectively prolong the storage life of the mussel up to 5 weeks, this assertion was corroborated by Adams and Moses (2008) and Mhongole (2009).

Generally, there was a strong correlation at ambient and refrigeration temperature between the total bacterial, fungal count and the Trimethylamine nitrogen and total volatile base nitrogen values but at frozen temperature, no correlation existed and that explained why the sensory and microbiological analysis did not tally with the Trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) values at frozen temperature. In a similar study, Hozbor *et al.* (2006) found a strong correlation between the microbiological changes in sea salmon stored in ice and other quality indices like Trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) and histamine but no correlation existed at frozen temperature (-25°C).

We conclude that the shelf life of cooked brown mussel (*Perna perna*) at ambient and refrigeration temperature were 12 hours, and 6 days respectively and the sensory, microbiological and chemical approach used in the analysis were all in agreement with the recommended international limits for acceptability, however at frozen temperature, no correlation existed between the total viable counts of

bacteria and the values of the Trimethylamine nitrogen (TMA-N) and total volatile base nitrogen (TVB-N) values which were still within the recommended acceptability limits. This is understandable because at frozen temperature, microbial spoilage is very unlikely because very few microbial species can grow at such temperatures, fish spoilage however can be due to biochemical and enzymatic changes in fish tissues and muscles which progresses slowly at such temperatures. We intend to investigate further, the survival of human pathogens like *Vibrio parahaemolyticus* under experimental conditions after cooking the meat of the mussel as well study the difference between the bacterial species in the raw meat and cooked meat so as to ensure food safety by maintaining the correct cooking temperature and proper storage conditions.

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