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Physiochemical Changes in Tissue of Edible Mussels *Perna viridis* at Refrigerator Temperature (7 ± 2°C)

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Abstract

Quality of stored mussels tissue depends on the temperature and storage time. Investigations on factors responsible for spoilage of mussel *(Perna viridis)* meat in refrigerator (7 ± 2°C) in term of biochemical indices indicated that Trimethyle amine (TMA), Total volatile basis (TVB), water content and pH significantly increased ($p < 0.001$) with storage time. Results concluded that mussel meat could be preserved for 4 days up to acceptable at refrigerated temperature.

Introduction

The rate of deterioration or spoilage in fish and shell fish occurs during low temperature storage, is due to invasion of specific microorganism, enzymatic action and autolytic action. They all chemically convert the tissue components, protein, carbohydrate and fat) into simpler metabolites (TMA, TVB, lactic acid, Dimethyle amine (DMA), Thiobarbituric acid (TBA). Increase in concentration of these metabolites in tissue leads to the changes in odour, texture, physical and chemical properties of tissues (Mathen and Thomas, 1988; Perigreen *et al*., 1988). They can be used as index of quality of fish.

Information is scanty on the spoilage pattern of mussel meat at refrigerated temperature. The present study discuss the changes occurring in mussel, muscle stored at refrigerated temperature in term of biochemical characteristics (pH, total protein, water content, TMA, TVB, salt soluble protein and total lipid). Production of fish and shell-fish in Pakistan has increased from 279263 tonnes in 1980 to 446231 tonnes in 1989 and international trade in fish and fish products is now important source of foreign exchange (Mahmood *at al.,* 1995; Anonymous, 1990; Beg *et al*., 1992). The result of present study will provide useful information for assessment of meat quality of mussel during storage at the aforementioned temperature.

Materials and Methods

Commercially important mussels were purchased from local market. These were cleaned, cut open and their shells were removed, wet tissue were excised on absorbent paper and the weight was recorded on an electrical balance then it was randomly divided in to two aliquots. One aliquot was freshly analyzed to determine the physical and chemical properties. Second was kept at 7 ± 2°C for seven days. The changes in physical and chemical properties were analyzed (by electrical pH meter), water content (AOAC, 1970), total protein (Dyer *et al*., 1950), total lipid were determined by modified method of Bligh and Dyer (1959), THAN (modified picrate method described by Murray and Gibson, 1972) and TVB-N (Cobb *et al*., 1973) respectively. The data obtained from experiment, were subjected to appropriate statistical analysis by using star personal XT computer. Analysis of ANOIA (F-test), degree of correlation, regression lines were drown by calculating with the help of least square method (Walpole, 1982).

Results and Discussions

The changes occurred in the water, total protein, salt soluble protein, total lipid, TVB, TMA of mussel meat during storage at 7 ± 2°C are presented in Table 1 and Fig. 1 and 2.

pH: The pH of freshly caught mussels (after immediate processing of oyster) 6.60 + 0.17 non significantly changed during 7 days storage. One the 7th day it was decreased to 6.33 ± 0.033 from its fresh value. The decrease in pH of oyster tissue may be due to the high lactic acid production from the glycogen degradation. Present study shows that glycogen content is significantly decreased from fresh value 4.5 ± 2.9 g percent showing lactic acid production (Akber *et al*., 1989) from anaerobic glycogen metabolism in oyster meat

Water content: The water content in fresh tissue of mussel was 7670 ± 31.12 g percent. It increased at 7 ± 2°C gradually with the increase Of storage time. After 7 days it reached to the highly significant ($p < 0.001$) value of 85 ± 1.0 g percent.

The changes in water content occurred during storage at 7 ± 2°C may be due the loss of water holding capacity of fish tissue or due to the reaction between formaldehyde or malanoaldehyde (breakdown products of TMA and Total lipid) and tissue protein with the release of water (Williams *et al*., 1983).

