3. QUALITY IMPROVEMENT METHODS

3.1. INTRODUCTION

Globally, seafood export industries meet a huge problem while transporting the products. The time taken for any mode of transportation may enhance the chance of contamination of seafood and the quality may reduce from catch level to storage and distribution. Crab industries face not only the problems of transport but also proper preservation technique because of the difficulties of keeping crabs alive more than two to three days at tropical climate. The quality of fresh products declines rapidly due to microbial growth and cross contamination from various sources (Graham, 1997). Lee and Pefifer, (1975) have isolated aerobics, heterotropic microorganisms from raw and cooked crab meats of Dungeness crab (*Cancer magister*) during commercial processing. Rippey (1994) and Jayasree et al., (2006) have reported that *Vibrio* species account for a significant proportion of human infections from the consumption of raw or undercooked shellfish.

Though the traditional methods such as freezing (Hansen and Aagaard, 1969) chilling and ice storage (Himelbloom et al., 1994), modified atmosphere packaging (Davies, 1997) high pressure treatment (Hugas et al., 2002) etc., are probably contributing to minimise the microbes in food products, but these techniques create certain technical problems. Normally, sea food and sea food products have been traditionally washed in chlorine solution to minimize the microbial level. White (1986) noted chlorine is one of mankind’s most trusted weapons in the war against the infectious waterborne diseases. Studies on chlorine usage on microbial reduction were carried out by Hong and Rapson, (1968), Brungs, (1973) and Collins, (1997).
However, its relative ineffectiveness produce certain by-products cause health problems (Xu, 1999). Ozone has been used in several studies to decontaminate freshly caught fish (Goche and Cox., 1999). Effects of ozone have been studied on a wide variety of organisms (Restaino et al., 1995) and are considered as less health risk to humans compared to those formed by chlorine treatment (Graham, 1997). The potential utility of ozone in the food industry lies in the fact that ozone is a 52% stronger oxidant than chlorine and acts over a wider spectrum of microorganisms than does chlorine and all other common disinfectants (Katz, 1986).

In order to meet consumer’s demand at global market, the seafood export industry is currently in need of innovating a better processing technology. According to the present observation, the newly moulted soft shell mud crabs harbour lower microbial load than wild crab. Further minimizing the microbial load in newly moulted crab is necessary one to promote its market value. Hence, a modified existing technique would be necessarily employed to improve the quality on exportable soft shell mud crabs *S. serrata*. Hence the treatment study was carried out for a period of 12 months.
3.2. TREATMENT TECHNIQUES

3.2.1 Chlorine (100ppm) Treatment

3.2.1.1 Materials & Methods

Sterile trough, 10% chlorine, 1 litre of RO water and Chlorine level monitoring strip.

To prepare 100ppm of chlorine, the following formula was used (Ritenour et al., 2002).

\[
\text{Volume of water} \times \frac{\text{Required ppm}}{10 \times \text{Strength of Chlorine}} = 1 \text{ litre}
\]

Volume of water = 1 litre

Required ppm = 100

Strength of Chlorine = 10%

\[
1 \times \frac{100}{10 \times 10} = 1 \text{ ml}
\]

100ppm chlorine concentration was made by adding 1ml of chlorine in 1 litre of water. The dissolved chlorine concentration was checked by the chlorine level monitoring strips. For active microbial reduction, the water temperature was maintained at below 15\(^{0}\) C (Reshma Zamir, 1998). The newly moulted crabs were introduced into chlorinated water (Plate-3), the initial time was noted and the crabs were taken out from the trough after half an hour. Then the treated crabs were checked for microbial load, nutritional status and pH.
3.2.2. Ozone Treatment Method

3.2.2.1. Materials & Methods

Ozonator, Sterile trough, Tintometer, DPD No.4 Tablet and 1 litre of RO water.

For the present study, ozone gas mixture was produced using an Ozonator by bubbling method. The ozone gas was pumped into the system using the aquatic air pump. One litre of water was taken in a trough, then air stones were connected with ozonator and introduced in to the water and the ozone started to flow as bubbles. The ozone gas started to dissolve slowly in the water, after one hour the dissolved ozone level was checked by Photometer (Tintometer) by using DPD.No.4 tablets. The treatment process was started, when the Photometer showing the dissolved ozone level of more than 1mg/l. Firstly, soft shelled crabs were introduced into the ozonated water (Plate - 4) and the initial time was noted. After that, the trough was covered with a lid immediately to avoid escape of the crab from trough. The water temperature was maintained at below 15°C for the active microbial reduction (Reshma Zamir, 1998). After half an hour of treatment, the crabs were taken out from the water and analysed the microbial, nutritional and pH. In order to avoid contamination, sterile gloves were used while handling the crabs by hand.

