

Physiochemical Changes in Tissue of Edible Oyster *Crassostrea glomerata* at Refrigerated Temperature

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Abstract: Oysters are bivalve molluscs in the family *Osteridae* of the order *Osteroidea* and are found throughout the world. Quality of stored muscle of oyster, *Crassostrea glomerata* depends on the temperature and storage time. Investigation on factors responsible for spoilage of oyster meat in refrigerator ($7\pm 2^{\circ}\text{C}$); in term of biochemical indices, indicated that in 1-2 days the following changes will occur: oyster tissue total protein from $7.6\pm 0.44\text{g}\%$ to $7.06\pm 0.64\text{g}\%$, salt soluble protein from $3.46\pm 0.52\text{mg}\%$ to $2.53\pm 0.14\text{g}\%$. Total lipid from $3.0\pm 0.38\text{g}\%$ to $2.5\pm 0.29\text{g}\%$, TMA from $1.1\pm 0.11\text{mg}\%$ to 1.46 ± 0.09 and TVB, from $9.46\pm 0.29\text{mg}\%$ to 19.33 ± 0.06 and on 7th day of storage, total protein to $4.5\pm 0.32\text{g}\%$, salt soluble protein to $1.1\pm 0.21\text{g}\%$, total lipid to $1.4\pm 0.19\text{g}\%$, TMA, to $4.3\pm 0.41\text{mg}\%$ and TVB to 83.3 ± 3.5 changed. Amount of Glycogen in fresh meat was $5.3\text{g}\%$ which decreased to $3.2\text{g}\%$ during 7 days storage.

Water content in fresh tissue was $79.0\pm 0.57\text{g}\%$ and increased at $7\pm 2^{\circ}\text{C}$ gradually with the increase of storage time. After 7 days it reached to the highly significant ($p<0.001$) value of $89.1\pm 0.54\text{g}\%$. pH in fresh tissue was also noted 6.60 ± 0.17 and slightly non significant change was observed during 7 days of storage. On the 7th day it decreased to 6.3 ± 0.05 from its fresh tissue value significantly ($p<0.001$) with storage time.

Results concluded that oyster meat could be preserved for 4 days up to acceptable refrigeration temperature.

KEY WORDS: Physiochemical, Edible oyster, Refrigerated temperature, Refrigeration period

Introduction

Oysters are bivalve molluscs in the family *Osteridae* of the order *Ostereoida* and are found throughout the world. *Crassostrea glomerata* which occurs in great abundance on stones and pilings in backwaters and creeks in the vicinity of Karachi. The raw crustacea reflect the quality of water from where the animals were harvested, and is also affected by the on-ship and inplant environment as well as the duration and type of refrigeration storage (Faghri *et al.*, 1984, Heinsz *et al.*, 1988). Shellfish flesh provides an excellent substrate for the growth of most heterotrophic bacteria which attributes the effect of bacterial growth and the related biochemical activities, (Durve & Bal, 1961; Labarta, 1999; Wyte & Englar, 1982). The primary factor affecting rate of spoilage is storage temperature and the substantial extension of shelllife; which can be achieved with storage, at -20 to 0 °C (Boyd *et al.*, 1992). The rate of deterioration or spoilage changes in fish and shellfish occur during low temperature storage is mainly 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) Trimethyleamine, (TVB) total volatile base, (DMA) Dimethyleamine, (TBA) Thiobarbutaricacid, increased in concentration of these metabolites in tissue leads to the changes in odour, texture, physical and chemical properties of tissues and can be used as index of quality of fish (Mathen & Thoms, 1988; Murray & Gibson, 1972); (Nester, *et al.*, 1979); (Perigreen, *et al.*, 1988).

