IV. RESULTS

The results obtained from different experiments are briefly explained in this section.

4.1. Physical characteristics

Important physical characteristics like total length, total weight, dorsal mantle length and meat yield of different batch are given in Table 6. The length of octopus varied between 19.8 to 71.0 cm and the weight of whole octopus ranged from 30.0 to 642.0 g. The dorsal mantle length varied from 5.5 - 14.0 cm. The dressed octopus yield was ranged from 65.6 to 77.9%.

4.1.1. The meat composition and characteristics:

a) Bio-chemical, microbiological and sensory characteristics

The composition of fresh meat of different batches of octopus used in various studies is given in Table 7. The moisture content, crude protein (total nitrogen x 6.25), total lipids / fat and ash contents of the fresh octopus of batch I were 86.16%, 10.54%, 0.85% and 2.15% respectively while that of fresh octopus of batch II were 85.34%, 12.02%, 0.92% and 2.08% respectively. The pH of fresh octopus meat of I batch was 6.13 while the pH of fresh octopus of batch II was 6.18.

The nitrogenous compounds such as salt soluble nitrogen, water soluble nitrogen, non protein nitrogen and alpha amino nitrogen of fresh octopuses of batch I were 1.05 g%, 412 mg%, 306 mg% and 216.62 mg% respectively while that of fresh octopuses of batch II were 1.15 g%, 419 mg%, 344 mg% and 208.6 mg% respectively. The total volatile base nitrogen and tri-methylamine nitrogen of fresh octopus of batch I were 2.35 mg% and 0.92 mg% respectively while that of fresh octopus of batch II were 1.42 mg% and 0.84 mg% respectively.

The lipid quality as measured by peroxide value and free fatty acid investigated for the meat of fresh octopuses of batch I registered a value of 9.03 millimoles of O₂ / kg fat and 4.57 as % of oleic acid respectively while that of fresh octopuses of batch II were 4.28 millimoles of O₂ / kg fat and 1.62 % of oleic acid respectively.

The microbiological quality of fresh meat of different batches of octopus used in various studies is given in Table 7. The total plate count of fresh octopus of batch I was 4.20x10⁴ colony forming unit/gram (cfu/g) while the fresh octopuses of batch II was 1.15x10⁵ cfu/g. The detection of pathogenic micro organisms such as Vibrio cholerae and Vibrio parahaemolyticus confirmed their presence but Salmonella spp. was found to be absent in the raw octopus in both the batches.
The sensory characteristics of fresh octopus meat of different batches used in various studies are given in Table 7. The individual sensory parameters such as appearance, colour, odour, texture and flavour of the dressed and steam cooked octopus of batch I and II showed that the raw materials were in good condition and have scored an average overall acceptance score of 8.25 and 8.30 on 9.0 point hedonic scale.

b) Amino acid composition of fresh octopus

The total amino acid composition of fresh octopus meat was analyzed to know the nutritional status. The total amino acid compositions obtained are presented in Table 8, which indicated that Glutamic acid was present in larger proportion (17.36 g / 16 g nitrogen) and Tryptophan was present in the lowest quantity (0.97 g / 16 g nitrogen). The amino acid sequence in term of weight are as follows; Glutamic acid> Aspartic acid> Leucine> Arginine> Glycine> Alanine> Isoleucine> Threonine> Serine> Lysine> Proline> Phenyl alanine> Cysteine> Methionine> Histidine> Tyrosine> Valine> Tryptophan.

c) Heavy metals concentration

The analysis of some important heavy metals such as zinc, lead, cadmium and mercury contents of fresh octopus are presented in Table 9. Zinc was present in high proportion (15.46 ppm) followed by cadmium (0.97 ppm), lead (0.52 ppm) and mercury (0.01 ppm). All the heavy metals analyzed were with in the tolerable limits / standards prescribed by EU.

4.2. Ice storage studies of octopus meat

In this section the results obtained from ice storage of octopus in different forms viz., whole octopus iced directly (WOI), dressed octopus iced directly (DOI) and dressed octopuses are then packed in polyethylene bag and ice stored (DPI). The changes in quality parameters during ice storage are presented in this section;

a) Moisture

The changes in moisture content of ice stored octopus are presented in Table 10 and Fig. 10. Slight increase in moisture in WOI and DOI samples were noticed in 15 days of storage, whereas in DPI not much change was noticed. However, the changes in moisture content among different methods of icing was almost remained a constant except whole octopus iced at the end of 21 days where moisture level increased (3.24%) from 86.02 to 88.81%.

b) Protein

The changes in protein of ice stored octopus are presented in Table 11 and Fig. 11. There was considerable decrease in protein content of all the samples. The decrease was more in DPI (39.30%) than WOI (34.25%) and DOI (35.72%). The protein content of all the samples ranged from 10.54 to 6.28% during storage.
c) Total lipids
The changes in total lipids of ice stored octopuses are presented in Table 12 and Fig. 12. Since the lipid content of octopus is less, not much change in all the samples was observed. The changes in lipids were very marginal and varied from 0.81% to 0.95% in all the samples during the ice storage study.

d) Ash
The ash content of ice stored octopuses at the beginning and at the end of experiment is given in Table 13. The average initial ash content of the octopus on the dry weight basis was 3.56 which decreased to 2.83%, 2.79% and 2.93% in WOI, DOI and DPI respectively at the end of storage period.

e) Salt soluble nitrogen
The changes in SSN of ice stored octopuses are presented in Table 14 and Fig. 13. It was the major nitrogenous constituent which contributed about 59.62% of total nitrogen which showed decrease in all the samples. The rate of decrease was more in dressed samples DPI (loss of 23.29% of TN) and DOI (loss of 22.47% of TN) in 15 days of ice storage when compared to undressed sample WOI (loss of 14.07% of TN) at the end of 21 days of ice storage.

