PREPARATION OF PRAWN PICKLE AND ITS STORAGE LIFE

A DISSERTATION SUBMITTED IN THE PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF

MASTER OF FISHERIES SCIENCE
IN
INLAND AQUACULTURE

BY

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B.F.Sc.
(1997-99)

Under the guidance of

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Indian Council of Agricultural Research
Versova, Mumbai-400061

1999
Dedicated to

My Beloved parents and sisters
This is to certify that this dissertation entitled "PREPARATION OF PRAWN PICKLE AND ITS STORAGE LIFE" is a record of bonafide research work done by Mr. SHAMBHU KUMAR, during 1997-99 under my guidance and it has not previously formed the basis for any other publication for the award of any other degree, diploma or other similar titles. He is submitting this dissertation in partial fulfilment of Master of Fisheries Science in INLAND AQUACULTURE to Central Institute of Fisheries Education (ICAR), Deemed University, Mumbai-400061.

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I do hereby declare that this dissertation entitled
"PREPARATION OF PRAWN PICKLE AND ITS STORAGE LIFE" is a
bonafide record of research work done by me under the guidance of
Dr. Subrata Basu, Associate Professor, Department of Post-harvest
Technology and that the dissertation has not previously formed the
basis for the award of any degree, diploma associateship, fellowship of
other similar title, of any other university or society.

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( SHAMBHU KUMAR )

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Place : CIFE, Mumbai
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INTRODUCTION
1. **INTRODUCTION**

Fish and shell fish are among the choicest food items under marine food resources. Apart from the internal demand, they support a very valuable trade for export. With the introduction of sophisticated processing techniques of freezing and canning, Indian products have gained importance in the foreign market. Prawn is one of the most important commodity in the present day Indian Fishery Industry due to its taste, colour and flavour. All the modern mechanised fishing are sophisticated methods of preservation activities are concentrated on it.

World fish production was 112 million tonnes in 1996-97 and India’s share is 5.14 million tonnes, which places the India at 6th rank in world scenario. The Inland fish production during 1996-97 was 2.28 million tonnes and marine fish production 2.85 million tonnes (Fishery Statistics CIFT, 1998). The shrimp production during 1997-98 was 1.0 lakh tonnes. Shell fish and fish exported from India according to MPEDA is 3,79,585 tonnes in 1997-98 which values Rs.4,697.48 crores.

The following table gives year-wise marine, inland and total production of fish from 1950-51 to 1996-97.
### Table 1: Fish Production of India

(Figures in lakh tonnes)

<table>
<thead>
<tr>
<th>Year</th>
<th>Marine</th>
<th>Inland</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1950-51</td>
<td>5.34</td>
<td>2.18</td>
<td>7.52</td>
</tr>
<tr>
<td>1960-61</td>
<td>8.80</td>
<td>2.80</td>
<td>11.60</td>
</tr>
<tr>
<td>1970-71</td>
<td>10.86</td>
<td>6.70</td>
<td>17.56</td>
</tr>
<tr>
<td>1980-81</td>
<td>15.55</td>
<td>8.87</td>
<td>24.42</td>
</tr>
<tr>
<td>1982-83</td>
<td>14.27</td>
<td>9.40</td>
<td>23.67</td>
</tr>
<tr>
<td>1983-84</td>
<td>15.19</td>
<td>9.87</td>
<td>25.06</td>
</tr>
<tr>
<td>1984-85</td>
<td>16.98</td>
<td>11.03</td>
<td>28.01</td>
</tr>
<tr>
<td>1985-86</td>
<td>17.16</td>
<td>11.60</td>
<td>28.76</td>
</tr>
<tr>
<td>1986-87</td>
<td>17.13</td>
<td>12.29</td>
<td>29.42</td>
</tr>
<tr>
<td>1987-88</td>
<td>16.58</td>
<td>13.01</td>
<td>29.59</td>
</tr>
<tr>
<td>1988-89</td>
<td>18.17</td>
<td>13.35</td>
<td>31.52</td>
</tr>
<tr>
<td>1989-90</td>
<td>22.75</td>
<td>14.02</td>
<td>36.77</td>
</tr>
<tr>
<td>1990-91</td>
<td>23.00</td>
<td>15.36</td>
<td>38.36</td>
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<td>1991-92</td>
<td>24.47</td>
<td>17.10</td>
<td>41.57</td>
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<tr>
<td>1992-93</td>
<td>25.76</td>
<td>17.89</td>
<td>43.65</td>
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<td>1993-94</td>
<td>26.49</td>
<td>19.95</td>
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<td>20.97</td>
<td>47.89</td>
</tr>
<tr>
<td>1995-96</td>
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<td>22.42</td>
<td>49.49</td>
</tr>
<tr>
<td>1996-97</td>
<td>28.57</td>
<td>22.83</td>
<td>51.40</td>
</tr>
<tr>
<td>1997-98</td>
<td></td>
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</tbody>
</table>

The world catch of fishes and shell fishes has progressively increased from about 1.5 million tonnes in 1950 to around 112.91 million tonnes in 1993.

By 2000 A.D., the projected demand would be about 130 million tonnes. In India, over 60% of population are fish eater with per capita consumption of only 3.7 kg which is very low in comparison to Iceland, Japan (Shrivastava et al., 1991). World average per capita consumption 12 KG. (Table 2) gives the details of per capita consumption of fish in different countries.

Table 2 : Per capita consumption of fish per annum in the year 1992

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Region and country</th>
<th>Estimated weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Iceland</td>
<td>92.1</td>
</tr>
<tr>
<td>2.</td>
<td>Japan</td>
<td>72.0</td>
</tr>
<tr>
<td>3.</td>
<td>Portugal</td>
<td>60.2</td>
</tr>
<tr>
<td>4.</td>
<td>Norway</td>
<td>41.1</td>
</tr>
<tr>
<td>5.</td>
<td>Spain</td>
<td>38.0</td>
</tr>
<tr>
<td>6.</td>
<td>Maldives</td>
<td>33.1</td>
</tr>
<tr>
<td>7.</td>
<td>Bangladesh</td>
<td>7.1</td>
</tr>
<tr>
<td>8.</td>
<td>India</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Source: Fisheries Statistics, Govt. of India, 1996.
According to the data published by Govt. of India, the total estimated potential in the marine sector is 3.9 million tonnes and 4.5 tonnes in inland sector total being 8.4 million tonnes, but the total production in 1996-97 is only 5.14 million tonnes. There exists a wide gap between the estimated potential and present production. Efforts must be directed towards increase in the production, proper processing and preservation to minimise wastage and to develop value added products so that they can be properly distributed throughout the country. Thus, through increased production and improved processing and distribution, per capita consumption of fish and shell fish may be increased.

