EFFECT OF SODIUM BI-CARBONATE CONTAINING CITRIC ACID AND SODIUM CHLORIDE ON YIELD AND CHARACTERISTICS OF TIGER SHRIMP (*Penaeus monodon*)

A Thesis

Submitted to the

West Bengal University of Animal and Fishery Sciences

In partial fulfillment of the requirement for the degree of

Master of Fishery Sciences

In

FISH PROCESSING TECHNOLOGY

By

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2015

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled **"Effect of sodium bi-carbonate containing citric acid and sodium chloride on yield and characteristics of Tiger Shrimp (*Penaeus monodon*)"** submitted by **Arka Guha** in partial fulfillment of requirement for the degree of **Master of Fishery Sciences (Fish Processing Technology)** in the Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, is the faithful and bonafide research work carried out under my supervision and guidance. The results of the investigation reported in this thesis have not so far been submitted for any other degree or diploma. The assistance and help received during the course of investigation have been duly acknowledged.

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APPROVAL SHEET

Approval of the examiners for the award of the degree of Master of Fishery Science

(Fish Processing Technology)

We, the undersigned have been satisfied with the performance of **Arka Guha,** in the viva–voce examination, conducted today. the 06th October, 2015. recommended that the thesis be accepted for the award of the degree.

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ACKNOWLEDGEMENT

I feel honoured to convey my deepest sense of gratitude and fathomless regards to my reverend guide Dr. Sreekanta Sarkar, Associate Professor, Department of Fish Processing Technology, Faculty of Fishery Sciences for his consistent guidance, constructive suggestions, motivation, patience and enthusiasm. His guidance made me capable of planning and conducting the present study and preparation of this thesis.

I express my deepest gratitude and proud gratefulness to the members of the Advisory Committee Prof. K. C. Dora, Department of Fish Processing Technology, Department of Aquatic Environment Management, Dr. Somen Sahu, Department of Fisheries Economics and Statistics, Dr. Batabyal, Department of Veterinary Biochemistry for their useful suggestions rendered throughout the course of this work.

I express my deepest sense of appreciation and indebtedness to Dr. Supratim Chowdhury, Assistant Professor, Department of Fish Processing Technology.

I sincerely acknowledge the Vice-Chancellor and Registrar, West Bengal University of Animal and Fishery Sciences, for providing opportunity and necessary facilities for the successful completion of the research work.

I sincerely acknowledge the Dean, Prof. B. K. Das for providing opportunity and necessary facilities for the successful completion of the research work.

I would like to acknowledge Mr. Monoj K. Das and Papai Da, Librarian, Faculty of Fishery Sciences for extending all help to avail the library facility.

I would like to express my sincere thanks to all the departments and AICRP staffs, especially Partha Da, Jhantu Da, Jayanta Da, Alok Da, Taposh Da, Piyali Di, Mithu Di for all their kind help that supported me during this study.

I wish to extend my indebtedness and heartiest thanks to my seniors Tirtha Da, Suman Da, Anniruddha da, Prasanta Da, Amit Da, Ranjit Da, Tanushri Di and Kuheli Di for all those immortal moments that we spent together in this campus, which I will carry with me throughout my life.

I sincerely extend my gratefulness to Nazrin, Pritam and Antara for their continuous support to me throughout all these years in this faculty and during this study.

Specially I express my heartiest thanks to Sayantani for helping me in every bit of time to complete my thesis work.

I extend my heartiest thanks to my friends Sudeshna, Madhurima, Supriti, Tanushree, Puspendu, Nandan, Susovan, Pulak, Sourav, Puspendu, Nihar, Bongo, soham for their kind co-operation rendered to me from time to time throughout the period of study.

I extend my heartiest thanks to all of my juniors for their heartiest co-operation.

Last but not the least, I find no words to express my sincerest gratitude and affection to my beloved Maa, Baba, Dida, Chotojethu, Pisimoni, Pisamosai, Dada, Boumoni, Nanga, Rangakaki, Piku, Sonakaka, Sonakaki, Pukku, Rajesh & all family members for their patience, inspiration, sacrifice, faith, mental support and silent prayer for my success without which it would not have been possible to achieve this goal.

I ascribe all glory to the gracious almighty from whom all good things come, who has showered all his kind blessings and benevolent graces upon me in life, for bestowing me with the blessings to see this day.

God is kind enough to bless and guide me throughout my work.

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LIST OF ABBREVIATIONS:-

ANOVA	Analysis of variance
AOAC	Association of analytical chemist
APHA	American public health association
BSA	Bovine serum albumin
°C	Degree centigrade
CFU	Colony forming unit
cm	Centimeter
EU	European Union
FDA	Food and Drug Administration
g	Gram
GMP	Good Manufacturing Practice
GRAS	Generally recognized as safe
HL	Headless
hr	Hour
MDA	Malonaldehyde
mEq	Milli equivalent
mg	Milligram
min	Minute
MPEDA	Marine Product Export Development Authority
NPN	Non protein nitrogen
PV	Peroxide value
RO	Reverse osmosis
SAPP	Sodium acid pyrophoaphate
SD	Standard deviation
SHMP	Sodium hexa-metaphosphate
STPP	Sodium tripolyphosphate
TBARS	Thio barbituric acid reactive substances
TCA	Tri chloro acetic acid
TMAN	Trimethyl amine nitrogen

TMAO	Trimethyl amine oxide
TPC	Total plate count
TSP	Trisodium phosphate
TSPP	Trisodium polyphosphate
TVBN	Total volatile base nitrogen
US	United States
WHC	Water holding capacity

CHAPTER – I

INTRODUCTION

The Indian food processing industry is one of the largest in the world in terms of production, consumption, export and growth prospects. A major portion of processing industry deals with the seafood processing. Earnings increased 90% in export of shrimp during April-December in the financial year 2014. The share of shrimps in total seafood exports earnings scaled to 65% from 51% in a year according to Marine Products Export Development Authority (MPEDA).

Shrimp is very popular in the world, with high nutritional value, what explains the high demands on the part of consumers. On the other hand, seafood are highly susceptible to both chemical and microbiological deterioration due to its high water content, neutral pH, large quantities of free amino acids, and naturally presence of autolytic enzymes (Fang *et al.*, 2013). Shortly after the capture, a series of complex alterations occurs on the surface and inside the edible portion of all seafood, resulting in a decrease of its quality (Tsironi *et al.*, 2009). Therefore, shrimp should be frozen to limit the microbial and enzymatic activity which causes deterioration and consumers should be able to obtain "frozen seafood products" of high quality, best appearance and little weight loss (Gonçalves *et al.*, 2008).

The quality deterioration in frozen shrimp is mainly due to lipid oxidation, protein denaturation, and dehydration (Sundararajan, 2010). These changes can result in the development of off-flavors, toughening and reduced water-holding capacity of shrimp (Boonsumrej *et al.*, 2007). Industry is therefore vitally interested in factors controlling these problems because reduced product quality results in reduced consumer acceptance (Benjakul *et al.*, 2001). Thus, the seafood processing companies have a great concern in retaining this water, first for economic reasons (seafood is sold by weight) and secondly, for the quality of the final product, because an excessive loss of water generates an undesirable appearance. (Masniyom, 2011).

Functional ingredients include a variety of additives or ingredients from vegetable and animal sources which are used to achieve different functionalities on the final product. Main functionalities to be achieved by means of those ingredients are presented by water

holding capacity and binding properties (i.e. increase adhesion among meat parts or in minced meat systems), texture modulation (i.e. increase tenderness). Last but not the least, functional ingredients offer possibilities to lower formulation cost by means of adding water to the meat, increase processing yield in product formulation.

The ability of muscle to absorb the added water during processing and capacity of retaining the water after cooking and freezing are the important factors governing the quality of seafood and seafood products. Commercial practices have evolved to control, add and retain moisture during harvest, processing, distribution, storage and preparation (Schubring *et al.*, 2003; Toldra, 2003).

To overcome all those problems seafood processors use additives. The most commonly used additive is compound of phosphates like Sodium tri-polyphosphate ($Na_5P_3O_{10}$). Phosphate is widely used to promote water-binding capacity and reduce cooking loss on meat, fish and seafood products (Kijowski *et al.*, 1988; Tehnet *et al.*, 1981; Thorarinsdottir *et al.*, 2004). The effectiveness of phosphates on water-holding properties of meat products depends on the type and quantity of phosphate on the specific food product. The role of phosphate may be due to the effects on pH and ionic strength and the interaction of phosphate ions with divalent cations and myofibrillar proteins (Thorarinsdottir *et al.*, 2004).

Among the functional properties changed by the treatment of phosphates in seafood and its products are:

(a) The retention of the moisture and natural flavor, inhibiting the loss of fluids during the distribution and the commercialization,

(b) Stabilize the protein structure of seafood, form a surface layer of coagulated (solid) protein, swell muscle fibers and solubilize muscle proteins,

(c) Enhancing WHC and tenderness of seafood by restricting protein denaturation,

(d) The inhibition of the process of lipid oxidation (by the chelation of metallic ions),

(e) The stabilization of the color, and

(f) The cryo-protection, which contributes to the extension of its shelf life (Love *et al.*, 1966; Applewhite *et al.*, 1993).

Jantranit and Thipayarat (2009) reported that a film of Sodium tripolyphosphates (STPP) and protein at surface formed to increase diffusion of phosphate and develop water binding capacity. Some part of these phosphate molecule binds with protein while others get attached with water molecule. But as per the European Union regulation phosphate in terms of P_2O_5 residue is limited to 0.5g/100g sample for all seafood product (Thorarinsdottir *et al.*, 2004). Therefore, there is a need to search for non-phosphate additives capable of exhibiting similar properties as polyphosphates.

The non-phosphate additive, sodium bicarbonate was reported to be effective in improving the water-holding capacity, color, and organoleptic properties of fresh meats, beef, pork and poultry (Ahn *et al.*, 1992; Boles *et al.*, 1993; Kauffman *et al.*, 2000; Kauffman *et al.*, 1998).

The present study deals with tiger shrimp (*Penaeus monodon)* which were treated with a solution of sodium bicarbonate with traces of citric acid and salt to reduce cook loss and increase weight. 1%, 2%, 3%, 4% sodium bicarbonate solution was prepared and citric acid and salt was added. Each concentration had four different time schedule from 1hr to 4 hr. The best result from each concentration was considered for frozen storage study. The objective of the research are:

1. To study the effect of sodium bicarbonate containing citric acid and sodium chloride on yield of headless (HL) *Penaeus monodon* using different levels of sodium bicarbonate.
2. To study the sensory parameters due to application of sodium bicarbonate.
3. To study the quality changes during frozen storage.

CHAPTER – II

REVIEW OF LITERATURE

Shrimp, one of the most important sources of animal protein, has been adopted for the maintenance of healthy body (Ravichandran *et al.*, 2012), but it is also one of the most perishable of all the foods (Ojutiku *et al.*, 2009). Shrimp is very popular in the world, with high nutritional value, what explains the high demands on the part of consumers (Moawad *et al.*, 2013), but are highly susceptible to both chemical and microbiological deterioration due to its high water content, neutral pH, large quantity of free amino acids and naturally presence of autolytic enzymes. (Fang *et al.*, 2013). Freezing and frozen storage is able to prevent microbial spoilage, but it cannot terminate chemical and Physical deteriorations (Ali, 2011). The quality deterioration in frozen shrimp is mainly due to lipid oxidation, protein denaturation and dehydration (Sundararajan, 2010). Which results in the development of off-flavours, toughening and reduced water binding capacity. To overcome these problems, some components are used in the time of processing of shrimps to reduce deteriorations and increase water holding capacity, reduce drip loss and facilitate weight gain. Some of them are:

1. Phosphate compounds
2. Carbonate and Bicarbonate compounds
3. Citrates and citric acid compounds
4. Common salt

2.1. Phosphate Compounds:

STPP, with formula $Na_5P_3O_{10}$ is the sodium salt of tri-phosphoric acid. It is used in various applications such as a preservative for sea foods, meats, poultry feeds and other foods. Phosphates have the ability to stabilize proteins against denaturation, increase water holding capacity, improve emulsification and buffering capacity (acid-base relationships), contribute nutrients, chelate metal ions and functions as antioxidants are among their contributions to a variety of foods and food products (Paul *et al.*, 2012).

Reddy *et al.* (1986) found that Phosphates are effective on fish and shellfish in preventing drip loss when frozen products are thawed and in enhancing tenderness by restricting protein denaturation during freezing and frozen storage. Sutton *et al.* (1969)

reported that a light treatment (0.5 min in 4% STPP solution) markedly improved water retention and textural properties of fish muscle.

Unal *et al.*, (2004) reported that phosphates did not penetrate the shell and therefore to improve the shrimp quality the shell would need to be removed before the phosphate solution was added. Among the functional properties changed by the treatment of phosphates in seafoods are:

a) The retention of moisture and the natural flavour, inhibiting the cross of fluids during the distribution and the commercialization,
b) Stabilize the protein structure of seafood, form a surface layer of coagulated (solid) protein., swell muscle fibres and solubilize muscle proteins,
c) Enhancing WHC and tenderness of seafood by restricting protein denaturation,
d) Inhibition of the process of lipid oxidation (by the quelation of metallic ions),
e) The stabilization of the colour, and
f) The cryoprotection (by decreasing populations of pathogens, and prevent growth of spoilage microorganisms), which contributes to the extension of its shelf life (Love and Abel, 1966; Applewhite *et al.*, 1993; Gonclaves *et al.*, 2008; Klinic *et al.*, 2009; Rajkowski and Sommers, 2012).

Phosphate compounds have been accepted as additives in fishery products to improve functional properties during processing and storage, because phosphate can increase the water retention in fresh products, reduce thaw loss and prevent cook loss of frozen fishery products (Chang and Regenstein, 1997; Masniyom *et al.*, 2005). Phosphates have mostly been used with NaCl in fish and seafood to increase moisture retention (Chang and Regenstein, 1997; Thorarinsdottir *et al.*, 2004) and to improve taste of products.

Wangtuei *et al.*, (2014) reported that the frozen Nile Tilapia fillets gave the highest weight gain and cooking loss and least drip loss when dipped in 2% STPP solution containing 2.5% NaCl compared to SAPP, TSPP and SHMP at 2% solution. Rattanasatheirn *et al.*, (2008) shows that SAPP mixed with STPP or TSPP resulted in a slightly lower cooking yield and higher cooking loss than STPP or TSPP alone in peeled and devined shrimp.

Moaward *et al.*, (2013) reported that White Marine Shrimp treated with 5% food grade TSP exhibit higher moisture retention, protein content, protein solubility, WHC, tenderness, bound water and cooking yield at any given time of frozen storage compared with control samples. The data also demonstrates lower values of drip loss, TVBN, TMAN, TBARS, NPN, total plate counts (TPC) and phychrotrophic bacterial counts (PBS) are recorded in phosphate treated samples.

Gonclaves *et al.* (2008) showed that Searobin (*Prionotus punctatus*) fillets treated with 2% blend and STPP and Pink Cuskeel (*Genypterus brasiliensis*), mussel (*Perna perna*) and red shrimp (*Pleoticus muelleri*) treated with 5% of blend (containing STPP, TSPP and NaCl) and STPP produced the greatest weight gains, lower phosphate concentration in the final product treated with blend than STPP only.

Faithong *et al.* (2006) reported that compared to control, the cooked White Shrimp treated with TSPP, STPP and SHMP showed weight gain and reduced cook loss. The results on the effects of immersing the shrimp in 2% TSPP for 8 hours were similar to those in 2% STPP for 10 hours. The addition of 1-3% sodium chloride increased the weight gained in all raw samples immersed in different phosphate solutions. The combined effect of 2% salt and all types of phosphates yielded a good quality of cooked shrimp with high acceptability.

The synergistic effect between NaCl and phosphate can improve WHC and cooking yield (Young *et al.*, 1987). However the excessive using of phosphate will gently result in the formation of slimy texture, translucency, and a soapy taste (Rattanasatheirn *et al.*, 2008). It might also leave illegally high phosphate residues in the final products.