f) Water soluble nitrogen
The changes in WSN of ice stored octopuses are presented in Table 15 and Fig. 14. The initial WSN content was 24.61% of TN. Considerable decrease in water soluble nitrogen of all the samples was noticed. The loss of WSN was more in DPI (11.24% of TN) and DOI (9.63% of TN) than the WOI (6.98% of TN) sample at the end of ice storage.

g) Non protein nitrogen
The changes in of ice stored octopuses are presented in Table 16 and Fig. 15. The initial NPN content was 19.30 % of TN which showed decrease throughout the storage, the NPN loss was more in DOI (7.99% of TN) sample which was dressed and in direct contact with ice followed by DPI (7.95% of TN) which is indirectly iced for 14 days and then by WOI (6.29% of TN) sample at the end of 21 days of ice storage.

h) Peroxide value
The oxidation of lipid in the meat during ice storage was monitored by measurement of PV. The changes in PV content of ice stored octopuses are presented in Table 17 and Fig. 16. This indicated a gradual increase through out the ice storage period in all the samples. The increase in PV was from 9.03 to 43.43 millimoles of O₂ / kg fat in WOI, 9.03 to 51.27 millimoles of O₂ / kg fat in DOI and 9.03 to 53.21 millimoles of O₂ / kg fat in DPI.
i) Free fatty acid

In the same way of PV, the FFA content also showed an increasing trend during the ice storage period (Table 18, Fig. 17). The initial FFA was 4.57 % of oleic acid in fat which rose to 28.79, 36.73 and 37.67 % of oleic acid in fat respectively in WOI, DOI and DPI samples.

j) Total volatile base nitrogen

The changes in TVBN content of ice stored octopuses are presented in Table 19 and Fig. 18. The TVBN content showed gradual increase which was proportional to the ice storage period. A maximum value 39.04 mg% in DPI, 22.60 mg% in DOI was recorded at the end of 15 days of storage period and 19.52 mg% in WOI during 21 days of ice storage.

k) Tri-methyl amine nitrogen

The changes in TMAN content of ice stored octopuses are presented in Table 20 and Fig. 19. Significant increase in TMAN also observed in all the samples. The rate of increase was much higher in DOI and DPI samples from initial value of 3.08 mg% to 9.25 mg% and 11.04 mg% respectively at the end of 15 days. On the other hand WOI reached 10.79 mg% at the end of 21 days of ice storage.

l) pH

The changes in pH of ice stored octopuses are presented in Table 21 and Fig. 20. A very small fluctuation from initial pH of 6.16 to maximum of 6.80 was observed in all the samples during iced storage of octopus. There was slight increase in pH up to 15 days. There after in DPI showed more rapid increase in pH, whereas not much changes in pH throughout the storage period was shown by DOI. WOI showed not much variation in pH and was almost constant up to 15 days, thereafter it showed marginal increase up to the end of (21 days) ice storage.

m) Hypoxanthine

The changes in hypoxanthine of ice stored octopus are presented in Table 22 and Fig. 21. Hx showed gradual increase in all the samples. WOI sample showed lower values and lower rate of Hx when compared to DOI and DPI samples. The raw octopus initially had hypoxanthine content of 0.86 μmole / g of meat which rose to 3.37 in WOI, to 3.96 in DOI and to 4.08 in DPI samples.

n) Total plate count

The changes in of ice stored octopus are presented in Table 23 and Fig. 22. In the present experiment, initial decrease in count with brief latency period was observed in all the samples. Initial total plate count of octopus was 4.20x10^4 which reached to 9.93x10^5, 6.50x10^5 and 8.76x10^5 cfu/g in WOI, DOI and DPI respectively at the end of
the storage periods. But none of the samples have reached maximum prescribed limits for the safe consumption of sea foods.

**o) Psychrophiles**

The changes in psychrophilic count of ice stored octopuses are presented in Table 24 and Fig. 23. Similar to TPC, initial decrease in count was noticed with brief latency phase which last in about 3-5 days, after latency period gradual increase in counts was noticed. Initial average psychrophiles count was 3.39 log cfu/g which showed increase after latency period in all the samples, the counts at the end of storage period were lower in DOI (4.28 log cfu/g) followed by DPI (4.34 log cfu/g) and WOI (4.58 log cfu/g) samples.

**p) Organoleptic evaluation**

The sensory quality characteristics of ice stored octopuses as a function of ice storage period is given in Table 25, 26 and Fig. 24 and 25. The sensory score for various attributes like appearance, flavour, taste and texture registered a value of more than 8.00 for fresh sample. With increase in ice storage period the score for different attributes decreased and the overall acceptability score was found to be 3.1 in WOI in 21 days, 6.5 in DOI and 6.2 in DPI in 15 days of ice storage. The reduction of overall acceptability was steep after 15 days of ice storage in WOI sample.

### 4.3. Pearson correlation significance (P) between sensory scores and quality parameters of ice stored WOI, DOI and DPI samples

#### 4.3.1. Whole octopus

The observations on biochemical and microbiological parameters of whole octopus iced which were drawn in 3 day intervals and the overall acceptability scores with respect to the storage days are analysed for Pearson correlation matrix to know significant parameters of whole octopus iced (Table 27). There was a highly significant +ve correlation between the overall acceptability score and bio-chemical parameters viz., NPN \(r=0.940\), protein \(r=0.898\), SSN \(r=0.810\), WSN \(r=0.922\) were observed. Similarly highly significant –ve correlation between the overall acceptability score and bio-chemical parameters viz., FFA \(r=-0.762\), TPC \(r=-0.864\), lipids \(r=-0.881\), psy \(r=-0.897\), pH \(r=-0.898\), PV \(r=-0.908\), moisture \(r=-0.911\), TMA \(r=-0.961\), VBN \(r=-0.966\) and Hx \(r=-0.967\) were observed.