The change in water content is well negatively correlated (Fig. 1) with the change in total protein ($r = 0.98$), salt soluble protein ($r = -0.94$) and total lipid ($r = -0.91$) and positively correlated with TVB ($r = 0.97$), TMA ($r = 0.93$)

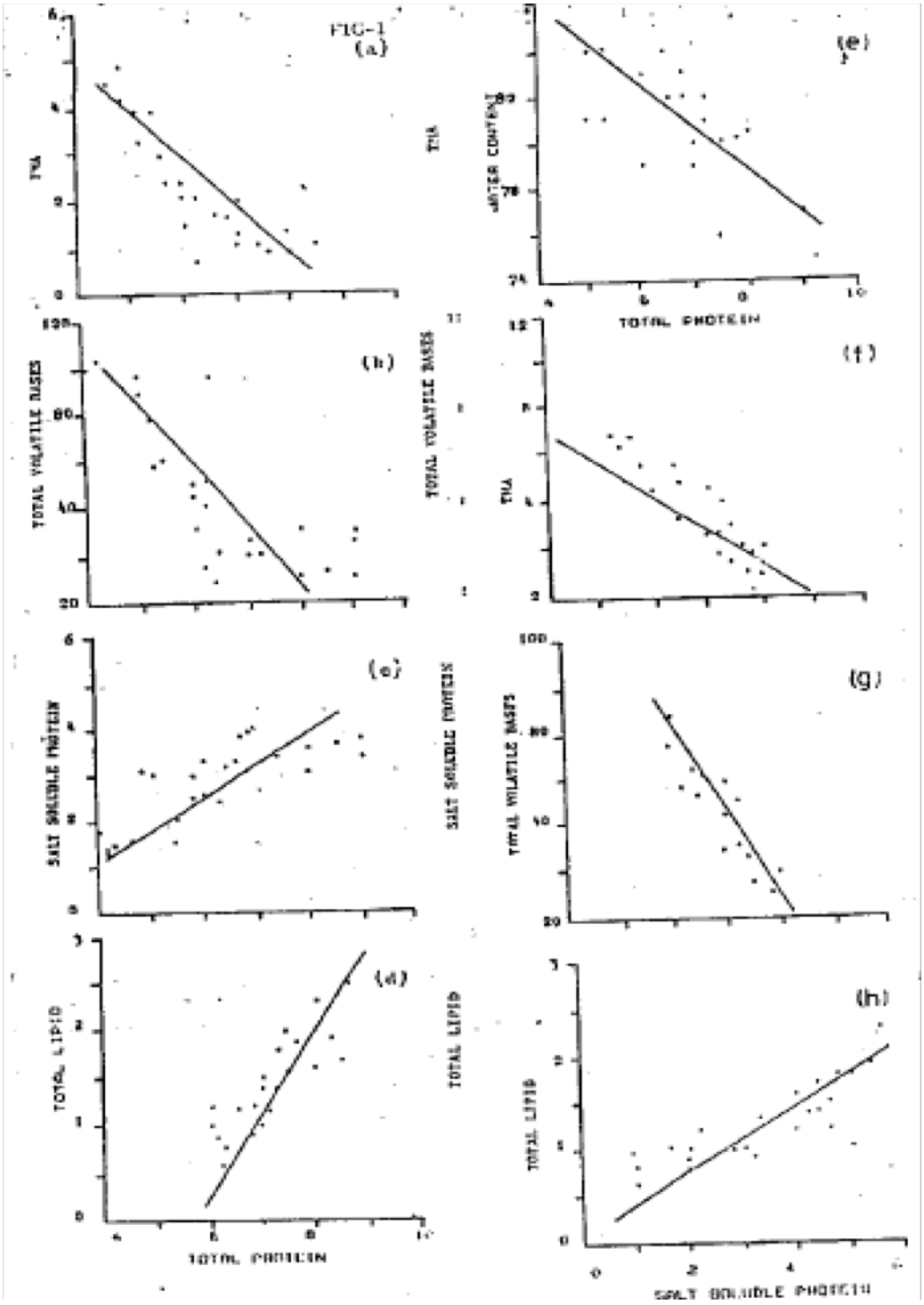

Fig. 1: Relationship between changes in total protein and TMA, total protein and TVB, total protein and salt-soluble protein, total protein and total lipid total protein and water, salt-soluble protein and TMA, salt-soluble protein a TVB, salt-soluble protein and total lipid content of mussel meat. The positive and negative correlation found 7 ± 2°C are described by the regression

