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Analysis of biochemical parameters of green mussel (*Perna viridis*)

N.Arun and M.Shamsheer

Department of Biochemistry, Thanthai Hans Roever College, Perambalur-621212.

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ABSTRACT

Green mussel (*Perna viridis*) was brought from Fort- Cochin Fish market to the laboratory was shucked and collected the meat. Mussel meat packed in the Tin Free Steel can (130gm in a can) and were thermally processed in an over pressure retort. Heat penetration characteristics, process time, nutritional and sensory quality parameters etc were analyzed. F_0 value of the product was 8.79 when thermal processed at 121oc for 48.94 minutes. The processed product was commercially sterile. The present study was to find out the changes in the biochemical constituents of green mussel (Perna viridis) when thermally processed in brine in an over pressure retort. Texture attributes such as hardness, chewiness, springiness, and cohesiveness did not show any marked variation in the processed meat. Proximate composition canned mussel meat showed a slight decrease when compared with fresh meat sample.

Introduction

Fish and shellfish including molluscs are highly nutritious and highly perishable food items. They have to be preserved properly. The preservation of food was practiced before the beginning of recorded history and some of the methods adopted by the ancients are still in use in a modified form. The preservation of food is a traditional field for research activities.Fish and shellfish are preserved and processed in many forms like icing, freezing, canning, drying, curing. These methods have their own advantages and disadvantages. Consumers prefer convenient, ready to cook/eat, wholesome, safe, preservative free products, which can be achieved by canning.

The Value of fish and shellfish for human nutrition lies in the relatively high protein content, good digestibility and high biological value of fish proteins (Geiger and Borgetrom, 1962). Fish provides well balanced amino acids and fatty acids in the diets and all this makes fish to occupy a important place in human nutrition.

Recent reserch has revealed many more beneficial uses of fish, namely prevention of cardiovascular diseases, arthritis, hypertension, improvement in vision, brain development, etc. Because of its wide range of advantages the per capita consumption is seen increasing in both developing and under-developed countries.

The food preservation methods are aimed at preventing undesirable changes in the wholesomeness, nutritive value and sensory quality of food by controlling growth of microorganism and obviating contamination by adopting economic methods. Thermal processing is one of such methods, by which food is given sufficient heat treatment in a hermetically sealed container to destroy pathogenic and/or spoilage causing microorganism and their spores, antinutrients and enzymes that cause degradation in the food. The spoilage of fish is effected by autolysis, oxidation and bacterial activity. Among them the bacterial activity is the most important factor in producing the most striking and undesirable alteration in the flavour, odour and appearance of fish (Ratnakumar. K and Ninawe, A.S; (2008).

Fish and shellfish are consumed in many forms in the developed countries; the value added fish products are consumed in substantial quantity. In today's affluent society, people prefer to buy read-to-cook and ready-to-serve convenience products from supermarkets than buying raw fish, which is cumbersome to prepare for the table. All over the world, the tendency now is to take convenience foods, such as assembling meals, rather than preparing from basic ingredients. Such change is due to change in food concept and increasing working woman, who finds shortage of time.

Besides exports, demand in internal market for value added product is increasing in India. The introduction of microwave oven also helped in increasing market for ready to cook products in urban areas. Among different value added food products, canned product is one of the most important one.

Primary purpose of heat preservation is to destroy microorganisms. It has been possible to develop heat treatments that produce good quality foods in which there is no danger of spoilage or food poisoning even after lengthy storage. These treatments destroy those microorganisms that could proliferate in the milieu concerned, when the correctly packed foodstuff is stored under given condition.

Canning is defined as the process of heating hermetically sealed foods to a temperature that kills harmful microbes. The containers may be made of metal, glass or any other material that is airtight and heatable. Commercial sterility may be defined as that condition in which all *Clostridium botulinum* spores and all other pathogenic bacteria, as well as more heat resistant organism, which if present could produce spoilage under normal conditions of storage and distribution have been destroyed (Denny, 1970).

For the propose of canning, different containers are used such as tin, aluminium etc. Several workers have tried to preserve the fish products in tin containers. The metal cans used

Tele:
E-mail addresses: aamaravian@gmail.com

as containers impart an undesirable taste to the product on storage. Secondly as far as India is concerned tinplate for making cans is imported and hence it is disadvantageous economically. Many of these containers have the problems of disintegration of lacquers, expensive and are difficult to open. One of the recent developments in rigid containers is Tin Free Steel cans. These are Drawn and Re Drawn steel cans with chromium coating and are manufactured with easy open ends and also coated with polyester inside which does not react with products. Now these TFS cans are manufactured in India and not much work has been carried out on the suitability of these cans for processing fish and fishery products.

