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Effect Of Frozen Storage -20, On Total Protein Content Of The Sea Lobster (Panulirus Homarus)

Priyanka Sinha, Aditi Singh & Sadhna Malgam

St. Aloysius College Autonomous Jabalpur India

Abstract – Present study was to examine the change of nutritional value such as protein in sea lobster at freezing time. It has some effect on the protein compositions of the selected lobster, which was taken under the experiment. To observe this study, it revealed that there were few changes in the in the protein composition of lobster in frozen condition after three weeks. This kind of deterioration was occurred by freezing time in addition to protein denaturation. Lobster enriched with protein, but the protein content dropped with processing and storage time; however, for long-time preservation, freezing is the best method when a nutrient is a focus. To conclude that a significant difference between the freezing days as the freezing period increases, the presence of protein in the muscle decreases because of the process of protein Denaturation.

Key words-Sea lobster, protein & sea food

INTRODUCTION

Any life form in the sea is seen as food by man as seafood. This can range from fish, shellfish, shrimps, oysters, and so on. Demand for seafood is increasing worldwide due to increasing health consciousness. Millions of people suffering from malnutrition in our India, Protein deficiency may be minimized to some extent by making available cheaper fish meal items which are available to local communities. Edible Crustaceans, such as Crab, Prawn, Cray fish and Lobster constitute one of the major sources of nutritious food for human beings (Bugel et al., 2001)[1]. Since seafood is recognized as a healthy food in terms of protein, unsaturated fatty acids and minerals, the demand for seafood in the global market is increasing.

Lobster can serve as the main source of protein in a meal. Lobsters are invertebrates that feed mainly on fish and molluscs, but also consume plant life especially algae. Lobsters are rich in copper, selenium, zinc, phosphorus, vitamin B12, magnesium and vitamin E. They are highly prized, economically important seafood. A report suggests that individuals consuming shellfish like lobsters may reduce their

risk of myocardial infarction by more than 50% (Yuan et al., 2001)[2]. The nutritive values of crustaceans depend upon their biochemical composition such as protein, amino acids, lipids, fatty acids, carbohydrates, vitamins and minerals. Among the seafood, prawns and shrimps contribute about 20-22% by volume of the world seafood market (FAO, 2014). Due to their nutritious nature, apart from the supply of good quality proteins, lipids, they also contain several dietary minerals such as calcium and iron, which are beneficial and vital and play a chief role in the maintenance of physiological and biochemical activities in human beings.Therefore prawns and shrimps are considered to be most popular species as it is a part of almost every nation's traditional meal rich in protein and minerals.

There is a lot of research on proximate composition (moisture, ash, lipid, crude protein, and carbohydrate contents) of fish. However, there is a neglect of other edible aquatic sea foods. The nutritional benefits of seafood have encouraged its continuous consumption. It may therefore be imperative to determine their nutritional composition in order to help make informed decision when aquatic products are to be consumed. Therefore, the present study is aimed to probe into the aspects for the evaluation of proximate composition of basic biochemical constituents including proteins, lipids, carbohydrates, ash and moisture to assess the nutritional significance of lobster (Thenus orientalis), shrimp (Penaeus semisulcatus) and puffer fish (Arothron immaculatus,) Merline et.al 2020 [3].

In general, fishery products do not suffer much quality loss as a consequence of the freezing process if it is done rapidly. However, significant deterioration has frequently been observed during frozen storage. Detrimental changes include development of off-odors and off-flavors as well as changes in texture, moisture, and appearance (Connell, 1975[4]; Shenouda, 1980)[5]. A major consideration in judging the quality of frozen seafood is texture. As a consequence of protein denaturation during frozen storages frozen seafood can change considerably from the fresh product (Dyers 1951[6]; Shenouda 1980)[7]. Protein denaturation factors related to changes in moisture include formation of ice crystals dehydration of protein moleculesand an increased salt concentration in the remaining unfrozen water (Shenouda1980). These denaturing conditions are initiated during the freezing process and continue to effect changes in muscle tissue during frozen storage. Salt concentrations in unfrozen water of fish muscle tissue are increased roughly 1 0 -fold at commercial storage temperature (- 10° to - 20°C). At these temperatures only the water is converted to ice. Increased salt concentrations have been shown to denature protein through aggregation or dissociation caused by disruption of tertiary and quaternary macromolecule configuration.