(a) Y = 13.70 -1.58 (x), (r = -0.98)
(b) Y = 219.70 -26.80 (x), (r = -0.99)
(c) Y = 1.56 - 0.68 (x), (r = -0.96)
(d) Y = -1.50 - 0.45 (x), (r = -0.96)
(e) Y = 41.91 - 0.44 (x), (r = -0.98)
(f) Y = 8.94 - 1.94 (x), (r = -0.96)
(g) Y = 141.09 - 33.3 (x), (r = -0.55)
(h) Y = -0.04 - 0.48 (x), (r = -0.97)

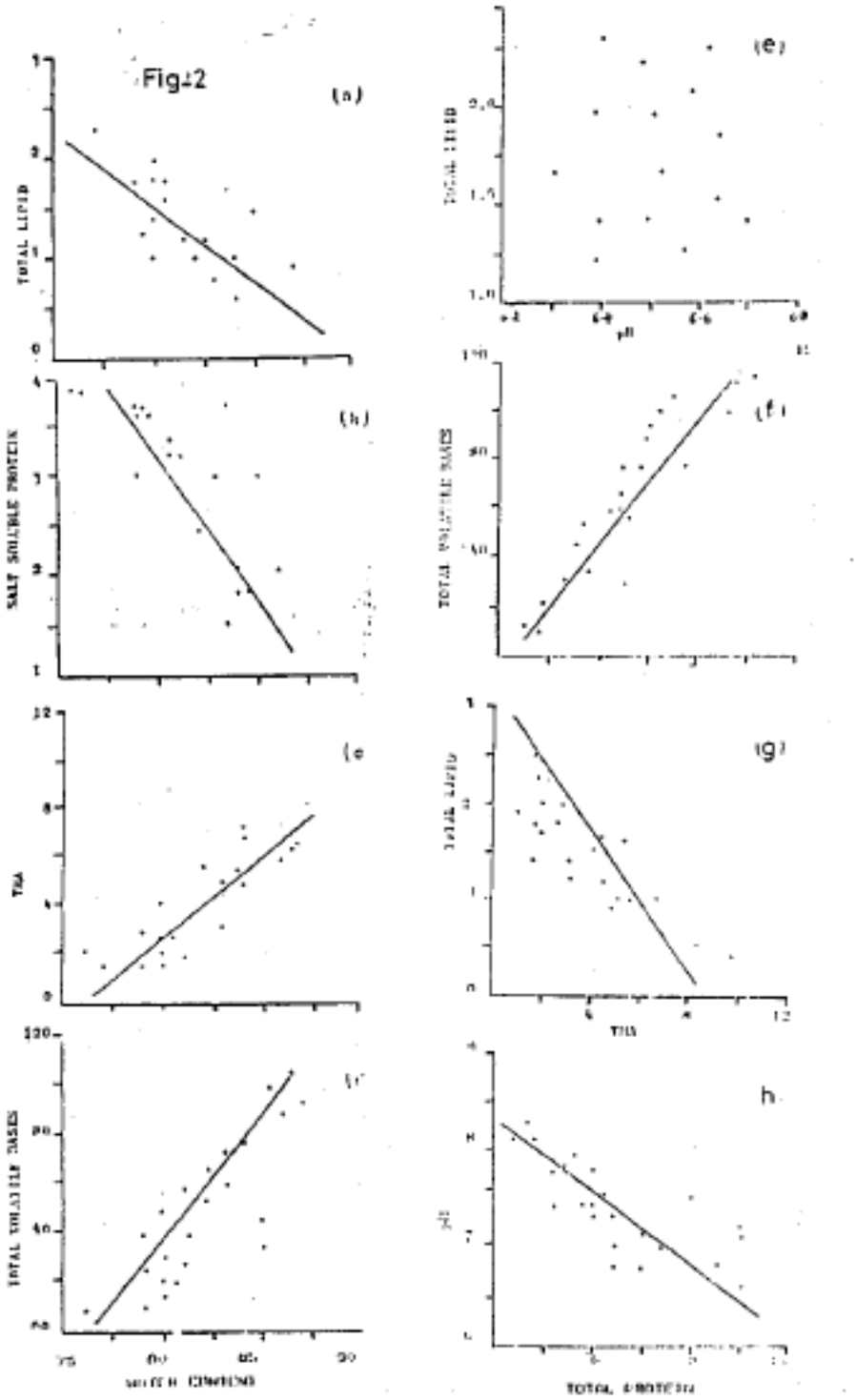

Fig. 2: Relationship between changes in water and total lipid, water and salt-soluble protein, water and TMA, water and TVB content pH and total lipid, total protein and TVB, total protein and total lipid, total protein and pH of muscle meat. The positive and negative correlation found at 7 ± 2°C are described by the regression.

(a) Y = 13.05 - 0.14 (x), (r = -0.91)
(b) Y = 25.70 - 0.28 (x), (r = -0.94)
(c) Y = 50.64 + 0.66 (x), (r = 0.93)
(d) Y = 892.80 + 11.57 (x), (r = 0.97)
(e) Y = 219.7 - 26.8 (x), (r = 0.99)
(f) Y = -1.50 - 0.45 (x), (r = -0.96)
(g) Y = 25.8 - 2.60 (x), (r = 0.91)

Table 1: Changes in chemical constituents of Mussel meat during storage at refrigerator temperature (7 ± 2°C)

Storage time in days	pH	Water (g%)	Total protein (%)	S.S.P (g%)	TMA (mg/100g)	TVB (mg.100g)	Total Lipid (g%)
0	6.60 ± 0.17	77.00 ± 1.22	8.06 ± 0.67	3.81 ± 0.06	1.37 ± 0.28	10.00 ± 1.15	2.30 ± 1.22