Information is scanty on the spoilage pattern of oyster meat at refrigeration temperature. The present studies discuss the changes occurring in oyster muscle stored at refrigeration temperature (7 ± 2 °C) which are in terms of biochemical characteristics (pH, total protein, water content, TMA (Trimethyleamine), TVB (total volatile base), salt soluble protein and total lipid and also studies will provide useful information for the assessment of quality of oyster meat during the storage at the aforementioned temperature.

Material and Methods

Commercially important edible oyster were purchased from local market. It were cleaned, cut open and their shells were removed, wet tissues were dried on absorbent paper and the weight was recorded on an electrical balance, then it was

randomly divided into two equal aliquots, One aliquot was freshly analyzed to determine the physical and chemical properties and the other was kept at $7\pm 2^{\circ}\text{C}$ for seven days. The changes in physical and chemical properties were observed; pH by electrical pH meter, water content by standard method of association of official analytical chemist (A.O.A.C, 1970), Glycogen by Dubois method (Dubois *et al.*, 1956), total protein by Lowery's method 1951 and salt soluble protein by Dyer method (Dyer *et al.*, 1950), total lipid were determined by modified method of Bligh and Dyer (Bligh & Dyer, 1959), TMA_N by modified picrate method described of Murray and Gibson (Murray & Gibson, 1972), and TVB-N by Cobb method (Cobb *et al.*, 1973) respectively.

The data obtained from experiment were subjected to appropriate statistical analysis by using star personal XT computer. Analysis of ANOVA (F-test), degree of correlation, regression lines were drawn by calculating with the help of least square method of Walpole (Walpole, 1982).

Results and Discussions

The changes occurred in the water content, total protein salt soluble protein, total lipid, TVB & TMA of oyster meat during storage at $7\pm 2^{\circ}\text{C}$ are presented in table 1 and Fig. 1 & 2.

Table 1: Changes in chemical constituents of Oyster meat during storage at Refrigerator temperature ($7\pm 2^{\circ}\text{C}$)

Storage time in day's	pH	Water (g%)	Total Protein (g%)	S.S.P. (g%)	TMA (mg/100g)	TVB (mg/100g)	Total Lipid (g%)
0	6.46 \pm 0.035	79.0 \pm 0.57	7.6 \pm 0.44	3.46 \pm 0.52	1.10 \pm 0.11	9.46 \pm 0.24	3.0 \pm 0.38
1	6.45 \pm 0.13	80.00 \pm 0.05	7.40 \pm 0.54	2.83 \pm 0.08	1.30 \pm 0.34	10.60 \pm 0.70	2.70 \pm 0.21
2	6.40 \pm 0.06	80.00 \pm 1.00	7.06 \pm 0.64	2.53 \pm 0.14	1.46 \pm 0.09	19.33 \pm 0.66	2.50 \pm 0.29
3	6.33 \pm 0.04	82.66 \pm 1.45	6.60 \pm 0.18	2.50 \pm 0.14	1.53 \pm 0.15	20.00 \pm 1.15	2.16 \pm 0.16
4	6.35 \pm 0.03	84.00 \pm 2.00	6.33 \pm 0.33	2.10 \pm 0.06	2.16 \pm 1.45	27.66 \pm 1.45	1.73 \pm 0.12
5	6.30 \pm 0.035	84.00 \pm 3.20	5.86 \pm 0.09	1.86 \pm 0.13	2.86 \pm 0.18	40.00 \pm 2.88	1.66 \pm 0.06
6	6.26 \pm 0.02	87.66 \pm 1.00	5.10 \pm 0.22	1.53 \pm 0.08	3.93 \pm 0.23	74.63 \pm 3.70	1.40 \pm 0.05
7	6.30 \pm 0.05	89.10 \pm 0.54	4.50 \pm 0.32	1.10 \pm 0.21	4.33 \pm 0.41	83.33 \pm 3.50	1.40 \pm 0.19

Values are statistically significant at the level of $P < 0.001$.
(Each value is $X \pm \text{S.E.M.}$)