The world fish production statistics shows that 80% is from capture fisheries and 20% from culture fisheries. Capture fisheries has reached stagnation point and aquaculture is a promising field. In the recent decade, the contribution from the aquaculture to the world fish production is increasing. Aquaculture is practiced both in salt water and in freshwater. The green mussel *Perna viridis* is abundantly found in west coast of India particularly in the Malabar region extending up to Cochin area.The green mussel is cultured in the estuaries of central and northern Kerala, south west coast of India.As lot of importance is given for aquaculture activities, the catch of green mussel and other freshwater fish varieties is going to increase tremendously in the increasing demand for value added products. In this study an attempt is made to utilize green mussel Perna viridis for developing canned mussel meat in Tin Free Steel (TFS) cans.

First canning factory in India was established in the year 1911 in Chaliyam in Kerala. This was closed after functioning for about one and half decades for several reasons like non-availability of cheap containers, poor and interrupted supply of raw materials, high processing costs and inability to compete with better quality imported products. The industry was later revived in India in the late 50's, mainly intended for canning of prawns for export market. Export of this commodity started in the year 1959 which after touching the highest recorded a steep fall due to the very same reasons mention above that caused the closing down of the first fish canning factory in the country.

India exported very insignificant quantity of canned shrimps amounting to 3.5 tonnes valued at 4.4 lakh rupees in 1996. This quantity was less than 1% of the total seafood export of 353675 tonnes during that year.

Thereafter no signficant export of canned seafoods has taken place. There are 25 canning units registered with MPEDA having a production capacity of 84 tonnes per day. Out of these only two units are well equipped and in good working condition. Except the canning plants run by the Lakshadweep Corporation at Minicoy island, Integrated fishery project at Cochin and three units run by the private companies all other units have been declared sick due to high cost of production.

One of the major factors for downfall of the Indian canning industry is inadequacy of indigenous tin cans with respect to their quality and price.

The quality of lacquer, the finish and diversity are the elements essential to ensure shelf life and consumer appeal. In India, the price of the can alone works out to nearly 30% of the cost. Added to the cost of labour, cartons and other items shoot upto 50% of the total production cost making it ill affordable to the average Indian consumers and not competitive in most of the overseas market. Under Indian condition TFS cans are advantageous in terms of low cost, good quality lacquer and are locally available.

Preservation of Green mussel (Perna viridis) in Brine Media in cans is expected to have great demand in India and abroad. In order to develop a product for better utilisation of Green mussel and also to standardize the preparation of Green mussel in TFS cans. This could help in reviving the canning industry in India.

Materials and methods

Materials

Green mussel (Perna viridis)
Fresh meat of Green mussel
TFS cans
Thermocouple
TFS can with thermo couple

Methods

Can Examination
Measurement of the Seam(Balachandran, 2003)

Air pressure test

In order to check the pressure holding capacity and also for any leakage through seams, the cans were subjected for air pressure test as described in IS: 2471- 1963 and IS: 93969-1979
Lacquer for food Contact Application (FDA: 1983)
Resistance to Sulphur Staining and Impermeability (IS: 5818-1970)
Lacquer Delamination Test
Canning
Thermal processing/ process Evaluation

Analysis

Green mussel (*Perna viridis*) was brought in to the laboratory and was shucked. A good amount (987.51 gm) of fresh meat was used to canning process. Seven cans were prepared (135+-3 gm meat in a can). About 200gm fresh mussel meat was minced.

After canning, seal was opened, meat was minced. This minced fresh and thermally processed meat used for all the Physical, Chemical and Microbiological analysis.

Physical parameters

pH Measurement

5g of the samples was dispensed in 10 ml water and was pH was measured by using pH meter.