1	6.60 ± 0.057	79.66 ± 0.33	7.46 ± 0.76	3.63 ± 0.18	1.96 ± 0.27	20.00 ± 2.88	1.86 ± 0.06
2	6.60 ± 0.07	80.50 ± 0.28	7.00 ± 0.91	3.26 ± 0.06	2.00 ± 0.28	26.00 ± 2.76	1.60 ± 0.11
3	6.60 ± 0.115	81.33 ± 1.85	6.50 ± 0.88	3.10 ± 0.10	3.16 ± 0.44	40.00 ± 5.72	1.43 ± 0.12
4	6.50 ± 0.05	82.00 ± 2.23	6.33 ± 1.45	2.36 ± 0.31	3.33 ± 0.60	51.60 ± 1.45	1.20 ± 0.11
5	6.46 ± 0.035	82.50 ± 1.56	5.76 ± 0.14	2.50 ± 0.13	4.76 ± 0.14	64.33 ± 6.98	1.03 ± 0.08
6	6.43 ± 0.03	83.66 ± 1.81	5.36 ± 0.08	1.96 ± 0.26	5.40 ± 0.26	76.50 ± 6.94	0.90 ± 0.07
7	6.33 ± 0.03	85.00 ± 1.00	4.60 ± 0.45	1.60 ± 0.10	6.60 ± 0.30	100.00 ± 2.88	0.83 ± 0.12

Values are statistically significant at the level of $p < 0.001$. (Each value is X ± S.E.M.)

of mussel tissue. Similar correlation's were reported by LeBlanc *et al*. (1988) in Cod fish during storage at low temperature. They suggested that increase information of TMA and TVB caused an increase in tissue water and loss of protein and lipid contents.

Total protein: Loss in total protein content from its fresh value was not markable during initial period of storage (1-2 days in mussel tissues (8.06 ± 0.67 g to 7.0 ± 1.0 g%). After that it gradually decreased to 4.6 ± 0.45 g percent on 7th day of storage.