3.2.3. Combined use of Chlorine and Ozone Treatment:

3.2.3.1 Materials & Methods

Three Sterile troughs, 0.5ml, 0.2ml & 0.1ml of 10% Chlorine, 3 litres RO water, Chlorine level monitoring stripe, Ozonator, Tintometer and DPD No.4 Tablet.

Three uniform sized troughs (I, II & III) were selected and filled with distilled water. The ozonator was introduced in to these three troughs and ozone started to flow. After one hour, the dissolved ozone level was checked by using Photometer and the level
of dissolved \( \text{O}_3 \) was noted up to 1mg/litre. The water temperature was reduced to \(15^0\text{C} \) for active microbial reduction. Then three different concentrations such as 50ppm, 20ppm and 10ppm of chlorine water were prepared and introduced into the troughs I, II & III respectively.

Crabs were introduced into the I trough containing of chlorine (50ppm) with ozone and the initial time was noted. After 10 minutes, the crabs were removed from the first trough and introduced into the II trough containing chlorine (20ppm) and ozone. After 10 minutes, the crabs were removed and reintroduced into the III trough of Chlorine (10ppm) and Ozone concentration and kept for another 10 minutes (Plate – 5). After the treatment the crabs were taken out for further bacterial, nutritional status and pH. Based on the result obtained from the three treatment methods, the amino acids sequences of the Chlorine + Ozone treated crabs were also carried out to confirm the presence of amino acids without any drastic change due to the treatment. The amino acids were analyzed in the SGS Laboratories, Chennai.
3.3 RESULT

The results of total bacterial load, biochemical components, and pH for the chlorine (100ppm), ozone and chlorine + ozone treated soft shell crabs are given in the figure (8-14). The result of amino acid profile of newly moulted crab is given in the figure (15) and Table (10 and 11).

3.3.1. Microbial analysis

Among the three treatments given (Chlorine (100ppm), Ozone and Chlorine + Ozone), the maximum number ($9.8 \times 10^3$ cfu/gm) of bacterial flora was isolated in Chlorine treated crabs during monsoon (September) season. Whereas, the minimum number ($3.5 \times 10^3$ cfu/gm) of bacterial flora was observed in Chlorine + Ozone treated crabs during summer (March 2010) season. Besides, the pathogenic bacteria such as Coliforms sp., and *Vibrio* sp., could not be traced in the crabs treated with Chlorine + Ozone combination.

3.3.2. Biochemical Components

In the present study, protein was found to be higher than carbohydrate and lipids. The maximum (9.6 g/100g) value of protein was recorded in summer season (March 2010). Likewise, the maximum levels of carbohydrate (2.17g/100g) and lipid (0.5g/100g) were found in Chlorine + Ozone treated soft shelled crabs during June (Monsoon season). The minimum value of protein (8.32 g/100g) was observed in chlorine treated soft shelled crabs in July (Monsoon season). Whereas the minimum carbohydrate level of 1.36 g/100g (May / Summer season) and minimum lipid level of 0.26 g/100g (April / Summer season) in ozone treated soft shelled crabs.

Totally, 20 amino acids (Inclusive 12 essential and 8 non-essential amino acids) were found in soft shelled crabs treated with Chlorine + Ozone. The presence of essential
aminoacids showed 54.36% and non essential aminoacids showed (45.64%). Among amino acids, glutamic acid recorded the maximum level (14.20%) and Methionine was the minimum level (0.08%) in Chlorine + Ozone treated soft shelled crabs.

3.3.3. pH

The pH range was from 6.72 to 7.07 in Chlorine (100ppm) treated crabs, between 6.80 and 6.97 in Ozone treated crabs and from 6.83 to 6.96 in Chlorine + Ozone treated crabs.

3.4. Statistical Analysis

ANOVA showed statistically significant difference in the pathogenic Coliforms bacteria between Chlorine (100ppm), Ozone and Chlorine + Ozone treated soft shell crabs at 1% level (Table 12-18).