Chemical parameters

Determination of Moisture (A.O.A.C, 2000)
Determination of crude protein (A.O.A.C, 2000)
Estimation of crude Fat (A.O.A.C, 2000)
Determination of Ash Content (A.O.A.C, 2000)
Preparation of Trichloroacetic Acid (TCA) Extract.
Determination of TVB- N (Conway, 1950)
Determination of TMA (Conway, 1950)
Estimation of Non -Protein (AOAC, 2000)
Determination of Total Amino acid profile (Ishida,1981)
Determination of Tryptophan (Sastry and Tummuru, 1985)
Determination of Fatty Acid Composition (AOCS, 1989)
1. Fat Extraction
2. Fatty acid composition of Fat
Determination of cholesterol
Determination if Minerals

The minerals like sodium, potassium, calcium are estimated using flame photometer after dissolving the ash in dilute hydrochloric acid (6N).

Studies on the Texture

Sensory Test (IS: 6273 (II)-1971)

Result and Discussion

Examination of Can

TFS cans were examined for various parameters to test their suitability for canning Mussel in brine medium and the results are presented in Table 3.

Table 1. Physico –chemical properties of tin Free Steel Can the can size is 301X206. Can appearance was good both internally and externally.

The air pressure test showed no signs of leakage. The test for lacquer delamination showed no delamination and the result of sulphur staining was no blackening in the test panels.

Air Pressure Test

No signs of leakage and deformation of cans were observed, when subjected to internal air pressure of 100 k pa for 15 seconds. This ensures that there won't be any chances of leakage through seams during heat processing and cooling operations

Test For Lacquers

Lacquer For Food Contact Application

The overall migration residue i.e. water extractive and chloroform extractive at 210C for 48 hrs of the sample was 0.0775 mg/lit and 0.0244 mg/lit respectively, which is below the acceptable limit for food contact application. This indicates that TFS cans are suitable for processing food products.

Sulphur Staining

The test for resistance to sulphur staining indicated that there was no blackening on the test panels and the cans were sulphur resistant.

Analysis of fresh Green Mussel (*Perna viridis*)

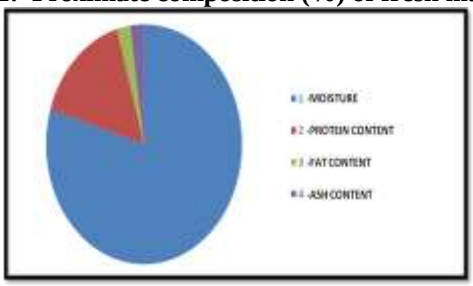
The green mussel (Perna viridis) was used in the present study intended for the thermal processing. The mussel was very fresh with a characteristic fresh odor, color, and shell was intact to the flesh.

Proximate composition

The proximate composition of raw fresh mussel is presented in Table

The fresh mussel had moisture content 79.75%, crude protein 15.83%, and crude fat 2.24%.

Fig 2: Proximate composition (%) of fresh mussel



Quality evaluation of fresh Mussel

Various quality parameters analyzed for the fresh mussel are given in Table 6. The pH was 6.59, which indicated mussel as a low acid food.

The amount of trimethyl amine and total volatile base nitrogen was found to be less than i.e. 5.5 mg/100gm and 10.39mg/100g respectively.

The amount of cholesterol present in the fresh mussel meat was 161.30 mg/100gm.The minerals composition of fresh mussel was evaluated using flame photometer.

Composition of minerals and its variation in the canned meat are presented in the Table.00. The composition of fatty acid and amino acid were also analyzed.

Fatty acid composition is presented in the Table.00.The amount of tryptophan was 1.033 mg/100gm in the fresh mussel. The total amino acid profile of raw mussel is presented in Table.00.

Thermal processing of Green mussel Perna viridis

The fresh meat of green mussel (*Perna viridis*) was collected by hand and pack weight was maintained 130 gm in a can. The processing was carried out in the section 3.4.3.The study was aim to determine the changes in the biochemical constituents of mussel meat during thermal processing. The target lethality was set around 8.0 minutes. The processing parameters were obtained by plotting the graph and process time was calculated by using the formula method (Stumpo, 1973).The parameters are presented in Table 7. The processing was carried out at 121.1^0C. In can, eating lag factor (J_h) was found to be 0.567, whereas the cooling lag factor was 1.07.Although the lethality was targeted at around 8.0 minutes, slight variation in the targeted value were observed. The process time (B) was calculated using the formula method (Stumbo, 1973) and is presented in Table 7.The retort temperature and core temperature data with corresponding time in combination with F_0 value and cook value is shown in the figure given below.

