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*Research Article*

# Impact of traditional and dip in-freezing in the refrigerator on the quality of sardines

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**Freezing is the method used for preserving fresh fish and other seafood products in home. In this study two different freezing such as dip in and traditional freezing method was followed to increase the shelf life of fish stored in a refrigerator. The quality were analyzed by sensory, microbial and biochemical methods. The results showed that dip in freezing fish can remain healthy and nutritious for a longer time without deteriorating its quality. All the quality parameters are within the acceptable limit in dip in freezing method than the traditional freezing. The best method for storing fresh fish in the refrigerator by dip in freezing method which increase the shelf life and reduce the seasonal wastage of a large amount of fish.**

**Key words:** Sardines, traditional freezing, dip in freezing, quality indicators, shelf life.

## INTRODUCTION

Freshly caught fish spoil easily so they must be preserved properly to retain their quality. Freezing is the method used for preserving fresh fish and other seafood products. Fish spoilage occurs rapidly with increased temperature, and spoilage is slowed down as temperature is reduced and it approaches the freezing point. The freezing method aims to extend the safe shelf-life of fishery products; it largely relies on the control of temperature, moisture, and oxygen. However, this preservation technique is effective only if the product is handled in such a way that its quality is kept near its peak freshness. Four most popular methods of fish preservation are freezing, canning, smoking, and pickling. The different types of cooling systems used in the fish processing industries are liquid ice, flow-ice, flake ice, and combined blast and contact or dip in chilling. Two main tasks in cooling are the fast reduction of the product temperature down to the desired low point and maintenance of the temperature over a longer period at that point. The fast reduction of the temperature is achieved by cooling equipment, in connection to some processing operation or storage and the maintenance at a constant low temperature over a longer period during storage or transport (Aberoumand, 2013). At home, people depend on the refrigerator for freezing fish so that the shelf life of the product is extended. For this, they simply keep the cleaned fish in a vessel or they add salt or spices to the fish and keep it as such in the freezer for the preservation for a few hours or days.

Commonly people think that the quality of frozen seafood product will not change. The mere fact that a product has been frozen does not ensure that its quality is protected. Precautions must be taken to guard against the changes in flavor and texture that can take place during frozen storage of the product and this is especially true for seafood held in the freezer of the refrigerator at homes. Fish has to be properly stored during the interval between its purchase in the market and its actual cooking. Doing it right is not difficult. Normally if we don't plan to eat the fish within a couple of days of purchase, we freeze it in a refrigerator by simply depositing the fish in a container kept inside the freezer and this is the traditional freezing method generally followed in homes. This project studies the quality status of this traditionally frozen fish. In place of the traditional freezing method a new type of freezing called dip in freezing may be employed in which the fish is kept in a container immersed in water and then it is frozen. This study compares the qualities of traditionally frozen and dip in-frozen sardines by sensory, chemical and microbiological analysis.

## MATERIALS AND METHODS

### Sample collection

The fish *Sardinella fimbriata* (n=200; 15-22 g) was selected for the present study and it was collected from the fish landing

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center of Tuticorin. The fishes were transported to the laboratory in an icebox. In the laboratory, the fish were gutted and cleaned as soon as possible and they were divided into two sample groups of 100 individuals each for two independent storage trials, namely the Traditional and dip in-freezing methods (Figure 1).


**Figure 1.** (A) Sardines are stored under Dip in freezing and (B) Traditional freezing methods.

**Traditional freezing method**

A hundred sardines, freshly harvested, were brought to the laboratory, washed and placed in 6 containers each with 16 fishes and stored in the freezer of Whirlpool 408 L refrigerator. Sampling was done once in 15 days for 3 months. On the day of analysis one container was taken out and defrosted by keeping it in the room temperature, and used for further quality analysis. 5 fish were used for microbiological evaluation, 5 were used for biochemical analysis and the remaining fish were used for sensory evaluation.

**Dip in freezing method**

Another hundred fish were utilized in the dip in freezing study and they were also placed in 6 containers each with 16 fishes. The containers were filled with water completely submerging the fish, and kept inside the freezer of a refrigerator. Sampling was done every 15 days for 3 months. On the day of analysis, one container was taken out and kept in room temperature until it defrosted and the fish samples were taken for analysis, 5 fish were used for microbiological evaluation, 5 were used for biochemical analysis and the remaining fish were used for sensory evaluation.