**NUTRITIVE VALUE OF PRAWNS**

Prawns are considered as good food item as, they are low in calories, devoid of bones and spines, easily digestible and a very good source of protein. It also contain all kinds of vitamins (except vitamin C), unsaturated fatty acids and minerals. Shellfishes are considered rich in iodine and copper. Prawns have low calorific value due to their low fat contents.
1.1 Body Composition:

The important constituents of the prawn muscle are moisture, proteins, fats, non-protein nitrogenous compound, vitamin and minerals. Water is the principal component of fish and shellfish body (Govindan, 1985)

1.1.1 Moisture:

Water is the principal component of the edible portions of seafood. The average percentage of moisture in raw prawn, summarized from various sources, is 77.39% (Mukundan, M.K. et al., 1981).

1.1.2 Protein:

Protein is the important constituent of prawn muscle. Protein content in prawn muscle is 20.90% (Mukundan, M.K. et al., 1981). The quality of seafood protein is high comparable with meat and poultry. Like other sea food proteins, prawn protein has high PER and high digestibility.

1.1.3 Carbohydrate:

Prawn has got about 0.80 g/100 g glycogen content in prawn muscle (Mukundan, M.K. et al., 1981).
1.1.4 Ash:

The ash content of prawn is 1.4%. Ash is mainly composed of inorganic material like sodium, zinc, iron, calcium, potassium, sulphur, iodine, phosphorous and copper etc. (Mukundan, M.K. et al., 1981).

1.1.5 Fat:

Shellfish are generally low in lipid content, with ranges of less than 1.0% of the edible portion of some species of crab and shrimp (Sidwell, 1981). Prawn muscle contains 0.35% fat (Mukundan, M.K. et al., 1981).

1.1.6 Non-protein nitrogenous compounds:

These compounds comprise of ammonia, TMAO, guanidine, imidazole derivatives and miscellaneous substances like urea, purines and pyrimidines. In crustaceans, non-protein nitrogen is 23% of the total nitrogen i.e. total protein nitrogen is 2.7 to 2.8% while non-protein nitrogen is 0.77 to 0.79% of original weight of the muscle (Govindan, 1985).

1.1.7 Minerals:

The minerals in seafood are found in slightly higher concentration than in meat. Shellfish contain nearly twice the amount
as finfish. Shrimp (prawn) contain more calcium than other fish and meat (Bennion, 1950). Shell fish are considered to be low sodium foods, with 209 mg/100 gm. Prawn is rich in potassium and calcium, providing 382.2 mg/100 gm and 323 mg/100 gm respectively (Mukundan, M.K. et al., 1981).

### 1.1.8 Vitamin:

Fat soluble vitamins A, D, K and E are present in seafood in varying amount often in higher concentration than in land animal. Prawn contain vitamins A, D and K.

### 1.2 SPOILAGE MECHANISM

Spoilage is defined as the unacceptable changes occurring in fish and shellfish muscle, post-mortem (Mukundan et al., 1986). Comparatively quicker rates of spoilage occur in prawns than in teleost. This may be attributable to the presence of large quantities of free alpha-amino acid in their muscle which provide excellent substrate for rapid proliferation of spoilage microbes, especially in the earlier stages of the same (Govindan, 1985). Hence, prawn retain their prime quality for about 4 hours at atmospheric temperature (28°C) in India. Rapid deterioration sets in after this period making the material almost inedible after 6-8 hours (Pillai et al., 1964).
There are three main causes of spoilage which occur simultaneously:

i. Enzymatic action

ii. Microbial action

iii. Chemical spoilage

During spoilage, the fish and shellfish passes through three different stages, namely pre-rigor, rigor, post-rigor.

Pre-rigor

Flesh of freshly caught prawn is soft, flexible and tending not to retain finger impression. This stage is called pre-rigor.

Rigor

After a while the muscle become firm, elastic and stiff. This stiffness which develops shortly after death is called as rigor mortis or stiffness after death.

Post-rigor

After some time, depending on a number of other factors, the muscle becomes pliable, soft and end to retain finger impression. This stage is called post-rigor.
These changes taking place in shellfish/fish from death till it becomes spoiled, are termed as post-mortem changes. Process of spoilage is mainly dependent on temperature. Lowering the temperature will reduce the rate of spoilage.

1.2.1 Enzymatic action:

Once the prawn/fish is dead the ability of its body to regulate enzymes are lost. This leads to catabolic reactions, catalysed by these enzymes. Hence the reactions are termed as “Autoysis or self digestion”. Autolysis is defined as degradation of body component by endogenous enzymes (Mukundan et al., 1986). Enzymes are extracellular or intracellular.

Extracellular enzymes are secreted in the gut cavity and comprise of protease, lipases and carbohydrases (Govindan, 1985). The neutral pH at the time of death, flavours, the carbohydrase and lipase activity. This produces acidic pH favourable for protease activity. The enzyme hydrolyse and perforate the gut and seep into the surrounding tissues causing general hydrolysis (Mukundan et al., 1986). The spoilage due to gut is negligible in prawn.
The intracellular enzymes those which are present in small organelles within every cell are called lysosomes. The lysosomes contain about 86 hydrolytic enzyme systems capable of degrading almost all the tissue components, such as carbohydrates, fat, proteins, nucleic acids etc. The breakage of lysosomal membrane results in self digestion, hence, it is rightly called “sucidal bags”. The final product of enzymatic reactions are ammonia indole, skatol, hydrogen sulphide, free fatty acids, glycerol etc. (Mukundan et al., 1986).

1.2.2 Microbial action:

The flesh of fresh prawn contains bacterial load unlike flesh of fish which is sterile (Pillay et al., 1961). More over, the gut and surface of fish are naturally inhabited by native microbes. All these, at the time of death invade the flesh from around and within. As spoilage progresses, more amino acids and free fatty acids are liberated due to the enzymatic and chemical spoilages, which further enrich the bacterial growth. These bacteria liberate ammonia due to deamination and produce off odours (Mukundan et al., 1986). These end products adversely affect the odour and flavour of the finally processed fish product (Empey, 1958). In prawns, the spoilage is fastest in whole prawn, since the head region contains the maximum number of bacteria (Feiger, 1950). The surface spoilage is more significant than gut
spoilage since the peeled prawns keep better than the headless prawn (Velankar et al., 1962).