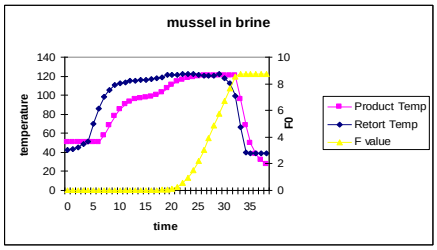


Fig 3: Heat penetration characteristics and F_0 value.

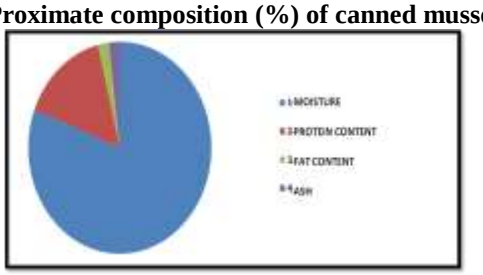
Analysis of canned Mussel meat

Study of thermally processed meat in the can was conducted**.**

Proximate composition

The proximate composition of canned mussel is presented in Table 1. The canned mussel had moisture content 78.27%, crude protein 14.80%, and crude fat 1.94%.

Fig 1: Proximate composition (%) of canned mussel meat



Changes in moisture content

Changes in moisture content of Mussel meat during processing is given in Fig. It is seen from the results that there is no significant change in moisture during thermal processing in brine. The reason is, thermal processing was in brine (1%). The initial moisture level decreases slightly during processing. The changes in the moisture content of mussel meat are presented in Fig:

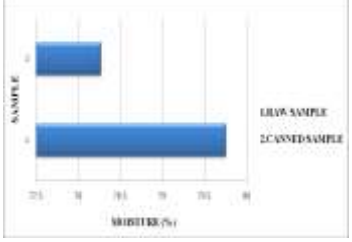


Fig: changes in the moisture

Changes in pH

The pH value is decreased from an initial value of 6.59 after processing to 5.47.

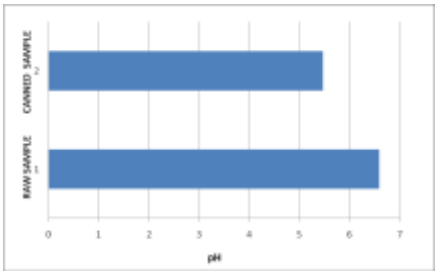


Fig 12: Changes in the pH

Changes in the TVBN

Total volatile base nitrogen is mainly seen in marine water fishes. There is a permittable level of TVBN in fishes. It should increase after processing because it is a volatile substance. The changes in TVBN are shown in the following table

Changes in the TMA

The value of Trimethylamine in fishes showing its freshness. For fresh fishes it should be below 10.If the value of TMA coming higher than 15, it showing that the material has spoiled. The TMA value for fresh Green mussel meat was 5.50 that mean it was fresh. The value of TMA after processing (10.39) indicating that thermal processing was not affected greatly to the freshness of the meat.

Changes in Amino acid profile

The total amino acid content (% as mg/g protein) of raw mussel meat and processed mussel meat after process is presented in Table:6

Changes in the minerals composition showed no big difference in the case of Sodium. It was found that fresh mussel meat had 2.2 g% of sodium where as in the canned mussel it was 1.78 g%. In the case of Potassium and Calcium, there was a decrease in the values in g% in the canned meat.

The slight difference in the minerals composition is due to the decrease in the ash content after thermal process in the brine. The decrease in the ash content was due to release of ash content from the meat to the brine.

Sensory evaluation

Instrumental texture profile analysis

The Texture is the combination of the physical structure of the food and characteristics of the food during various treatments. Texture attributes were analyzed for the fresh mussel and canned mussel.

Various parameters analyzed includes hardness 1 and 2, Area 1 and 2, Cohesiveness, Springiness, Springiness index, Gumminess, Chewiness, Fracture force, Adhesive force, Adhesiveness, Stiffness which are given in Table 11.