**Sensory evaluation of raw and cooked fish**

Sensory analyses were performed by a panel of five experienced assessors. Raw fish was evaluated using the Quality Index Method (QIM) shown in Table 1. This structured category scale is based on the freshness quality grading system developed by (AOAC, 1995) for whole iced fish. The QIM involves specifying the characteristics of appropriate sensory attributes of the raw fish. Once the characteristics of a sensory attribute were determined, it is assigned a demerit score ranging from 0 to 3. The scale gives zero scores for absolutely fresh fish, while increasingly larger totals result as fish deteriorate.

The Torry scores sheet for cooked fish is shown in Table 2. Cooked fish were assessed according to the simplified Torry Sensory Scheme for white fish fillets (Aubourg, et al., 2005). In the preparation of cooked samples, small portions of skinned flesh were wrapped in aluminum foil and steam-cooked for 12 min. The scoring was carried out in individual booths and all samples were evaluated when hot (60°C) within 15 min of cooking. The scores of the simplified Torry scale and what they signify are as follows:10-fresh, sweet flavours characteristic of the species; 9-some loss of sweetness; 8-slight sweetness and loss of flavours characteristic of the species; 7-definite loss of flavour but no off-flavours; 6-absolutely no flavour; 5-trace of off-flavours, some sourness but no bitterness; 4 to 2 increasing off flavours; 1-strong bitterness, but not nauseating; and 0-putrid flavours. The fish were judged unfit for consumption when the mean value for a sensory score was below 5.

**Microbiological analyses**

Approximately 10 g of the flesh were sampled using sterile forceps. Tenfold dilution in 0.1% peptone water was prepared from the flesh samples and 1 ml aliquots were plated in triplicate in Iron Agar (Beatty, 1937). Total viable counts and selective counts of hydrogen sulfide-producing bacteria were enumerated after 3 days' incubation at 20°C. Black colonies were recorded as sulfide-producers.

**Biochemical quality**

**pH:** 5 g of minced meat was homogenized with 45 ml of distilled water and was transferred to a sterile glass beaker and kept in the refrigerator for 30 min. The pH of the homogenate was measured with the help of combined electrode pH meter after calibration with standard pH 4.2 and 9.0 buffer solutions.

**Moisture:** The moisture content of the sample was determined by following the hot air oven method (Bimbo, 1998). 5 g of minced meat samples were taken into each plate of known weight and weighed in a digital balance. The samples were allowed to dry into the oven at 105°C for 24 hours in order to remove the moisture till constant weight. Then the plates were taken out of the oven, cooled in a desiccator and weighed by a digital balance.

Moisture (%)=Weight loss/Original weight of taken sample × 100

**Lipid:** About 5 g sample was taken into conical flasks and 10 ml of Folch reagent (Chloroform: Methanol=2:1) was added into the sample and homogenized properly and kept in air-tight condition for 24 hours. Lipid contents of the fish muscle react with that solvent and remain in the solution. After 24 hours the solution of the flask was filtered into another weighed conical flask. Then these flasks were placed in a hot water bath to dry up and the solvent removed. Then the flasks were kept in an oven for an hour to get the actual lipid content. Then the flasks were weighed by an electronic balance to get the amount of lipid content (Chomnawang, et al., 2007).

Fat (%)=Weight of the residue/Weight of taken sample × 100

**Protein:** Protein was estimated by following the method of (Chytiri, et al., 2004). To 10 mg of sample, 1 ml of 1N solution of NaOH was added for protein extraction in a water bath for 30 minutes. Thereafter it was cooled to room temperature and neutralized with 1 ml of 1N solution of HCl. The extracted sample was centrifuged at 2,000 rpm for 10 minutes, and an aliquot of the sample (1 ml) was further diluted with distilled water (1/9 v/v). From the diluted sample, 1 ml was taken and treated with 2.5 ml of mixed reagent (carbonate-tartrate-copper) and 0.5

ml of 1N solution of Folin's reagent. After 30 minutes, sample absorbency was read at 750 nm using a spectrophotometer. Bovine serum albumin was used as a standard for this analysis. The results were expressed as a percentage.

Protein%=X × V × 100/W ×100

X=Amount of protein obtained from the graph, V=Volume of the dip in natant, W=weight of the sample.

**Lipid quality:** Free Fatty Acids (FFA) and Thiobarbituric Acid Reactive Substances (TBARS) were estimated to measure the lipid quality.