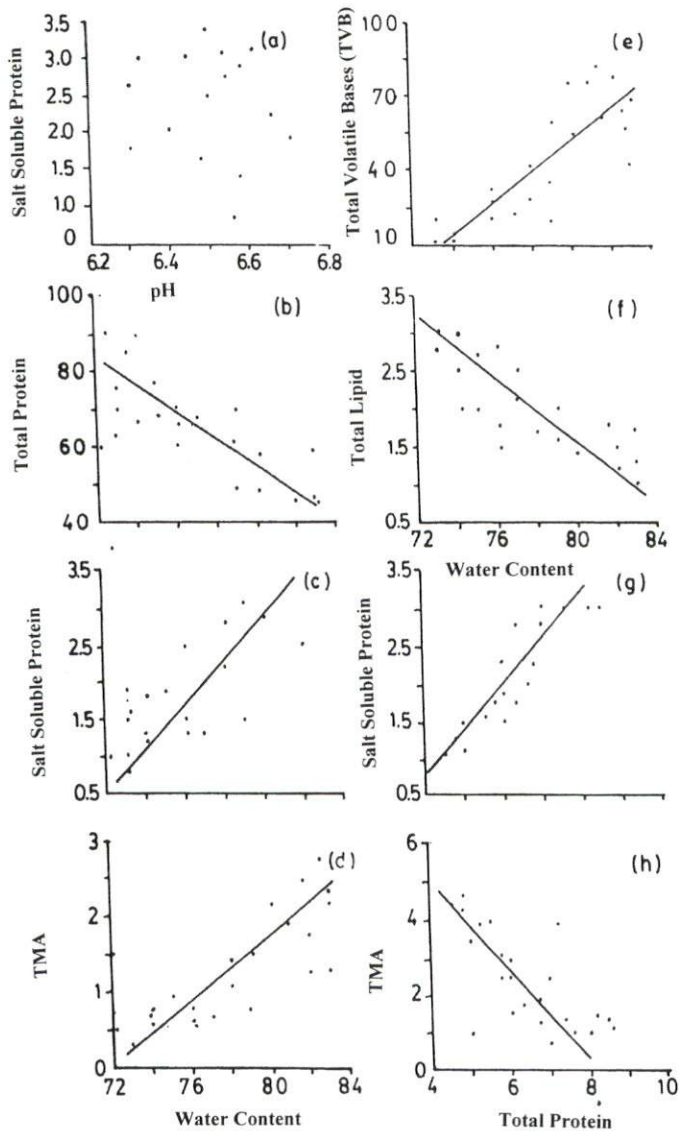


Figure 1: Relationship between changes in pH and salt soluble protein, water and total protein, water and salt-soluble water and TMA and TVB, water and total lipid, total protein and salt soluble proteins, total protein and TMA content of oyster meat. The position and negative correlation (but not between pH and TVB found at $7\pm 2^{\circ}\text{C}$ are described by the regression