The variation in these parameters are presented in the figures (17, 18, 19,)

Fig: Changes in the Hardness 1

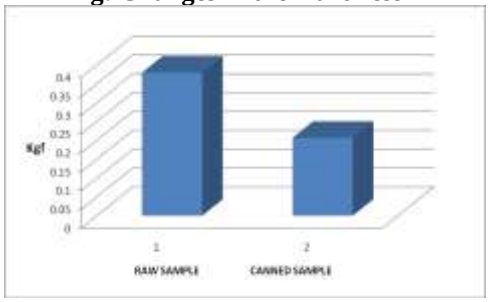


Fig: Changes in the Hardness 2

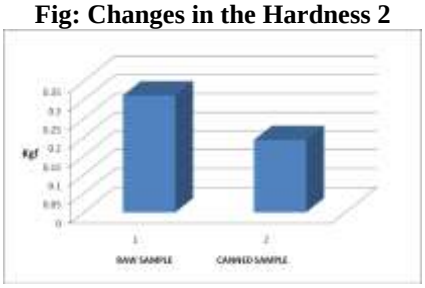


Fig Changes in the Area

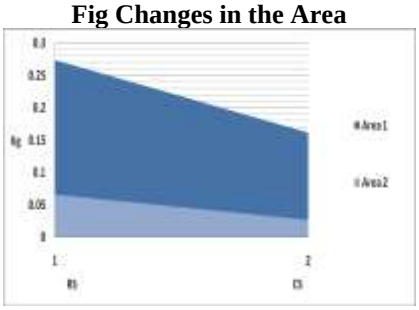


Fig Changes in the springiness and springiness index

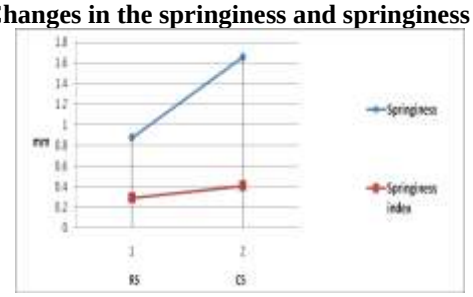


Fig Changes in the cohesiveness

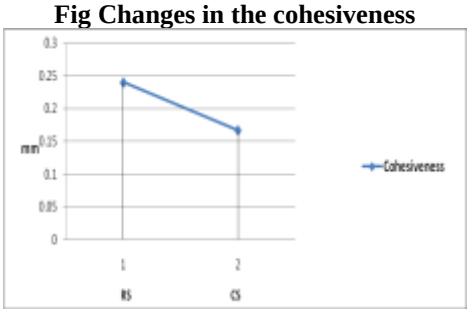


Fig Changes in the Gumminess, Chewviness and Adhesiveness

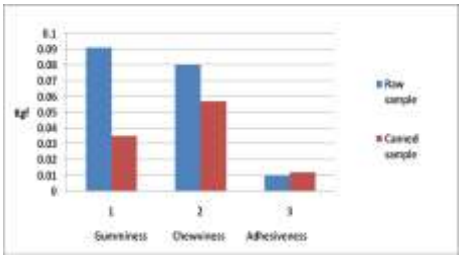


Fig Changes in the Fracture force and Adhesive force

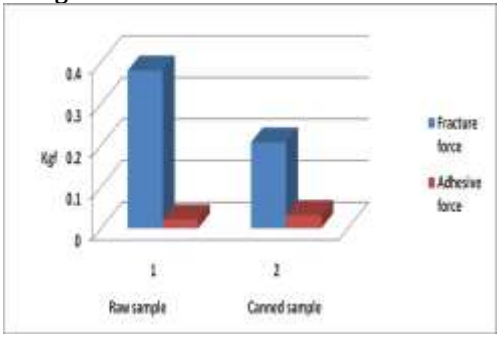
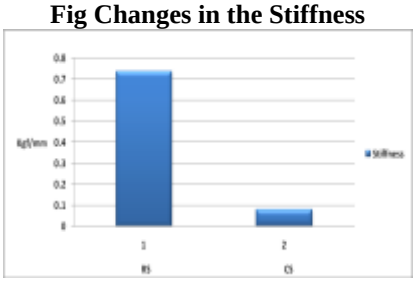


Fig Changes in the Stiffness



Summary and conclusion

The present study deals with the suitability of polymer coated tin free steel cans (TFS) for the canning of Green mussel (Perna viridis) in the Brine. Fresh Green mussel were collected from a Fort-Cochin fish market and brought to the laboratory which were shucked and collected the meat. The fresh meat were then washed; cold blanched in water for 10 minutes, then can was filled with 130g mussel meat. Then 1% brine was added in to the cans. It was then exhausted and seamed using double seaming method. The can were processed to Fo 8.0 in an over pressure autoclave with a retort temperature at 121°C. The selected tin free steel cans were subjected to test for various physical properties ant its suitability. Heat processed cans were stored at room temperature. The samples were subjected to various biochemical, physical and sensory evaluation.