The bacterial species of vibrio, *Aeromonas psuedomonas*, *Enterobacteriaceae*, *micrococcus*, *Bacillus* and *Corynbacterium* produce off odour and are the main spoilers of prawns (Chandrasekaran et al., 1987).

Since spoilage in prawns begins immediately after death, the retardation of spoilage from the site of harvest till the prawn is processed becomes essential. Temperature is the major factor influencing spoilage. Reducing the temperature enhances the preservation of prawn. Moreover the processing industry requires almost all the entire quantity of raw material in an iced form.

1.2.3 Chemical action:

Chemical spoilage consists of oxidation of fat by atmospheric oxygen resulting in rancidity, melanosis and hydrolytic changes in fats and proteins (Govindan, 1985). Since prawn contains higher concentrations of free amino acids and non-protein nitrogen, volatile bases are more easily formed during putrifactive processes. The
oxidation of fat is not very prominent due to the low content of unsaturated fatty acids.

Melanosis in prawn is very prominent. This is due to the interaction of three substances.

i. Tyrosine or hydroxyphenylalanine

ii. Molecular oxygen

iii. Enzyme tyrosinase

The discolouration first develops in the tail and head regions, then at the lower side of the abdomen along the shell, ultimately spreading slowly upwards and finally on to the meat.

1.3 PRODUCTION

Our country has vast water resources. We can increase our fish and shell fish production by utilizing of water resources in proper way. Proper fishing technique should be used by the fishermen. We can increase our production by proper exploitation of Inland water body as well as marine water body.
1.3.1 Inland Sector:

River, reservoirs, flood plains and estuaries comprise inland capture fisheries. The inland fish production can be increased very much by better aquaculture technique. As the potential is about 4.5 million tonnes in inland sector while the present production is 2.34 million tonnes, a wide gap exists which can be bridged by using good management practices.

1.3.2 Marine Sector:

Large quantity of fish/shellfish are discarded at sea because it is currently uneconomic to preserve and bring them ashore. Shrimp bycatch to the best known example of such fish. It has been estimated that the global amount of discard of bycatches is in the range of 17-39 million tons/year with an average of 27 million t/year (Alvason et al., 1994). Factors discouraging the landing of the shrimp bycatch are the low market value of the material, the size and species composition, the lack of suitable refrigerated storage space on-board and the possible reduction in shrimping efficiency.

Considering optimum and over-exploitation of most of the available species in the inshore areas, the gap between the maximum potential and present exploitation in India is expected to be met mostly
from the deep sea fishing (Anon. 1997). Due to the recent interest in
deep sea fishing in the EEZ, lot of deep sea fishes are expected to land
which may not be immediately acceptable to the consumer due to
unfamiliarity with the shape, size, colour and flavour of the new
varieties. The rapid development of the minced fish technology over the
last four decade could make a major contribution to the increased
exploitation of these deep sea varieties and the by-catches.
Consumption of fish may be greatly increased by making better use of
the existing catch. Due to lack of infrastructural facilities like ice plants,
landing facilities and training etc., the quality of the fish is downgraded
particularly in developing countries leading to their use as aquaculture
feed. Through improvement in infrastructural facilities, the quality of the
landings can be upgraded for direct human consumption. The
upgradation of these species may be achieved on the one hand by use
of improved, handling and processing technique and on the other hand
developing different products. Preparation of value added product
using a species in glut is a sure way of better utilization and
distribution of the species when the landing is scanty.

1.4 PRODUCT DEVELOPMENT

We can reduce the wastage of fish/shellfish by promoting
product development. Many fish/shell fish products have been
developed in India. Prawn pickle is one of them. Preservation of food in common salt or vinegar is called pickling, species and oil may also be added.

Pickling of fruits, vegetables, meat, fish and shellfish to enhance their keeping quality, is an ancient practice, and their are historical evidences to suggest that it was followed by the ancient Indians, Egyptians and Chinese. Sauerkraut, a salted, sliced and fermented cabbage was manufactured in ancient China. Pickling is practised today in India and other Asian countries in quite a large scale.

Pickling is one of the safest means of easy preservation of fish/shell fish. Pickles prepared from finfish, crustaceans in various styles are gaining acceptance in recent days. Pickles are now used by the people as an important side dish. When used as an adjunct to prawn/fish pickle, they make them very appetising and digestible. Pickles are good appetizers and add to the platability to starch based bland tasting Asian dishes.

The technology is simple and can be adopted by the rural people/fisher folk after short training. No costly equipment is involved. So investment is low. Cottage industry can be started. However,
knowledge of hygienic handling is essential. This product has got good export potential, particularly in those countries where a large number of Asian live. A variety of fish/prawn pickles are produced and marketed in Germany, North European countries, China, Japan, Phillipines and several other countries (Borgstrom and Pares, 1965). At present there is an expanding export market for the pickles. Domestic market is also expanding.

An attempt has been made to develop acceptable pickle and study its storage characteristics at ambient temperature.
REVIEW OF LITERATURE
2. REVIEW OF LITERATURE

Much work have been reported on pickling of different food materials like fish, vegetable, egg, prawn, clam, meat etc.

A great variety of fishery products prepared with vinegar and spices enjoy wide popularity in Germany and other north European countries. Initially the fish is preserved in 1 to 2% acetic acid and 2 to 4% salt, and sold in market (Regenstein and Regenstein, 1991).

Processing of pickles from different varieties of fish and shellfish has been reported from India (Chandrasekhar et al., 1978; Chandrasekhar, 1979).