$$(b) Y=31.20 - 0.29 (x), (r = -0.99)$$

$$(d) Y=117.10 + 7.50 (x), (r = -0.97)$$

$$(f) Y=14.98 - 0.15 (x), (r = -0.95)$$

$$(h) Y=9.39 - 1.13 (x), (r = -1.00)$$

$$(c) Y = 16.85 - 0.18 (x), (r = -0.97)$$

$$(e) Y = -592.0 + 7.5 (x), (r = -0.96)$$

$$(g) Y = 1.53 + 0.59 (x), (r = -0.87)$$

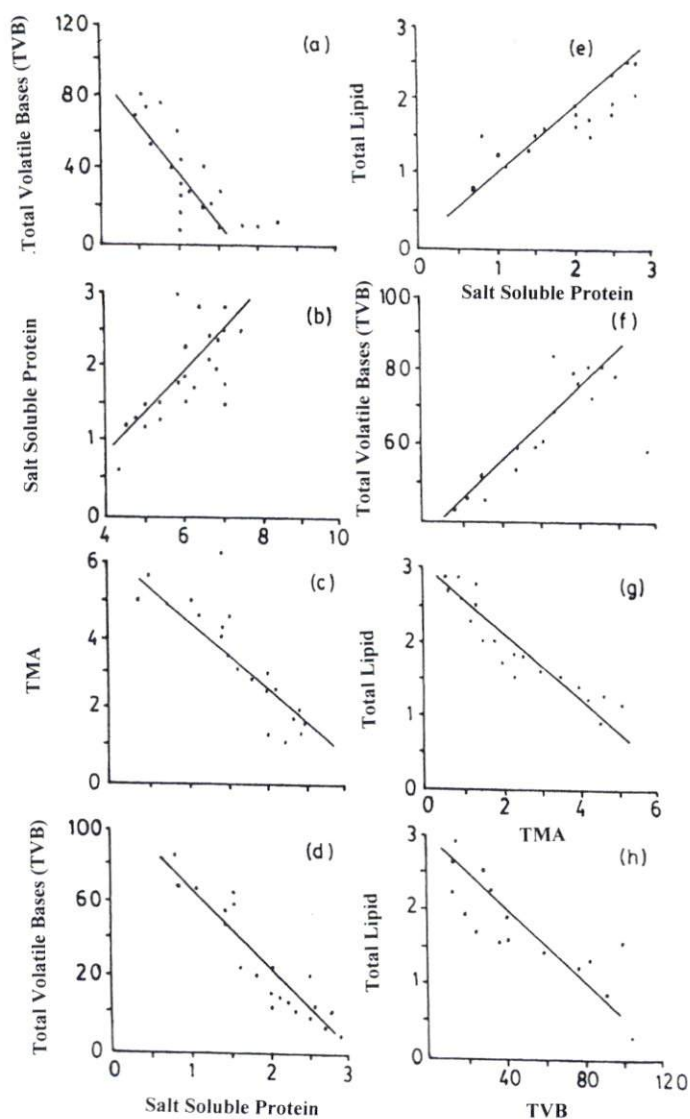


Figure 2: Relationship between changes in total protein and TVB, total protein and ssp, salt-soluble protein and TMA, salt-soluble protein and TVB, salt soluble protein and total lipid, TMA & TVB, TMA and total lipid, TVB and total lipid content of oyster meat. The position and negative correlation found at $7\pm 2^\circ\text{C}$ are described by the regression

(a) $Y = 193.00 - 25.24 (x)$, ($r = -0.98$)

(b) $Y = -1.10 + 0.50 (x)$, ($r = 0.94$)

(c) $Y = 6.40 - 1.88 (x)$, ($r = -0.98$)

(d) $Y = 126.70 - 0.84 (x)$, ($r = -0.96$)

(e) $Y = -0.22 + 0.84 (x)$, ($r = 1.0$)

(f) $Y = 29.47 + 28.05 (x)$, ($r = 0.99$)

(g) $Y = 3.10 - 0.43 (x)$, ($r = 0.97$)

(h) $Y = 2.6 - 0.01 (x)$, ($r = -0.60$)

pH:

pH of freshly caught oyster (after immediate processing of oyster) was noted 6.60 ± 0.17 with non significant change during 7 days storage time. On the 7th day it decreased to 6.3 ± 0.05 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 studies show that glycogen contents significantly decreased from fresh value 5.3 to 3.2, showing lactic acid production, from anaerobic glycogen metabolism in oyster meat (Akber, *et al.*, 1989).

Glycogen:

Glycogen content of freshly caught oyster was 5.3g% which decreased to 3.2g% significantly ($p \leq 0.001$) of storage period. It shows that the tissue glycogen used in an aerobic glycolysis and lactic production. This lactic acid decrease the pH of oyster tissue on 7th day of storage.