2.2. Bicarbonate and Carbonates Products:

Salt and phosphates are commonly used in combination to exploit their synergistic action (Murphy and Zerby, 2004). However, small peeled and deveined shrimp can be over-treated by those compounds (Henson and Kowalewski, 1992). Over-treatment generally results in the formation of a translucent and slimy texture. Due to the strict regulation for the limit of the residual phosphate in fish (0.5% for EU and Japan) and frozen seafood (0.5% for EU and 0.2% for Japan; Department of Fisheries, 2004), the processors have to search for other potential alternative, which have the ability in improving the quality and yield of seafood products.

Sodium bicarbonate was reported to be effective in improving the water-holding capacity, color, and organoleptic properties of fresh meats, beef, pork and poultry (Ahn, *et al.*, 1992; Boles *et al.*, 1993; Kauffman *et al.*, 2000).Sodium bicarbonate is widely used as a marinade in Chinese cookery (Skurray *et al.*, 1986), but it has been largely overlooked in the West, where acidic marinades have received much greater attention (Gault, 1991). Bicarbonate has been used to minimize the problem of pale, soft and exudative pork (Wynveen *et al.*, 2001) and to mask the typical aroma and flavor in sow meat (Sindelar *et al.*, 2003).

Bicarbonate was shown to reduce drip loss and sheer force of pork (Wynveen *et al.*, 2001) presumably because of the improved water holding capacity at elevated pH (Bouton et al., 1973). This effect could be explained because bicarbonates have higher buffering capacity and ionic strength than phosphates (Petracci *et al.*, 2014; Sheard *et al.*, 2004; Bertram *et al.*, 2008). Due to differences in buffering capacity and ionic strength, bicarbonate showed a greater ability to increase meat pH (0.7 vs. 0.3 pH units) and higher yield in comparison with sodium tripolyphosphate. Low-resolution nuclear magnetic resonance technique showed that combined use of bicarbonate with sodium chloride determined a remarkable increase of proportion of entrapped water into myofibrillar spaces (Petracci *et al.*, 2012).

Alkalinization effect of bicarbonate moved pH of meat away from isoelectric point of myofibrillar proteins and increased net negative charge. Electrostatic repulsion forces cause expansion of muscle fibres which allow more water to be immobilized in the myofibrillar lattice. Low-resolution nuclear magnetic resonance technique showed that combined use of bicarbonate with sodium chloride determined a remarkable increase of proportion of entrapped water into myofibrillar spaces (Petracci *et al.*, 2012).

Lopkulkiaert *et al.*, (2009) reported that The treatment of sodium bicarbonate containing traces of citric acid at 4 g/100 ml with sodium chloride at 3 g/100 ml lead to the increase of yield thus reduced the freezing loss by about 6.83–10.28 and 6.41–12.4 g/100 g fresh shrimp for the frozen–thawed samples frozen as uncooked and cooked products, respectively. The toughening of shrimp was observed while sodium bicarbonate containing traces of citric acid treatment with sodium chloride could reduce the texture change occurred during the freezing.

Mudalal *et al.*, (2014) found that Bicarbonate marinated fillets showed higher ability to retain water (67.3% *vs.* 65.7%) during severe heat treatment and lower cook losses (30.7% *vs.* 33.4%) when compared with phosphate-marinated fillets. Bicarbonate marinated fillets showed significant differences in the percentage of bound water, latent heat, and water activity after cooking in comparison to phosphate-marinated fillets. The results of this study revealed that phosphate-marinated fillets interacted with heat treatments in different patterns in comparison with bicarbonate marinated fillets.

According to Chantarasuwan *et al.*, (2011) Shrimp soaked in 2.5% NaCl containing both compounds at different levels of pH (5.5, 7, 8.5, 10 and 11.5) showed an increase in the weight gain and cooking yield and a reduced cooking loss as pH of solutions increased. pH of solutions above 8.5 led to the pronounced leaching of pigments, associated with the lowered redness of cooked shrimp. Solution containing 2.5% NaCl and 2.0% NaHCO3 (pH 8.5) was recommended for treatment of white shrimp as a promising alternative for phosphates to increase the yield and to lower cooking loss without any negative effect on sensory properties.

Petracci *et al.*, (2012) reported that broiler breast meat marinated with Samples marinated With sodium bicarbonate alone or in combination with sodium chloride and sodium pyrophosphate significantly increased the meat pH by approximately 0.7 units compared with that of the control, whereas phosphate alone or in combination with salt increased the pH by 0.2 units. The combination containing all of the ingredients produced the highest marinade performances; however, sodium bicarbonate was able to guarantee a better marinade uptake and water retention ability with respect to that of sodium pyrophosphate.

The use of bicarbonate or other mild alkaline compound should be as alternative to improve the yield and quality of shrimp, both raw and cooked, and to reduce the phosphate residue in treated shrimp. As a consequence, the exporting problem associated with the strict regulation of using phosphate can be alleviated. Nevertheless, the little information regarding the use of bicarbonate as well as carbonate in shrimp exists. The mechanisms of those compounds in the improvement of yield and quality should be elucidated in order to have an effective application.

2.3. Citrates and Citric acid compounds:

Citrates are widely used in poultry meat product formulation to improve water binding capacity by increasing the ionic strength and swelling of muscle fibre structure. Alkaline citrates (e.g. trisodium citrate) are the most common salts used in meat industry to improve WHC by raising the pH value (Feiner, 2006, chap. 5). Citrates also reduce the oxidative processes by chelating the oxidizing metals.

Ke *et al.*, (2009) reported a significant increase in water-binding capacity of bovine muscle at pH 3.52 upon the addition of citric acid (0.2 M). Burke and Monahan (2003) noted increased moisture uptake, fibre swelling, and collagen solubility along with a reduced cooking loss of shin beef upon marination treatments with 31% citric juices (orange and lemon). The substantial reduction in the pH (from 5.7 to 3.1) was thought to be responsible for the improved water-binding capacity in the muscle tissue. In addition, a reduced pH level can activate cathepsins, thereby promoting proteolytic degradation of myofibrils and increasing the solubility of collagen fibrils (Berge *et al.*, 2001; Aktas *et al.*, 2003).

2.4. Sodium Chloride:

The most commonly used salt in meat products is NaCl. The main functions of NaCl, other than providing flavor, are to solubilize proteins thereby eliciting their functionalities, to improve meat dehydration, and to alter osmotic pressure so as to inhibit bacterial growth and subsequent spoilage. NaCl at an application level of ~2% or higher raises the ionic strength of the sarcoplasm to above 0.5, enabling myosin filaments to depolymerize and myofibrils to swell resulting in improved hydration and water-holding capacity (Hamm, 1986). Marinades consisting of sodium chloride and polyphosphates are used to improve the texture and yield of muscle food products (Young and Lyon, 1997; Xiong and Kupski, 1999; Smith and Young, 2007).

Salt addition assists in protein solubilising by extracting the salt-soluble myofibrillar proteins in raw meat. Main effects of extracted myofibrillar proteins in a complex meat system are:

i) Increased WHC and binding among whole muscles and/or meat particles;
ii) Assisting fat emulsification in finely comminuted products due to coating of fat globules with a thin layer of extracted myofibrillar proteins;

iii) Increased meat batter viscosity which counteracts fat segregation during processing.

Upon heating, the extracted proteins coagulate and provide binding, water and fat holding (Barbut, 2002).

The theory about the role of sodium chloride in improving WHC of meat products has extensively been reviewed (Puolanne & Halonen, 2010; Ruusunen & Puolanne, 2005). In solution, sodium chloride hydrolyses into sodium (Na+) and chloride (Cl-) ions, however the effect on meat proteins is most likely caused by the fact that Cl- ions are more strongly bound to the proteins that the Na+ ions (Sebranek, 2009). Chloride ions tend to bind to the thick (myosin) and thin (actin) filaments and increase the electrostatic repulsive forces between them. With increasing the repulsive forces, the protein structure matrix unfolds, the gaps between actin and myosin increase and then transverse swelling occurs (Offer and Trinick, 1983; Hamm, 1986). Moreover the adsorption of Cl- ions with positively charged groups of myosin results in a shift of the isoelectric point towards a more acidic pH value. As a result, increased levels of water can be bound without changing the pH value of the meat itself.

In meat industry, sodium chloride is considered as multifunctional ingredient enabling to improve texture and WHC by solubilisation /extraction of the salt-soluble myofibrillar proteins in raw meat, promoting the taste, enhancing the flavours of meat and ameliorate microbiological stability.

CHAPTER – III

MATERIALS AND METHODS

3.1. Materials:-

3.1.1. Shrimp:-

Tiger shrimp/ Bagda chingri (*Penaeus monodon)* was used as sample for the present study and were bought from the freshly harvested lots from Malancha, North 24-Paraganas and transported to the laboratory within two hours in insulated containers with ice.

3.1.2. Chemicals and glasswares:-

The chemicals used in the analysis were either of 'Analytical' or 'Guaranteed' reagent grades. The glassware used were all of 'Borosil' made.

3.1.3. Equipments:-

3.1.3.1. Processing equipments:-

a) Refrigerator: Maintaining a constant temperature of 4 ±1°C.

b) Plate freezer: Maintaining a constant temperature of -40 ± 1°C.

c) Deep freezer: Horizontal model deep freezer maintaining a constant temperature of -20±2°C was used.

d) Display freezer: Maintaining a constant temperature of 4 ± 1°C.

e) Vessels and utensils: All the vessels and utensils were made of stainless steel.

f) Processing tables: Stainless steel.

3.1.3.2. Analytical instruments:-

1. Kjeldahl digestion system,
2. Kjeldahl distillation unit,
3. Sartorius Digital weighing balance.
4. Stainless steel table,
5. pH meter.

6. Plate freezer.
7. Muffle Furnace.
8. Soxhlet apparatus
9. Homogenizer.
10. Hot air oven.

3.2. Methods:-

3.2.1. Raw material processing:-

The iced shrimp was deiced and washed properly with potable water. Beheading was done in stainless steel table under good hygiene and sanitary condition so that no contamination would occur. Then, shrimps were randomly segregated into batches based on their size grades (60/80) and weight of individual fishes were taken.

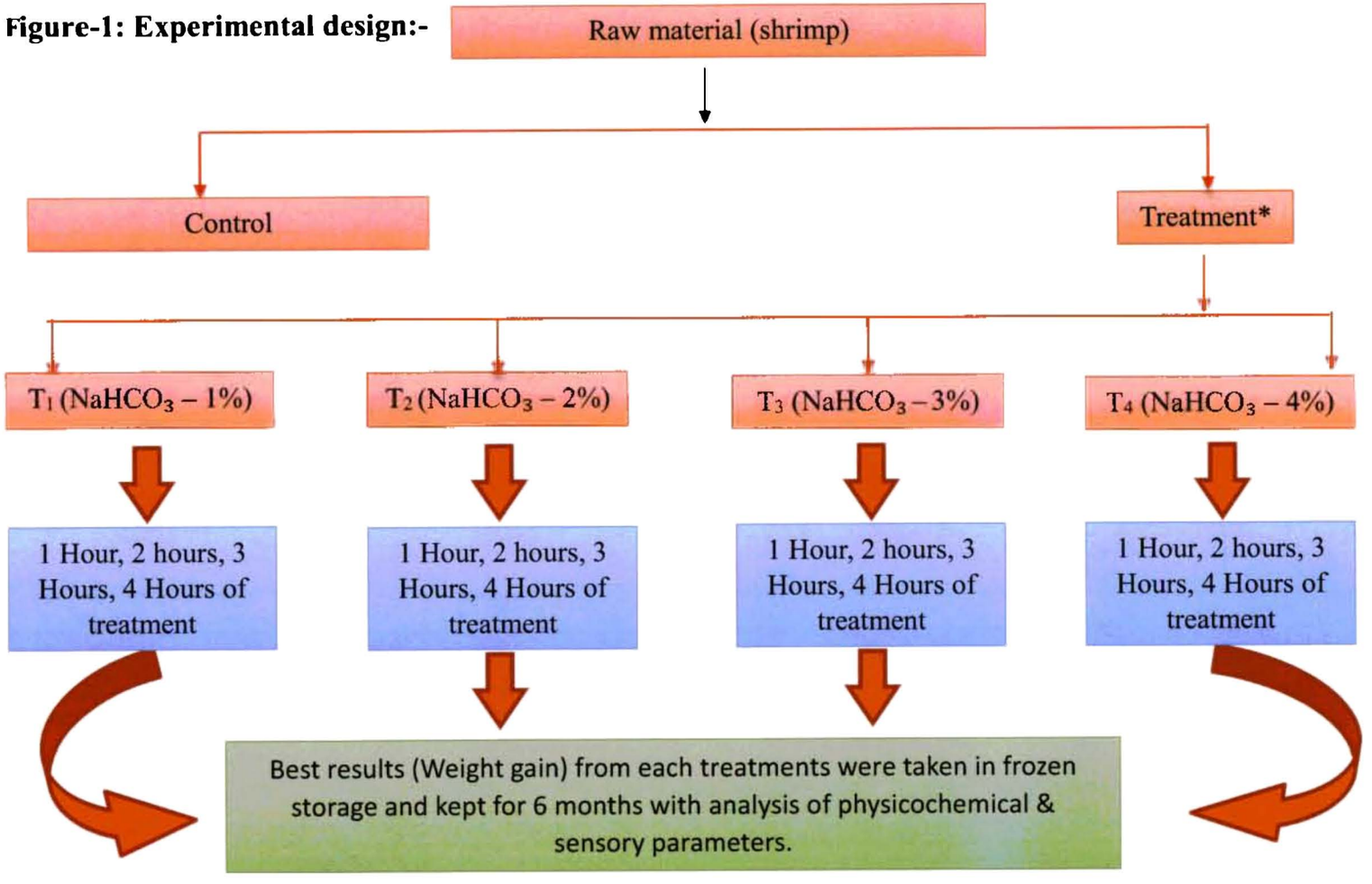
3.2.2. Preparation of sodium bicarbonate solution:-

Sodium bicarbonate solution was used to prepare four different concentrations of solution i.e. 1%, 2%, 3% and 4% (w/v). Traces of citric acid and common salt (2% w/v sodium chloride) was also added to each concentration to maintain the pH of the solution at 7.4. Near neutral pH is maintained so that the absorption of the solution to the shrimp does not affect its taste and flavor. Shrimps were dipped in each concentration for four different time durations like 1hour, 2hour, 3hour, 4 hour respectively. As a result 16 different solutions were made. Reverse osmosis (RO) water was used to prepare the solutions. One control was also maintained using RO water.