The outcome of the result showed that of all the three methods of treatment, Ozone + Chlorine treatment could be considered the better one. Due to this treatment, the shelf life also increased considerably and also mass reduction or complete eradication of pathogenic bacterial load.
3.5. DISCUSSION

The three different methods of treatment of soft shelled crabs showed some interesting trends. In the present study, the use of Chlorine + Ozone resulted in better microbial reduction and extended shelf life, based on, the appearance were observed in the soft shelled crabs. Besides, the pathogenic bacteria such as Coliforms and *Vibrio* spp were untraceable. The effectiveness of Ozone + Chlorine treatment method was found to be a remedial method in improving the quality of the soft shelled crabs. Garcia et al., (2003) reported the effective microflora reduction and potential shelf life on lettuce when it was treated with the combined use of Chlorine and Ozone. Kuangii et al., (2007) proved that the ozone and chlorine treatment extended the shelf life of the agricultural product. The present observation was also in confirmation with the studies of Korich et al., (1990), Richardson et al., (1996), Collins (1997) and Beuchat et al., (1998). In the present work, instead of using Ozonized water directly, Ozone molecules were delivered as bubbles slowly in water to reduce microbial reduction. Bubbling ozone was significantly better treatment and also more efficient way of lettuce disinfection than ozonated water treatment (Garcia et al., 2003).

The use of Chlorine(100ppm) treated crabs also showed the minimal microbial next to combined use of Chlorine and Ozone on soft shelled mud crab and the shelf life (appearance and flavour) was not better than the Chlorine and Ozone treated crabs. This may be due to the fact that residual chlorine appeared on the surface of the soft shelled crabs which made the different appearance and unpleasant flavour on the product. Zhou (1995) and Kim et al., (1999) stated that the use of high dosage of chlorine could result in the formation of various chlorinated by-products, some of which are considered to be carcinogenic. Beuchat and Brackett, (1990) found some of the food borne pathogens were resistant to chlorination.
Though the residual products were not formed on the surface of the Ozone treated crabs, but higher bacterial load (lower than Chlorine treated crab) was observed in the present study. This could be compared with the unstable nature of Ozone molecules as Ozone is immediately dissociated into O and O$_2$ so that, the disinfection process is not effective. Graham (1997) observed that, residuals are not formed because of ozone is reduced down to oxygen. The other by-products of ozone are considered as less health risk to human compared with those formed by chlorine (100ppm) treatment. Besides, the ozone is not much effectively kills the bacteria in dirty water. The present observation is confirmed with the findings of Blogoslawski et al., (1979) and Haraguchi et al., (1969). Effects of particulate matter on virus inactivation by ozone were reported by Sproul et al., (1985). The lower bacterial density in ozone treated crabs was also confirmed with Kinman (1972), Glaze (1986), Katz (1986), Mitstuda et al., (1990) and Restaino et al., (1995). Nathapol et al, (2007) observed that the soaking the soft shell mud crab with ozonated water for 20 minutes resulted in microbiological changes.

The results of the present study showed that there is no major variation on the proximate composition in the combined use of Chlorine and Ozone treated crabs. A lower TVB-N value indicates good quality of the products. It could be correlated with the lower number of bacteria which might have reduced the Total Volatile Basic Nitrogen (TVBN) as reported by Kyrana and Lougouvious (2002) and Kayim and Can (2010). The present observation also confirms the reports of Sootrawat and Nuntapol (2008).

The pH was found to increase when the samples were treated with Chlorine with ozone combination. The pH value between 6.8 and 6.9 is considered as an ideal pH for maintaining the quality of meat when the crab is treated with combined use of Chlorine
and Ozone. Reshma Zamir et al., (1998) recorded the pH <7.7 are considered as good in maintaining the quality of tissue of fishes.

In the present study, amino acids profile was not changed in combined use of chlorine and ozone treated crabs after processing. It could be correlated with the maximum percentage of protein in the study animal which reflects the quality of meat. The quality of the protein in a food depends on its aminoacid content and on the physiological utilization of specific aminoacids after digestion, absorption and oxidation (Friedman, 1996). The essential aminoacid, methionine was found to be detected after treatment which could be correlated with the interaction of amino acids residue in protein molecules by peptide bond. Vithayathil and Richards (1960) who reported that certain salts interfere the repulsive force peptides in the protein and decreased binding constant. The methionine residue usually contributes to the binding energy, but the sulfonium salt decreased the binding force of methionine in protein molecule. As a result, the methionine residue might have freely available in the peptide to protein complex and is not”masked”.

It could be concluded that the combination of Chlorine + Ozone treatment bestowed significant result on microbial reduction on the palatable mud crab S. serrata. Besides, the dosage used in this study did not affect the biochemical composition, pH and aminoacid profile of the samples. Even though the quality of the meat depends on physico-chemical and biological factors, the consumer acceptance centralizes the organoleptic attributes of the product. Hence, the organoleptic characters were analyzed in the soft shell crabs and presented in the ensuing chapter.