#### 4.3.2. Dressed and iced octopus during iced storage

The observations on biochemical and microbiological parameters of dressed and iced octopus which were drawn in 3 day intervals and the overall acceptability scores with respect to the storage days are analysed for Pearson correlation matrix to evaluate significant parameters of dressed and iced octopus (Table 28). There was a highly significant +ve correlation between the overall acceptability score and bio-chemical
parameters viz., protein \( r=0.995 \), pH \( r=0.986 \), moisture \( r=0.981 \), lipids \( r=0.958 \), NPN \( r=0.929 \), psy \( r=0.928 \), WSN \( r=0.922 \), TPC \( r=0.899 \), SSN \( r=0.861 \), Hx \( r=0.723 \), TMA \( r=0.670 \), FFA \( r=0.569 \) and significant +ve correlation with PV \( r=0.539 \) were observed. Whereas the overall acceptability score showed no significance with VBN \( r=0.366 \).

4.3.3. Dressed, packed and iced octopus

The Pearson correlation matrix to evaluate significant parameters of dressed, packed and iced octopus with respect to overall acceptability score is indicated in Table 29. There was a high significance between the overall acceptability score and biochemical parameters viz., protein \( r=0.991 \), moisture \( r=0.964 \), NPN \( r=0.962 \), lipids \( r=0.940 \), WSN \( r=0.931 \), pH \( r=0.914 \), psy \( r=0.879 \), SSN \( r=0.858 \), TPC \( r=0.850 \), Hx \( r=0.667 \), TMAN \( r=0.616 \), FFA \( r=0.516 \) and PV \( r=0.494 \) were observed. Whereas the overall acceptability score showed no significance with VBN \( r=0.323 \) during ice storage.

4.4. DEVELOPMENT OF VALUE ADDED PRODUCTS FROM OCTOPUS

4.4.1. PRODUCTION OF BATTERED AND BREADED OCTOPUS

The results of the studies on battered and breaded octopus are presented in this section.

4.4.1.1. Standardisation of spice mixture

The composition of different spice mixtures is given in the Table 30. Standardised spice mixture (chilli powder (2%), ginger (1%), garlic (1%) and salt (2%)) was selected from the taste panelist’s inference. Table 30A indicates ANOVA of different spice mixtures on the organoleptic quality of battered and breaded octopus.

4.4.1.2. Standardisation of batter

The amount of water that has to be added to make a batter of desired consistency and its flow rate is shown in Table 31 and the composition of standardised batter is given in Table 32. Maida gave batter of better consistency and was well accepted by the most of the panelists and the same was selected and adopted. Addition of 650 ml of water for 500 g maida flour gave better viscosity for the preparation of batter. This gave better adhesion to the fillets.

4.4.1.3. Changes in weight of meat during mixing and coating

On an average 46.47% increase in weight of meat during mixing and coating was observed (Table 33). The standard recipe for the production of battered and breaded octopus is given in Table 34. The changes in temperature of the meat during freezing are shown in Table 35 and Fig. 26.
4.4.2. PRODUCTION OF SMOKED OCTOPUS

The standardisation of smoking process, quality evaluation of smoked product as a function of frozen storage period was carried out. In this section the results of smoked octopus meat and it’s frozen storage studies are presented.

4.4.2.1. Standardisation of brining concentration and brining time for smoking of octopus

The sensory evaluation was done after smoking of brine salted octopus with reference to their salty taste. Based on the panelists inference 10% brine treatment for 4 min. was found to be optimum and acceptable (Table 36 & 36A), which had a salt content of 1.60%, as the salt concentration increases more salty taste was observed and products were less accepted by the panelists. The brining treatments were significant at p=0.01.

4.4.2.2. Standardisation of smoking time and temperature

Initially smoking was done at 80 °C for varying timings, resulted in insufficient cooking in bigger octopus. Later, smoking was tried at 90 °C for varying timings. The results obtained are shown in Table 37 & 37A. The smoking treatments were significant at p=0.01. Based on the opinion of the panelists smoking time of 135 min. was selected for smoking of octopus. Further smoking led to drying of surface and formation of tough texture.

Table 38 shows the results of analysis of variance on the mean sensory scores of smoked octopus meat due to attributes and smoking time and temperature combination. From the table it is seen that there was no significant difference among different smoking time (p < 0.05) and among different attributes.

4.4.2.3. Selection of wood saw dust

Smoke was generated by burning saw dust of mango, acacia and teak separately and in combination (Table 39 & 39A). Octopus mantles and tentacles were smoked and tested for organoleptic characteristics by a panel of 10 members, when the smoke from mango, acacia and teak saw dust were used separately; the product not only acquired the undesirable colors but also possessed unpleasant taste and flavour. The organoleptic characteristics of the products smoked by using the combination of the saw dust of mango, acacia and teakwood (in equal proportion) yielded product having good organoleptic characteristics such as golden yellow colour, acceptable taste and flavour. The different smoke treatments were significant at p=0.01. Hence this combination of saw dust was selected for further experiments.

Using standardized brine concentration and time, smoking temperature and time and mixture of saw dust, smoked octopus was prepared. During smoking the
temperature of kiln and meat core were measured and the results are shown in the Table 40 and Fig. 27.

Table 41 indicates the yield % of octopus meat at different unit operation of smoking. From the results it was observed that the final meat yield after smoking was 63.85%.

4.4.2.4. Effect of smoking on the bio-chemical composition and freshness parameters of octopus meat

The effect of smoking on the bio-chemical composition was reported in the Table 42. There was a reduction in moisture content, increase in protein content and nitrogenous compounds such as SSN and NPN. There was increase in VBN, decrease in TPC and FFA were also observed. Only marginal changes were observed in total lipids and PV of smoked octopus.