Changes during frozen are caused by processes known as freeze denaturation of proteins, involving usually denaturation proper, followed by interactions of the denatured proteins with various reactive components of the fish tissues. Fish muscle proteins, mainly myosin, are more susceptible to abuse conditions of freezing and frozen storage than those from land animals. (Z. E. Sikorski et al. 1994)[8].frozen storage may cause lysis of organelles, such as mitochondria and lysosomes, and disintegration of membranes, resulting in a loose, disorganised fish muscle structure(Karvinen et al., 1982)[9]. The activity of alpha-glucosidase in fluids obtained by centrifugation, 'centrifugal tissue fluids', and the protein

content of the fluids have been found to correlate with the cellular damage caused by the length of time a frozen fish remains in frozen storage. after the storage, myofibrillar protein denaturation and lipid degradation products showed that storage time also affected the integrity of muscles, and caused structural changes to myosin and hydrolysis and oxidation of lipids (Maria Makri, 2009)[10]. Nowadays, one of the most active areas of nanoclays research is their employ in food packaging. Then critical issues in developing food packaging materials are reviewed to minimize gasses and other small molecules transfer between out-side packaging environment and food[11].The extension of shelf life can be achieved by freezing, chilling, salting, smoking, glazing, etc. Among the various techniques of preservation, freezing is considered to be the only long term method which can preserve a fish with a minimum change in its quality these mean that fish if necessary should be stored, for a short period to retain the taste, and provide both the protein. Bugel, S.H. et.al studied on Absorption and retention of selenium from shrimps. was undertaken to evaluate the bioavailability of selenium in shrimps, a possible good source of selenium, by measurements of the absorption and retention of selenium and the effects on plasma selenium concentration and glutathione peroxidase activity. Yuan, J. M., et.al, 2001 studied on dietary intake of n-3 fatty acids derived from seafood also was significantly associated with reduced mortality from myocardial infarction. Neither dietary seafood nor n-3 fatty acid intake was associated with a reduced risk of death from stroke or ischemic heart disease other than acute myocardial infarction. Merline, et.al 2020,investigated that edible sea foods such as prawn (Penaeus semisulcatus), lobster (Thenus orientalis) and puffer fish (Arothron immaculatus). And their proximate composition including protein, carbohydrate, lipid, moisture and ash contents varied among the three organisms. From the present results prawn and puffer fish had higher amount of protein content while lobster were rich in lipid and carbohydrate content. The study suggest that sea foods have shown to be good sources of nutrients while the prawn and puffer fish are particularly rich in protein, the lobster is rich in lipid content. Connell, 1975, have studied on control of fish quality with principles of fish and fishery product quality and measures to regulate, and not with handling, processing or treating fish. It is written for fish technologists starting out in the field, industrial technical managers, and fishery administrator. Soliman Y.K.Shenouda,1980, have studied on theories of protein denaturation during frozen storage to quantify the undesirable deteriorative characteristics mentioned, scientists have tried to correlate them with various analytical parameters, mostly associated with the phenomena of protein denaturation. Changes in the physical and chemical properties of extracted fish proteins give more in-depth information on the changes that have occurred at the molecular level during frozen storage. Dyers 1951, have studied on protein denaturation in frozen and stored fish and observe that the taste panel on frozen and fresh halibut showed that freshly samples were somewhat firmer and drier than the unfrozen ones and were preferred by many people and the taste panel were very definitely in disagreement. Zdzislaw E. Sikorski and Anna Kolakowska 1994, have studied on changes in proteins in frozen stored fish and observe that the rate of freezing and the temperature of storage affect the size and distribution of ice crystals in the tissues and may change the microstructure of fish muscle. These changes are caused by processes known as freeze denaturation of proteins, involving usually denaturation proper, followed by interactions of the denatured proteins with various reactive components of the fish tissues. Veli-Pekka Karvinen et.al 1982, The autolytic enzymic

degradation of the muscle of Baltic herring (Clupea harengus membras) was studied by investigating the muscle fractions after two different types of storage commonly used in fish handling. freezing and storage at - 30°C, and storage at +5°C, comparing them with the muscle fractions of fresh fish.Maria Makri, 2009, have studied on Biochemical and textural properties of frozen stored and found A slight decrease in salt soluble proteins was observed after 266 days of frozen storage suggesting that storage time hardly affected the formation of aggregates. The water holding capacity of the stored frozen fillets decreased with storage time and was associated with the damage in muscle structures (protein content in centrifugal tissue fluids), denaturation of myofibrillar proteins and lipid degradation products free fatty acids and peroxide value. Afsharian et.al have studied on one of the most active areas of nanoclays research is their employ in food packaging. In this paper, at first, the reasons of nanoclay applications are introduced. Then critical issue in developing food packaging materials is reviewed to minimize gasses and other small molecules transfer between out-side packaging environment and food

MATERIALS AND METHOD

The aquatic sea foods, lobster were bought from the Ansari fish centre, sadar, Jabalpur in a poly bag to Zoology laboratory of St. Aloysius college Autonomous, the samples were washed thoroughly and then it was dissected with a cleaned stainless steel knife. The head and viscera were discarded and cut the muscle. The muscle was cut into small pieces and crushed with mortal Pestel. Then the flesh was measured into six same quantity and made six packets wrapped with aluminium foil.