3.2.3. Standardization of time:-

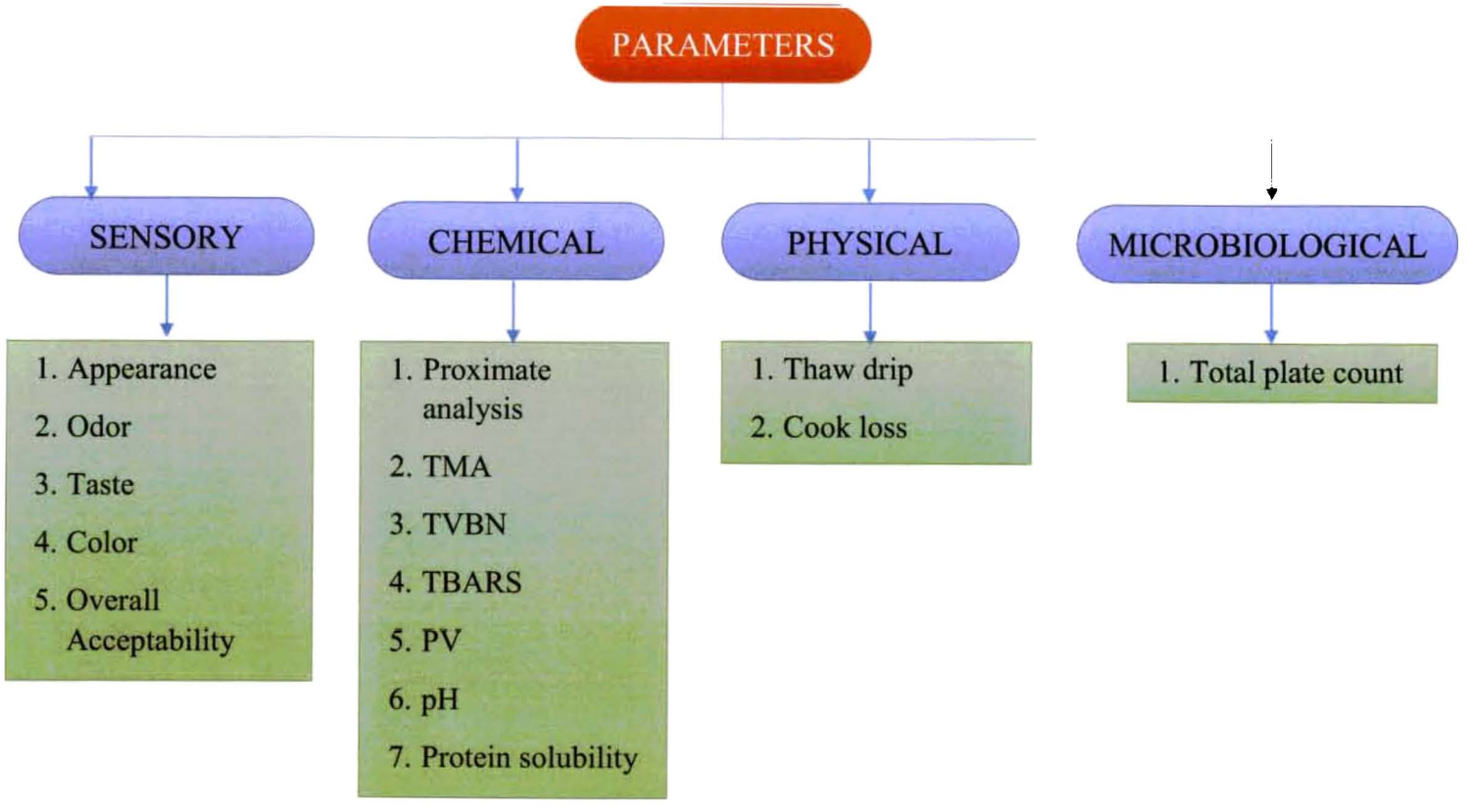
For each concentration weight gain (%) was calculated in intervals of 1 hour up to 4 hours. The time interval in which the weight gain (%) was maximum was determined.

Figure-1: Experimental design:-



*NaCl 2% (w/v) was added to each solutions. Citric acid was added to standardise the pH of the solution to 7.4.

Figure-2: Frozen Storage Study:-



3.4. Determination of weight gain after treatment:-

Weight of individual shrimp was taken before and after treatment. Weight gain percentage was determined by the method described by AOAC, 1995.

$$\text{Weight gain (\%)} = \frac{100 \text{ X } (B-A)}{A}$$

Where,

B = weight in gram after treatment

A = weight in gram before treatment

3.5. Frozen storage:-

3.5.1. Processing before storage:-

Shrimps were deiced and washed with potable water. After washing the shrimps were beheaded immediately and batched into the required size grade (60/80). Few randomly selected shrimps were separated for freshness test. All of the procedures were done in a good hygiene and sanitary condition to avoid any contamination and loss of freshness and flavor.

3.5.2. Dipping of shrimp:-

Four different solutions of sodium bicarbonate of 1%, 2%, 3%, 4% (w/v) were prepared. Traces of citric acid and 2% common salt (sodium chloride) were added to each of the four concentration. Shrimps were dipped in each of four concentration for time interval that gave highest weight gain (%). Finally, frozen blocks were prepared in plate freezer and stored for 6 months in frozen condition.

3.6. Frozen storage study:-

3.6.1. Determination of drip loss during storage:-

Drip loss is the loss of weight of food products during the thawing of the frozen product. Drip loss of frozen shrimp was determined by the method described by Gonclaves

et al. (2008). Drip loss of thawed samples were determined through the known weights of samples before and after thawing and expressed as % thawing loss. Samples were thawed in a cold room (4°C) for 24 hours (according to Chang *et al.*, 1997) and weighed to calculate drip loss after thawing.

$$\text{Drip loss (\%)} = \frac{100 \times (B-A)}{A}$$

Where,

B = weight in gram before Thawing

A = weight in gram after thawing

3.6.2. Determination of cooking loss :-

Cooking loss was determined by the method described by Applewhite *et al.* (1993). The determination was done by taking the known weights of samples before and after boiling in water for 1 minute and expressed as cooking loss (%).

$$\text{Cooking loss (\%)} = \frac{100 \times (B-A)}{A}$$

Where,

A = initial weight of sample

B = weight of sample after cooking.

3.6.3. Sensory analysis:-

Sensory panel evaluation of boiled shrimp samples was applied by aid of 10 untrained panelists according to the method described by Botta (1995). Panelists familiarized in shrimp quality assessment were asked to score appearance, odor, taste, colour and overall acceptability of cooked (boiled in water/5 min) shrimp. Rating was assigned separately for each parameter on a 9 point descriptive hedonic scale (Annexure- I). A score of 5 of sensory acceptability was taken as the average score for minimum acceptability.

3.6.4. Proximate analysis:-

Proximate composition of the HL shrimps was determined before frozen storage and after 6 months of frozen storage. The parameters include protein, fat, ash and moisture content.

3.6.4.1. Estimation of Moisture content:-

Determination of moisture content was done by the method described by AOAC (1995). At first an empty dish and lid were dried in 105°C for 3 hours and then transferred to the desiccator for cooling. After that weight of the empty dish and lid was taken. 3 grams of sample was taken in the dish and uniformly spread. Then the dish with the spread sample was dried in a hot air oven for 3 hours at 105°C. After drying the dish was transferred with partially covered lid to the desiccator for cooling. The weight of the dish was taken after cooling.

$$\textbf{Moisture content (\%)} = \frac{100 \times (W_2 - W_1)}{W_1}$$

Where,

W_1 = Weight (g) of sample after drying.

W_2 = Weight (g) of sample before drying.

3.6.4.2. Estimation of Protein:-

Protein content (%) was determined by Kjeldahl method in a Kjeltec Auto sampler system (N x 6.25) (AOAC, 1995). In kjeldahl digestion flask 1-2 g of sample with a pinch of digestion mixture and 20 ml of sulphuric acid was taken. Then flask was heated first slowly and then vigorously for 4-6 hours to make it colorless and clear to ensure that complete conversion of nitrogen to ammonium sulphate was done. Cooling was done and in a volumetric flask in which digested sample was poured and distilled water was added to make it 250ml. After washing the distillation unit a 50ml conical flask was taken in which 10ml of

2% boric acid and a pinch of Tashiro's indicator was given. The flask was placed in such a way that the tip of condenser outlet may be dipped into the boric acid. Then, 5 ml of digested sample from volumetric flask with 10 ml of 40% NaOH was added to the distillation chamber and distilled water was added to rinse the walls of chamber. When the distillate become 30ml it was taken for titration against 0.02N H_2SO_4. Titration value was recorded to calculate total protein content of the sample.

$$\% \text{ Total nitrogen} = \frac{14 \times N \times X \times 250 \times 100}{1000 \times V_1 \times W}$$

Where,

N = Normality of H_2SO_4,

X = ml of H_2SO_4 required for titration,

V_1 = aliquot of digested sample taken for distillation,

W = weight of sample in gram.

Hence, % protein = % total nitrogen X 6.25 (conversion factor). Because, average nitrogen content of fish protein is 16%, so 1g nitrogen = 100/16 = 6.25g protein.

3.6.4.3. Estimation of Fat:-

Fat content was estimated through the method described by Bligh and Dyer (1959) by using Soxhlet apparatus. Initially, hot air oven was used to dry the sample. After fully drying, weight of the moisture free sample was taken (2g). the sample was transferred in an extraction thimble. The thimble was then placed in the extraction chamber in straight direction so that the condensed petroleum benzene may drop into it. The heating unit was adjusted in such a way that solvent siphons 5-6 times per hour. Extraction process was continued for 16-20 hrs. Then the thimble was taken out from the chamber and again dried in the hot air oven. After drying the weight of sample with thimble was taken. The fat content was determined by using the following formula:

$$\text{Fat content (\%)} = \frac{\text{Weight of fat in the sample X 100}}{\text{Original weight of the sample}}$$

3.6.4.4. Estimation of Ash content:-

The inorganic constituents like sulphites, phosphates, chlorides present after incinerated sample is regarded as ash content. Ash content was determined by the method described by AOAC (1995). A clean dried porcelain crucible was taken and weighed. 5-10g of sample was taken in the crucible and marked as W. The crucible with sample weight was recorded as W_1. The sample containing crucible was then heated for 6 to 8 hours in 550-600°C. After that, the crucible was cooled and weight of crucible with ash content was taken and marked as W_2. Estimation of ash was done by the following formula:

$$\text{Ash content (\%)} = \frac{100 \times (W_2 - W_1)}{W_1}$$

3.6.5. Quality test:-

3.6.5.1. TVBN:-

TVBN was measured by the method described by AOAC (1995). Three Conway's units were taken which had been thoroughly cleaned with detergent to remove any containment. To the edge of the outer ring of each unit paraffin wax was applied. 10g sample was macerated with 20ml of 10% TCA (Tri Chloro Acetic Acid) extract. The extract then filtered in volumetric flask and extraction continued in a volumetric flask to make it 100ml. Using a micropipette, 1ml of concentrated H_2SO_4 was added to inner chamber of Conway cup and 1ml of TCA extract (sample) and 1ml of saturated potassium carbonate was added to outer chamber. A blank was also run using 10% TCA solution in case of TCA extract. 1 ml of saturated potassium carbonate solution was carefully pipetted into the outer chamber of each unit carefully. After that immediately the units were covered and closed with lid. The solution of the units was then mixed gently, to prevent any solution mixing from one ring to other. After then the units were placed.in an incubator at 45°C for 45 mins. After this the units covers were removed and the inner chamber solution was titrated with 0.02N H_2SO_4

using a pipette until green color solution turned to blue. An average titrated volume of H_2SO_4 was found from the result of three titration for each muscle sample. For each volume the TVB-N volumes were calculated. A blank test was also carried out using 1 ml of 1% TCA, instead of sample extract.

$$\text{TVBN (mg \%)} = \frac{14 \times N \times (X-Y) \times 50 \times 100}{W}$$

Where, N = Normality of H_2SO_4, X = ml of NaOH required for titration of sample, Y = ml of NaOH required for titration blank, W = weight of sample taken.

3.6.5.2. TMA:-

TMA was measured by the method described by AOAC (1995). The procedure is same as TVBN. The only difference was 1ml of 40% (w/v) formaldehyde solution was added to the outer chamber along with other reagents.

$$\text{TMA (mg \%)} = \frac{14 \times N \times (X-Y) \times 50 \times 100}{W}$$

Where, N = Normality of H_2SO_4, X = ml of NaOH required for titration of sample, Y = ml of NaOH required for titration blank, W = weight of sample taken.

3.6.5.3. Peroxide Value:-

The peroxide value was determined as previously reported by Kirk & Sawyer, (1991). Approximately 4 g of shrimp meat that had been homogenized with 10 ml chloroform and 15 ml glacial acetic acid and was filtered using Wattman's filter paper (125 mm diameter). The homogenate was shaken vigorously for 30 seconds and drops of fresh saturated aqueous potassium iodide (KI) solution were added to the mixture which was allowed to stand in the dark for 5 min. An equivalent (50 mL) amount of distilled water was added to the mixture to release the iodine and solution titrated with 0.01 M sodium thiosulphate solution against blank. The PV was calculated and expressed as mEq active O2/kg lipids.

$$\text{PV (mEq } O_2\text{/kg of fish)} = \frac{1N \times (V-X) \times 1000}{N}$$

Peroxide value (milli-equivallent peroxide O2 per 1000 g) = 1000 x (V-X) N /W

Where, V = Volume of sodium thiosulphate used for sample

X = Volume of sodium thiosulphate used for blank

N= Normality of sodium thiosulphate

W= Weight of sample.

3.6.5.4. TBARS:-

TBARS (Thio- Barbituric Acid Reactive Substances) was estimated by EZAssay™ TBARS Estimation kit of Hi- Media Cell Culture following the method as described in the package insert.

3.6.5.5. Protein Solubility:-

Protein solubility was determined according to the method of Lee *et al.* (1992), with some modification. 2 g homogenized fillet sample was added to 40 ml of distilled water and the mixture was stirred using a magnetic stirrer at speed 2 at room temperature. The pH of slurry was adjusted to desired pH (11.0) by the addition of 1 or 0.1 N HCl and 1 or 0.1 N NaOH to desired pH value. The volume was adjusted to 50 ml with distilled water. It was shaken for 1h at room temperature (about 270C), centrifuged at 5000 rpm for 20 min at 40C and the pH of the supernatant noted. Protein content of supernatants was determined by the Biuret method (Layne, 1957) and (Torten and Whitaker, 1964) by combining 1 part sample, 4 parts Biuret reagent, and 1 part 10% deoxycholic acid. The deoxycholic acid was added to eliminate lipids from the test solution that could interfere with the measurement. Bovine serum albumin (BSA) was used as a standard. The protein content in the initial homogenate before solubilization was determined by the Kjeldahl method. Percentages of soluble protein in the supernatant compared to the total protein were calculated as follows:

$$\textbf{Protein Solubility (\%)} = \frac{\textbf{Protein concentration in Supernatant}}{\textbf{protein concentration in homogenate}} \; x\,100$$

3.6.5.6. pH:-

The pH value was determined for homogeneous mixtures of 1g shrimp flesh and 10mL distilled water, as described by Ozyurt *et al.* (2009) using pH meter FiveEasy TM FE20, made by Mettler Todelo AG, Switzerland.

3.6.6. Total plate count:-

TPC was determined according to standard American Public Health Association method (APHA, 2001). It was determined as mean log $_{10}$ $CFUg^{-1}$ of shrimp samples. Physiological saline was prepared and 10g sample was mixed into it. Nutrient agar was used to make the media. Serial dilution process was done and pour plate technique was followed. Plates were incubated at 37°C for 24hrs. All materials used were sterilized except sample and all the procedure was done under aseptic condition.

3.6.7. Statistical analysis of data:-

All the data were checked for normal distribution with standard deviation and normality plots prior to analysis of variance (ANOVA) to determine significant differences among means at $\alpha = 0.05$ level, using statistical tools of Microsoft Office Excel (2013).

Plate-1: Fresh fish brought for experiment

Plate-2: Frozen block prepared for storage

Plate-3: 1% treated shrimp after 6 months of storage.

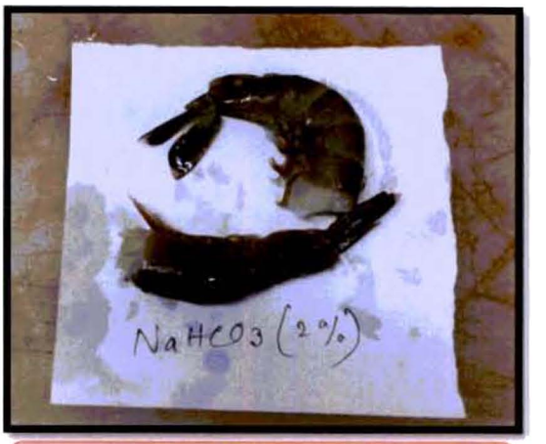


Plate-4: 2% treated shrimp after 6 months of storage.

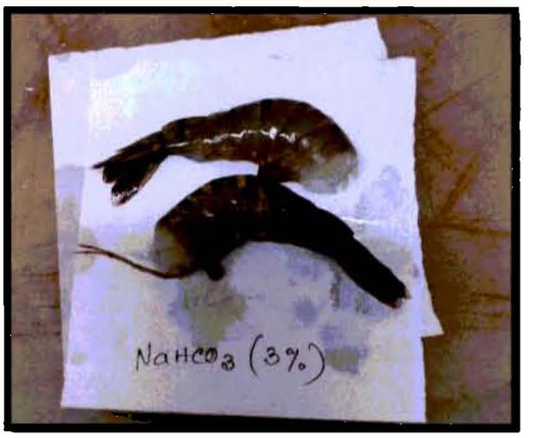


Plate-5: 3% treated shrimp after 6 months of storage.