After smoking the smoked products were packed in polyethylene bags frozen at -35 °C and stored at -20 °C and its quality was evaluated at regular intervals.

4.5. Changes in quality of dressed octopus (control), battered & breaded octopus and smoked octopus during frozen storage

In this section the results obtained from the frozen storage studies of dressed octopus, battered & breaded octopus and smoked octopus are presented.

4.5.1. Bio-chemical changes:

4.5.1.1. Moisture

The changes in moisture content of dressed octopus, battered & breaded octopus and smoked octopus during frozen storage are given in Table 43 and Fig. 28. There was decrease in moisture content of all the samples, the decrease was very marginal (86.27 to 83.11%) in control and (67.10 to 64.44%) in smoked octopus but considerable (70.80 to 63.94%) in battered and breaded octopus. The decrease in moisture content were about 3.66%, 3.96% and 9.69% respectively in control, smoked octopus and battered & breaded octopus during 12 months of frozen storage at -20 °C. The results of ANOVA of changes in moisture content of prepared products during frozen storage are presented in Table 43A.

4.5.1.2. Total Nitrogen (TN)

The changes in total nitrogen content of dressed octopus, battered & breaded octopus and smoked octopus during frozen storage is given in Table 44 and Fig. 29. The total nitrogen content increased gradually from 1.91 to 2.31 g% in control, from 2.38 to 3.56 g% in battered & breaded octopus and from 4.54 to 5.00 g% in octopus. The increase in total nitrogen content were 20.94 %, 49.57 % and 10.13 % respectively in control, battered & breaded octopus and smoked octopus during 12 months of frozen storage.
storage at -20 °C. The results of ANOVA of changes in Total nitrogen content of prepared products during frozen storage are presented in Table 43A.

4.5.1.3. Total lipids

The variation in total lipids content of dressed octopus, battered & breaded octopus and smoked octopus during frozen storage is given in Table 45 and Fig. 30. The changes in total lipids of all the samples were very marginal. The total lipids varied from 0.97% to 0.62% in control, from 1.30% to 0.85% in battered & breaded octopus and from 1.25% to 0.88% in smoked octopus, the changes in lipids were 36.56%, 34.89% and 29.89% respectively in control, smoked octopus and battered & breaded octopus during 12 months of frozen storage at -20 °C. The results of ANOVA of changes in total lipids during frozen storage are presented in Table 45A.

4.5.1.4. Salt soluble nitrogen

Changes in salt soluble nitrogen content of stored samples during 12 months of frozen storage at -20 °C are given in Table 46 and Fig. 31. In control sample the SSN decreased from 61.75% of to 57.78 % of TN, in battered and breaded octopus the SSN decreased from 60.34 to 58.65 % of TN and in smoked octopus the SSN decreased from 59.74 to 57.22 % of TN contributing total SSN loss of 3.97%, 1.69% and 2.52% of TN respectively. Table 46A represents the ANOVA of changes in SSN content of prepared products during frozen storage.

4.5.1.5. Non protein nitrogen

The pattern of changes in NPN of dressed octopus, battered & breaded octopus and smoked octopus during frozen storage at -20 °C is given in Table 47 and Fig. 32. Gradual decreases in NPN of all the samples were noticed. The NPN decrease was 1.54% of TN in control, followed by 1.59% of TN in battered and breaded octopus and 0.52% of TN in smoked octopus. However, the decreases in NPN of all the samples during frozen storage were very marginal. Table 47A indicates the ANOVA of changes in NPN of prepared products during frozen storage.

4.5.1.6. Peroxide value

The changes in PV of dressed octopus, battered & breaded octopus and smoked octopus during frozen storage at -20 °C is given in Table 48 and Fig. 33. There was increase in PV of all the samples were noticed. The increase from 7.21 to 33.59 millimoles of O₂ / kg fat in control, from 13.00 to 35.76 millimoles of O₂ / kg fat in battered and breaded octopus and 7.57 to 29.46 millimoles of O₂ / kg fat in smoked octopus were recorded. Higher PV was noticed in battered and breaded octopus at the end of storage period of 12 months. Table 48A represents the ANOVA of changes in PV of prepared products during frozen storage.
4.5.1.7. Free fatty acids

The Table 49 and Fig. 34 represent the changes in FFA value during the frozen storage at -20 °C. The FFA value increased from 2.84 to 17.91 % of oleic acid in control sample, 1.19 to 13.56% of oleic acid in battered and breaded octopus and from 1.21 to 20.93 % of oleic acid in smoked octopus. The rate of increase was more in smoked octopus and followed by battered and breaded octopus and the least was recorded by the control at the end of 12 months of frozen storage. Table 49A indicates the ANOVA of changes in free fatty acids of prepared products during frozen storage.

4.5.1.8. Alpha amino nitrogen

The variation in the alpha amino nitrogen of the prepared products is shown in Table 50 and Fig. 35. Table 50A indicates the ANOVA of changes in AAN of prepared products during frozen storage. Considerable decrease in AAN during frozen storage at -20 °C was noticed in all the samples, in control the AAN decreased from 189.45 to 63.45 mg%, in battered and breaded octopus decreased from 203.25 to 71.60 and in smoked octopus decreased from 280.43 to 122.24 mg%. The decrease (66.51%) was more in control sample followed by battered and breaded octopus (64.77%) and then by smoked octopus (56.41%).

4.5.1.9. Total volatile base nitrogen

The Table 51 and Fig. 36 indicate the changes in VBN of octopus prepared products during frozen storage at -20 °C. Table 51A indicates the ANOVA of changes in TVBN of prepared products during frozen storage. Gradual increases in the VBN of all the samples were observed. The rate of increase was more at the beginning of the storage up to 7 months later there was decrease in the rate was recorded. In control sample the values increased from 2.35 to 15.64 mg%, the VBN increased from 3.18 to 18.53 mg% in Battered and breaded octopus and the VBN increased from 8.40 to 19.69 mg% in smoked octopus during 12 months frozen storage.