Various other workers also have described methods for the development of pickles from blood clam (Anadara granosa) meat (Gupta and Basu, 1983). Pickles from clam meat (Velosita sp. was studied by Vijayan, et al., 1982). Pickle from green mussel Perna viridis (Muraleedharan et al., 1982). Pickles from edible oyster, Crassostrea madrasensis (Sugmar et al., 1994) and pickle from Chank meat, Xancus pyrum (Dhamapal et al., 1994). However at lower ranges of salt
1 to 3.5% and 1 to 2% acetic acid the stability of pickled fish is lower (Meyer, 1990 and Kreuzer, 1990). Comparison of laboratory and commercial prawn pickle with special reference to nature of microorganisms in pickle with salt content 3.5 to 4% and pH 4.71 to 4.79 were studied extensively by Abraham and Jaya Chandran (1994, 1993 and 1992), while Indrani et al. (1989), reported the micro-organisms associated with spoilage of prawn pickle. Chandrasekhar (1979) reported a TVC (Total Viable Count) in prawn pickle in the range of $10^3$ to $10^5$ gm$^{-1}$. But Erichsen (1967), reported that pickled fish, unless spoiled, normally carry low levels of bacteria in the range of $10^1$ to $10^3$ gm$^{-1}$. *Staphylococci* have been reported in spoiled prawn pickles (Karunadasagar et al., 1988).

Vinegar was used in ancient Rome as a food additive for preservation purposes on its own or in admixture with salt, wine or honey. Vinegar made from palm wine was known in the east as long ago as 5,000 BC. If they have identical acetic acid contents, vinegar obtained by fermentation and that derived from synthetic acetic acid do not differ in their toxicological properties (Bornmann et al., 1952). Only above a concentration of some 0.5% does acetic acid display an antimicrobial action.
In penetrating the cell wall and denaturing the protein of the cell plasma (Reynaulds, 1975). If the food is adjusted to a pH of about 3 by the addition of acid, the anti-microbial effect of the acetic acid is 10 to 100 times as powerful as the other acids, such as hydrochloric acid (Reynaulds, 1975). Acetic acid increases the heat sensitivity of bacteria but not that of yeasts or molds. Between pH 6 and pH 5 the action of acetic acid only doubles (Woolford, 1975), whereas the undissociated portion increases about seven fold over this range.

Benzoic acid or sodium benzoate is used as a preservative for prawn pickle. The preservative action of benzoic acid was first described in 1875 by H. Fleck (Strablmann, 1974). Most yeast and moulds can be controlled using 0.05 - 0.1% undissociated acid (Baird - Parker, 1980). While some bacteria associated with food poisoning are inhibited by 0.01 - 0.02% undissociated acid, the control of many spoilage bacteria requires much higher concentrations (Baird - Parker, 1980). Benzoates are most effective at pH 2.5 - 4.0 and least effective above 4.5 (Lloyd and Drake, 1975; Chichester and Tanner, 1981). Rahn and Conn (1944) reported that the compound was 100 times as effective in acid solutions as in neutral solutions and that only the undissociated acid had anti-microbial activity. Macris (1975) studied the effect of benzoic acid on Saccharomyces cerevisiale. Several
researchers have found that benzoic acid inhibits amino acid uptake in moulds and bacteria (Sheu et al., 1975; Freese, 1978; Eklund, 1980). Freese et al. (1973) hypothesized that benzoic acid uncoupled both substrate transport and oxidative phosphorylation from the electron transport system.
MATERIALS AND METHODS
3. MATERIALS AND METHODS

Prawns were procured from the Versova landing centre. Several varieties were available such as Tiger prawn, white prawn, *Parapenaeopsis stylifera* (Karikadi), *Metapenaeus affinis* etc. Some were very costly like tiger prawn, white prawn etc. This work was carried out mainly using *Parapenaeopsis stylifera* which was available in plenty during September to December and was very cheap. The size of the prawn used was between 40 to 60 mm.

After procurement from landing centre the prawns were brought to the laboratory and washed repeatedly with portable water to make it free from sand and any other extraneous material. After washing, the prawns were drained and peeled hygienically. The weight of the peeled prawns were taken. The 50% of the salt from the recipe was mixed with the peeled prawns and left for 1-1½ hours for the salt to penetrate into the muscle. Garlic, ginger and green chilli was made into a paste. Dry chilli powder, turmeric powder and cumin powder were made into another paste separately. 50% of the oil was added in frying pan and the prawn mixed with salt were fried and fried prawns were kept aside. The remaining amount of oil is added to the frying pan and half broken mustard seed and menthy seed was added and fried for 1-2 minutes.
Then the paste containing crushed ginger, garlic and green chilli were added and fried for a while. When half fried, the second paste contains turmeric, red chilli and cumin powder was added and frying continued until characteristic odour emerged. Sugar is added during the frying of pastes. Fried prawns were added into the fried paste and both together fried for some more time at low flame. Frying is stopped when characteristic smell emerged. Then the frying pan was taken out of the flame and allowed to cool under fan. When the temperature is little higher than room temperature, vinegar and benzoic acid is added and mixed thoroughly and allowed to cool to room temperature. The pickle so prepared was packed in glass container having acid proof lid. The glass container was washed with hot water and dried in oven before use for packaging. Care is taken that a layer of oil remains in the top of the pickle in the bottle. At regular intervals samples were drawn and analysed to study its storage characteristics.

3.1 BIOCHEMICAL TEST

3.1.1 Estimation of Total volatile bases by Conway (1947) methods:

Principle:

Known weight of sample is de-protenized with 7% TCA. This solution is allowed to react with potassium bicarbonate which liberates
the volatile bases. This liberated volatile bases are absorbed by an acid. The excess acid is titrated with an alkali.

Procedure:

5 gm of sample is taken and grounded in mortar using 10 ml of 20% TCA solution. The mixture is grounded well. It is filtered and residue is washed with distilled water containing a few drops of TCA. This protein free filtrate was made upto 100 ml by using distilled water.

Estimation:

Conway cups and lids are washed with distilled water and dried. Paraffin wax and vaselin in ration 1:3 is melted and cooled. This is applied on the rim of cup. 1 ml of N/70 HCl is added into the inner chamber of cup. Lid is placed over the conway cup, covering part of outer chamber and complete inner chamber. 1 ml of TCA extract is poured into the outer chamber with a new pipette and then 1 ml of K_2CO_3 is added to outer chamber with another pipette. The cup is completely covered with lid immediately after this. The contents were mixed rotating the unit gently and then unit was left overnight for reactions at room temperature (it can be kept inside an incubator at 36°C for 2 hours). The excess acid left in inner chamber is titrated
against N/70 NaOH using a drop of Tashiros indicator till the greenish end point is obtained. A blank is run simultaneously.