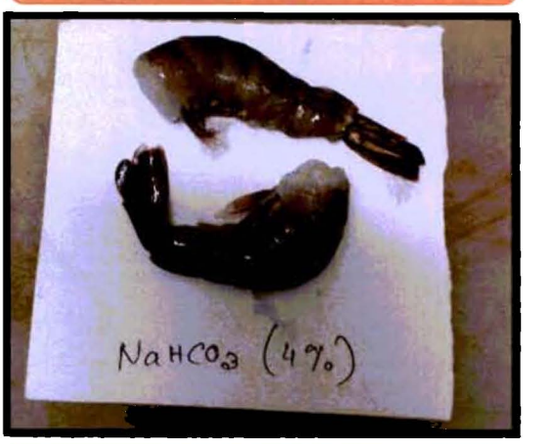


Plate-6: 4% treated shrimp after 6 months of storage.

CHAPTER - IV

EXPERIMENTAL RESULTS

4.1. Characteristics of fresh shrimp:-

4.1.1. Sensory characteristics:-

Table- 1. Sensory characteristics of raw tiger shrimp.

Components	Descriptive terms	Score
Appearance	Like extremely	8.50±0.48
Odour	Like very much	8.60±0.96
Taste	Like very much	8.60±1.25
Colour	Like extremely	8.70±0.98
Overall acceptibility	Like very much	8.60±0.66

**Results are mean of ten determinations (n=10) with s.d.*

4.1.2. Proximate composition:-

Table-2. Proximate composition of raw tiger shrimp.

Parameters	Value in %
Moisture	78.13±0.81
Protein	17.85±1.52
Fat	2.50±0.92
Ash	1.41±0.31

**Results are mean of three determinations (n=3) with s.d.*

4.1.3. Physical and chemical parameters:-

Table-3. Table-3 shows the physical and chemical parameters of raw tiger shrimp.

Parameters	Value
Size grade	60/80
TMA	0.81±0.57 mg%
TVBN	12.83±1.27 mg%
TBARS	0.92±0.34 mg MDA/kg
PV	1.99±0.47 mEq of O_2/kg
pH	7.05±0.91
Protein solubility	81.10±1.37%

**Results are mean of three determinations (n=3) with s.d.*

4.1.4. Microbiological parameter:-

Table-4. Table-4 shows the microbiological parameter of raw tiger shrimp.

Prameters	Value
TPC	5.45±0.96 log CFU/g

**Results are mean of three determinations (n=3) with s.d.*

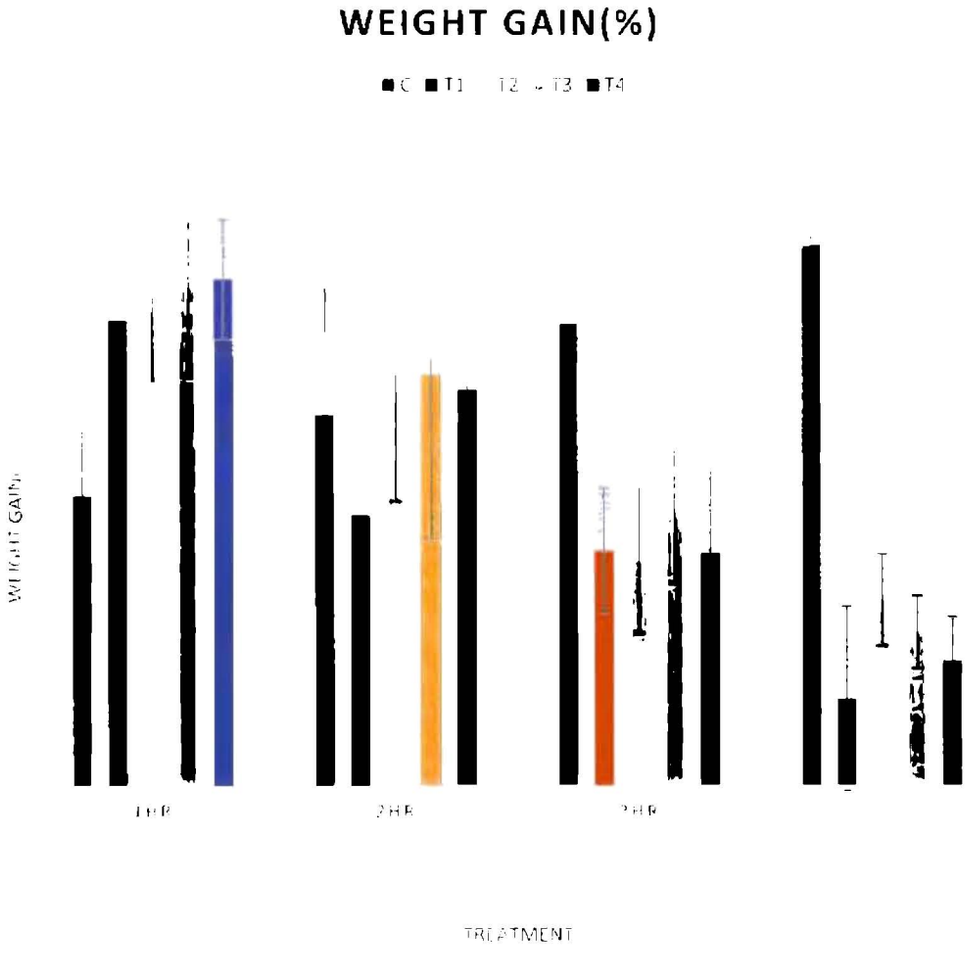
4.2. Weight gain (%) of shrimps after dipping in sodium bicarbonate solution:-

Table-5. Weight gain (%) of shrimps after dipping in sodium bicarbonate solution:-

Time	C	T1	T2	T3	T4
1hr	2.23±0.70	3.57±0.28	3.64±0.53	3.81±0.69	3.88±0.45
2hr	2.85±0.80	2.08±0.30	3.15±0.97	3.15±0.25	3.04±0.96
3hr	3.55±0.65	1.80±0.48	1.72±0.55	2.07±0.51	1.79±0.95
4hr	4.15±0.51	0.67±0.70	1.42±0.35	1.18±0.28	0.96±0.33

**Results are mean of three determinations (n=3) with s.d.*
 # Values of weight gain vary insignificantly (p>0.05) among the treatments.
**C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

Figure-3. Weight gain (%) of shrimps after dipping in sodium bicarbonate solution:-



**Results are mean of three determinations (n=3) with s.d.*
 # Values of weight gain vary insignificantly (p>0.05) among the treatments.
***C= Control, T1 = Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

.4.3. Sensory scores of shrimps after dipping in sodium bicarbonate solution:-

Sensory scores of appearance, odor, taste, colour and overall acceptability of shrimps after dip treatment in sodium bicarbonate solutions. The results are shown below.

Table-6 (a-e):- Sensory scores of appearance, odour, taste, colour and overall acceptability of shrimps after dipping in treatments. The results are shown below:-

Table-6.a. Sensory scores of untreated samples (Control):

Time (Hour)	1hr	2hr	3hr	4hr
Components				
Appearance	8.80±0.42	8.90±0.53	8.60±0.70	8.50±0.71
Odour	8.90±0.31	8.80±0.42	8.60±0.96	8.60±0.70
Taste	8.70±0.67	8.70±0.48	8.60±0.96	8.40±0.84
Colour	8.80±0.42	8.50±0.70	8.50±0.85	8.60±0.70
Overall acceptability	8.90±0.31	8.70±0.48	8.60±0.51	8.50±0.85

**Results are mean of ten determinations (n=10) with s.d.*
 # Values of sensory scores vary significantly ($p<0.05$) among the hours.

Table-6.b. Sensory score of shrimps treated with sodium bicarbonate 1% solution (T1):-

Time (Hour)	1hr	2hr	3hr	4hr
Components				
Appearance	8.70±0.67	8.50±0.53	8.40±0.70	8.30±0.82
Odour	8.80±0.42	8.80±0.42	8.60±0.51	8.50±0.52
Taste	8.70±0.48	8.50±0.71	8.60±0.96	8.60±0.51
Colour	8.60±0.97	8.70±0.48	8.50±0.85	8.60±0.70
Overall acceptability	8.80±0.42	8.60±0.51	8.50±0.52	8.30±0.95

**Results are mean of ten determinations (n=10) with s.d.*
 # Values of sensory scores vary significantly ($p<0.05$) among the hours.

Table-6.c. Sensory score of shrimps treated with sodium bicarbonate 2% solution (T2):-

Time (Hour)	1hr	2hr	3hr	4hr
Components				
Appearance	8.70±0.48	8.600±0.516	8.500±0.527	8.500±0.707
Odour	8.70±0.67	8.800±0.422	8.700±0.483	8.500±0.080
Taste	8.70±0.48	8.600±0.843	8.500±0.849	8.400±0.966
Colour	8.40±1.07	8.500±0.971	8.500±0.850	8.400±0.843
Overall acceptability	8.70±0.67	8.500±0.707	8.600±0.516	8.400±0.966

**Results are mean of ten determinations (n=10) with s.d.*
 # Values of sensory scores vary significantly ($p<0.05$) among the hours.

Table-6.d. Sensory score of shrimps treated with sodium bicarbonate 3% solution (T3):-

Time (Hour)	1hr	2hr	3hr	4hr
Components				
Appearance	8.60±0.84	8.50±0.70	8.60±0.51	8.60±0.70
Odour	8.70±0.48	8.60±0.51	8.40±0.96	8.50±0.70
Taste	8.60±0.70	8.60±0.84	8.50±0.70	8.40±0.51
Colour	8.50±0.52	8.40±1.26	8.50±0.97	8.40±1.07
Overall acceptability	8.60±0.70	8.50±0.71	8.40±0.97	8.20±1.23

**Results are mean of ten determinations (n=10) with s.d.*
 # Values of sensory scores vary significantly ($p<0.05$) among the hours.

Table-6.e. Sensory score of shrimps treated with sodium bicarbonate 4% solution (T4):-

Time (Hour)	1hr	2hr	3hr	4hr
Components				
Appearance	8.60±0.97	8.50±1.08	8.50±0.97	8.40±1.07
Odour	8.70±0.67	8.60±0.699	8.50±0.85	8.50±0.70
Taste	8.70±0.48	8.60±0.51	8.50±0.71	8.40±0.70
Colour	8.60±0.52	8.50±0.53	8.30±0.95	8.30±0.82
Overall acceptability	8.70±0.48	8.60±0.70	8.40±0.52	8.20±0.63

**Results are mean of ten determinations (n=10) with s.d.*
 # Values of sensory scores vary significantly ($p<0.05$) among the hours.

4.4. Frozen storage study:-

4.4.1. Sensory quality study:-

Table-7 (a-e):- Sensory scores of appearance, odour, taste, colour and overall acceptability of cooked shrimps after frozen storage of treated samples. The results are shown below:-

Table-7.a. Sensory scores of untreated (control) shrimps during frozen storage:-

Months	1st	2nd	3rd	4th	5th	6th
Components						
Appearance	8.00±1.13	7.20±0.44	6.50±0.82	6.00±1.22	5.40±0.96	5.20±0.94
Odour	7.90±1.28	7.10±0.11	6.50±0.38	5.90±0.67	5.50±1.29	5.10±0.97
Taste	8.00±0.44	7.30±0.92	6.60±1.10	5.80±0.89	5.60±1.22	5.30±1.23
Colour	7.90±0.96	7.00±0.71	6.80±0.52	6.00±0.32	5.40±0.51	5.10±1.13
Overall acceptability	7.90±0.67	7.10±1.20	6.40±0.94	5.80±0.52	5.60±0.94	5.10±0.70

**Results are mean of ten determinations (n=10) with s.d.*
 # Values of sensory scores vary significantly ($p<0.05$) among the months.

Table-7.b. Sensory score of shrimps treated with sodium bicarbonate 1% solution (T1) during frozen storage:-

Months	1st	2nd	3rd	4th	5th	6th
Components						
Appearance	8.10±0.67	7.70±0.67	7.20±0.89	6.80±0.48	6.50±0.97	6.10±0.53
Odour	8.20±1.05	7.80±0.82	7.20±1.11	6.90±1.23	6.30±1.29	6.20±0.95
Taste	8.20±0.85	7.70±1.06	7.30±0.48	6.80±0.70	6.50±1.22	6.20±0.48
Colour	8.30±0.97	7.50±1.10	7.20±1.07	6.70±1.16	6.40±0.51	6.20±0.69
Overall acceptability	8.20±0.82	7.80±0.99	7.10±0.67	6.80±0.96	6.40±0.94	6.10±1.07

**Results are mean of ten determinations (n=10) with s.d.*
 # Values of sensory scores vary significantly ($p<0.05$) among the months.

Table-7.c. Sensory score of shrimps treated with sodium bicarbonate 2% solution (T2) during frozen storage:-

Months	1st	2nd	3rd	4th	5th	6th
Components						
Appearance	8.00±0.85	7.70±0.82	7.20±0.52	6.70±0.82	6.40±1.08	6.10±1.08
Odour	8.30±1.10	7.70±1.06	7.30±1.28	6.90±0.45	6.30±0.67	6.20±0.44
Taste	8.20±0.70	7.70±0.70	7.20±0.99	6.80±0.51	6.50±0.63	6.20±0.84
Colour	8.20±0.71	7.60±1.28	7.30±0.31	6.80±0.44	6.40±1.23	6.20±0.96
Overall acceptability	8.20±1.10	7.70±0.51	7.10±1.16	6.80±0.63	6.50±0.70	6.10±1.07

**Results are mean of ten determinations (n=10) with s.d.*
 # Values of sensory scores vary significantly ($p<0.05$) among the months.

Table-7.d. Sensory score of shrimps treated with sodium bicarbonate 3% solution (T3) during frozen storage:-

Months	1st	2nd	3rd	4th	5th	6th
Components						
Appearance	8.00±0.85	7.80±0.70	7.30±1.16	6.80±0.99	6.50±0.53	6.20±1.10
Odour	8.30±1.25	7.80±1.40	7.30±1.07	7.00±1.22	6.40±0.44	6.20±1.16
Taste	8.30±0.96	7.80±0.92	7.30±1.10	6.90±0.89	6.40±0.44	6.20±0.44
Colour	8.30±0.92	7.60±0.44	7.20±0.45	6.80±1.28	6.50±1.07	6.10±0.63
Overall acceptability	8.30±1.03	7.80±0.71	7.20±0.44	6.90±1.22	6.50±0.70	6.20±1.03

**Results are mean of ten determinations (n=10) with s.d.*
 # Values of sensory scores vary significantly ($p<0.05$) among the months.

Table-7.e. Sensory score of shrimps treated with sodium bicarbonate 4% solution (T4) during frozen storage:-

Months	1st	2nd	3rd	4th	5th	6th
Components						
Appearance	8.10±0.79	7.90±0.87	7.30±0.31	6.90±0.67	6.60±0.31	6.20±0.48
Odour	8.40±1.28	7.90±1.10	7.40±0.71	7.00±0.71	6.40±1.16	6.20±0.32
Taste	8.30±1.40	7.80±0.94	7.30±0.82	6.90±1.07	6.50±0.70	6.30±1.29
Colour	8.40±0.44	7.60±0.67	7.30±1.06	6.80±1.11	6.50±0.48	6.20±0.82
Overall acceptability	8.20±0.94	7.90±1.03	7.20±1.29	6.90±0.53	6.50±0.97	6.30±1.40

**Results are mean of ten determinations (n=10) with s.d.*

 # Values of sensory scores vary significantly ($p<0.05$) among the months.

4.4.2. Drip loss (%) of (untreated and treated) shrimps during frozen storage:-

Table-08 shows the changes in drip loss (%) of shrimps (untreated and treated)during frozen storage.