Water content:

The water content in fresh tissue of oyster was 79.0 ± 0.57 g% and increased at $7 \pm 2^\circ\text{C}$ gradually with the increase of storage time. After 7 days it reached to the highly significant ($p \leq 0.001$) value of 89.1 ± 0.54 g%.

The changes in water content which occurred during storage at $7 \pm 2^\circ\text{C}$, 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 (Murray, *et al.*, 1972 ;Galebeck, *et al.*, 1977 ; Kramer, *et al.*, 1977 ; Nester, *et al.*, 1979 ; Siddiqui, *et al.*, 1979; Vyncke, 1980; Walpole, 1982; Kelleher, *et al.*, 1982; William, *et al.*, 1983; Koizumil, *et al.*, 1985; Kolakowski, 1986; Han & Listen, 1987; LeBlanc, *et al.*, 1988; Mathen & Thomas, 1988; Perigreen, *et al.*, 1988; Riaz, *et al.*, 1990).

The changes in water content is well negatively correlated (Fig. 1) with the change in total protein ($r = -0.99$), salt soluble protein ($r = -0.99$) and total lipid ($r = -0.95$) and positively correlated with TVB ($r = 0.96$), TMA ($r = 0.97$) of oyster tissue. Similar correlations were reported by LeBlanc, *et al.*, 1988 in Cod fish during storage time at low temperature. They suggested that the increase in formation of TMA and TVB is due to 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 in 1-2 days in oyster tissues ($7.6 \pm 0.44\text{g}\%$ to $7.06 \pm 0.64\text{g}\%$) but it gradually decreased after that to $4.5 \pm 0.32\text{g}\%$ on 7th day of storage time.

It is evident from our results that the quality and quantity of oyster tissue protein is changed significantly ($p < 0.001$) during 7 days of storage at $7 \pm 2^\circ\text{C}$. Our results are in good agreement with those reported by Antunes, *et al.*, (1982) 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 decreases. Siddiqui & Ali, (1979) reported that decrease in protein contents of prawn during ice storage is due to the leaching effect, the amino acids and water soluble protein leach out with melting ice.

Our results show (Fig.1 & 2) that changes in total protein contents of oyster meat directly correlate with salt soluble protein (0.87) and total lipid (0.94) and reciprocally with TMA (- 1.0), TVB (-0.98). Similar relationship was also reported by Gagon & Feller, (1958) during low temperature storage of shrimp, they found correlation between denaturation of tissue protein and lipid with the formation of TMA & TVB as degraded products.

Salt soluble protein:

The solubility of tissue protein has been used as an index of alteration in protein quality during low temperature storage. Our result showed that SSP content of freshly caught oyster is decreased considerably with the increase in storage period at $7 \pm 2^\circ\text{C}$. During first two days of storage, slight change in ssp. ($2.53 \pm 0.14\text{g}\%$) has been noted and on 7th day of storage it decreased from $3.46 \pm 0.53\text{g}\%$ to $1.1 \pm 0.21\text{g}\%$.

It is apparent from the results that the protein solubility changes significantly ($p < 0.001$) after each day interval. The results of present studies are very much similar to the finding of Bhohe & Pai 1986 in shrimp meat during storage at 0°C

and frozen temperature (Riaz & Qadri, 1990). They stated that at low temperature storage an increase in salt concentration of tissue is mainly due to the loss of free water which ultimately decrease the solubility of protein by changing the ultrastructure of myofibril protein. LeBlanc (1988), stated that this loss in SSP indicate the denaturation of protein which may be due to the interaction of formaldehyde or dimethyl ammine with tissue protein.

The changes in SSP content of oyster during storage at $7\pm 2^{\circ}\text{C}$ is well correlated with TMA, TVB & total lipid. The results show statistically reciprocally correlation with TMA, TVB and bacterial count ($r = -0.98, -0.96$ and -0.81) and positive correlation with lipid ($r = 1.0$). Similar correlation is reported by Riaz & Qadri (1990). They have suggested that increased 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 (hydrolyzed products).