**FFA:** Free Fatty Acid was determined by the method described by Olley and Lovern (Devadasan, et al., 1977). 5 ml of the chloroform extract was evaporated in a water bath, dissolved in 50 ml of hot neutral alcohol and titrated against standard alkali and expressed as an oleic acid percent in total lipids.

FFA (% of oleic acid)=28.2 × (Titre value of sample-TV of blank) × N of NaOH/Weight of fat taken.

**TBARS:** The value for Thiobarbituric Acid Reactive Substances (TBARS) was determined in fish to evaluate the oxidation stability during storage. Samples were homogenized with chloroform: methanol (2:1, v/v). Two parts of water were then added to make a two-phase system and an aliquot of the methanol-water phase, containing the short-chain aldehydes, was heated in the presence of excess thiobarbituric acid. The complex created was measured spectrophotometrically at 532 nm using *malondialdehyde* as standard (Elliot, 1952).

**Freshness indices:** Total Volatile Base Nitrogen (TVB-N) and Tri-Methylamine Nitrogen (TMA-N) were estimated for freshness indices.

**Preparation of TCA extract:** About 10 g of the sample was ground well with 20 ml of 20% TCA. The homogenate was filtered through a Whatmann No.1 filter paper and the filtrate was made up to 100 ml with distilled water.

**Total Volatile Base Nitrogen (TVB-N):** 1 ml of the extract was put in an outer chamber of Convey's unit and 2 ml of boric acid in the inner chamber. The lid was partially closed and 1 ml of saturated potassium iodide was added to the outer chamber and then the lid was closed and the contents incubated at 37°C for 90 min. After incubation, the inner chamber was titrated against the standard $H_2SO_4$ solution. Blank was carried out using 2% TCA instead of the sample (Folch, et al., 1957).

TVB-N (mg%)=14.01 × N of $H_2SO_4$ × (Titre value of sample-TV of blank) × 100 × 100/1000 × weight of sample × 1000.

**Tri-Methylamine-Nitrogen (TMA-N):** The TMA-N content of the fish samples was estimated by the method of Beatty and Gibbons using Convey's micro diffusion technique. 1 ml of the extract was taken in the outer chamber and 2 ml of 2% boric acid containing mixed indicator was put in the inner chamber of the Convey's unit and the lid was partially closed. 1 ml of formalin and 1 ml of saturated potassium carbonate were added to the outer chamber and the lid was immediately closed and incubated at 37°C for 90 min. After incubation, the inner chamber was titrated against the standard $H_2SO_4$ solution. Blank was carried out using 2% TCA instead of the sample.

TMA-N (mg%)=14.01 × N of $H_2SO_4$ × (Titre value of sample-TV of blank) × 100 ×100/weight of sample × 1

## RESULTS AND DISCUSSION

The raw and cooked fish were evaluated using the Quality Index Method (QIM) shown in Tables 1 and 2. The mean results obtained from the panelists for the study of the total quality index score for the raw and cooked fish are presented in Tables 3 and 4. The QIM scores summarize the results for each quality parameter. An overall sensory score of zero is for very fresh fish and the values increase in proportion to the storage period as the fish deteriorates with time. The average quality index scores fluctuated considerably for a few quality parameters throughout the storage time both in the traditional as well as the dip in freezing of whole fish. This depended on the type of freezing system used and also the method of handling the fish from catch to processing and storage. From day 0 to 15, in both types of freezing methods the fish appeared bright pearly and shining, had blood-reddish gills, convex eyes and without any odor. During and following the storage period in the dip in freezing method up to 60 days of storage the gill appearance was bright red in color and body stiffness was slightly reduced from the 45th day onwards. On days 60, 75 and 85 the eyes were clear and translucent. Up to 75 days most of the panelists give zero scores of absolutely fresh fish. But fishes stored under the traditional freezing method had bright skin, very stiff body, fresh odor, clear, translucent eye cornea and bright red gills only up to 15 days. The fishes appeared less bright with slight off-odor, had slightly grayish skin with less pearly shine, sunken eyes and brownish gills from the 30th day onwards. From the 45th day onwards the fishes had putrid smell, opaque eyes with brown color around, faded gills, pale appearance and grey-white pupil. Dull yellowish skin with thick mucus, brown and grayish-brown gills with milky clotted mucus and sunken dark grey pupils appeared in the fishes stored under the traditional freezing from the 60th day onwards but it did not appear in the fishes stored under the dip in freezing method until the end of the storage period Table 5.