Table-8. Drip loss (%) of (untreated and treated) shrimps during frozen storage:-

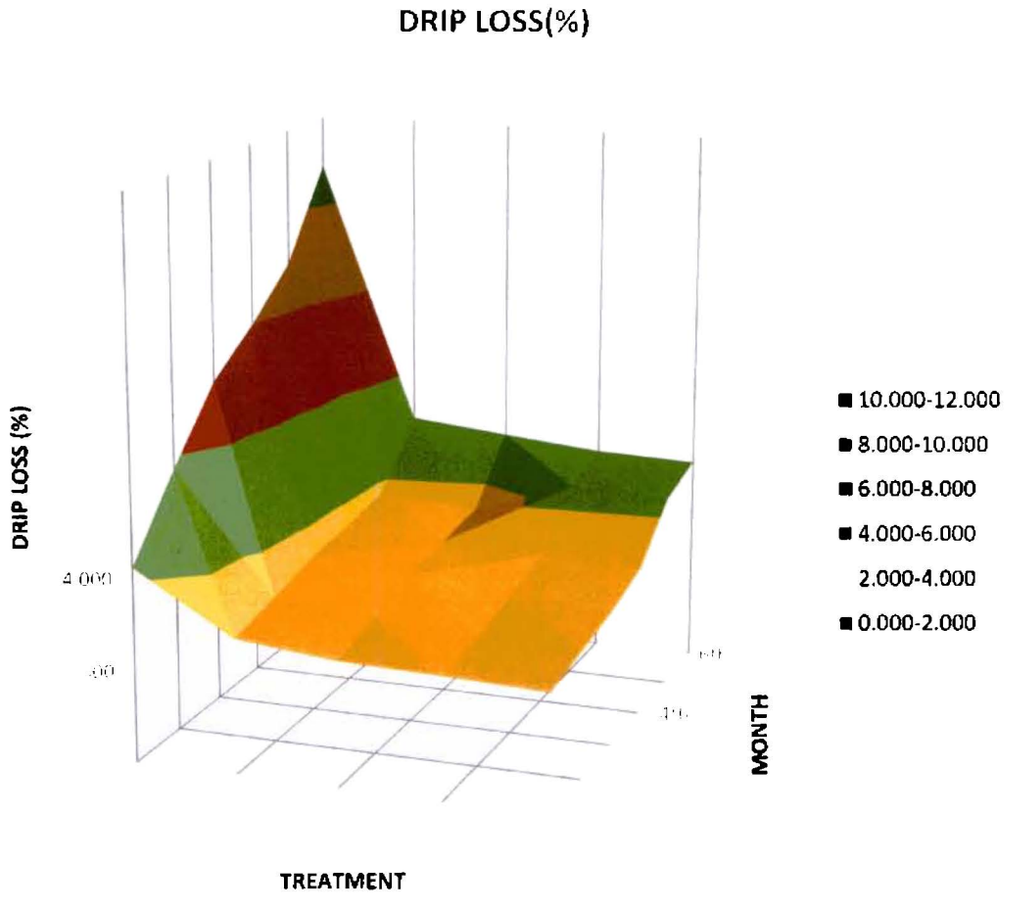
Month	C	T1	T2	T3	T4
1	4.22±0.35	2.95±0.16	2.73±0.09	2.67±0.39	2.59±0.18
2	5.78±0.19	3.18±0.12	2.99±0.26	2.83±0.10	2.79±0.16
3	7.17±0.32	3.47±0.29	3.28±0.29	3.08±0.33	2.97±0.16
4	7.98±0.46	3.59±0.11	3.44±0.20	3.36±0.16	3.28±0.11
5	8.96±0.36	3.79±0.17	3.61±0.32	4.35±0.12	4.28±0.10
6	10.84±0.24	5.04±0.12	4.83±0.20	4.64±0.12	4.58±0.11

**Results are mean of three determinations (n=3) with s.d.*

 # Values of drip loss vary significantly ($p<0.05$) among the treatments.

***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

Figure-4. Drip loss(%) of shrimps (untreated and treated) during frozen storage:-



**Results are mean of three determinations (n=3) with s.d.*
 # Values of drip loss vary significantly ($p<0.05$) among the treatments
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%), T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

4.4.3. Cook loss (%) of (untreated and treated) shrimps during frozen storage:-

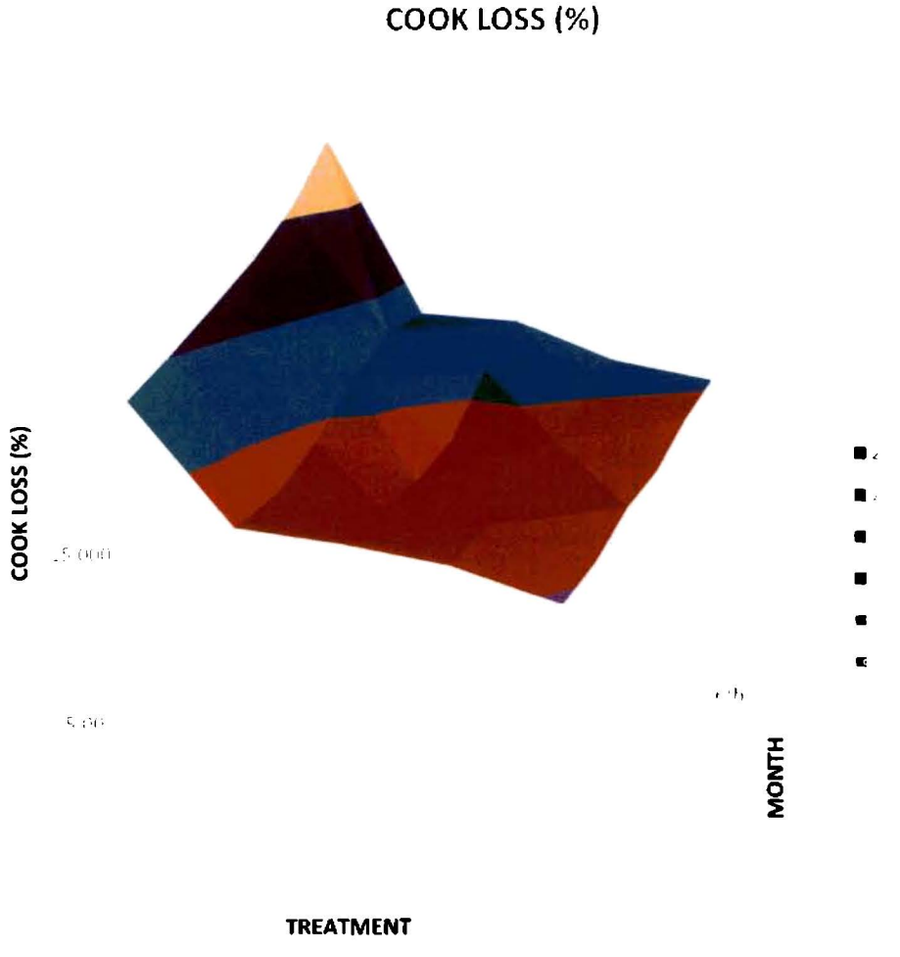
Table-09 shows the changes in cook loss (%) of shrimps (untreated and treated) during frozen storage.

Table-9. Cook loss (%) of (untreated and treated) shrimps during frozen storage:-

Month	C	T1	T2	T3	T4
1	23.77±0.21	17.14±0.80	16.83±0.23	16.23±0.25	14.70±0.46
2	25.15±0.57	17.48±0.45	17.28±1.10	16.86±1.34	15.43±0.13
3	26.74±0.23	19.09±0.52	17.37±1.03	17.11±0.44	16.94±0.78
4	28.36±0.25	21.45±1.13	18.32±1.65	18.85±0.13	17.58±0.38
5	30.52±0.27	23.13±2.39	21.28±1.06	20.05±1.40	19.22±1.26
6	33.82±0.63	23.15±0.74	23.09±1.49	21.09±0.28	20.31±0.85

**Results are mean of three determinations (n=3) with s.d.*
#Values of cook loss vary significantly ($p<0.05$) among the treatments.
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%). T4= Sodium bicarbonate (4%)*

Figure-5. Cook loss (%) of shrimps (untreated and treated) during frozen storage:-



**Results are mean of three determinations (n=3) with s.d.*
 # Values of cook loss vary significantly ($p<0.05$) among the treatments.
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

4.4.4. Proximate composition analysis before and after frozen storage:-

Proximate analysis was done with fresh shrimp and treated shrimps in sodium bicarbonate solution before frozen storage and in the last month of frozen storage.

Table-10. Changes in moisture (%) of (untreated and treated) shrimps before and after frozen storage:-

Month	C	T1	T2	T3	T4
0	78.14±0.82	78.14±0.83	78.13±0.82	78.14±0.70	78.14±0.70
6	75.03±1.33	75.02±1.33	75.10±1.07	75.12±1.07	75.33±1.01

**Results are mean of three determinations (n=3) with s.d.*
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

Table-11. Changes in protein (%) of (untreated and treated) shrimps before and after frozen storage:-

Month	C	T1	T2	T3	T4
0	18.00±0.32	17.59±0.80	17.91±0.74	17.73±0.53	18.04±0.31
6	17.05±0.69	16.83±0.64	17.20±0.35	17.37±0.29	17.89±0.73

**Results are mean of three determinations (n=3) with s.d.*
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

Table-12. Changes in fat (%) of (untreated and treated) shrimps before and after frozen storage:-

Month	C	T1	T2	T3	T4
0	2.53±0.92	2.56±0.95	2.40±0.81	2.96±1.05	2.42±1.10
6	2.36±0.98	2.39±1.02	2.25±0.73	2.81±1.13	2.32±1.04

**Results are mean of three determinations (n=3) with s.d.*
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

Table-13. Changes in ash (%) of (untreated and treated) shrimps before and after frozen storage:-

Month	C	T1	T2	T3	T4
0	1.39±0.84	1.43±0.88	1.43±0.88	1.38±0.95	1.43±0.88
6	1.41±0.82	1.44±0.79	1.42±0.87	1.34±0.71	1.41±0.78

**Results are mean of three determinations (n=3) with s.d.*
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

4.4.5. Changes in TMA content (mg N/100g) of shrimps (untreated and treated) during frozen storage:-

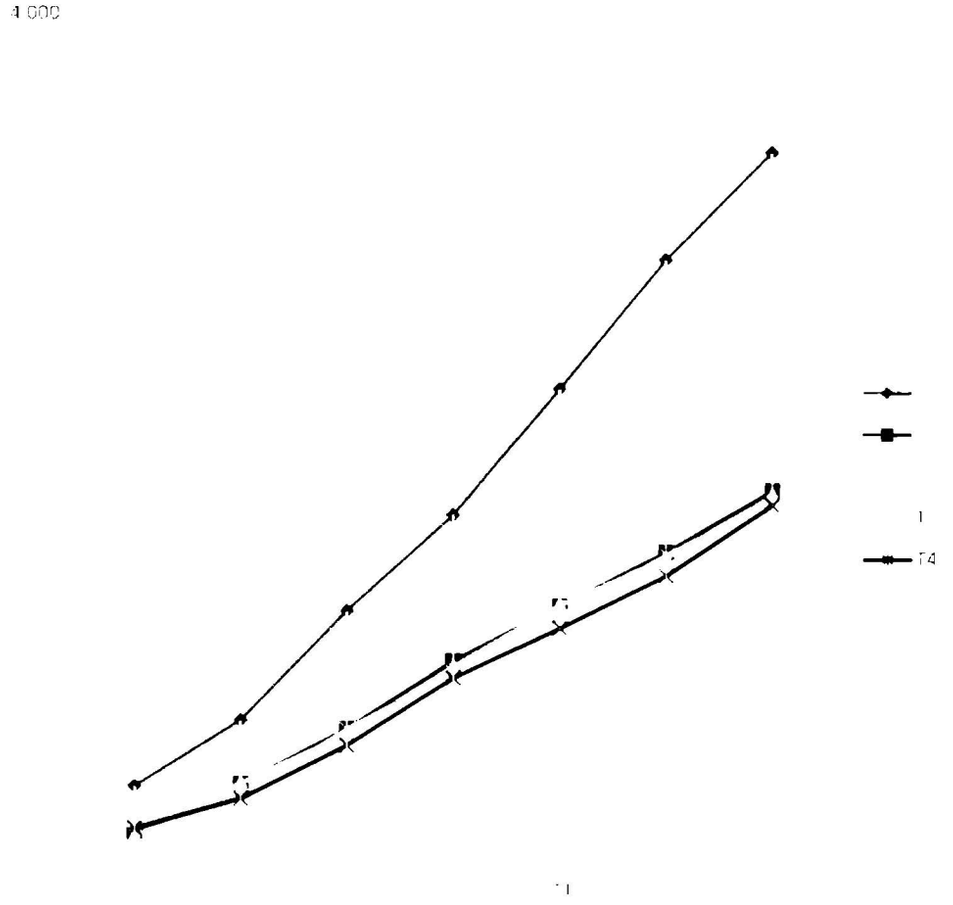
Table-14 shows the changes in Tri-Methyl Amine (TMA) content (mg N/100g) of shrimps (untreated and treated) during frozen storage.

Table-14. Changes in TMA content (mg N/100g) of shrimps (untreated and treated) during frozen storage:-

Month	C	T1	T2	T3	T4
0	0.82±0.05	0.62±0.15	0.62±0.14	0.66±0.08	0.65±0.20
1	1.09±0.06	0.83±0.11	0.83±0.15	0.79±0.15	0.77±0.13
2	1.54±0.05	1.06±0.13	1.04±0.14	1.06±0.23	0.99±0.21
3	1.94±0.18	1.34±0.15	1.32±0.15	1.29±0.15	1.27±0.14
4	2.45±0.41	1.56±0.21	1.57±0.15	1.52±0.11	1.47±0.06
5	2.98±0.55	1.78±0.18	1.76±0.12	1.78±0.15	1.69±0.18
6	3.42±0.31	2.04±0.17	2.02±0.14	1.99±0.17	1.97±0.18

**Results are mean of three determinations (n=3) with s.d.*
 # Values of TMA vary significantly ($p<0.05$) among the treatments.
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

Figure -06. Changes in TMA content (mg N/100g) of (untreated and treated) shrimps during frozen storage:-



**Results are mean of three determinations (n=3) with s.d.*
 # Values of TMA vary significantly ($p<0.05$) among the treatments.
***C= Control, T1 = Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

4.4.6. Changes in TVB-N content (mg N/100g) of shrimps (untreated and treated) during frozen storage:-

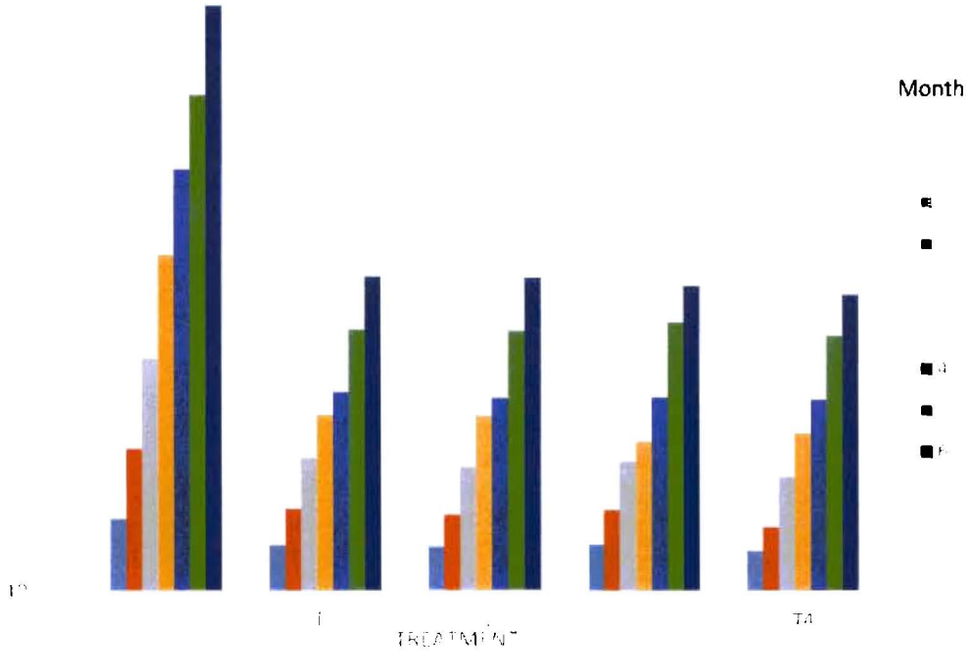
Table-15. Shows the changes in Total Volatile Base Nitrogen (TVB-N) content (mg N/100g) of shrimps (untreated and treated) during frozen storage.