Then for proteins estimation one packet of flesh were kept in oven for drying at 60^{O}c for 48 hours. Then it was ground into powder by the help of mortal pestle. And rest of the five packet was kept in deep freezer (-20^{O}C) for the protein estimation after freezing.

Rest of the samples (2,3,4,5 and 6) were determined after the freezing of 3 days, 5days, 7days, 10 days and 13 days of freezing, respectively. To estimate the amount of protein 10mg of fine dried powder was taken to which to 5ml of IN sodium hydroxide (NaOH) was added and homogenized the content and centrifuged for about 15 mints at 2500rpm. The supernatant was taken separately and kept for further analysis. 0.1ml of solution was taken into a Test tube and 0.9ml of distilled water was added to get 1ml of solution. Then 4ml of Lowry's mixture (Lowry A + Lowry B. Lowry A is a mixture of 10gm anhydrous Sodium carbonate and 2gm of sodium hydroxide in 500ml of distilled water. Lowry B is also a mixture of 0.5gm of copper sulphate and 1gm of sodium potassium tartrate in 50ml of distilled water each). Then the contents are shaken well and kept for incubation for about 15 mints. To this 0.5ml of Folin phenol reagent (phenol and distilled water at 1:1 ratio) was added and shake the contents once again for the development of colour. The colour concentration (optical density) of the samples was measured with an U.V Spectrophotometer and the readings of absorption were noted.

Result & Discussion

BIOCHEMICAL ANALYSIS - The protein content of the samples was estimated by following the method of Lowry et al., (1951)[12]. protein was estimated by Protein Estimation Kit by GeNei. Before the estimation of protein, a standard curve was required, whose sample was given in the kit which is Bovine Serum Albumin (BSA). The protein given within the instruction guide was followed, and a standard curve was prepared, from which the protein values in serum samples were inferred.

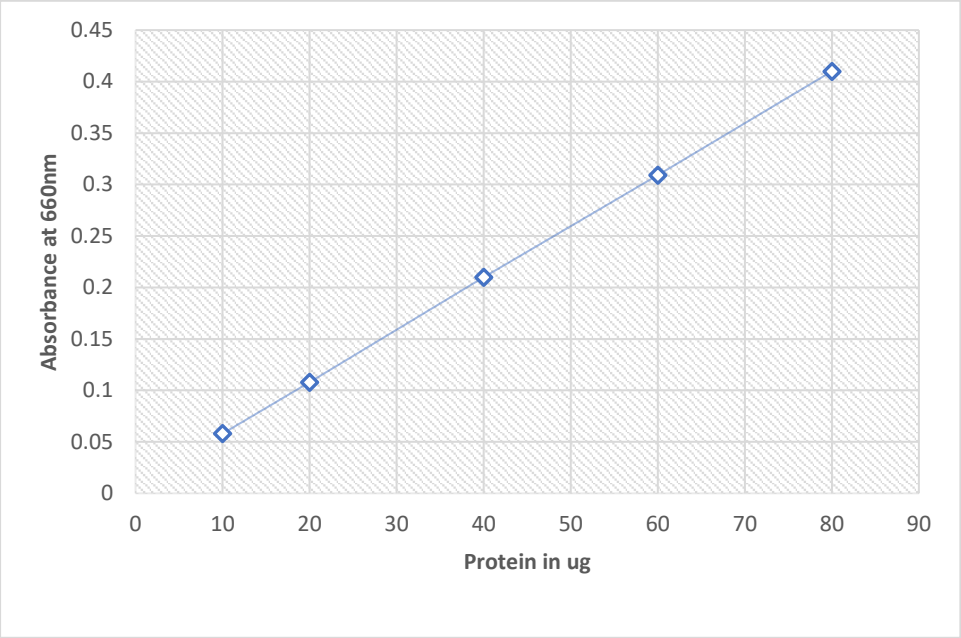
Preparation of standard protein (BSA)

1. BSA (5 mg) was reconstructed during a vial by dissolving in 1 ml distilled water to get 5 mg/ml. 0.1 ml of 5 mg/ml BSA solution was diluted with 0.9 ml water to get 0.5 mg/ml, just before use.

Complex forming reagent

2. To at least one volume of solution I, 100 volumes of solution Il were added just before use. Solution Ill was used directly.
3. Reconstituted BSA (0.5 mg/ml) was pipetted, test sample and water were mixed according to the protocol and therefore the volume was adjusted to 0.2ml with water.
4. Then added 2ml of complex forming reagent, mixed and kept for 10 minutes at temperature.
5. Solution Ill (0.2 ml) was mixed thoroughly and incubated for 20 minutes.
6. The O.D was read during a spectrophotometer at 660 nm.