Change in moisture content was observed during storage study at room temperature. Sensory evaluation of the product by panel members rated the product as GOOD based on overall acceptability scores. Texture parameters such as hardness, chewiness, springiness and cohesiveness did not show any marked variation.

The result of the present experiments showed that Tin free steel cans (TFS) are suitable for processing fish products. The TFS cans were found to withstand all the conditions of thermal processing. Mussel processed in these cans could be kept in good condition at room temperature for long time. The TFS cans are now available in India, can be used for packing various fish products as an alternate to tin and aluminum cans. Keeping in view with the increase in demand for ready to eat products in both domestic and export markets, there is a need for developing such products. The present work on development of canned Mussel in the brine will help in utilizing Green mussel in a better way for developing ready to serve convenience product.

References

1. AOAC. (2000), Official methods of analysis of association of official analytical chemists INTERNATIONAL, 17th , Suite 500,481North Frederick Avenue, Gaithersburg, Maryland 20877-2417, USA.
2. AOAC (1998), Official methods and recommended practices of American oil chemist's society, AOAC, Chepaign, USA.
3. AOAC. (2000), Official methods of analysis of association of official analytical chemists, Virginia, USA.
4. Balachandran K.K, (2003), Fish canning – Principles and Practices, C.I.F.T., (ICAR), Cochin, India, Pp180,
5. Charbonneau, J.E., (1997), Recent case histories of food product- metal container interaction using scanning electron microscope- X- ray microanalysis, Scanning,19(7), Pp512-518.
6. Colomenero, F.J., Tejada, M and Borderias, A.J., (1998), Effect of seasonal variation in protein functional properties fish during frozen storage, J. Food Biochem. , 12, Pp 159-170.
7. Denny, C.B. (1970), collaborative study of procedure for determining commercial sterility of low acid canned foods, J.A.O.A.C., 53, Pp713-715.
8. Desreason, N.W. (1995), Principle of food preservation by canning in: The technology of food preservation, The AVI publishing Company, Inc., Westport, Connecticut,Pp171-255.
9. Geiger, E and Borgstorm, G (1962). Fish protein- nutritive aspects. In fish as food (Borgstorm, G; Ed) Academic press, New York.
10. IS : 6093(1971), Indian Standard, Method for determining the capacity and dimensions of hermetically sealed metal containers, Indian Standards Institution, Manak Bhavan, 9 Bahadur Saha Zafar Marg, New Delhi-1
11. IS: 2471(1963), Indian Standard, Method for test for metal containers , Indian Standards Institution, Manak Bhavan, 9 Bahadur Saha Zafar Marg, New Delhi-1
12. IS: 9396,(1979), Indian Standard, Specification for mackerel canned in curry, Indian Standards Institution, Manak Bhavan, 9 Bahadur Saha Zafar Marg, New Delhi-1
13. IS: 5818,(1970), Indian Standard, Specification for lacquers and decorative finishes for food cans, Indian Standards Institution, Manak Bhavan, 9 Bahadur Saha Zafar Marg, New Delhi-1
14. IS:3366,(1968), Indian Standard, Specification for prawns/shrimps canned in brine, Indian Standards Institution, Manak Bhavan, 9 Bahadur Saha Zafar Marg, New Delhi-1
15. IS: 6273 [11],(1971), Indian Standard, Guide for sensory evaluation of foods, Indian Standards Institution, Manak Bhavan, 9 Bahadur Saha Zafar Marg, New Delhi-1
16. IS : 2168(1971), Indian Standard, Method for test for metal containers , Indian Standards Institution, Manak Bhavan, 9 Bahadur Saha Zafar Marg, New Delhi-1
17. IS:594,(1962), Indian Standard, Specification for common salt fish curing(revised), Indian Standards Institution, Manak Bhavan, 9 Bahadur Saha Zafar Marg, New Delhi-1.
18. Ratnakumar. K and Ninawe, A.S; (2008), Fish Processing Technology and value added product development, Pp 1951-1955.