It is evident from our results that the quality and quantity of mussel tissue protein is changed significantly ($p < 0.001$) during 7 days storage at 7 ± 2°C. It showed that rate of bacterial decomposition is higher during refrigerated temperature storage. Our results are in good agreement with those reported by Antunes *et al*. (1981) and French *et al*. (1988). According to the finding at low temperature storage (above freezing), the rate of denaturation and autolytic hydrolysis of fish protein is markable. The autolysis helps the bacteria to invade the tissue rapidly, the free amino acids and water soluble protein of tissue serve as an excellent source for their growth and as a results not only the quality but also the quantity of protein is decreased. Riaz and Qadri (1979) report that decrease in protein contents of prawn during ice storage is due to the leaching affect, the amino acids and water soluble protein leach out with melting ice.

The results of the present study showed that changes in total protein content of mussel meat is positively correlated Fig. 1 and 2 with salt soluble protein (0.96) and total lipid (0.96) and negatively with TMA (-98), TVB (-0.99). Similar relationship was also reported by Gagnon and Fellers (1958) during low temperature storage of shrimp, they found the correlation between denaturation of tissue protein and lipid with the of TMA & TVB as degrative products.

Salt soluble protein: The solubility of tissue protein has been used as an index of alteration in protein quality during the low temperature storage. Our results show that SSP contents of freshly caught mussel decrease considerably with the increase in storage period at 7 ± 2°C. During first two days of storage slightly change (3.26 ± 0.06 g%) has been noted. On 7th day of storage SSP decreases from 3.8 ± 0.06 g to 1.6 ± 0.1 g percent.

It is apparent from the results that protein solubility is changed significantly ($p < 0.001$) after each day interval. The results of present study are very much consent with findings of Bhobe and Pai (1986), in shrimp meat during storage at O°C and frozen temperature (Riaz and Qadri, 1990). They stated that low temperature storage causes an increase in salt concentration of tissue due to the loss of free water which ultimately decrease the solubility of protein by changing the ultrastructure of myofibril protein. LeBlanc *et al*. (1988) stated. This loss in SSP indicate the denaturation of protein which may due to the interaction of formaldehyde or diethyl amine with tissue protein.

The changes in SSP content of mussel during storage at 7 ± 2°C is well correlated with TMA, TVB & total lipid. The results shows statistically negative correlation with TMA. TVB and bacterial count (r - -0.96, -0.91 and -0.75) and positive correlation with lipid (r = 0.97). Similar correlation is reported by Riaz and Qadri (1990), they have suggested that increase in denaturation of protein in shrimp tissue at low temperature storage is because of bacterial decomposition of protein to nitrogenous metabolite (TMA & TVB) and lipid to free fatty acids (hydrolysed products).

TMA: The TMA content of fresh mussel tissue is found as 1.37 ± 0.28 mg percent, during first 2 days of storage, it changes negligibly and after that the rate of TMA formation is increases and on 7th day it reached to a value of 1.37 ± 0.28 to 6.60 ± 0.30 mg percent.

Our results shows that at 7 ± 2°C increased in TMA content is highly significant ($p < 0.001$). Vyncke (1980) found that as the TMA content is increased in fish tissue during ice storage and the bacterial load is also on enhance rate. The Duncans multiple range test (Table 1) also proves the same result in connection with the rate of TMA formation after interval of more than 2 days. Similar findings have been observed in fish tissue during storage at low temperature by Kramer *et al*. (1977) and Kelleher *et al*. (1982), an increase 5 to 13 mg percent in TMA during storage. From the present study it is evident that increase in TMA-N of oyster is within the range of above reported value 4.5 ± 0.4 mg percent.

The quality of fish and shell-fish is classified in to different grades on the basis of their TMA content. The meat containing 0-2.5 mg percent TMA-N as considered as grade 1 quality (prime or good), 1-5 mg percent TMA-N is as

grade II quality (acceptable) and meat having more than 5 mg percent is graded as grade III (spoiled or rejected). Our results showed that at 7±2°C storage the quality of mussel (2.0±0.2 mg% TMA) tissue was prime of grade 1 up to two days of storage. On 5th day of storage quality of mussel (4.76±0.14 mg%) was of grade II. After that the meat of mussel had become spoiled or considered as grade III quality.