4.5.1.10. pH

The changes in pH of dressed octopus, battered & breaded octopus and smoked octopus during frozen storage at -20 °C is given in Table 52 and Fig. 37. Table 52A indicates the ANOVA of changes in pH during frozen storage. Marginal increases in pH of all the prepared products after small initial decrease were recorded (6.13 to 6.51). More decrease in control sample at the beginning and slight increase at the end of the experiment was observed. In battered & breaded octopus and smoked octopus the increase in pH was very negligible. Although a very marginal increase in pH of octopus was noticed in all the samples during frozen storage.
4.5.2. Total phenols

The changes in total phenol content of smoked octopus during frozen storage at -20 °C are given in Table 53 and Fig. 38. There was a decrease in steam volatile phenols and steam non volatile phenols of smoked octopus were observed. Initially the steam volatile phenol content was 10.70 mg / 20g which decreased to 5.21 mg / 20g. The steam non volatile phenols decreased from 5.01 mg / 20g to 2.53 mg / 20g at the end of 12 months of frozen storage.

4.5.3. Microbiological analysis:

4.5.3.1. Total plate count

The changes in TPC of dressed octopus, battered & breaded octopus and smoked octopus during frozen storage at -20 °C is given in Table 54 and Fig. 39. Table 54A indicates the ANOVA of changes in TPC of dressed octopus, battered & breaded octopus and smoked octopus during frozen storage. There was decrease in TPC in all the samples. The decrease in TPC was rapid in the initial stage up to 4 months. Initially, much lower count was observed in smoked octopus (4.10x10² cfu/g) followed by control (1.69x10³ cfu/g) and higher TPC was observed in battered and breaded octopus (5.80x10³ cfu/g). Finally the counts were 9.00x10¹, 5.50x10² and 2.20x10¹ respectively in control, battered and breaded and smoked octopus samples.

4.5.3.2. Salmonella spp.

The results of the analysis of Salmonella spp. in prepared products during frozen storage are presented in Table 55. Salmonella spp. were absent in the raw material. Analysis for the presence of Salmonella spp. in raw material, after freezing and during frozen storage of 12 months showed absence in all the products and confirmed their absence by biochemical tests.

4.5.3.3. Vibrio cholerae and Vibrio parahaemolyticus

The results of the analysis of Vibrio cholerae and Vibrio parahaemolyticus in prepared products during frozen storage are presented in Table 56. It is clear from the biochemical tests of Vibrio cholerae and Vibrio parahaemolyticus species were present in the raw material. In smoked product both the species were absent throughout the frozen storage. Whereas, both the species were found to be present in control & battered and breaded product after freezing and before keeping in to the frozen storage but found to be absent in the subsequent 12 months of frozen storage.

4.5.4. Sensory quality characteristics

The sensory quality characteristics of control, battered & beaded octopus and smoked octopus as a function of frozen storage period is given in Table 57 and Fig. 40. Table 57A indicates the ANOVA of changes in overall acceptability scores of prepared products during frozen storage. With the increase in frozen storage period the scores for
different attributes decreased. The sensory score for different attributes like appearance, flavour, taste, texture and overall acceptability registered a value of more than 8.30 for the control sample which decreased to 7.14. Initial score 8.40 for battered & breaded octopus which decreased to 7.60 and initial score of 8.30 for smoked octopus which decreased to 7.30 at the end of 12 months of frozen storage at -20 °C. Much lower acceptance was observed in control sample throughout the storage period and battered & breaded octopus could retain its acceptability for longer time.

4.5.5. The Pearson correlation significance (P) between sensory scores and biochemical and microbiological parameters of prepared and frozen stored octopus products.

4.5.5.1. Control sample

The Pearson correlation matrix to evaluate significant parameters of control sample is indicated in Table 58. There was a highly significant +ve correlations between the Overall acceptability score and biochemical, microbiological parameters viz., moisture ($r=0.970$), SSN ($r=0.958$), lipids($r=0.940$), NPN ($r=0.935$), AAN ($r=0.849$), protein ($r=0.834$) and TPC ($r=0.774$) were observed. Similarly there was a highly significant –ve correlation was found between the overall acceptability scores and biochemical parameters like PV ($r=-0.990$), FFA ($r=-0.989$), DL ($r=-0.983$) and VBN ($r=0.910$). This clearly indicate that different biochemical and microbiological parameters have their own influence either the way on the overall acceptability score given by the panelists. Whereas the overall acceptability score showed –ve association with pH ($r=-0.24$) and was non significant.

4.5.5.2. Battered and breaded octopus

The Pearson correlation matrix to evaluate significant parameters of battered and breaded octopus is indicated in Table 59. There was a highly significant +ve correlations between the overall acceptability score and biochemical parameters viz., NPN ($r=0.952$), SSN ($r=0.942$), AAN ($r=0.913$), protein ($r=0.907$), moisture ($r=0.902$), lipids($r=0.890$), and TPC ($r=0.886$) were observed. Similarly, there was a highly significant –ve correlation between the overall acceptability scores and biochemical parameters like FFA ($r=-0.950$), PV ($r=-0.915$), VBN ($r=-0.897$), and pH ($r=-0.0581$). This clearly indicate that different biochemical and microbiological parameters have their own influence either the way on the overall acceptability score given by the panelists.