Calculation:

\[ \therefore 1 \text{ ml of TCA extract} = V \text{ ml of N/70 HCl} \]

\[ \therefore 100 \text{ ml of TCA extract} = \frac{V \times 100}{1} \text{ ml of N/70 HCl} \]

\[ 5 \text{ ml N/70 HCl} = 1 \text{ mg. of N}_2 \]

\[ \therefore V \times 100 = \frac{1 \times V \times 100}{5} \text{ mg. of N}_2 \]

\[ W \text{ gram sample} = \frac{V \times 100}{5} \text{ mg. of N}_2 \]

\[ \therefore 100 \text{ gram sample} = \frac{V \times 100 \times 100 \times 1}{5 \times W \times 1} \text{ mg. of N}_2 \]

\[ \therefore \text{TNV} = \underline{\text{_____________ mg\%}} \]

3.1.2 Estimation of α-Amino-Nitrogen of a sample (C.G. pope and M.F. Steven’s Methods)

Principle:

The method depends on the formation of soluble copper compounds through the reaction between the amino acid and excess of copper in the form of CuSO₄. The amount of Cu taken into the solution by amino-acid is then determined lodometrically.
Regent:

i. 20% TCA solution and 5% TCA solution.

ii. Cupric Chloride (CuCl₂): 27.3 gm of CuCl₂ dissolved in one litre of distilled water.

iii. Tri sodium phosphate (Na₃PO₄): 65.5 gm of di-sodium hydrogen phosphate (Na₂HPO₄) dissolved in 500 ml of D/W and 7.2 gm of NaOH added to it then dissolved. The solution was made up to 1 litre.

iv. Borate buffer: 57.21 gm of sodium borate is dissolved in 1500 ml of distilled water and 100 ml of normal HCl was added to it and their volume made up to two litre.

v. Thymolphthalein: 0.25 gm of Thymolphthalein is dissolved in 100 ml of 50% Ethyl alcohol.

vi. sodium thiosulphate solution: 0.248 gm of Na₂S₂O₃ is dissolved in 100 ml of water. This solution was standardized by using standard dichromate solution (Iodometry).

vii. Normal NaOH: 4 gm of sodium Hydroxide is dissolved in 100 ml of distilled water.
viii. Starch indicator: 1 gm of starch powder was added to 100 ml of boiling water and boiled for five minutes.

ix. Glacial acetic acid.

x. Potassium iodide (AR)

**Preparation of TCA extracts:**

5 gm of sample was taken and grounded in the mortar with 10 cc of 20% TCA (Trichloro acetic acid) was added. The mixture was grounded well and the residue was washed with distilled water containing few drops of TCA. The protein free filtrate was made up to 100 cc with the help of distilled water. That TCA extract solution is used for the determination of $\alpha$-amino-N$_2$.

**Procedure:**

10 cc of TCA extract was taken into a 50 ml volumetric flask. Added a few drop of thymolphthalein and the extract was made alkaline by adding normal NaOH, till distinct blue colour appeared one part by volume of CuCl$_2$ solution was mixed with two parts by volume of trisodiumphosphate and two parts of volume of Borate buffer. The solution was mixed well and 30 ml of suspension was added to alkaline solution in standard flask (extract). The volume was made up to 50 ml with distilled water. After shaking, it was allowed to stand for 15 minute
and then filtered 10 ml. of the filtrate was pipetted out into a conical flask. 0.5 cc of glacial acetic acid was added followed by addition of 1.0 gm. KI. The liberated iodine was titrated against N/100 Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} using starch as indicator. When yellow, few drops of starch solution was added and titration was continued till blue colour disappeared.

\textit{Calculation}:

\[ \therefore 1 \text{ ml of N/100 Na}_2\text{S}_2\text{O}_3 = 0.28 \text{ mg of N}_2 \]

\[ \therefore V \text{ of N/100 Na}_2\text{S}_2\text{O}_3 = 0.28 \times V \text{ mg of N}_2 \]

\[ \therefore 10 \text{ ml of buffer extract contain } 0.28 \times V \text{ mg of N}_2 \]

50 ml of buffer extract contain \( \frac{0.28 \times V \times 50}{10} \text{ mg of N}_2 \)

\[ \therefore 50 \text{ ml of buffer extract contain } \frac{0.28 \times 50 \times V}{10} \text{ mg of N}_2 \]

\[ \therefore 10 \text{ ml of TCA extract contain } \frac{0.28 \times 50 \times V \times 100}{10} \text{ mg of N}_2 \]

\[ \therefore W \text{ gm of sample contain } \text{TNV} = \frac{0.28 \times 50 \times V \times 100 \times 100}{20 \times 10 \times W} \text{ mg of N}_2 \]

\section*{3.1.3 Estimation of Peroxide value (Lima \textit{et al.}, 1981)}:

\textit{Principle}:

Sample contain some unsaturated fatty acid. They can easily undergo oxidation and gives rise to peroxy or hydroxy radicals by free
chain mechanism which gives peculiar rancid flavour. Saturated Potassium iodide solution after reaction liberates iodine, which can be titrated against N/500 Na$_2$S$_2$O$_3$.

**Procedure:**

2 gm of sample was taken in stoppered bottle. 30-40 ml of chloroform was added and kept in a dark place for half hour with occasional shaking, then filtered the extract through Whatman no.1 filter paper.

The volume of chloroform extract is made to 30 ml and it is divided into two parts. In 1st part 15 ml of chloroform extract, 15 ml of glacial acetic acid, 1 ml of saturated KI solution were added and kept in a dark place for 10 minutes with occasional shaking. After that 50 ml of distilled water and 1 ml of starch solution were added and the liberated iodine was titrated with 0.002 N Na$_2$S$_2$O$_3$ solution. Disappearance of pale brown to white colour were noted. Remaining 15 ml of chloroform extract were transferred into a beaker and evaporated over a water bath to dryness and dried at 100°C in hot air oven for 30 minutes.
The beaker was cooled and weighed for fat content present in the extract. The amount of peroxide present in the given sample was calculated which expressed as millimole of oxygen per kg of fat.