The results of the present study shows statistically that TMA has well direct correlation with TVB (r = 0.99) and indirect correlation with total lipid (r = -0.94).

TVB: Total volatile basis are considered as marking compound for the assessment of fish quality. Like other spoilage indicator (TMA, SSP. pH) the change in TVB content of mussel tissue has very much significant ($p<0.001$) during storage at 7±2°C. The results showed that it increased from 10.0±1.1 mg to 100±2.88 mg, showing an increase of approximately 10 to 20 mg percent TVB-N per day.

A prominent increase in TVB content is reported by Boyd and Wilson (1977), Cho *et al*. (1984) and Kolakowski (1986) in shrimp and lobster at various temperatures. They found direct correlation in TVB content with storage time. It is suggested that this increase in TVB may be due to the endogenous enzymatic and bacterial activities which ultimately affect the quality of meat.

On the basis of TVB content the quality of fishery products are classified in to three grades, an excellent grade having TVB-N value less than 30 percent, acceptable grade with the value of TVB more than 40 percent (Koizumi *et al*., 1985). Our result showed that at 7±2°C the quality of mussel is excellent up to 2 days of storage 126.0±2.88 mg). The quality of mussel meat is decreased to acceptable limit on 3rd day (40±5.7 mg%) after that period the quality of mussel meat decreased to rejected grade.

TVB content has negative correlation with total lipid content (r = -0.96). The similar correlation are reported by Riaz and Qadri (1990) in shrimp during low temperature, they observed that TVB increase with the decrease in lipid content.

Total lipid: Total lipid content was decreased with an increase in storage time. During first two days storage it changed from 2.3±0.22 g to 1.6± 0.119 percent in mussel tissues. On 7th day it declined significantly ($p<0.001$) to the value 0.83±0.12 g percent in mussel meat.

A non significant percent loss (50 to 70%) in total lipid content of fish at 0°C was noted by Bottino *et al*. (1979). The percent decrease in total lipid content of tissue noted in our study is 50 to 65 percent at refrigerated temperature. This loss in tissue lipid occurres during storage is due to the oxidative rancidity. The rancidity in term of thiobarbutaric acid number (TBAN) is used as an indicator of quality of fish, it has been found by many workers that TBAN is increased linearly with the increase of storage period (Han and Liston, 1987).

From the present study it is concluded that the refrigerator temperature can only be recommended for 3 days storage.

References

AOAC., 1970. Official Method of Analysis. 11th Edn., Association of Official Analytical Chemists, Washington, DC., USA., pp: 350.

Akber, Z., P.J.A. Siddiqui and R. Qasim, 1989. Thinking of crab meat. Pak. Agric. J., 11: 26-29.

Anonymous, 1990. Handbook of Fisheries Statistics 1986-1989. Vol. 15, Marine Fisheries Department, Karachi, Pakistan.

Antunes, S.A., A.F. Novak and R. de Camargo, 1981. Variation in the chemical composition of shrimp *Penaeus* (M.) *brasiliensis* Latreille during iced storage. Boletim Instituto Oceanografico, 30: 1-8.

Beg, M.A.A., S.N. Mahmood and A.H.K. Yousufzai, 1992. Heavy metals pollution in the coastal environment of Karachi. Pak. J. Mar. Sci., 1: 117-126.

Bhobe, A.M. and J.S. Pai, 1986. Study of the properties of frozen shrimps. J. Food Sci. Technol., 23: 143-147.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Bottino, N.R., M.L. Lilly and G. Finne, 1979. Fatty acid stability of Gulf of Mexico brown shrimp (*Penaeus aztecus*) held on ice and in frozen storage. J. Food Sci., 44: 1778-1779.

Boyd, N.S. and N.D.C. Wilson, 1977. Hypoxanthine concentrations as an indicator of freshness of iced snapper. New Zealand J. Sci., 20: 139-143.