4.5.5.3. Smoked octopus

The Pearson correlation matrix to evaluate significant parameters of smoked octopus is indicated in Table 60. Highly significant +ve correlations between the overall acceptability scores and biochemical parameters viz., moisture ($r=0.957$), SSN ($r=0.937$), protein ($r=0.933$), lipids ($r=0.920$), AAN ($r=0.913$), NPN ($r=0.906$), phenols
and TPC \((r=0.820)\) were observed. Similarly, there was a highly significant \(–\text{ve}\) correlation was found between the overall acceptability scores and bio-chemical parameters like FFA \((r=-0.970)\), PV \((r=-0.968)\), VBN \((r=-0.879)\), and pH \((r=-0.685)\). This clearly indicate that different bio-chemical and microbiological parameters have their own influence either the way on the overall acceptability score given by the panelists.

4.6. EFFECT OF PRETREATMENT ON THE FROZEN STORAGE STABILITY OF OCTOPUS

Octopus was dip treated in 5\% salt solution for firming the meat before giving further pre treatments with different chemicals.

4.6.1. Standardisation of sodium tri-poly phosphate treatment

The concentration of sodium tri-poly phosphate treatment was standardised, the results obtained are presented in Table 61. Based on the drip loss and panelist’s inference, 4\% sodium tri-poly phosphate treatment for 5 min. was found to be acceptable. However, the different treatments were non significant (Table 61A).

4.6.2. Standardisation of sodium citrate treatment

The concentration of sodium citrate treatment was standardised, the results obtained are presented in Table 62 & 62A. Based on the drip loss and panelist’s inference, 3\% sodium citrate treatment for 5 min. was found to be acceptable.

4.6.3. Changes quality of pretreated octopus during frozen storage:

In this section the results obtained from the frozen storage studies of pretreated octopus and control octopus are presented.

4.6.4. Bio-chemical changes:

4.6.4.1. Moisture

The change in moisture content is presented in Table 63 and Fig. 41. There was a minimum fluctuation in moisture content during frozen storage. However, decrease in moisture content was observed in all the samples during frozen storage. The decrease was 2.23\%, 2.56\%, 2.83\% and 3.66\% respectively in STPP, SC, \(H_2O_2\) and control samples. The results of analysis of variance on the changes in moisture content of pretreated meat stored at \(-20\ °\text{C}\) are given in Table 63A. From the table it is clear that there was no significant difference among storage months and treatments.

4.6.4.2. Total Nitrogen

The changes in total nitrogen content are presented in Table 64 and Fig. 42. An increase in total nitrogen content in all the samples during frozen storage was observed. The TN increased from 1.93 to 2.46 g\% in STPP, from 1.93 to 2.62 g\% in SC, from 1.91 to 2.42 g\% in \(H_2O_2\) and from 1.91 to 2.31 g\% in control. The increase was 27.46\%, 34.19\%, 26.70\% and 20.94\% respectively in STPP, SC, \(H_2O_2\) and control samples during 12 months of storage. The rate of increase was less at the initial stages of storage.
and more after 3rd month. The increase in TN is mainly due to reduction in moisture content of all the samples during frozen storage. The results of analysis of variance on the changes in total nitrogen content of pretreated octopus meat stored at -20 °C are given in Table 64A. From the table it is clear that there is no significant difference among storage months and between treatments / samples.

4.6.4.3. Total lipids

The change in total lipids of pretreated octopus is presented in Table 65 and Fig. 43. There was a gradual decrease in total lipids during frozen storage. However, the decrease was very marginal (0.97% - 0.63%) in all the samples after 12 months of frozen storage. The results of analysis of variance on the changes in total lipids of pretreated meat stored at -20 °C are given in Table 65A. From the table, it is clear that there is significant difference among storage months and no significant difference among the treatments were observed.

4.6.4.4. Salt soluble nitrogen

The salt soluble nitrogen extracted at different period of frozen storage is given in Table 66 and Fig. 44. There was gradual reduction in SSN from 59.87 to 58.44% of TN in STPP, from 60.89 to 58.62% of TN in SC, from 59.70 to 57.82 % of TN in H₂O₂ and from 60.75 to 57.8% of TN in control samples were observed. The rate of reduction was more in control than in the dip treated samples. Between the treated samples the reduction in SSN during frozen storage was high in H₂O₂ sample and the least was in SC. Table 66A shows the results of analysis of variance on the changes in SSN of pretreated and frozen octopus stored at -20 °C for 12 months. There was significant difference in the SSN among treatments / samples at 5% level of significance.

4.6.4.5. Non-Protein nitrogen

The changes in NPN content as a function of frozen storage period is presented in Table 67 and Fig. 45. The NPN content of all pretreated samples showed gradual decreasing trend during 12 months of frozen storage at -20 °C. The reductions were 1.36, 1.08, 1.03 and 1.52% of TN respectively in STPP, SC, H₂O₂ and control. The results of analysis of variance of NPN changes during frozen storage, clearly indicated that there was significant difference (p=0.05) in NPN value among different treatments / samples (Table 67A).

4.6.4.6. Peroxide value

The changes in peroxide values of pretreated and frozen storage samples are presented in Table 68 and Fig. 46. Gradual increase in peroxide value of all the samples was recorded. The increase was more in control and H₂O₂ treated samples compared to STPP and SC samples. STPP recorded the least change during frozen storage. The increases in PV from 6.42 to 27.87 in STPP, from 6.25 to 29.19 in SC, from 6.81 to 32.81
and from 7.21 to 33.59 millimoles of O\textsubscript{2} / kg fat were recorded at the end of 12 months of frozen storage. The results of analysis of variance of PV changes in pretreated octopus during frozen storage, clearly indicated that the PV in relation to the storage months are significantly different (p = 0.01) and not between the samples (Table 68A).