**Table 1.** Sensory evaluation of raw fish by Quality Index Method (QIM).

| Parameter | Sensory characters | Demerit points |
|---|---|---|
| Appearance | Bright, shining, iridescent | 0 |
| | Less bright, some loss of iridescence | 1 |
| | Pale, dull | 2 |
| Body stiffness | Very Stiff, hard (in rigor) | 0 |
| | Firm, elastic (post-rigor) | 1 |
| | Some softening | 2 |

| | | |
|---|---|---|
| Odour | Fresh | 0 |
| | Neutral | 1 |
| | Slight off-odours | 2 |
| | Spoiled | 3 |
| Eyes cornea | Clear, translucent | 0 |
| | Cloudy | 1 |
| | Opaque | 2 |
| Pupil | Black, bright, shiny | 0 |
| | Slightly grayish | 1 |
| | Grey-white | 2 |
| Gills appearance | Uniformly dark red | 0 |
| | Brownish red | 1 |
| | Discoloured/faded | 2 |

**Table 2.** Sensory evaluation of cooked fish.

| Score | Odour | Flavor |
|---|---|---|
| 10 | Initially weak odour of sweet, boiled milk, starchy followed by strengthening of these odours | Watery, metallic, starchy. Initially no sweetness but meaty flavours with slight sweetness may develop |
| 9 | Shellfish, sea weed, boiled meat | Sweet, meaty characteristics |
| 8 | Los of odour, neutral odour | Sweet and characteristic flavor but reduced in intensity |
| 7 | Wood shavings | Neutral |
| 6 | Condensed milk, boiled potatoes | Insipid |
| 5 | Milk jug odours, boiled cloths-like | Slight sournes, trace of off flavours |
| 4 | Lower fatty acids composed grass, soapy, turnip, tallow | Strong bitter, rubber, slight sulphide |

**Table 3.** Sensory evaluation of dip in freezing raw fish during storage.

| Parameters | Days of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 85 |
| Appearance | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Body stiffness | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| Odour | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Eyes cornea | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| Pupil | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Gills appearance | 0 | 0 | 0 | 0 | 0 | 0 | 1 |

**Table 4.** Sensory evaluation of traditional freezing raw fish during storage.

| Parameters | Days of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 85 |
| Appearance | 0 | 0 | 1 | 2 | 3 | 3 | 3 |
| Body stiffness | 0 | 0 | 1 | 3 | 3 | 3 | 3 |
| Odour | 0 | 0 | 1 | 2 | 3 | 3 | 3 |
| Eyes cornea | 0 | 0 | 1 | 2 | 3 | 3 | 3 |
| Pupil | 0 | 0 | 2 | 3 | 3 | 3 | 3 |
| Gills appearance | 0 | 0 | 2 | 3 | 3 | 3 | 3 |

**Table 5.** Sensory evaluation of dip in freezing cooked fish during storage.

| Parameters | Days of storage | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 85 |
| Odour | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Flavour | 10 | 10 | 10 | 10 | 10 | 10 | 9 |

The panelists evaluated the attributes of odor and flavor of the cooked fish samples. The cooked dip in frozen fish showed the good score of 9 and 10 which indicate that the fishes have maintained the freshness until the end of the storage period. According to the scores obtained for the traditionally frozen cooked fish from day 0 to day 15 the fish was acceptable based on flavor and odour, and following the length of storage it reached the end of shelf life (i.e. Torry Score 7-4). The score of 5 is the Torry limit for sardines because at this score most of the panelists started to detect spoilage attributes in the fish, and spoilage started from day 30 onwards which is indicative of the poor quality of edible fish. Thus, the changes in the appearance of the eyes, gills, and skin depend on the type of the freezing effects. At the beginning of storage when the sardine were very fresh, the gill odor was described as 'fresh seaweed' or 'metallic' and then the odor became neutral. During the later stages, the odor was described as 'sour' and finally as 'rotten' and it was observed in the traditionally frozen fish Table 6. The scores for the quality attributes of eyes, skins, and gills increased rather at a constant pace throughout the storage period in traditional freezing, even though the scores varied somewhat with storage types, especially for the form of eyes and cornea in both traditional and dip in-freezing. Fluctuation in scores for the cornea and color of eyes was observed, especially in traditional freezing. At the end of the storage period, the scores reached values close to the maximum score. The quality of the cornea showed considerable fluctuations throughout the storage time for both groups. The low scores at the beginning of the sampling days indicate the best quality. This information is a useful feedback to homemakers concerning the quality of their fishes, which may induce them to use the improved storage method.