Cho, Y.J., H. Shinano and M. Akiba, 1984. Studies on the microbiological ecology of mackerel stored by the method of partial freezing, 1: Changes in microflora and chemical pompounds in mackerel stored by partial freezing. Bull. Fac. Fisher.-Hokkaido Univ., 35: 271-286.

Cobb, III B.F., I. Alaniz and C.A. Thompson Jr., 1973. Biochemical and microbial studies on shrimp: Volatile nitrogen and amino nitrogen analysis. J. Food Sci., 38: 431-436.

Dyer, W.J., H.V. French and J.M. Snow, 1950. Proteins in fish muscle.: I. Extraction of protein fractions in fresh fish. J. Fish. Res. Board Can., 7: 585-593.

French, J.S., D.E. Kramer and J.M. Kennish, 1988. Protein hydrolysis in coho and sockeye salmon during partially frozen storage. J. Food Sci., 53: 1014-1017.

Gagnon, M. and C.R. Fellers, 1958. Biochemical methods for determining shrimp quality. 1. Study of analytical methods. Food Technol., 12: 340-343.

Han, T.J. and J. Liston, 1987. Lipid peroxidation and phospholipid hydrolysis in fish muscle microsomes and frozen fish. J. Food Sci., 52: 294-296.

Kelleher, S.D., E.M. Buck, H.O. Hultin, K.L. Parkin, J.J. Licciardello and R.A. Damon Jr., 1982. Chemical and physical changes in red hake blocks during frozen storage. J. Food Sci., 47: 65-70.

Koizumi, C., T. Ohshima and S. Wada, 1985. Preservative effect of NaCl in salted and dried fish products. Bull. Jpn. Soc. Scient. Fisher., 51: 87-90.

Kolakowski, E., 1986. Changes of non-protein nitrogens fractions in Antarctic krill (*Euphausia superba* Dana) during storage at 3° and 20°C. Zeitschrift Lebensmittel-Untersuchung Forschung, 183: 421-425.

Kramer, D.E., D.M.A. Nordin and L.G. Gardener, 1977. A comparison of quality changes in Alaska Pollock and pacific cod during frozen storage at -28°C. Fisheries and Marine Service Technical Report No. 753, Indian Technical and Inspection Directorate Department of Fisheries, India.

LeBlanc, E., R.A.J. LeBLanc and I.L.E. Blum, 1988. Prediction of quality in frozen cod (*Gadus morhua*) fillets. J. Food Sci., 53: 328-340.

Mahmood, S.N., S. Naeem, F.A. Khan and R.B. Qadri, 1995. Heavy metals in 15 species of Pakistani commercial fish. Trop. Sci., 35: 289-394.

Mathen, C. and F. Thomas, 1988. Solid loss and weight gain in prawns during storage in ice. Fish. Technol., 25: 110-111.

Murray, C.K. and D.M. Gibson, 1972. An investigation of the method of determining trimethylamine in fish muscle extracts by the formation of its picrate salt-Part I. Int. J. Food Sci. Technol., 7: 35-46.

Perigreen, P.A., J. Joseph and C. George, 1988. Studies on freezing and storage of *Psenopsis cyanea*. Fish. Technol., 25: 44-46.

Riaz, M. and R.B. Qadri, 1979. Effect of ice storage prior to freezing on the quality of frozen-shrimp. Pak. J. Scient. Ind. Res., 6: 327-333.

Riaz, M. and R.B. Qadri, 1990. Time-temperature tolerance of frozen shrimp. 2. Biochemical and microbiological changes during storage of frozen glazed shrimps. Trop. Sci., 30: 343-356.

Vyncke, W., 1980. Quality assessment of gutted and ungutted gurnard (*Trigla* spp.) by organoleptic and objective methods. Zeitschrift Lebensmittel-Untersuchung Forschung, 171: 352-354.

Walpole, R.E., 1982. Introduction to Statistics. 3rd Edn., Macmillan Publishing, New York, ISBN: 9780024241504, Pages: 521.

Williams, S.K., R. Martin, W.L. Brown and J.N. Bacus, 1983. Moisture loss in tray-packed fresh fish during eight days storage at 2°C. J. Food Sci., 48: 168-171.