S. no.	Std. (ug/ml)	Amount (in ul)	Water (in ul)	Absorbance (Abs 660nm)
1	Blank	0	200	0.000
2	10	20	180	0.058
3	20	40	160	0.108
4	40	80	120	0.210
5	60	120	80	0.309
6	80	160	40	0.410



Standard curve for the determination of total protein:

Absorbance of all the sample were estimated through the spectrophotometer by lowry method.

Observation table: -

Days of deep freezing (-20)	**Absorbance (660nm)**
Fresh sample	0.301
After 3 days of freezing	0.207
After 5days of freezing	0.180
After 7 days of freezing	0.127
After10 days of freezing	0.085
After 13 days of freezing	0.055

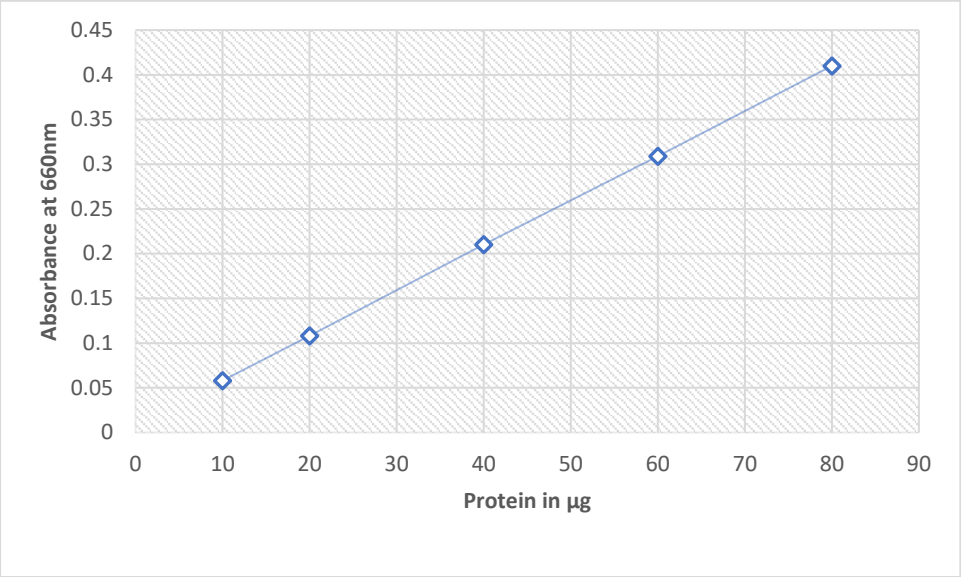


Table – 1.2 BSA standard curve with the sample absorbance to find out the protein in ug.

The results of protein composition of muscle tissue have been presented. Protein was measured by the standard methods of universally accepted. Protein content of seafood (lobster) was observed of the fresh sample, 3rd day after freezing, 5th day after freezing, 7th day of freezing, 10th day after freezing and 13th day after freezing is found 43ug, 37ug, 35ug, 18ug, 12ug and 7ug respectively, according to Table-1.2. The maximum value of protein content in muscle occurred as (43ug/g) during the time of before freezing, while the minimum value observed as (7ug/g).

The highest protein content of Labeo rohita was recorded for fresh (unfrozen) samples, and the lowest protein content was recorded for samples stored for 3rd weeks .it revealed that after three weeks of frozen storage, protein declined in a significant manner. In addition, protein content decreased for denaturation and loss in gelatine proteins. High protein content based on different protein sources develops hardness during storage.

All the above observations are further verified by subjecting the detail on protein composition. It was already reported the changes in the composition of biochemical constituents of biota vary not only with environmental changes, but also with the seasons. Such changes were also be attributed to various physiological and other factors like maturation, spawning, feeding etc. Bruce (1924)[12] and others reported that the protein content was more in fishes during early summer and winter months corresponding to their maturity stages. During maturity stages gonads get studded with proteins as well as fat and therefore the muscle generally contains relatively meager amount of protein and fat.

CONCLUSION

The main findings of this study were to examine the change of nutritional value such as protein in sea lobster at freezing time. It has some effect on the protein compositions of the selected lobster, which was taken under the experiment. To observe this study, it revealed that there were few changes in the protein composition of lobster in frozen condition after three weeks. This kind of deterioration was occurred with freezing time in addition to protein denaturation. lobster enriched with protein, but the protein content dropped with processing and storage time; however, for long-time preservation, freezing is the best

method when a nutrient is a focus. To conclude that significant difference among the freezing days as the freezing period increases, the presence of protein in the muscle decreases because of the process of protein Denaturation

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