**Calculation:**

\[
\text{Peroxide value} = \frac{\text{Volume of } \text{Na}_2\text{S}_2\text{O}_3 \text{ consumed} \times \text{Normality of } \text{Na}_2\text{S}_2\text{O}_3}{\text{Weight of fat}}
\]

**Result:**

\[
\text{PV} = \underline{\text{________________________}} \text{ milli equivalents/1000 gm of lipid}
\]

3.1.4 MEASUREMENT OF pH VALUE BY DIGITAL pH METER

**Instrument standardization:**

To standardise the glass electrode to the pH scale of the instrument, place a sample cup of fresh buffer solution of know pH under the two electrodes. Insert a thermometer into the solution and adjust the temperature compensating dial to the temperature shown on the thermometer. Adjust the asymmetry potential knob of the instrument. So that the instrument reads the same pH as the buffer. The electrodes should remain in the buffer solution until the reading becomes stable before adjustment is made. For maximum accuracy the meter should be standardized against a buffer having a pH close to that of the samples; most meters will agree reasonably closely between buffer solution in the pH 4 to 7 range.
Sample analysis:

Samples are tested for pH by inserting the electrodes into the sample solution. For greatest accuracy the electrodes should be allowed to remain in each sample until reproducible readings are obtained, which may take a third of a minute or more. Wipe the electrodes with cleaning tissue between samples. Whenever changing from one type of sample to another, rinse the electrodes with distilled water before wiping them. A uniform temperature must be maintained for the electrodes, buffer solutions and all samples to obtain accurate results. For buffered samples or those of high ionic strength, condition electrodes after cleaning by dipping them into sample for 1 minute. Blot dry, immerse in a fresh portion of the sample, and read pH. With dilute, poorly buffered solutions, equilibrate electrodes by immersing in three or four successive portions of sample. Take a fresh sample to measure pH.

3.1.5 MEASUREMENT OF TITRABLE ACIDITY (AOAC, 1975)

One ml of liquid portion was taken in a conical flask. Some amount of distilled water was added into conical flask. Some drops of Phenolphthalein was added into the conical flask. Then it was titrated
with the help of N/10 NaOH till the colour changes. The reading of titrate values were noted.

3.2 MICROBIOLOGICAL ANALYSIS

3.2.1 Estimation Of Total Plate Count (Apha, 1976):

Total bacteria count present per gram of sample was estimated by standard spread plate method.

Procedure:

Sterilization of petridishes and pipettes. After cleaning, the petridish and pipettes were dried and wrapped properly with brown papers. Small pieces of cotton were pressed inside the upper opening of the pipette and wrapped. Then all petridishes and pipettes were placed inside an electrically operated oven for sterilization by dry heat at 160°C for one hours.

Preparation of Nutrient Agar

Nutrient agar is the most commonly used media for growth of bacteria. The following ingredients were used in the given quantity weighed in an electric balance.

i. Peptone - 1.0 gm

ii. Sodium Chloride - 1.0 gm

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iii. Agar - agar - 1.5 gm
iv. Beef extract - 0.3 gm
v. Distilled water - 100 ml.

The weighed materials were transferred into a conical flask with the measured volume of distilled water (100 ml). The pH of the mixture is then adjusted at 7.2 by adding drop by drop either NaOH or HCl before adding the agar. After adding the agar, it is gently heated to melt it. The mouth of the conical flask is then plugged with cotton.

**Sterilization of Normal Saline and Nutrient Agar**

Some test tubes were taken with normal saline (0.85 gm of sodium chloride per 100 cc distilled water) 9 ml normal saline was taken in each of the test-tube. These test tubes will be used for preparing different dilution of the sample. These test tubes were also plugged with cotton.

Again some conical flask each with 90 ml normal, saline was plugged with cotton.

The conical flasks with nutrient agar and normal saline, and the test tubes with normal saline were wrapped with brown papers and kept
in a wire basket. This wire basket is placed into an autoclave and sterilized at 15 lbs pressure for 15 minutes.

After sterilization, about 10-12 ml media was poured into each of the sterilized petridishes and allowed to solidify. After solidification the petridishes were kept inside an incubator at 35-45°C for 15-20 minutes to dry in order to remove any condensed moisture.

**Preparation of homogenate from sample:**

For handling the sample, glass wares, knife etc. are sterilized by smearing with alcohol and setting fire to it. 10 gm of sample was weighed by taking it in a sterile steel container. The sample was transferred to a glass mortar which was earlier sterilized by pouring small quantity of alcohol into it and setting fire. The sample in the mortar was grounded into a paste by adding a small quantity of sterilized saline from 90 ml present in conical flask. Now the remaining saline is added and mixed it thoroughly. The dilution is known as original homogenate or 10⁻¹ dilution.

**Spreading:**

The dried petridishes with media, sterilized pipettes and test tubes were kept ready. The sterile petridishes were labelled in
duplicates for each dilution. From original homogenate, 1 ml was transferred by a sterile 1 ml pipette into 9 ml sterile saline tube. It is 10^2 dilution. From this dilution 0.1 ml was transferred to each of the two petridishes (marked as 10^2 ) followed by gently spreading with a sterile spreader. Similarly 10^3, 10^4 and 10^5 dilution were prepared and from each dilution 0.1 ml was transferred into the petridishes in duplicates followed by spreading. 0.1 ml saline from test tube was transferred into a petridish marked as C and spreaded which was served as control. All the Petridishes were kept in inverted position inside a bell jar and incubated at room temperature for 24-36 hours for multiplication of bacteria counting.

Plates where the colonies were more than 30 and less than 300 were considered for counting. Colonies in both the plates of dilution were counted. The numbers of bacteria per gram of prawn sample were calculated, as the average numbers of colonies in duplicate plates multiply by the inverse of the corresponding dilution and 10.

3.2.2 Estimation of Total Fungal Count:

Total fungal count present per gram of sample was estimated by standard spread plate method. The media which is used for fungal count is known as Rose Bengal Chlorophenicol Agar.
Agar - 15.0 gm
Glucose - 10.0 gm
Palpaic digest of Soyabean meal/Peptone - 5.0 gm
KH₂PO₄ - 1.0 gm
MgSO₄7H₂O - 0.5 gm
Rose Bengal - 0.05 gm
Chloraphenicol solution - 10.0 ml
pH - 7.0-0.2 at 25°C
Distilled water - 1 litre

Other procedures and counting is done same as Total Plate Count.

3.3 Sensory/Acceptability test

The sensory tests were carried out for the product by a panel of 8 assessors drawn from the laboratory staff.

Sequence of Observations:

i. General appearance (discolouration of any)
ii. The texture of prawn pickle
iii. The odour of prawn pickle
iv. The flavour of pickle
Organoleptic evaluation and overall acceptability rating of the product was done on a 9 point hedonic scale.