**4.6.4.7. Free fatty acid**

The changes in free fatty acid content of the pretreated octopus during frozen storage are presented in the Table 69 and Fig. 47. Steady increase in FFA of all the samples during frozen storage was noticed. The rate of increase was slightly higher in control than the treated samples. The increase in FFA from 1.25 to 13.65 in STPP, from 2.51 to 12.93 in SC, from 1.83 to 16.23 and from 2.84 to 17.91 % of oleic acid were recorded at the end of 12 months of frozen storage. Table 69A indicates the results of analysis of variance of FFA due to storage months and pretreated samples. The analysis indicated that there is significant difference between storage months (p = 0.05) but not between samples.

**4.6.4.8. Alpha amino nitrogen**

The AAN content of the pretreated and frozen stored octopus meat samples as a function of frozen storage period is given in Table 70 and Fig. 48. The AAN values decreased from an initial value of 184.64 to 99.96 in STPP, 189.45 to 99.96 mg% in SC, 198.24 to 119.56 mg% in H\textsubscript{2}O\textsubscript{2} and 198.24 to 113.68 in control samples respectively at the end of 12 months of frozen storage. The analysis of variances on the changes in AAN during frozen storage indicated that values were significantly different (p=0.01) between storage months and between treated samples (Table 70A).

**4.6.4.9. Total volatile base nitrogen**

The total volatile base nitrogen content of the pretreated and frozen stored octopus meat samples as a function of frozen storage period is given in Table 71 and Fig. 49. The TVBN values increased from an initial value of 1.57 to 13.90 mg% in STPP, 1.31 to 12.16 mg% in SC, 1.83 to 14.77 mg% in H\textsubscript{2}O\textsubscript{2} and 2.35 to 15.64 mg% in control samples during 12 months of frozen storage. The analysis of variances on the changes in TVBN of pretreated samples during frozen storage indicated that values were significantly different (p=0.01) between storage months and not between the treated samples (Table 71A).
4.6.4.10. pH

The pH of the pretreated and frozen stored octopus meat samples as a function of frozen storage period is given in Table 72 and Fig. 50. The pH values showed more fluctuation in all the samples. The values fluctuated between value of 7.01 to 7.36 in STPP, from 6.64 to 7.03 in SC, from 6.22 to 6.42 in H₂O₂ and from 6.13 to 6.30 in control samples during 12 months of frozen storage. The analysis of variances on the changes in pH of pretreated octopus during frozen storage indicated that values were not significantly different between storage months and between the samples (Table 72A).

4.6.4.11. Drip loss

Table 73 and Fig. 51 represent the changes in drip loss of pretreated octopus samples during frozen storage. The drip loss steadily increased in all the samples with time but the rate of increase was slightly higher in control than in treated samples. Comparatively lower drip losses were recorded in STPP and SC samples. The drip loss increased with an initial value of 7.11 to 13.43 % in STPP, from 5.00 to 12.34 % in SC, from 7.60 to 16.86 % in H₂O₂ and from 7.85 to 17.20 % in control sample.

Table 73A gives results of the analysis of variance of drip loss in pretreated octopus due to different storage months and samples. It was found that there was no significant difference between storage months and between samples.

4.6.4.12. Cook loss

Table 74 and Fig. 52 indicate the changes in cook loss of pretreated octopus samples during frozen storage. The cook loss showed decrease in all the samples with time but the rate of decrease was slightly higher in control and H₂O₂ than in treated samples. Comparatively lower cook losses were recorded in STPP and SC samples. The cook loss decreased with an initial value of 54.26 to 50.34 % in STPP, from 52.39 to 45.45 % in SC, from 63.24 to 55.43 % in H₂O₂ and from 66.37 to 56.24 % in control sample.

Table 74A gives results of the analysis of variance of cook loss due to different storage months and samples. It was found that there was significant difference between storage months and between samples (p=0.05).

4.6.4.13. SDS-PAGE analysis

The SDS-PAGE pattern of total proteins extracted from raw, frozen, pretreated and frozen stored octopus samples of different periods is given in Fig. 53. The concentration of proteins decreased with the increase in storage period. The emergence of protein components in the molecular weight range of 24 KD, 55 KD and 116 KD were prominent with SC sample. With this treatment the concentration of various molecular weight proteins the alteration or degradation was minimum during frozen storage. The disappearance of different molecular weight components were observed in STP followed
by H₂O₂ samples and very less bands was observed in control sample at the end of 12 months of frozen storage. Among the pretreated samples SC treatment has shown better cryoprotective effect during frozen storage.

4.6.5. Total plate count

The total plate counts estimated during frozen storage of the three samples are presented in the Table 75 and Fig. 54. The TPC showed a significant decreasing trend in all the samples during 12 months of frozen storage. The colony forming unit was decreased with initial counts from 1.11x10³ to 7.30x10¹ cfu/g in STPP sample, from 1.02x10³ to 8.80x10¹ in SC, from 1.54x10³ to 5.50x10¹ in H₂O₂ and from 1.69x10³ to 9.00x10¹ in control sample at the end of 12 months of frozen storage. The analysis of variances on the changes in TPC during frozen storage indicated that values were not significantly different between storage months and between the samples (Table 75A).

4.6.5.1. Salmonella spp.

The results of the analysis of Salmonella spp. in pretreated and frozen storage octopus are presented in Table 76. Salmonella spp. were absent in the raw material. Analysis of Salmonella spp. for their presence after freezing and during frozen storage of 12 months in all the samples showed absence and confirmed their absence by biochemical tests also.

4.6.5.2. Vibrio cholerae and Vibrio parahaemolyticus

The results of the analysis of Vibrio cholerae and Vibrio parahaemolyticus in pretreated octopus during frozen storage are presented in Table 77. Both Vibrio cholerae and Vibrio parahaemolyticus species were present in the raw material. In STPP and H₂O₂ both the species were absent after freezing and throughout the frozen storage. Whereas, both the species were found to be present in SC and Control samples after freezing and before keeping in to the frozen storage, but they were found to be absent in the subsequent frozen storage of 12 months.