Table 1

Can examination Test	Result
Can size	301*206
Can appearance internal	Good
Can appearance external	Good
Air pressure test	No leakage
Sulphur staining	No staining
Lacquer delamination	No delamination
Gross weight (gm)	200
Net weight (gm)	104
Drained weight (gm)	38
Drained liquid (ml)	90

Table 2: Proximate composition (%) of fresh mussel meat

MOISTURE	79.75
PROTEIN CONTENT	15.83
FAT CONTENT	2.24
ASH	2.72

Table 3: Quality parameters of fresh Mussel

PARAMETER	VALUE
pH	6.59
TVB-N (mg/100gm)	18.70
TMA (mg/100gm)	5.5
Cholesterol (mg/100mg)	161.30
Tryptophan (mg/100mg)	1.033

Table 4. Thermal processing parameters

PARAMETER	VALUE
Come up time (min)	11.02
Heat rate index F_h (min)	16
Heating lag factor (J_h)	0.567
Cooling lag factor (J_c)	1.07
F_o Value (min)	8.43
Temperature deficit (g)	1.92
Ball process time, B (min)	21.09
Total process time,T (min)	27.48
Operator process time (min)	16.47

Table 5: Proximate composition (%) of canned mussel meat

MOISTURE	78.27
PROTEIN CONTENT	14.80
FAT CONTENT	1.94
ASH	1.83

Table. Changes in the TVBN

Sample	**TVBN Value**
Raw meat	18.70
Canned meat	28.48

Table. Changes in the TMA

Sample	TMA Value
Raw meat	5.50
Canned meat	10.39

Table 6: Changes in Amino acid profile

Amino acids	Raw mussel	Canned mussel
Aspartic acid	4.31	7.75
Treonine	1.75	3.33
Serine	1.95	3.82
Glutamic acid	5.47	9.86
Proline	1.46	3.41
Glycine	3.92	4.44
Alanine	1.77	2.69
Valine	1.60	3.10
Methionine	0.55	1.43
Iso leucine	1.61	3.48
Leucine	2.46	5.08
Tyrosine	8.70	2.17
Phenylalanine	1.38	3.07
Histidine	3.56	3.65

Table 7: Changes in the fatty acid composition

Fatty acids	Raw sample	canned sample
Myristic Acid Methyl ester	4.13	3.02
Myristolcic Acid methyl esters	0.53	0.78
Pentadecanoic Acid	1.52	1.51
Palmitic Acid methyl Ester	22.20	19.64
Palmitoleic Acid Methyl Ester	6.31	6.44
Heptadecanoic Acid Methyl Ester	1.31	1.30
Stearic Acid Methyl Ester	4.62	4.65
Elaidic Acid Methyl Ester	1.84	2.13
Linoelaidic Acid Methyl ester	3.99	3.96
Linolenic Acid Methyl Ester	1.23	1.34
Arachidic Acid Methyl Ester	1.29	1.40
Cis-11-Eicosenoic Acid Methyl Ester	2.73	2.28
Heneicosanoic Acid Methyl Ester	3.17	3.78
Cis-5,8,11,14,17-Eicosapentaenoic Acid Methyl Ester	14.53	16.56
Cis-4,7,10,13,16,19-Docosahexaenoic Acid Methyl Ester	29.58	33.14

Table 8: Changes in the composition of Minerals

Minerals	Na	K	Ca
1.Raw sample			
In ppm	22834.15	9034.65	2029.70
In dry weight	2.2834g%	0.9034g%	0.2029g%
In wet weight	0.4623	0.1829	0.0411
2.Canned sample			
In ppm	17892.15	1470.588	511.029
In dry weight	1.7892g%	0.1470g%	0.0511g%
In wet weight	0.3887	0.0319	0.0111

The sensory ratings of Canned Mussel were analyzed after processing and are resented in the Table 10.

Sensory attributes	**Scores**
Can appearance internal	8.5
Can appearance external	8.3
Color	7.5
Flavour	7.7
Odour	7.5
Taste	7.5
Firmness	8
Overalll acceptability	7.5

Table 11. Texture profile analysis of fresh and canned mussel meat

Characteristics	**Raw mussel**	**Canned mussel**
Hardness 1 (Kgf)	0.380	0.207
Hardness 2 (N)	0.312	0.193
Area 1 (Kg)	0.274	0.162
Area 2 (kg)	0.066	0.027
Cohesiveness	0.240	0.167
Springiness (mm)	0.875	1.657
Springiness index	0.289	0.407
Gumminess (Kgf)	0.091	0.035
Chewiness (kgf)	0.080	0.057
Fracture force (Kgf)	0.380	0.207
Adhesive force (Kgf)	0.022	0.032
Adhesiveness (Kgf)	0.010	0.012
Stiffness (Kgf/mm)	0.739	0.082