The pattern of scoring:

9 - Extremely good
8 - Very good
7 - Moderately good
6 - Slightly good
5 - Neither good nor poor
4 - Slightly poor
3 - Moderately poor
2 - Very poor
1 - Extremely poor

A score of 5 was taken as limit of acceptability.
Plate No. 1 : Peeling of Prawn

Plate No. 2 : Peeled Prawn
Plate No. 3 : Preparation of Prawn Pickle

Plate No. 4 : Prawn Pickle
RESULTS AND DISCUSSION
4. RESULTS AND DISCUSSIONS

While attempting to prepare a traditional spiced prawn pickle, the advantages of the use of acetic acid (vinegar) for preservation and flavouring, have been made use of in the present method. In the traditional type of pickles prepared from fresh lemon and mangoes, the main preservatives are the salt and organic acids provided by the fruits. Since the prawn muscle do not contribute any such acids, it becomes essential to use some organic acid to provide the necessary texture and flavour to the product, in addition to bringing down the pH to the required level. While citric acid can be used for this purpose, it was thought better to use vinegar instead, as latter has got much higher anti-microbial activity at lower pH than the former. While the traditional pickle needed a minimum of 18 to 20% salt for good preservation, the same had been cut down in the present process to around 8% due to the addition of vinegar and benzoic acid.

A number of trials with different combinations of spices, salt, oil, vinegar were tried and the products were presented to the panel. A suitable combination of spices mixture which scored well with most of the panel members is shown in Table 3.
During shelf life studies it was observed that the pH of the product decreased slightly up to four months from 4.64 to 4.58 (Table 5). The change in pH value has been presented in figure (1). T.J. Abraham et al. (1996) observed that the pH in prawn pickle decreased initially slightly up to 90 days to 4.71 and then increased to 4.78 at the end of 270 days. Collins et al. (1989) were of the opinion that if the pH of the vinegar pickled fish is 4.5 or less, no further precaution is necessary against bacterial pathogens.

The change in the titrable acidity in the liquid fraction during storage is presented in figure (2). It was observed that the titrable acidity increased during storage from 0.36 to 0.72 during four months storage, in keeping with the change in pH described earlier. T.J. Abraham et al. (1996) observed a marginal increase in titrable acidity from 0.25 to 0.33% in prawn meat of the pickle after 120 day and remained constant thereafter.

Figure (3) shows the change in the total viable count (TVC) in the pickle during storage. The TVC increased initially up to 30 days and decreased gradually up to 90 days, finally showing an increasing trend thereafter. In the first month, the acid tolerant bacteria multiplied, showing the increase in TVC. Due to gradual increase in titrable acidity
and decrease in pH as described earlier, the less sensitive bacteria died, showing decrease in TVC. Probably at the end of fourth month the more acid sensitive bacteria started multiplying thereby showing an increasing trend in TVC. Chandrasekhar (1979) reported a TVC in prawn pickle in the range of $10^3$ to $10^5$ gm$^{-1}$. But Erichson (1967) reported that pickled fish, unless spoiled, normally carry low levels of bacteria in the range of $10^1$ to $10^3$ gm$^{-1}$. Karunasagar et al. (1988) have reported a viable count in the range of $10^6$ to $10^9$ gm$^{-1}$ in spoiled prawn pickle of which $10^6$ to $10^7$ gm$^{-1}$, were halophiles. The aerobic spore formers comprised more than 50% of the viable bacterial population throughout the storage period. These have been reported to be the viable bacterial population throughout the storage period. These have been reported to be the dominant group in fish pickle (Chandrasekhar et al., 1978). Lactic acid bacteria, Coliforms, Salmonellae, Vibrios and Clostridium perfringens were not encountered in prawn pickle immediately after preparation and up to 60 days. These organisms are reported to be either killed or fail to multiply in acetic acid fish preserves (Emberger, 1972) and in fish pickle (Chandrasekhar et al., 1978). No fungal colonies were observed up to 4 months. Behanan et al. (1990) did not find any fungal colonies in pickled fish up to 60 days.
The anti-microbial action of benzoic acid is based on various interventions in the enzymic structures of the cell of the micro-organism. In many bacteria and yeasts, for instance, enzymes that control the acetic acid metabolism and oxidative phosphorylation are inhibited (Bosund, 1960; Bosund, 1962). Benzoic acid appears to intervene various points in the citric acid cycle especially that of α-ketoglutaric acid and succinic acid dehydrogenase (Bosund, 1962). Benzoic acid also appears to inhibit tyrosinase (Menon et al., 1990). Besides its enzyme inactivating effects, benzoic acid also acts on the cell wall. To be able to develop its action inside the cell of the micro-organism, benzoic acid has to penetrate the cell wall. When this takes place, it is the undissociated part of the acid that more readily enters the cell. The action of benzoic acid is dependent on the pH value. Only the undissociated part of the acid has an anti-microbial action. Benzoic acid can be used only for preserving strongly acid products. Depending on the concentration, benzoic acid lowers the intracellular pH value as well. Thus effect, which likewise helps to inhibit growth and destroy the cells (Salmond et al., 1984), is more pronounced in the undissociated acid portion (Eklund, 1985).

On the whole, the action of benzoic acid is directly mainly against yeast and moulds, including aflatoxin forming micro-organisms
(Urain and Chipley, 1976; Uraihi et al., 1977). Bacteria are only partially inhibited. Benzoic acid has less effect on lactic acid bacteria and clostridia.

The change in TVN value during storage is presented in figure (4). There is a gradual increase in TVN value as the storage period progressed. The value increased from 12 to 24 after four months of storage. The use of TVN and TMAO values as criteria for judging the spoilage of fish pickle had been considered to be of no practical significance as observed by Nicholson, 1930. Our observation also show that TVN values do not correlate with organoleptic evaluation. However, the TVN values appear to be well around the acceptable limits. α-amino nitrogen values also increased slowly but steadily as shown in figure (5). The increase in the values of α-amino-nitrogen with time did not correlate with the bacterial load. The increase did not reflect the bacterial degradation of protein. Probably the increase was due to the acid hydrolysis of protein. Probably this α-amino-nitrogen was responsible for improvement of flavour of the pickle on maturation. It was found that the flavour of the pickle improved gradually as the storage period increased beyond one month.
Peroxide value increased slowly as depicted in figure (6) and at the end of fourth month, the value was 5.48. This amount did not impart any bad flavour to the product.