Changes in TVC and counts of H2S-producing bacteria in fishes stored under the dip in-and traditional freezing methods are represented in Table 7. The results show a low initial bacterial load on day 0 of storage and an increasing trend for the subsequent days of storage in both the lots. The TVC and H2S counts were higher for the traditionally frozen fishes than the dip in-frozen samples at the end of the experiment (day 85). In the dip in freezing method, the increase in TVC and H2S-producing bacteria was low throughout the study, while in traditionally frozen fish there was an exponential increase from day 15 onwards. This was in accordance with the results of sensory evaluation of the attributes of cooked and raw fish especially the attribute of taste. This could indicate that the dip in-frozen sardines have a higher resistance to microbial growth than the traditionally frozen ones. The initial H2S bacterial load in the fish muscle was zero in both the lots and no H2S bacteria could be detected up to 30 days on the dip in-frozen lots. Initially, TVC was only less than 10 colony-forming units per g of flesh (Too Low Too Count) in both the freezing methods. The total counts in early storage days are low because the flesh of newly caught fish is sterile for the immune system of the fish prevents the bacteria from growing in the flesh, but when the fish dies, the immune system collapses and consequently during storage the bacteria invade the flesh (Gram, et al., 1996). During the first 30 days of storage, H2S-producing bacteria are absent in the dip in-frozen fish. They were found in low numbers in the later stages of storage and the value did not exceed 105 limits proposed for the iced fish (Gram, et al., 1987) whereas in the traditionally frozen fish the values of both the TVC and H2S bacteria exceeded the acceptable limit of 105-106 CFU/g respectively (Huss, 1995). At the end of the storage period, higher total flora were found in the traditionally frozen fish than in the dip in-frozen samples, indicating spoilage, since H2S-producing bacteria associated with spoilage often constitute a major proportion of the microbial flora of spoiling fish. TVC in sardine muscle at the end of the traditional storage period observed in this study was considerably higher than what was found earlier in similar studies on 30 days of storage (Huss, 1988). This could be due to the high proportion of H2S-producing bacteria associated with spoilage observed in the traditionally frozen fish. However, these results are comparatively closely related in many characteristics to a previous study on salmon in which similar values were obtained. On the 40th day of storage in ice the salmon was considered unfit for consumption (Gram, et al., 1996). The same TVC values were recorded by Magnusson and Martinsdottir (Huss, 1971) when cod became unfit for consumption. The values of the present study are also, likewise, parallel to the findings of (Indira, et al., 2010) that when the number of TVC exceeds 106 CFU/g a significant amount of volatile sulfur-containing compounds are produced and spoilage becomes evident by sensory evaluation.

**Table 6.** Sensory evaluation of traditional freezing cooked fish during storage.

| **Parameters** | **Days of storage** | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 15 | 30 | 45 | 60 | 75 | 85 |
| Odour | 10 | 8 | 7 | 5 | 5 | 4 | 5 |
| Flavour | 9 | 8 | 7 | 5 | 5 | 4 | 5 |

**Table 7.** Changes in Total Viable Count (TVC), selective count of hydrogen Sulphide-Producing Bacteria (SPB) in sardines using dip in freeze and traditional freezing method at home refrigerator.

| | **Dip in freezing** | | **Traditional freezing** | |
|---|---|---|---|---|
| Days in freezer | TVC (CFU/g) | $H_2S$ (CFU/g) | TVC (CFU/g) | $H_2S$ (CFU/g) |
| 0 | TLTC | - | TLTC | - |
| 15 | TLTC | - | $0.94 \times 10^3$ | $1.0 \times 10^2$ |
| 30 | $0.9 \times 10^2$ | - | $1.9 \times 10^4$ | $2.2 \times 10^3$ |
| 45 | $1.1 \times 10^2$ | $3.5 \times 10^1$ | $2.6 \times 10^5$ | $2.0 \times 10^4$ |
| 60 | $1.5 \times 10^2$ | $3.6 \times 10^1$ | $3.5 \times 10^6$ | $2.2 \times 10^4$ |
| 75 | $1.8 \times 10^2$ | $3.9 \times 10^1$ | $6.8 \times 10^7$ | $2.6 \times 10^5$ |
| 85 | $1.85 \times 10^3$ | $3.8 \times 10^2$ | $2.2 \times 10^8$ | $1.9 \times 10^6$ |