Table-15. Changes in Total Volatile Base Nitrogen (TVB-N) content (mg N/100g) of shrimps (untreated and treated) during frozen storage:-

Month	C	T1	T2	T3	T4
0	12.95±0.33	11.88±0.92	11.81±0.51	11.85±0.58	11.88±0.81
1	15.82±0.46	13.03±0.83	12.89±0.42	12.99±0.77	12.71±0.42
2	19.49±0.45	14.58±0.63	14.71±0.39	14.42±0.60	14.61±0.67
3	23.74±0.23	16.20±0.77	16.39±0.81	16.11±0.66	15.82±1.01
4	27.22±0.29	18.48±0.56	18.17±0.74	18.47±0.59	18.13±0.77
5	30.24±0.37	20.69±0.93	20.62±0.78	20.67±0.80	20.07±0.62
6	33.87±0.17	23.36±0.59	23.01±0.73	22.81±0.61	22.13±0.36

**Results are mean of three determinations (n=3) with s.d.*
 # Values of TVBN vary significantly ($p<0.05$) among the treatments.
***C= Control, T1 = Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%). T4= Sodium bicarbonate (4%)*

Figure -07:- Changes in TVB-N content (mg N/100g) of shrimps (untreated and treated) during frozen storage:-



**Results are mean of three determinations (n=3) with s.d.*
 # Values of TVB-N vary significantly ($p<0.05$) among the treatments.
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

4.4.7. Changes in TBARS (mg MDA/kg) of shrimps (untreated and treated) during frozen storage:-

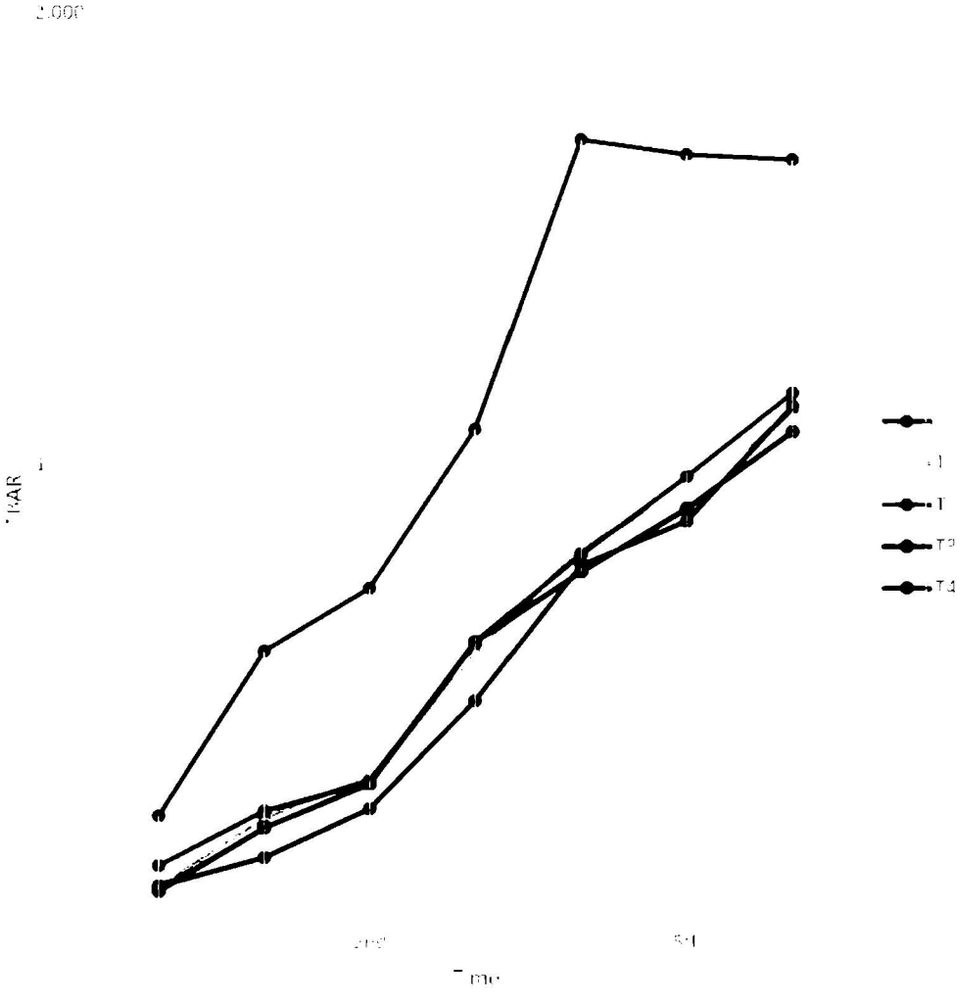
Table-16. Shows the changes in Thio Barbituric Acid Reactive Substances (TBARS) content (mg MDA/kg) of shrimps (untreated and treated) during frozen storage:-

Table-16. Changes in TBARS content of shrimps (untreated and treated) during frozen storage:-

Month	C	T1	T2	T3	T4
0	0.93±0.06	0.83±0.04	0.86±0.05	0.83±0.08	0.83±0.03
1	1.15±0.07	0.93±0.03	0.94±0.049	0.91±0.01	0.87±0.14
2	1.23±0.19	0.98±014	0.97±0.16	0.97±0.14	0.94±0.15
3	1.44±0.07	1.14±0.12	1.16±0.09	1.16±0.15	1.08±0.12
4	1.83±0.06	1.29±0.08	1.28±0.23	1.25±0.09	1.26±0.09
5	1.81±0.01	1.36±0.11	1.38±0.10	1.34±0.15	1.32±0.02
6	1.80±0.08	1.46±0.07	1.49±0.04	1.44±0.08	1.42±0.05

**Results are mean of three determinations (n=3) with s.d.*
 # Values of TBARS vary significantly ($p<0.05$) among the treatments.
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

Figure -08. Changes in TBARS content (mg MDA/kg) of shrimps (untreated and treated) during frozen storage:-



**Results are mean of three determinations (n=3) with s.d.*
 # Values of TBARS vary significantly ($p<0.05$) among the treatments.
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

4.4.8. Changes in PV (mEq of O2/kg) of shrimps (untreated and treated) during frozen storage:-

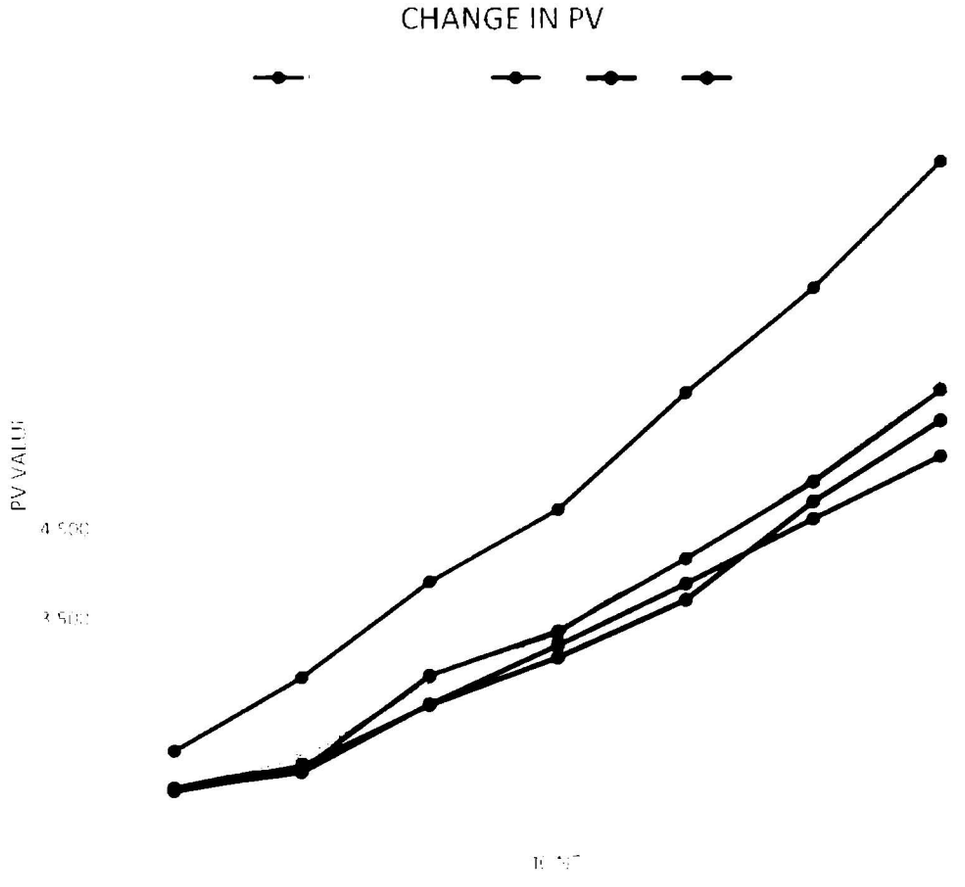
Table-17. Shows the changes in Peroxide Value (PV) (mEq of O_2/kg) the shrimps (untreated and treated) during frozen storage.

Table-17. Changes in PV (mEq of O2/kg) of the shrimps (untreated and treated) during frozen storage:-

Month	Control	T1	T2	T3	T4
0	2.02±0.24	1.61±0.16	1.57±0.16	1.60±0.17	1.60±0.18
1	2.84±0.18	1.95±0.12	1.81±0.07	1.85±0.04	1.78±0.05
2	3.91±0.31	2.77±0.09	2.87±0.06	2.54±0.12	2.55±0.24
3	4.72±0.33	3.31±0.10	3.36±0.23	3.07±0.16	3.21±0.18
4	6.03±0.20	4.37±0.30	4.17±0.39	3.71±0.37	3.89±0.10
5	7.12±0.19	5.06±0.08	5.03±0.21	4.82±0.62	4.62±0.57
6	8.61±0.50	6.15±0.16	6.05±0.13	6.05±0.13	5.32±0.50

**Results are mean of three determinations (n=3) with s.d.*
 # Values of PV vary significantly ($p<0.05$) among the treatments.
***C= Control, T1 = Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

Figure -09. Changes in PV content (mEq O2/kg) of shrimps (untreated and treated) during frozen storage:-



**Results are mean of three determinations (n=3) with s.d.*
 # Values of PV vary significantly (p<0.05) among the treatments.
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

4.4.9. Changes in pH of shrimps (untreated and treated) during frozen storage:-

Table-18. Shows the changes in pH of the shrimps (untreated and treated) during frozen storage.

Table-18. Changes in pH of the shrimps (untreated and treated) during frozen storage:-

Month	C	T1	T2	T3	T4
0	7.06±0.24	7.09±0.27	7.09±0.26	7.15±0.26	7.18±0.32
1	7.20±0.30	7.15±0.26	7.16±0.28	7.21±0.32	7.25±0.32
2	7.31±0.50	7.13±0.24	7.13±0.25	7.18±0.32	7.22±0.29
3	7.31±0.49	7.16±0.23	7.17±0.30	7.22±0.30	7.26±0.27
4	7.32±0.49	7.20±0.30	7.21±0.32	7.27±0.28	7.28±0.48
5	7.37±0.43	7.18±0.32	7.20±0.33	7.24±0.31	7.27±0.28
6	7.42±0.43	7.23±0.32	7.24±0.31	7.29±0.47	7.31±0.50

**Results are mean of three determinations (n=3) with s.d.*
 # Values of pH vary significantly ($p<0.05$) among the treatments and time.
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

4.4.10. Changes in protein solubility (%) of shrimps (untreated and treated) during frozen storage:-

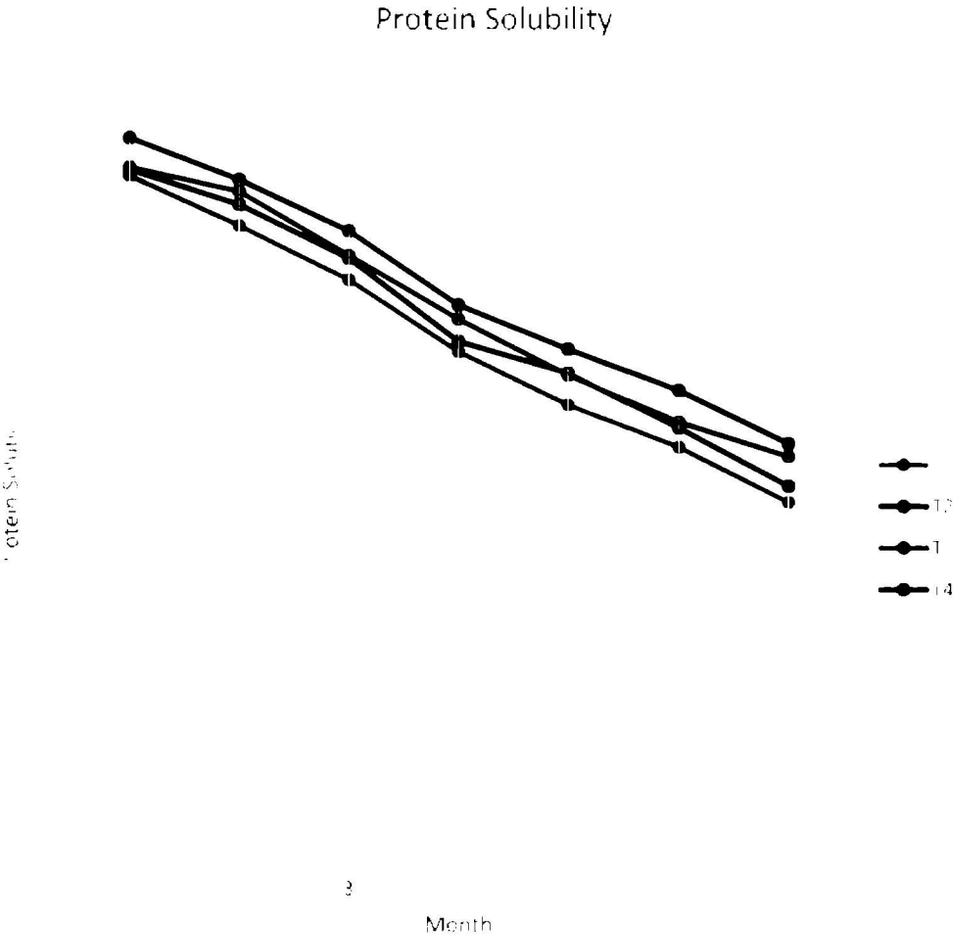
Table-19. Shows the changes in protein solubility (%) of the shrimps (untreated and treated) during frozen storage.

Table-19. Changes in protein solubility (%) of the shrimps (untreated and treated) during frozen storage:-

Month	C	T1	T2	T3	T4
0	80.95±0.48	84.65±1.24	84.96±0.89	85.10±2.33	86.57±1.64
1	75.73±0.46	82.13±3.21	83.20±3.58	83.85±1.60	84.46±3.54
2	71.77±0.27	79.38±2.19	80.48±0.72	80.62±1.46	81.87±0.61
3	66.25±0.27	75.75±2.31	76.28±1.01	77.41±1.05	78.11±0.34
4	61.45±0.41	73.07±0.88	74.66±0.52	74.56±1.18	75.89±0.46
5	56.74±0.44	70.92±0.45	71.89±0.33	72.19±0.48	73.80±1.77
6	52.34±0.38	68.13±0.64	68.95±0.62	70.45±0.86	71.10±0.46

**Results are mean of three determinations (n=3) with s.d.*
 # Values of protein solubility vary significantly ($p<0.05$) among the treatments.
***C= Control, T1= Sodium bicarbonate (1%). T2= Sodium bicarbonate (2%).T3= Sodium bicarbonate (3%). T4= Sodium bicarbonate (4%)*

Figure -10. Changes in protein solubility (%) of shrimps (untreated and treated) during frozen storage:-



**Results are mean of three determinations (n=3) with s.d.*
 # Values of Protein Solubility vary significantly ($p<0.05$) among the treatments.
***C= Control, T1= Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

4.4.11. Changes in total plate count (log CFU/g) of shrimps (untreated and treated) during frozen storage:-

Table-20. Shows the changes in total plate count (log CFU/g) of the shrimps (untreated and treated) during frozen storage.