Figure 7 depicts the overall acceptability rating of the product by a taste panel consisting of 8 members on a 9 point hedonic scale. The acceptability rating increased from first month onwards till the end of second month and then fall slightly. This was due to the improvement of the flavour of the product on maturation. The texture also became softer gradually as storage time increased. However acceptability rating showed a downward trend due to increase in peroxide value and TVN value. At the end of fourth month, the product was in very good acceptable condition.
<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peeled prawn</td>
<td>1 kg</td>
</tr>
<tr>
<td>Mustard seed</td>
<td>4 gm</td>
</tr>
<tr>
<td>Methi seed</td>
<td>4 gm</td>
</tr>
<tr>
<td>Peeled garlic</td>
<td>100 gm</td>
</tr>
<tr>
<td>Ginger (fresh)</td>
<td>25 gm</td>
</tr>
<tr>
<td>Green chilly.</td>
<td>30 gm</td>
</tr>
<tr>
<td>Chilli powder</td>
<td>30 gm</td>
</tr>
<tr>
<td>Turmeric powder</td>
<td>5 gm</td>
</tr>
<tr>
<td>Cumin powder</td>
<td>30 gm</td>
</tr>
<tr>
<td>Salt</td>
<td>80 gm</td>
</tr>
<tr>
<td>Sugar</td>
<td>5 gm</td>
</tr>
<tr>
<td>Oil</td>
<td>250 ml</td>
</tr>
<tr>
<td>Vinegar</td>
<td>300 ml</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>200 ppm</td>
</tr>
</tbody>
</table>
Table 4: Proximate Composition of *Parapeneopsis stylifera*

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture %</td>
<td>77.24</td>
</tr>
<tr>
<td>Protein %</td>
<td>20.71</td>
</tr>
<tr>
<td>Fat %</td>
<td>00.45</td>
</tr>
<tr>
<td>Ash %</td>
<td>1.45</td>
</tr>
</tbody>
</table>

Table 5: Changes in biochemical, bacterial and or ganoleptic characteristics of prawn pickle

<table>
<thead>
<tr>
<th>No of days</th>
<th>pH</th>
<th>x-amino nitrogen (mg/100g)</th>
<th>TVN (mg/100g)</th>
<th>PV (m.e.g/kg of fat)</th>
<th>Titrable acidity (as% acetic acid)</th>
<th>Total bacterial count (TVC/gm)</th>
<th>Organo-leptic scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.64</td>
<td>113.86</td>
<td>15</td>
<td>0</td>
<td>0.36</td>
<td>$3.6 \times 10^3$</td>
<td>8.0</td>
</tr>
<tr>
<td>30</td>
<td>4.63</td>
<td>121.33</td>
<td>16</td>
<td>0</td>
<td>0.51</td>
<td>$4.4 \times 10^3$</td>
<td>8.5</td>
</tr>
<tr>
<td>60</td>
<td>4.62</td>
<td>184.60</td>
<td>20</td>
<td>1.732</td>
<td>0.60</td>
<td>$1.4 \times 10^2$</td>
<td>8.7</td>
</tr>
<tr>
<td>90</td>
<td>4.60</td>
<td>203.00</td>
<td>22</td>
<td>3.673</td>
<td>0.66</td>
<td>$1.2 \times 10^3$</td>
<td>8.5</td>
</tr>
<tr>
<td>120</td>
<td>4.58</td>
<td>219.00</td>
<td>24</td>
<td>5.479</td>
<td>0.72</td>
<td>$1.5 \times 10^3$</td>
<td>8.3</td>
</tr>
</tbody>
</table>
Fig. 1: Changes in pH value during storage
Fig. 2: Changes in titrable acidity
Fig. 3: Changes in bacterial count during storage.
Fig. 4: Changes in total volatile base nitrogen

TVN (mg/100g)

Days of storage
Fig. 5: Changes in alpha-amino nitrogen of the product during storage.
Fig. 6: Changes in peroxide value during storage
Fig. 7: Organoleptic scores of prawn pickle
SUMMARY
5. SUMMARY

Prawn pickle was prepared using cheap smaller variety of prawns. Vinegar and salt was used to preserve the fish muscle against spoilage. Different spices were used to get desired attractive flavor. Benzoic acid to the extent of 200 ppm was used as preservative. Several trails were carried out using different amounts of spices and different methods of preparation. After each trial the sample was subjected to sensory evaluation by a panel of judges consisting of five members who had previous experience of acting as panel members. Suggestions for improvement put forward by the panel members were incorporated while next trial was carried out. After several trails, a final recipe was arrived at. Utilizing this final recipe, a product was prepared and subjected to large scale consumer acceptance trial and was found very much acceptable. The product was subjected to biochemical, bacteriological and organoleptic evaluation and found to remain quite acceptable after four months of storage in glass jar at ambient temperature.
सारांश

झींग का आचार सभी छोटे किस्म के झींगों में तैयार किया गया। आचार खराब न हो इसके लिए बिन्न गर्म नमक का इस्तेमाल किया गया। आकारित खुशाबु आने के लिए विभिन्न प्रकार के मसालों का प्रयोग किया गया। बेंजियोक रसायन का 200 PPM प्रयोग परीक्षण के रूप में किया गया। कई परीक्षण निकाले गए, जिसमें विभिन्न मात्रा में मसालों तथा विभिन्न प्रकार की विधि का प्रयोग किया गया। प्रत्येक परीक्षण के बाद नमूनों का मूल्यांकन पांच सदस्यों वाले उन जनों की पैनल द्वारा करवाया गया, जिन्होंने इससे पहले भी जनों की पैनल में सदस्य के रूप में काम किया। पैनल के सदस्यों द्वारा दिए गए सुझावो को आगे किया जाने वाले परीक्षण में सम्मिलित किया गया। कई परीक्षण करने के बाद अंतिम पदार्थ तैयार किया गया। इस आचार को छायां के लिए भी बड़ी मात्रा में तैयार किया गया जो कि बहुत अच्छा पाया गया।

इस पदार्थ को जैव-रसायन, जीवाणु विज्ञान तथा जैव तत्त्वों आदि में रखकर मूल्यांकन किया गया और यह देखा गया कि 4 महीने तक ग्लास जार में रखने के बावजूद भी वह आस-पास के तापमान के अनुकूल रहा।
REFERENCES
6. REFERENCES