TMA:

The TMA content of fresh oyster tissue is found as $1.1\pm 0.11\text{mg}\%$, during first 2 days of storage, and changed negligibly. After that the rate of TMA formation increased and on 7th day it reached to a value of $4.3\pm 0.41\text{mg}\%$.

Our results showed that at $7\pm 2^{\circ}\text{C}$ increase in TMA content is highly significant ($p \leq 0.001$) during 7 days storage. Same results was also be represented by Vyncke (1980), during ice storage of fish tissue.

The Duncan's multiple range test also provides the same 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. They observed that there is an increase of 5 to 13 mg% in TMA during storage. From the present study it is evident that the increase in TMA-N of oyster is within the range of above report value ($4.5\pm 0.4\text{mg}\%$).

The quality of fish and shell-fish is classified into the different grades on the basis of their TMA content. The meat containing 0-2.5mg% TMA-N is considered as grade I quality (prime or good), 2.5-5mg% TMA-N as grade II quality (acceptable) and meat having more than 5mg% as grade III (spoiled or rejected). Our results showed that at $7\pm 2^{\circ}\text{C}$ storage the quality of oyster ($1.46\pm 0.09\text{mg}\%$ TMA) tissue was prime of grade-I upto 2 days of storage. On 5th day of storage

quality of oyster ($2.86 \pm 0.18 \text{mg}\%$) was grade-II and after that, the quality of oyster meat became spoiled or considered as grade -III quality.

Our results show statistically that TMA has a well direct correlation with TVB ($r=0.99$) and indirect correlation with total lipid ($r= -0.97$).

TVB:

Total volatile bases are considered as marking compound for the assessment of fish quality. Like other spoilage indicators (TMA, SSP, pH); the change in TVB content of oyster tissue has very much significant ($p \leq 0.001$) during storage at $7 \pm 2^\circ\text{C}$. The results showed that it increased from $9.46 \pm 0.29 \text{mg}\%$ to $83.30 \text{mg}\%$, showing an increase of approximately 10 to $20 \text{mg}\%$ TVB-N per day. A permanent increase in TVB content is reported by Botta *et al.*, 1977, Cho *et al.*, 1984, 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 into three grades, an excellent grade having TVB-N value less than $30 \text{mg}\%$, acceptable grade with the value of TVB between $30-40 \text{mg}\%$ and for the spoiled or rejected grade the TVB-N value will be above $40 \text{mg}\%$ Koizumil, *et al.*, (1985). Our result showed that at $7 \pm 2^\circ\text{C}$ the quality of oyster is excellent up to 4 days of storage ($27.66 \pm 1.45 \text{mg}$). The quality of oyster meat on 5th day ($40 \pm 2.88 \text{mg}\%$) was acceptable and after that period, it was decreased to rejected grade.

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

Total lipid:

Oxidation of lipid is an important factor of deterioration of fish and shellfish. Total lipid content was decreased with an increase in storage time. During first two days storage it changed from $3.0 \pm 0.38 \text{g}\%$ to $2.5 \pm 0.29 \text{g}\%$ in oyster tissues. On 7th day it declined significantly ($p \leq 0.001$) to the value of $1.4 \pm 0.19 \text{g}\%$ in oyster meat.

A non significant percent loss (50 to 70%) in total lipid content of fish at 0°C was noted by Nester *et al.*, 1979, Riaz & Qadri, 1990. The percentage decrease in total lipid content of tissue noted in our study is 50 to 65% at refrigerated

temperature. This loss in tissue lipid occurred during the storage period, is due to the oxidative rancidity. The rancidity in term of thiobarbutaric acid number (TBAN) is used as indicator of quality of fish. It has been found by many workers that TBAN is increased linearly with the increase of storage period (Han, *et al.*, 1987).

From the present study it has been concluded that the refrigerator temperature could only be recommended for four days storage.

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