Table-20. Changes in total plate count (log CFU/g) of the shrimps (untreated and treated) during frozen storage:-

Month	C	T1	T2	T3	T4
0	5.51±0.03	4.08±0.03	4.01±0.05	3.98±0.02	3.90±0.05
1	3.90±0.05	3.11±0.09	3.04±0.04	3.02±0.06	3.00±0.04
2	4.20±0.02	3.08±0.03	3.01±0.06	3.00±0.04	2.97±0.02
3	4.47±0.05	3.20±0.02	3.17±0.03	3.15±0.04	3.13±0.05
4	4.61±0.06	3.42±0.02	3.40±0.02	3.40±0.02	3.37±0.03
5	4.83±0.01	3.56±0.02	3.55±0.02	3.52±0.03	3.51±0.04
6	5.17±0.11	3.63±0.02	3.59±0.03	3.58±0.02	3.58±0.03

**Results are mean of three determinations (n=3) with s.d.*
 # Values of total plate count vary significantly ($p<0.05$) among the treatments.
***C= Control, T1 = Sodium bicarbonate (1%), T2= Sodium bicarbonate (2%),T3= Sodium bicarbonate (3%), T4= Sodium bicarbonate (4%)*

CHAPTER - V

DISCUSSIONS

5.1. Raw material characteristics:

The raw material with size grade 60/80 bought from market had a high level of freshness. Sensory analysis (Table-1) of raw shrimp reveals an overall acceptability score of 8.60±0.66 which indicates that the sample initially was liked by the untrained panelists. Initially the sample had approximate moisture content of 78.13±0.81%, 17.85±1.52% of protein content, 2.50±0.92% of fat content and 1.41±0.31% of ash content (Table-2). The sample had initially 0.81±0.57 mg% of TMA content, 12.83±1.27 mg% of TVBN, 0.92±0.34 mg MDA/kg of TBARS and 1.99±0.47 mEq of O_2/kg of PV (Table-3). The initial pH of the raw material was 7.05±0.91 and the protein solubility was 81.10±1.37% (Table-3). All of the chemical parameters are within the critical limit, indicates that the raw material were in extremely fresh condition, which were suitable for further experiment. The total plate count of raw material was 5.45±0.96 log CFU/g (Table-4)

5.2. Weight Gain:

Weight gain was observed by dipping shrimps with different levels (1% to 4%) of sodium bi-carbonate solution with different time intervals (Table-5). Substantially a control sample is tested with same time intervals. In 1st hour 2.23±0.70% weight gain was observed in control sample and 3.57±0.28%, 3.64±0.53%, 3.81±0.69% and 3.88±0.45% weight gain was observed in T1, T2, T3 and T4 samples, respectively. Result shows that weight gain was highest in control sample for higher time intervals of dip treatment (from 1st hour to 4th hour) with the values of 2.23±0.70%, 2.85±0.80%, 3.55±0.65% and 4.15±0.51% for 1hour, 2 hours, 3 hours and 4 hours respectively. Whereas bi-carbonate treated samples shows highest weight gain in 1st hour, with highest in T4, i.e. 3.88±0.45%. This may be for some differences in the concentration of salt in shrimp muscle which affects the uptake of sodium bi-carbonate solution containing citric acid and sodium chloride. The results also indicated that best weight gain was obtained in 4% bi-carbonate solution. According to Chantarasuwan *et al.* (2011) weight gain and cooking yield of *P. indicus* was increased when pH increased, regardless of types of solutions i.e. carbonates or bicarbonates. ($p<0.05$). At the same pH

used, the higher weight gain was observed in shrimp treated with sodium carbonate, compared with those treated with sodium bi-carbonate ($p<0.05$), except at pH 11.5, where no difference was found ($p>0.05$) (Chantarasuwan *et al.*, 2011). At a pH above 11.5 or very alkaline pH, proteins have a negative charge, in which protein molecules repulse each other, resulting in the swollen muscle structure (Zayas, 1997). As a consequence, water could be more up taken. Increases in weight gain corresponded to the higher moisture content indicating a higher mobility of water into the muscle. At the pH lower than 11.5, some differences in weight gain of shrimp treated with both chemicals might be caused by the differences in ionic strength of the solution (sodium bicarbonate: 0.666 mol/L; sodium carbonate: 0.995 mol/L). The increasing ionic strength generally weakened the structural integrity of myofibrils to a greater extent (Wu and Smith, 1987).

Xiong *et al.* (2000) reported that the combination of NaCl and phosphorus influenced the physical changes in chicken muscle. This indicates that NaCl has a properties to enhance the functional properties of meat. Fennema (1990) and Offer and Knight (1988) reported that increasing salt concentration in the muscle up to about 6 g/100 g sample resulted in the increase of WHC. Similar result was obtained here as 2% NaCl is used and weight gain observed. The water that is taken during dipping was not lost at the time of thawing or cooking, thus lower drip loss and cook loss was obtained (Table-8 and Table-9) than control. Control samples have higher yield than treated samples over the time of dipping, but in case of thawing or cooking the up taken water was being removed in case of control samples. No significance difference ($p>0.05$) was found in weight gain as control shows tremendous weight gain than other treatments but lost its most up taken water in the form of cooking loss and drip loss.

5.3. Sensory evaluation:-

Changes in sensory scores of shrimp samples after dipping in sodium bi-carbonate solutions containing 1%, 2%, 3% and 4% sodium carbonate with traces of citric acid and salt were given in Table-6(a-e). Results shows that control had sensory scores of 8.80±0.42, 8.90±0.31, 8.70±0.67, 8.80±0.42 and 8.90±0.31 for 1 hour dipping, which was decreased to 8.50±0.71, 8.60±0.70, 8.40±0.84, 8.60±0.70 and 8.50±0.85 for 4 hours dipping in respect of Appearance, Odour, Taste, Colour and Overall acceptability. For 4% Bicarbonate solution

dipped samples, the results were 8.60±0.97, 8.70±0.67, 8.70±0.48, 8.60±0.52 and 8.70±0.48 for 1 hour and 8.40±1.07, 8.50±0.70, 8.40±0.70, 8.30±0.82, 8.20±0.63 for 4 hours in respect of Appearance, Odour, Taste, Colour and Overall acceptability. Taste panel evaluations for the cooked shrimp (boiled in water) indicated that all fresh cooked shrimp samples of four different time period were liked by them and they rated all the shrimps > 8, and at that time the samples had fresh odor; white color flesh; bright shining appearance and firm and elastic texture.

When compared to shrimps which were not dipped in sodium bi-carbonate solution (control) with the treated samples, sensory score is almost same. There is no significant difference between shrimp of control and treated shrimps. Chantarasuwan *et al.* (2011) reported no difference in colour likeness were found between the control (fresh shrimp) and those were soaked in brine containing 2% sodium bicarbonate solution at pH 8.5. In the present study there was no significant difference existed between shrimps of four different time period. All the shrimps had the score above the limit of acceptability (4-5) and were in a good condition.

Throughout the storage period the sensory attributes (appearance, odor, taste, texture, and overall acceptability) were significantly decreased. However, at the end of frozen storage overall acceptability scores were 5.20±0.94, 5.10±0.97, 5.30±1.23, 5.10±1.13 and 5.10±0.70 for control samples (Table-7a) and 6.20±0.48, 6.20±0.32, 6.30±1.29, 6.20±0.82, and 6.30±1.40 for 4% bi-carbonate treated samples (Table-7e) in respect of Appearance, Odour, Taste, Colour and Overall acceptability, indicated that control samples were organoleptically acceptable, they rated as "fair" quality, when there was some loss of neutral odor; the texture became soft and watery (spongy) and loss of elasticity; with definite dullness appearance and the degree of freshness of the shrimp having treated with bi-carbonate was higher than those treated in water. Bi-carbonate treated shrimp samples were organoleptically in "good" condition up to 6 months, at that time, the samples had natural odor and natural flesh color; the texture became less firm; and there was some loss of brightness. Similar trend of sensory changes were achieved for boiled shrimp by other authors (Applewhite *et al.*, 1993; Sriket *et al.*, 2007; Tsironi *et al.*, 2009 and Paul *et al.*, 2012; Moawad *et al.*, 2013). Results also showed that best sensory score was given on the shrimps treated with bicarbonate 4%

solution. As can be noticed from the results, no evidence of melanosis, off-flavor or undesirable changes was detected in all decapitated shrimp samples even after 6 months at 20°C. In this respect, Yamagata and Low (1995) and Sundararajan (2010) reported that samples of frozen shrimp remained acceptable after 6 months at -20°C. The sensory study results vary significantly ($p<0.05$) along with the time and the concentration of bi-carbonate.

5.4. Drip loss:-

Drip loss (%) of the control sample and sodium bi-carbonate are given in Table-8. The drip loss (%) of control was 4.22±0.35% and in T1, T2, T3 and T4 samples it was 2.95±0.16%, 2.73±0.09%, 2.67±0.39% and 2.59±0.18% respectively in first month, which was increased to 10.84±0.24% in control and 5.04±0.12%, 4.83±0.20%, 4.64±0.12% and 4.58±0.11% in T1, T2, T3 and T4, respectively in the 6th month. From the result it is cleared that significant relationship ($p<0.05$) is established between treatments and control samples. Also, there is an increase in drip loss (%) value along with period of frozen storage. But control samples have also higher rate of drip loss over time than other bi-carbonate treated samples. This confirms the findings of Moawad *et al.*, (2013), where the drip loss value of 1st month of control *P. indicus* was 4.46% and STPP treated *P. indicus* was 2.17% which increased to 11.8% in the control and 4.62% in the treated sample. In the treated samples, it was also noticed that the rate of drip loss is being lowered over time as bi-carbonate concentration increased, there was significant relationship ($p<0.05$) between the samples and the time. This may be explained as higher quantity of sodium bicarbonate cause higher water holding capacity for which lower drip losses were found. These results confirm the findings of Turan *et al.*, (2003), Kolbe and Kramer (2007), Boonsumrej *et al.*, (2007) and Goncalves et al., (2008). Moawad *et al.*,(2013) suggested that drip results from the inability of the thawed muscle to reabsorb all of the separated water, which had been previously frozen. Formation of drip brings about the loss of weight, nutrient and flavor components, an unpleasant appearance of seafood, and a tough texture.

5.5. Cook loss:-

Cook loss (%) of samples have been shown in Table-9. Control samples had a cook loss(%) of 23.77±0.21%, whereas T1, T2, T3 and T4 samples had cook loss(%) of 17.14±0.80%, 16.83±0.23%, 16.23±0.25% and 14.70±0.46% respectively in the 1st month,

which was increased to 33.82±0.63% for control and 23.15±0.74%, 23.09±1.49%, 21.09±0.28% and 20.31±0.85% for T1, T2, T3 and T4 respectively. Weight gain has a definite relation with cook loss. The water that was being absorbed in the muscle during dipping was lost during cooking. This may be the cause that cook loss (%) of control sample was high because the water absorbed, but due to low WHC, most of was removed during cooking, whereas in case of bi-carbonate treated samples the less water was removed as the treatment increase the WHC of samples. It matches with the findings of Moawad *et al.* (2013) where tri-sodium polyphosphate was used in place of sodium bi-carbonate on *P. indicus*, which had 23.7% cooking loss in control sample and 15.3% in the STPP treated sample on the 1st month, but increased to 29.2% for control samples and 20.5% in the STPP treated sample on the 6th month. Results also indicates that higher concentration of bi-carbonate treated samples have lower cook losses. The results were significant with treatments and times ($p<0.05$). It may prove that bi-carbonate may be effectively used as a cook loss reducing chemical in shrimp processing.

5.6. Proximate analysis:-

5.6.1. Moisture:

The proximate composition of raw fresh control and sodium bi-carbonate treated tiger shrimp were presented in Table-10 to Table-13. Black tiger shrimp meat had a higher moisture content but lower protein content than had white shrimp meat ($p< 0.05$). Table-6 showed initial moisture content of 78.14±0.82% in control and 78.14±0.83%, 78.13±0.82%, 78.14±0.70% and 78.14±0.70% in T1, T2, T3 and T4 respectively. After 6 month frozen storage at -20±2°C, sodium bi-carbonate treated samples of 1%, 2%, 3% and 4% showed moisture content of 75.02±1.33%, 75.10±1.07%, 75.12±1.07% and 75.33±1.01%, where control sample have moisture content of 75.03±1.33% which indicates that sodium bi-carbonate protected shrimp samples have higher moisture retaining capacity than controlled shrimps over time. Also among the treatments, moisture retention capacity is highest in T4. This may be due to more quantity of bi-carbonate was being absorbed by the shrimp muscle, as a reason, more moisture retention ability was noticed. Moawad *et al.* (2013) also showed the decreasing trend of moisture content of trisodium phosphate treated white shrimp samples from 77.104% to 72.19% in control and from 77.32% to 75.53% in treated shrimps over 6

month of storage period at -20°C, but this loss of moisture more in controlled samples. Wangtuei *et al.* (2014) showed that the frozen nile tilapia fillets treated with STPP showed highest moisture content than controlled fish fillets. This clearly defines that bicarbonates also have moisture retention capability like phosphates. Pacific white shrimp treated with sodium bi-carbonate containing traces of citric acid solution at 2% and 4% caused an increase in the shrimp water holding due to improvement in moisture retention resulting from the water holding capacity of the protein in the shrimp meat (Erdogdu *et al.*, 2004).

5.6.2. Protein:

As seen in table-11 there was a decreasing trend in protein content from 18.00±0.32% to 17.05±0.69% for control samples and 17.59±0.80% to 16.83±0.64% for T1, 17.91±0.74% to 17.20±0.35% for T2, 17.73±0.53% to 17.37±0.29% for T3 and 18.04±0.31% to 17.89±0.73% for T4 respectively, at the end of frozen storage of 6 months at -18±2°C. The results also indicated that decreasing of protein content was minimal at T4 sample than other treated samples. Concerning protein loss during frozen storage of white shrimp, Sundararajan (2010) reported that, water soluble nutrients, including proteins, minerals and B-vitamins can be lost along with the thaw exudates, and thus reduced nutritional value of frozen shrimp. Beklevik *et al.* (2005) achieved similar results for sea bass fish fillet frozen at -18±2°C where initial protein content was 19.75%, which was decreased to 19.31% after 9 months of frozen storage; respectively. However, the loss of protein could be attributed to the breakdown of proteins by proteolytic enzymes that are not completely inactivated during frozen storage (Pan and Yeh, 1993), and also due to the loss of the nitrogenous compounds either as volatile substances caused by microbial effect or separated in drip (Orak and Kayisoglu, 2008). In the present study higher protein retention may be explained by the effect of bi-carbonate to reduce the denaturation of protein during frozen storage.

5.6.3. Fat:

Table-12 showed the initial fat content of 2.53±0.92% for control and 2.56±0.95%, 2.40±0.81%, 2.96±1.05% and 2.42±1.10% for bi-carbonate treated samples i.e. T1, T2, T3 and T4 respectively. After 6 month of frozen storage fat content decreases from the initial

value i.e. 2.36±0.98% for control and 2.39±1.02%, 2.25±0.73%, 2.81±1.13% and 2.32±1.04% for T1, T2, T3, T4 respectively. From the result it is cleared that the loss of fat was due to oxidation which was the major cause of deterioration of quality. Also the loss of fat is minimal in T4 than other treatments. This also indicates that higher concentration of bicarbonate results in lower loss of fat during frozen storage. The reduction in lipid content may be associated with the oxidation of polyunsaturated fatty acids found in shrimp tissues to other products as aldehydes, free fatty acids, ketones, and peroxides (Horner *et al.*, 1992). Compared to control, bi-carbonate treated sample has shown lower loss of fat content. However in every treated sample the rate of decreasing of fat content in the samples from initial to 6th month was same.

5.6.4. Ash:

Table-13 showed initial ash content of 1.39±0.84% for control and 1.43±0.88%, 1.43±0.88%, 1.38±0.95% and 1.43±0.88% for T1, T2, T3 and T4 samples respectively. After 6 month frozen storage at -20±2°C ash content became 1.41±0.82% for control and 1.44±0.79%, 1.42±0.87%, 1.34±0.71% and 1.41±0.78% for T1, T2, T3 and T4 samples respectively. From the results it is clear that there was negligible difference ash content value after frozen period. So, it may be possible that bi-carbonate might not contribute any change in ash content of shrimp.