4.6.6. Sensory quality characteristics

The sensory quality characteristics of pretreated octopus as a function of frozen storage period are given in Table 78 and Fig. 55. The sensory score for different attributes like appearance, flavour, taste and texture registered a value of more than 8.5 for the fresh sample. With the increase in frozen storage period the scores for different attributes decreased, at the end of the 12 months the SC sample has scored comparatively higher score (7.28) than the other samples and Control sample has scored lower score (7.14) than the other samples. Table 78A gives results of the analysis of variance of overall acceptance scores due to different storage months and samples. It was found that there was significant difference between storage months (p=0.05) but not between the samples.

4.6.7. The Pearson correlation significance between sensory scores and biochemical and microbiological parameters of pretreated and frozen stored octopus:
4.6.7.1. STPP sample

The Pearson correlation matrix to evaluate significant parameters of STPP treated octopus is indicated in Table 79. There was a highly significant +ve correlations between the Overall acceptability score and bio-chemical parameters viz., moisture \( r=0.982 \), SSN \( r=0.978 \), NPN \( r=0.970 \), AAN \( r=0.928 \), protein \( r=0.897 \), lipids \( r=0.810 \) and TPC \( r=0.796 \) were observed. Similarly there was a highly significant –ve correlation between the overall acceptability scores and bio-chemical parameters like FFA \( r=-0.996 \), PV \( r=-0.986 \), DL \( 0.977 \), and VBN \( r=-0.944 \). These clearly indicate that different bio-chemical and microbiological parameters have their own influence either way (+ve or –ve) on the overall acceptability score given by the panelists. Whereas, the overall acceptability score showed –ve association with pH \( r=-0.195 \) and was non significant.

4.6.7.2. SC sample

The Pearson correlation matrix to evaluate significant parameters of sodium citrate treated octopus is indicated in Table 80. A highly significant +ve correlations between the Overall acceptability score and bio-chemical parameters viz., NPN \( r=0.986 \), moisture \( r=0.985 \), SSN \( r=0.984 \), AAN \( r=0.913 \), lipids \( r=0.862 \) and protein \( r=0.787 \) were observed. Even though TPC showed +ve correlation \( r=0.392 \) it was non significant. Similarly there was a highly significant –ve correlation between the overall acceptability scores and bio-chemical parameters like DL \( r=-0.991 \), PV \( r=-0.991 \), FFA \( r=-0.983 \), VBN \( r=-0.910 \), and pH \( r=-0.707 \). These clearly indicate that different bio-chemical and microbiological parameters have their own influence on the overall acceptability score given by the panelists.

4.6.7.3. H\(_2\)O\(_2\) sample

The Pearson correlation matrix to evaluate significant parameters of H\(_2\)O\(_2\) sample is indicated in Table 81. There was a highly significant +ve correlations between the overall acceptability score and bio-chemical parameters viz., moisture \( r=0.975 \), SSN \( r=0.966 \), NPN \( r=0.952 \), AAN \( r=0.894 \), lipids \( r=0.852 \), and protein \( r=0.785 \). Even though TPC showed +ve correlation \( r=0.381 \) it was non significant. Similarly, there was a highly significant –ve correlation was found between the overall acceptability scores and bio-chemical parameters like FFA \( r=-0.983 \), PV \( r=-0.978 \), DL \( r=-0.978 \), and VBN \( r=-0.942 \). The pH \( r=-0.442 \) showed –ve correlation with overall acceptability score and was not significant. These clearly indicate that different bio-chemical and microbiological parameters have their own influence either the way (+ve or –ve) on the overall acceptability score given by the panelists.
4.6.7.4. Control sample

The Pearson correlation matrix to evaluate significant parameters of control sample is indicated in Table 82. There was a highly significant +ve correlations between the overall acceptability score and bio-chemical parameters viz., moisture (r=0.970), SSN (r=0.958), lipids(r=0.940), NPN (r=0.935), AAN (r=0.849), Protein (r=0.834) and TPC (r=0.774). Similarly, a highly significant –ve correlation was found between the overall acceptability scores and bio-chemical parameters like PV (r=-0.99), FFA (r=-0.989), DL (r=-0.983) and VBN (r=0.910). These clearly indicate that different bio-chemical and microbiological parameters have their own influence on the overall acceptability score given by the panelists. Whereas the overall acceptability score showed –ve association with pH (r=-0.24) and it was non significant.

V. DISCUSSION

In the present investigation the composition of octopus, ice storage characteristics, effect of pretreatments on frozen storage stability, preparation of different value-added products and their storage stability has been attempted. In this section the results obtained have been critically discussed with reference to changes in biochemical, microbiological and sensory quality characteristics during different stages of processing and storage. Since references pertaining to above studies on octopus are scanty, wherever comparison is not possible with other cephalopods the results of the present study are compared with the changes already reported in other seafoods like fish and shellfishes.

5.1. Physical characteristics

Octopus used for different studies differed in their physical characteristics as indicated in Table 6. The whole octopuses used in this study were ranged from 19.8 to 71.0 cm and the weight ranged from 30.0 to 642.0 g. The dorsal mantle length varied between 5.5 -14.0 cm. and were average commercial size. The dressed octopus yield was ranged from 65.6 to 77.9%. Moral et al., (2002) have noticed 60-80% of edible portion in cephalopods depending on the species, size and sexual maturity stage. Further, they have noticed short life cycle (in most cases 8 months to 2 years) which can bring considerable change in muscle protein content. The average dressed octopus counts per kg were ranged from 5 to 25 numbers. It was found that the yield of edible meat from the octopus depends on various factors like size, season, sexual maturity stage, processing methods etc. Smaller specimens were tastier and having more moisture content and less yield. Females during breeding seasons yielded less due to loss of weight of gonads. Smaller specimens were used for the ice storage study and