The chemical indices (TMA, TVB-N, and pH of the flesh) were measured during both the storage trials and they provide valuable information as spoilage indices. PH was initially uniform in both the storages. It was increasing from 6.2 on day 0 to 6.54 on day 60 and thereafter gradually reduced to 6.41 on day 85 on dip in-frozen fish. In traditionally frozen fish exponential increase was found from 6.2 to 7.7 at the end of storage life on day 85. The low muscle pH early in the period of iced storage reflected the good nutritional state of the fish. The fluctuation in pH observed during storage is probably due to biological variation. pH was considered a valuable indicator as it is expected to increase as non-protein nitrogen and other volatile amines are produced as a consequence of bacterial activity. According to (Jeyasekaran, et al., 2004a), in cod the postmortem pH dropped following rigor mortis from 6.8 to 6.1 and then rose to 6.5. In the present trial, in both instances of freezing the pH was initially 6.2, and following storage it increased due to the formation of amines and reached to about 7.7 in the traditional method and 6.41 in the dip in freezing method. Love (Joseph, et al., 1988) indicated that loss of water in fish has a detrimental effect on the texture of fish muscle and that there is an inverse relationship between muscle toughness and pH. There were small variations in the pH of muscle tissue (6.2-6.41) with the length of storage in dip in freezing, and drastic variation in traditional freezing method (6.2 to 7.7). The variations were likewise observed in the odor and flavor of cooked flesh because according to Huss (Jeyasekaran, et al., 2005), the post-mortem pH is the most significant factor influencing the sour odor with rancidity. One of the reasons for this is that even minor changes in pH drastically affect the properties of the odor and flavor of the fish. Increase in pH value, as storage time progresses, coincides with the peak production of TVB-N (Kyrana, 2001).

Other chemical indices measured were TMA-N and TVB-N and the values vary between the traditionally frozen and dip in-frozen samples. TVB-N is mainly a composition of TMA and ammonia. In traditionally frozen fish, there was an increase in both TMA-N and TVB-N. The increases were from 0.01 to 22.58 and 1.1 to 57.73 mg N/100 g respectively, and they were due to the formation of ammonia during storage. In the dip in frozen fish, TMA-N and TVB-N did not increase much during storage. The value of increase for TMA-N was from 0.02 to 2.81 mg N/100 g and for TVB-N from 1.1 to 57.73 mg N/100 g during the late storage period. The TMA-N content of the dip in-frozen fish did not increase the acceptable limit of 10-15 mg N/100 g at the end of the storage (Huss, 1971) whereas in traditional frozen fish the values exceed the acceptable limit from 30 days storage onwards. The TVB-N values were higher from storage day 30 onwards in traditionally frozen fish than the dip in-frozen fish. The respective values are 57.3 and 11 mg/100 g at the end of the storage period (85 days). The values were below the EU limit for TVB-N which is 35 mg/100 g (Kyrana, et al., 1997) in dip in frozen fish and above the acceptable limit in traditionally frozen fish. The resulting chemical measurements show that there was a difference between the traditional and dip in freezing methods in terms of spoilage factor. Also there is longer shelf or storage life for the dip in frozen fish than the traditionally frozen one due to the freezing effects.

In both the methods, the moisture content increased during the initial period of frozen storage and later it decreased till the end of storage. The moisture content of the dip in-frozen fish increased from the initial value of 78.5% to 80.19% on the 45th day. This could be due to absorption of water from melting ice by the fish muscle. Similar results were observed by (Liu, et al., 2010) in rohu (*Labeo rohita*) stored in ice. The later gradual decrease of moisture content was observed up to 77.54% on the 85th day due to condensation of water. Aberoumand (Love, 1975) proposed that decrease in moisture is due to condensation of water during frozen storage. The traditional freezing method shows a similar trend of increase in moisture content up to day 30 (78.52%-79.22%) and then it decreased to 78.05% on the 85th day. It might be due to the evaporation of moisture from the surface which depends on various factors like geometric shape and the chemical composition of the products, the storage temperature and the relative humidity. This decrease in moisture content is attributed to the sublimation of ice in frozen storage and drip loss during the thawing process. The decrease in moisture content of fish during the later part of frozen storage may be due to the continuous exchange of water between fish muscle and its surrounding environment as observed by (Lowry, et al., 1951) in hybrid catfish.

The lipid content decreased with an increase in frozen storage period of traditional and dip in freezing methods. The lipid content decreased from 3.54% to 2.59% and 3.55% to 0.84% in dip in-and traditionally frozen samples respectively. A slight decrease in the content of lipid has been observed during the dip in freezing storage. The initial lipid content of fresh sardine fish stored under dip in freezing method was 3.54% and the content decreased immediately after freezing, and during frozen storage it was 3.54 to 2.59% till day 85 (Tables 8 and 9) whereas a rapid decrease was observed in the traditional freezing storage. Devadasan and Nair (Magnusson et al., 1995) observed a decrease in lipid content due to lipid breakdown in sardine stored at refrigeration temperature. Similar results are reported by (Milanes, 2004) in pink perch, (Meenakshi, et al., 2010) in *Cyprinus carpio* and (Chomnawang, et al., 2007) in hybrid catfish. Aberoumand (Love, 1975) found a decrease in total lipid content during frozen storage in Iranian fishes and reported that the loss must be due to hydrolysis of lipids during frozen storage. Lipid content decreases are observed normally in the frozen storage and it is very low in the dip in freezing storage method.

**Table 8.** Changes in pH, lipid and moisture content over the period of storage.

| | **Dip in freezing** | | | | **Traditional freezing** | | | |
|---|---|---|---|---|---|---|---|---|
| Days in freezer | pH | Moisture (%) | Lipid (%) | Protein (%) | pH | Moisture (%) | Lipid (%) | Protein (%) |
| 0 | 6.2 | 78.5 | 3.54 | 20.15 | 6.2 | 78.52 | 3.55 | 20.15 |
| 15 | 6.3 | 78.96 | 3.51 | 20.07 | 6.6 | 79.25 | 3.11 | 18.45 |
| 30 | 6.4 | 80.12 | 3.22 | 19.97 | 7.0 | 79.22 | 2.96 | 16.74 |
| 45 | 6.45 | 80.19 | 3.19 | 19.85 | 7.2 | 78.84 | 2.41 | 15.22 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 60 | 6.54 | 79.22 | 3.11 | 19.74 | 7.3 | 78.41 | 2.27 | 14.65 |
| 75 | 6.42 | 78.48 | 3.08 | 17.55 | 7.5 | 78.19 | 1.5 | 11.79 |
| 85 | 6.41 | 77.54 | 2.59 | 17.41 | 7.7 | 78.05 | 0.84 | 8.25 |

**Table 9.** Changes in quality indicator over the period of storage.

| | **Dip in freezing** | | | | **Traditional freezing** | | | |
|---|---|---|---|---|---|---|---|---|
| Days in freezer | TMA-N (mg N/100 g | TVB-N (mg N/100 g | FFA (g oleic/100 g fat) | TBA (mg MDA/kg) | TMA-N (mg N/100 g | TVB-N (mg N/100 g | FFA (g oleic/100 g fat) | TBA (mg MDA/kg) |
| 0 | 0.02 | 1.1 | 0.008 | 0.002 | 0.01 | 1.1 | 0.008 | 0.001 |
| 15 | 0.6 | 2.27 | 0.01 | 0.084 | 2.52 | 8.8 | 0.042 | 0.158 |
| 30 | 0.95 | 2.55 | 0.025 | 0.121 | 14.58 | 19.68 | 0.058 | 0.387 |
| 45 | 1.22 | 2.74 | 0.029 | 0.245 | 15.53 | 19.87 | 1.247 | 1.825 |
| 60 | 1.89 | 4.87 | 0.03 | 0.257 | 18.97 | 22.45 | 2.311 | 3.23 |
| 75 | 2.25 | 8.54 | 0.039 | 0.354 | 16.43 | 42.68 | 5.354 | 4.25 |
| 85 | 2.81 | 11 | 0.042 | 0.411 | 22.58 | 57.73 | 5.361 | 5.32 |

The protein content in fresh sardine meat was 20.15%. During dip in freezing, the protein content decreased from 20.15 to 17.41%, whereas in traditional frozen fish protein content decreased from 20.15% to 8.25% at the end of the 85th day of storage. The decrease in true protein content during the storage might be due to leaching out of water-soluble protein components and dilution effect caused by water uptake. It is further implicit that proteolytic enzymes might have caused such effect by splitting the peptide bonds. (Siddique, et al., 2011) reported that the protein loss must be due to oxidation. Aberoumand reported denaturation of proteins and loss of nitrogen as volatile bases and nitrogenous substances are formed by bacterial decomposition that escape from tissue during frozen storage. The leaching out of water-soluble protein, oxidation, and denaturation of protein were limited in the fish stored under the dip in freezing method.

Hydrolysis of lipid leads to the formation of FFA and it causes quality deterioration during storage (Nishimoto, et al., 1985). In the present study, the free fatty acid content increased with the length of storage in both the storage methods. In the case of dip in freezing, it increased from 0.008 (% of oleic acid) to 0.042 (% of oleic acid) after 85 days of storage; whereas in traditional freezing, the FFA content increased from 0.008%-5.361% of oleic acid after 85 days of storage. Bimbo (Olley, et al., 1960) suggested the acceptable limit of FFA as 2%-5%. However, the FFA content showed an increasing trend throughout the storage period and the content was very low in dip infrozen fish and exceeded the acceptable limit in the traditionally frozen fish. The results indicate that there is a relationship between FFA release and the degree of freshness. The increase in FFA content during ice storage of common carp has been attributed to enzymatic hydrolysis of esterified lipids (Ozygurt, et al., 2009). An increase in FFA during storage was observed by (Sarma, et al., 1994) in pink perch and oil sardine. (Jeyasekaran et al., 2004-2005) in Emperor bream, Lethrinus (*Epinephelus merra*), (Yesim et al., 2005) in European eel (*Anguilla anguilla*), (Aubourg et al., 2005) in farmed turbot, (Ozygurt et al., 2009) in red mullet (*Mullus barbus*) and gold band goatfish (*Upeneus moluccensis*). (Rodriguez et al., 2007) reported the release of lipase from liposomes during frozen storage which degrades the fats, thus increasing the free fatty acids and the present study proved that lipolysis is controlled in the dip in frozen fish.

Thiobarbituric acid value, which is considered as a secondary lipid oxidation product, increased with the increase of storage time. The value increased from 0.002 to 0.411 mg of *malonaldehyde*/kg of the sample dip in frozen while in the traditional freezing method the TBA value increased during the storage of fish from 0.001 to 5.32 mg of *malonaldehyde*/kg of sample. TBA value of <3 mg *malonaldehyde* kg 1 of a sample is considered to indicate good quality fishery products (Valtýsdóttir et al., 2010). Although a high degree of correlation was obtained between TBA values and storage time, the difference between initial and final values was very small in the dip in freezing method indicating that oxidative rancidity developed only to a limited extent. However, in the case of traditional freezing the difference between the initial and final values was high indicating occurrence of high rancidity in fishes stored in this method. In general, the increase in TBA indicated the formation of secondary lipid oxidation products, such as aldehyde and other volatile compounds responsible for rancid flavor, off-odors as well as color and texture deterioration. A large amount of polyunsaturated fatty acid molecules found in fish lipids makes them highly susceptible to oxidation by an autolytic mechanism. (Kyrana, et al., 2001) reported that icing of whole and gutted fish tends to slow down the production of *malonaldehyde*. The results of the present study are similar to the results obtained by (Vogel, 2005) in sea bass and by (Chytiri et al., 2004) in aqua-cultured rainbow trout. (Liu, et al., 2010) reported that the TBA values in tilapia meat increased during ice storage at a very low rate. In the present study, the lower TBA values observed in dip in-frozen fish compared with the traditionally frozen one might be the result of the direct microbial utilization of *malonaldehyde* and other amine compounds, during bacterial metabolism.

## CONCLUSION

Fish preservation is a vital part of the life of the common people and they use refrigerators for freezing fishes for periods of some hours to a few days. Dip in freezing is a good method of storing fish. Fish frozen in this method remain healthy and nutritious for a longer time without any deterioration in its quality. This storing system helps to reduce the seasonal wastage of a large amount of fish all around the globe. On the basis of the results of the present study, the dip in freezing

method is recommended for freezing fish in refrigerator at home to get an extended shelf life of the fish without any loss of good flavour and quality.

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