5.7. TMA assessment:-

Changes in TMA was represented in Table-14. The initial TMA value was 0.82±0.05 mg N/100g for control and 0.62±0.15 mg N/100g, 0.62±0.14 mg N/100g, 0.66±0.08 mg N/100g and 0.65±0.20mg N/100g for T1, T2, T3 and T4 samples respectively. After 6 month frozen storage at -18±2°C TMA content increased steadily ($p<0.05$) to 3.42±0.31 mg N/100g for control and 2.04±0.17 mg N/100g, 2.02±0.14 mg N/100g, 1.99±0.17 mg N/100g and 1.97±0.18 mg N/100g for T1, T2, T3 and T4 samples respectively. This confirms the findings of Moawad *et al.* (2013) where initial TMA value of control white shrimp and TSP treated white shrimp were 0.67 mg N/100g and 0.48 mg N/100g respectively, while after 6 month of frozen storage, the value was increased to 2.73 mg% in control and 1.87 mg N/100g in TSP treated shrimp. This increase may be due to action of spoilage bacteria and endogenous enzyme, which impart an unpleasant "fishy" odour (Kilinc and Cakli, 2004). It is clearly

noticed that there is a higher increase in TMA value of control than the bi-carbonate treated samples. Also, the least increase in TMA is observed in T4 compared to other treatments. This may be due to the ability of bi-carbonate to reduce microbial load, thereby decreasing protein breakdown and hence lower values of TMAN were formed. However, it is worth mentioning that a level of 5 mg N/100g flesh for TMAN; is usually regarded as the limit beyond which seafood will develop an objectionable odor/taste (Cobb *et al.*, 1976 and EOS, 2005). Consequently, control samples also were of acceptable quality, while bi-carbonate treated shrimp exhibit better quality after 6 months of frozen storage at -18±2°C.

5.8. TVB-N assessment:-

Table-15 showed the changes in TVB-N content from 12.95±0.33 mg N/100g to 33.87±0.17 mg N/100g for control and 11.88±0.92 mg N/100 g to 23.36±0.59 mg N/100 g for T1, 11.81±0.51 mg N/100g to 23.01±0.73 mg N/100g for T2, 11.85±0.58 mg N/100g to 22.81±0.61 mg N/100g for T3 and 11.88±0.81 mg N/100g to 22.13±0.36 mg N/100g for T4 respectively. From the result it is shown that there was a significant difference ($p<0.05$) in the TVB-N value throughout the storage period and among the treatments. The higher increase rate observed in control than bi-carbonate treated samples, also the increase is minimal in T4. The same results obtained by Moawad *et al.* (2013) where white shrimps were dipped in tri-sodium phosphate in place of sodium bi-carbonate, TVBN Value increased from 11.9 mg N/100g to 28.6 mg N/100g in control sample and from 10.2 mg N/100g to 21.0 mg N/100g in TSP treated shrimp. This may be due to activity of spoilage bacteria and endogenous enzymes. The result also shows that the increased concentration of sodium bicarbonate dipping results in lower value of TMA after 6 months of frozen storage. Sodium bi-carbonate treated samples may have same effect on TMA and TVBN as observed in tri-sodium phosphate treated samples. Another explanation may be due to the ability of bi-carbonate to reduce microbial load, thereby decreasing protein breakdown and hence lower values of TVB-N were formed. However, a level of 30 mg N/100g flesh for TVB-N; is usually regarded as the limit beyond which seafood will develop an objectionable odor/taste (Cobb *et al.*, 1976 and EOS, 2005). Consequently, control samples were at acceptable level of quality, while bi-carbonate treated shrimp exhibit better after 6 months of storage at -18°C.

5.9. TBARS assessment:-

Table-16 showed the change in TBARS content of shrimps. The initial value was 0.93±0.06 mg MDA/kg flesh for control and 0.83±0.04 mg MDA/kg flesh, 0.86±0.05 mg MDA/kg flesh, 0.83±0.08 mg MDA/kg flesh and 0.83±0.03 mg MDA/kg flesh for T1, T2, T3 and T4 samples respectively which was increased to 1.80±0.08 mg MDA/kg flesh in control and 1.46±0.07 mg MDA/kg flesh, 1.49±0.04 mg MDA/kg flesh, 1.44±0.08 mg MDA/kg flesh and 1.42±0.05 mg MDA/kg flesh in T1, T2, T3 and T4 respectively, after 6 months of frozen storage. As seen in the result there was a significant increase in the TBARS ($p<0.05$) value during the storage period and within the treatments it is significantly decreased. Also the TBARS value decreases with increasing of dipping concentrations, T4 gives the lowest TBARS value. Sundararajan (2010) reported that TBARS values of white shrimp increased from 0.51 to 2.96 mg MDA/kg after 180 days of frozen storage at -20°C. TBARS values first increased during frozen storage due to accumulation of products from lipid oxidation especially Malonaldehyde (MA) and malondialdehyde (MDA) and then a lower increase rate due to interaction of MA and MDA with protein, amino acid, glycogen etc resulting in lower amount of free MDA, which was reported earlier (Goulas and Kontominas, 2007). MA formed during lipid oxidation has been reported to form cross-links with protein and can form insoluble protein aggregates (Buttkus, 1970). Control when compared to the, bi-carbonate-treated samples had significantly ($p<0.05$) lower TBARS values at each step during the of frozen storage, indicating that bi-carbonate dipping treatment was effective in retarding the lipid oxidation process, due to its ability to complex "quelation" pro-oxidant metallic cations (Goncalves *et al.*, 2008). Mentioning that, seafood products of good quality will have TBARS values less than 2 mg MDA/kg, while consumption limits should be less than 5 mg MDA/Kg flesh (EOS, 2005; Goulas and Kontominas, 2007). So it could be said that the treated samples possess good quality after 6 month of storage, whereas control samples would not be considered as good quality but it was good for consumption purpose.

5.10. Peroxide Value:-

High levels of polyunsaturated fatty acid are present in the tissue membranes of many crustaceans. The processing of crustaceans has been suggested to inflict damage on the tissues and induce lipid oxidation (Chaijan, 2011; Morrissey *et al.*, 1998). It has been reported that the PV typically quantifies the primary lipid oxidation of products particularly the hydroperoxides (Chaijan, 2011). Other authors have reported initial PV ranges in other sea foods also such as fresh sardine (0.36-1.11 mmol of O_2/kg) and herring (Clupea harengus) (0.1-0.15 mmol of O_2/kg) (Erkan & Ozden, 2008).

The change of PV in shrimps were monitored during frozen storage (Table-17). The initial PV was observed 2.02±0.24 mEq of O_2/Kg of shrimp for control sample and 1.61±0.16 mEq of O_2/Kg of shrimp, 1.57±0.16 mEq of O_2/Kg of shrimp, 1.60±0.17 mEq of O_2/Kg of shrimp and 1.60±0.18 mEq of O_2/Kg of shrimp for T1, T2, T3 and T4 samples, respectively. After a frozen storage of 6 months, it was observed that the PV is increased over the time in all samples. After 6 months, control sample had a PV of 8.61±0.50 mEq of O_2/Kg of shrimp and T1, T2, T3 and T4 had a PV of 6.15±0.16 mEq of O_2/Kg of shrimp, 6.05±0.13 mEq of O_2/Kg of shrimp, 6.05±0.13 mEq of O_2/Kg of shrimp, and 5.32±0.50 mEq of O_2/Kg of shrimp. This findings are similar with Okapala *et al.* (2014) where PV increased from 1.52 mEq O_2/Kg of shrimp to 32.52 mEq O_2/Kg of shrimp in 32 days of iced storage. It was noticed that the significant change of PV ($p<0.05$) is higher in control samples than bi-carbonate treated samples. Also, the differences between the treatments varies significantly ($p<0.05$) with the least increase in T4 after 6 months of frozen storage. It may be due to the oxidation of fat, several peroxides and hydro-peroxides were formed which increases the value. Results also indicated that bi-carbonated dipping treatment was effective in retarding the lipid oxidation process.

5.11. pH value:-

Table-18 showed the changes in pH throughout the frozen storage period. The initial pH value was 7.06±0.24 for control and 7.09±0.27, 7.09±0.26, 7.15±0.26 and 7.18±0.32 for T1, T2, T3, T4 samples. Here the result showed significant differences ($p<0.05$) in respect to sodium bi-carbonate concentration, and also shows significant relationship ($p<0.05$) with time. It can be explained by the say that as the solutions buffered at pH 7.4, The pH changes

of shrimp meat more likely determined the changes in muscle, particularly, the modification of charge as well as conformation of proteins (Chantarasuwan *et al.*, 2011). After 6 months frozen storage the value increased to 7.42±0.43 for control and 7.23±0.32, 7.24±0.31, 7.29±0.47 and 7.31±0.50 for T1, T2, T3 and T4 respectively. This proves the findings of Moawad *et al.*, (2013) where, pH valoe increased from 6.83 to 7.36 in control white shrimps and from 6.95 to 7.13 in TSP treated white shrimps during frozen storage of 6 months. Similar results were achieved in seafood after phosphate treatments (Goncalves *et al.*, 2008; Kilinc *et al.*, 2009 and Etemadian *et al.*, 2012). However, the increase in pH is related to accumulation of alkaline compounds such as TMA, TVB, volatile amines and ammonia (Sriket *et al.*, 2007). Generally, pH value of 7.8 is reported to be a critical value in determining the acceptability of shrimp (Cobb *et al.*, 1976 and Sundararajan, 2010). So, the treated shrimps possess good quality and is acceptable.

5.12. Protein solubility:-

Protein solubility of sodium bi-carbonate treated samples and control samples have shown in Table- 19. The protein solubility of control sample initially was 80.95±0.48%, whereas for the treated samples those were 84.65±1.24%, 84.96±0.89%, 85.10±2.33% and 86.57±1.64% in T1, T2, T3 and T4 samples respectively. After 6 months of frozen storage solubility decreased to 52.34±0.38% in control and 68.13±0.64%, 68.95±0.62%, 70.45±0.86% and 71.10±0.46% in T1, T2, T3, T4 samples respectively. The solubility decreases significantly ($p<0.05$) with time and increases significantly ($p<0.05$) among the treatments. The increase in trimethyl amine, the interaction of lipid oxidization products and the breakdown of trimethyl amine oxide (TMAO) have all been associated with the denaturation and aggregation of proteins and subsequently loss of their solubility during frozen storage (Buttkus, 1970; Torrejon *et al.*, 1999; Kolbe and Karmer, 2007). The decrease in the solubility of protein has been used as an indication of oxidative deterioration of muscle protein (Decker *et al.*, 1993; Srinivasan and Hultin, 1997; Xiong and Decker, 1995). Vojdani (1996) had shown that a decrease in protein solubility is the result of shift from a balance of protein intermolecular interaction and protein-water interaction resulting in a situation where protein intermolecular interaction is forced where protein water interaction is weakened.

5.13. Total plate count:-

Shrimp is considered a suitable medium for the growth of many organisms (Nirmal and Benjakul, 2010). The changes in total plate count of shrimp samples have been summarized in Table- 20 where it is clearly seen that initially control samples has total plate count of 5.51±0.03 log CFU/g and T1,T2,T3 and T4 has 4.08±0.03 log CFU/g, 4.01±0.05 log CFU/g, 3.98±0.02 log CFU/g, 3.90±0.05 log CFU/g respectively, where after 6 months of frozen storage the increased value was 5.17±0.11 log CFU/g for control and 3.63±0.02 log CFU/g, 3.59±0.03 log CFU/g, 3.58±0.02 log CFU/g and 3.58±0.03 log CFU/g for T1, T2, T3 and T4 respectively, after 6 months of frozen storage. It is clearly noticed that the total plate count value is significantly ($p < 0.05$) higher in control sample than the treated samples. Similar results were achieved by Moawad *et al.* (2013) where control *P. indicus* had an initial value of 5.16 log CFU/g and tsp treated white shrimp had a value of 3.45 log CFU/g, which was decreased after 6 months to 4.76 log CFU/g for control and 3.12 log CFU/g for TSP treated white shrimp, after 6 months of frozen storage. It can be explained by the antimicrobial activity of sodium carbonate. There is a steady decrease in the total plate count in first month due to frozen shock, subsequently slight increase upto the six month. Similar trends in TPC were found in white shrimp during frozen storage. (Sriket *et al.*, 2007 and Ali, 2011). This may be due to the death of thermophilic and mesophilic bacteria present in the shrimp. Lopez-Caballero *et al.* (2007) reported that microbial spoilage was retarded during frozen storage, but continued in defrosted shrimp, it was postulated that the nutrient released after thawing could promote the growth of bacteria in shrimp. However, total plate count in all examined sample is lower than the critical limit of 10^6 CFU/g recommended by EOS (2005).

CHAPTER - VI

SUMMARY AND CONCLUSION

The objective of the present study was to study the effect of sodium bi-carbonate in yield and characteristics of tiger prawn (*Penaeus monodon*). The findings are summarized below:

1. The tiger prawns was dipped in each of the four different concentration (1% to 4%) of sodium bi-carbonate with traces of citric acid and salt in four different time period (1hr to 4hr) and weight gain of each sample was taken. Simultaneously a blank sample was runned.

2. The highest weight gain was observed in 1hr for each of the sodium bi-carbonate solution and in control sample weight gain (%) was maximum in ^{4th} hr. It is because the control sample which absorb highest quantity of water in 4^{th} hour will lost highest amount of water during thawing and cooking as drip loss and cook loss simultaneously. Bi-carbonate treated samples will have lower amount of drip loss and cook loss due to its properties to increase WHC and moisture retention capacity. The shrimps of control and each of the concentration dipped in sodium-bicarbonate for 1hr was taken for frozen storage.

3. The sensory quality of the samples was taken initially after dipping and during frozen storage of 6 months. All the shrimps had the score above the limit of acceptability (4-5) and were in a good condition initially and during storage period. Throughout the storage period the sensory attributes (appearance, odor, taste, texture, and overall acceptability) were significantly ($P<0.05$) decreased. However, at the end of frozen storage all shrimps were a sensory score above 5 and regarded as "fair" quality.

4. Drip loss and cook loss during frozen storage was determined and it is observed that both parameters has higher value in control than the treated samples. This is because the water entered during dipping was lost during thawing and cooking. Whereas, treated samples have higher WHC and moisture retention capacity.

5. In proximate composition analysis the moisture content of treated samples was higher than the control samples due to moisture retention capacity of sodium bi-carbonate. The protein content decreased significantly ($P <0.05$) in the 6 month of frozen storage. It

may be due to the loss of the nitrogenous compounds either as volatile substances caused by microbial effect, and also the action of proteolytic enzymes. Those enzymes are not completely deactivated during frozen storage. The change in fat content is observed and explained as a result of lipid oxidation to form peroxides, aldehydes etc, resulting in higher TBARS value. In case of ash content there is no significant change when compared to the initial value to the final value in 6 month. So, it can be said that sodium bi-carbonate do not have any effect in ash content.

6. The quality parameters like TMA, TVBN, TBARS was calculated during frozen storage. All the samples (control and treated) are within the critical limit of each parameter. It is shown in TMA and TVBN value that the value decreased with increasing sodium bi-carbonate concentration and increased with the period of frozen storage. This may be due to activity of spoilage bacteria and endogenous enzymes. TBARS value increased with the period of frozen storage due to peroxides formed in lipid oxidation process.

7. Result showed slight increase in pH with the increase in sodium bi-carbonate concentration and the pH value was highest in 4% sodium bi-carbonate solution. It could be explained by the say that with higher concentration of sodium bi-carbonate the higher is the pH.

8. Protein solubility is the result in shift to lower protein water interaction. Thus the result showed a significant ($P<0.05$) decrease in solubility throughout frozen storage.

9. PV increased in respect of time but reduced in respect of treatment ($p<0.05$). 4% bi-carbonate solution gives the best result.

10. Total plate count showed in Table-13 indicated that control samples have higher TPC than the treated sample. In first month there is sudden decrease due to frozen shock, but afterwards it increased. All the samples are within the critical limit of 10^6 CFU/g recommended by EOS.

CHAPTER - VII

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ANNEXURE-I

I. ORGANOLEPTICALLY EVALUATION OF THE SHRIMPS BY TEN UNTRAINED PANELIST BASED ON 9-POINT HEDONIC SCALE:-

Points	Remarks
9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely