1. GENERAL INTRODUCTION

1.1. Preamble

The problems of ever increasing human population have induced mankind to think on sustainable utilization of marine resources. Marine food has traditionally been a popular diet in many parts of the world and some countries constituted the main supply of animal protein. From the nutritional point of view, seafood is considered as very valuable food item and contains a high percentage of easily digestible animal protein. Seafood also contributes a lot to national economy and foreign exchange earnings for any developing maritime country.

India is bestowed with a long coastal line of over 8,129 km, two million sq. Km of Exclusive Economic Zone with 1.2 million hectares of brackish water bodies (Marine and Earth Sciences Group, 2009). Before 1960’s, the markets of Indian Marine Products were dominated by dried items and were exported largely to neighbouring countries like Srilanka, Myanmar, Singapore etc., The quality of dried fish never receives much attention (Gupta and Samuel, 1985) due to improper handling while processing, storage, transporting and marketing. This situation gradually changed after the development of number of techniques such as canning, freezing etc. As a result, the major seafood importers from our country are Japan, USA, Europe, Middle East, Australia etc. India exports more than Rs. 8000 crores worth of seafood every year. The South East Asia is the largest importer of seafood from India with a major share (25.75%) followed by European Union (22.02%), US (19.17%), Japan (14.09%), China (7.06%), the Middle East (4.39%) and other countries (7.51%), (FISHING CHIMES, 2012).
The crustaceans, particularly shrimps and crabs are harvested in huge quantities annually and are being used largely for human consumption. The global estimates of the described species of crustaceans is 40,000 of which 2934 species have been reported in India, amongst 705 species are brachyuran crabs (Venkataraman and Krishnamoorthy, 1998). The common edible crabs are *Portunus pelagicus*, *P. sanguinolentus*, *Podopthalmus vigil*, *Charybdis charybdis*, *C. cruciata*, *C. lucifera*, *Ocypoda ceratopthalmia*, *Matuta lunaris*, *Thalamita crenata* and *Scylla serrata*. Along Indian coasts, *P. pelagicus*, *P. sanguinolentus* and *S. serrata* are commercially important crabs. Usually crabs are exported in the form of live crabs, frozen whole crabs, chilled whole crabs, frozen cut-leg crabs and crab meat products. The mud crab, *S. serrata* is one of the popular economically important crustaceans for its larger size, quality meat and rich nutrition (Rameshkumar et al., 2009). They are abundantly found in tropical regions of the Indian and Pacific Oceans. It has been widely cultured in China, especially in the southern regions such as the Fujian, Guangdong and Hainan Provinces. Though the mud crabs culture and fattening units are largely started in India, Thailand, Bangladesh, Srilanka, Indonesia etc., the information is limited on the quality improvement of exportable mud crab.

Introducing quality food is a major concern to food processing industry and public health authorities. There are more than 76 million cases per annum of food-borne illnesses in the USA (American Medical Association, 2004) and in India about 400,000 children (<5 years) die every year due to various kinds of diseases such as hepatitis A, enteric fever, dihorrea etc., mainly because of poor hygiene and unsafe food (Ministry of Health and Family Welfare, 2006). The US National Research Council Committee (1985) estimated that one–fourth of the world's food supply is lost through microbial
activity alone. In India it is believed that 40% of all food production is wasted due to mismanagement, inadequate infrastructure, poor knowledge about the processing, improper storage etc., (Chris Nurko, 2011). The USFDA recently announced a program which is designed to establish a national baseline on the occurrence of food borne disease risk factors within the retail segment of the food industry. Risk factors outlined in the food borne illness are, food from unsafe source, inadequate cooking, improper holding temperature, contaminated equipment and poor personal hygiene (USFDA, 2000). Recently, Indian Government also guiding to the processors and retailers to provide hygienically processed and packed food to customers.

The quality and nutritional value of food are mainly depends on spoilage and biochemical composition. About one-third of the world’s food production is lost annually as a result of microbial spoilage. The major problems are the presence of higher level of pathogenic bacteria such as *Salmonella, Vibrio spp, Escherichia coli, faecal coliforms, Listeria monocytogens, Staphylococcus aureus* etc., in commercial seafood items would pose serious threat to human health. Though crabs are consumed as food by a large section of the people in various parts of the world, its bacterial load is still eye-catching at national as well as in international markets as suggested by Vijayalakshmi, (2007). The wild hard crab is likely to harbour more pathogenic bacteria and may encounter bacterial diseases like Shell disease and Vibriosis. Huss (1994) reported that *Salmonella* and faecal Coliformes are the common pathogenic bacteria harbouring on *S.serrata* during unhygienic handling operation.

Though wild crabs are used in various parts of the world, there is a huge demand for soft shelled mud crabs in the global market, particularly in USA and EU countries for its rich protein and minimal microbial load. But in India, they are discarded as such at
the landing centre itself, as people are unaware of the processing technology to promote its market value. Hence, it is the need of the hour to promote its market value as well as the consumption rate by adopting new techniques to minimize the harbouring of bacterial flora in newly moulted crabs. So controlling or reducing the bacterial load from this economically important exportable crustacean *S. serrata* is of paramount important to promote the quality thereby increasing the market value. Hence the present study is designed to find a suitable cost effective technique to improve the quality of the mud crab *S. serrata*.
1.2 REVIEW OF LITERATURE

There are almost 5,000 species of crabs among this 4,500 are true crabs and rests of about 500 are hermit crabs. Most of the crabs live in ocean but many live on land like the robber crab. The biggest ocean crab is the Japanese Spider crabs – *Macrocheira kaempferi*, which lives on the floor of the Pacific Ocean and it has about 3.7m leg span. The biggest land crab is the Coconut crab – *Birgus latro* lives on island of Pacific Ocean which has leg span up to 75cm.

In India, common edible crabs are belonging to the families Portunidae, Calappidae, Ocypodidae, Grapsidae and Xanthidae, each family has 9, 2, 3, 1 and 1 species respectively. Out of 16 species of crabs, the larger crabs belonging to the family Portunidae are *Scylla serrata* and *S. oceania*, the economically important species. Till 1948, the mud crabs were the only one species included in the genus *Scylla*. However, the studies made in Phillipines (Estampador, 1949), Vietnam (Serene, 1952) and in India (Kathirvel, 1981; Radhakrishnan and Samuel, 1982; Joel and Sanjeevaraj, 1983 and Kathirvel and Srinivasagam, 1992) have led to the conclusion that there were more than one species in the Genus *Scylla* namely, the larger species *Scylla oceania* and *S. tranquebarica*, and the smaller species *Scylla serrata* and *S. paramamosain*. Recently it has been confirmed in the revision of the genus *Scylla* which includes four species namely *S. serrata*, *S. tranquebarica, S. olivacea* and *S. paramamosain*, based on morphometric analysis, allozyme electrophoresis and mitochondrial DNA sequencing (Fuseya and Watanabe, 1996; Overton et al., 1997; Keenan et al., 1998 and Sugama and Hutapea, 1999).

Studies on the biology and related aspects of the genus *Scylla* were attempted by a number of researchers, Arriola (1940), Estampador (1949), George and Rao (1967),


The pH of meat depends on several physical and chemical factors such as buffering capacity and ionic composition. Tissue pH has been accepted as a parameter of spoilage was studied by several workers (Bailey et al., 1956; Bhobe and Pai, 1986 and Reshma Zamir et al., 1998). Kyrana et al., (1997), Kyraana and Lougouvis (2002) and Kayim and Can (2010) have found that the pH value is increased after fish harvesting.
which reflect the production of alkaline bacterial metabolites coincide with the increase of Total Volatile Basic Nitrogen (TVBN). Abbas et al., (2006) analyzed and reported that the pH of fish could be used to predict the shelf life of the fish.

Control or minimizing bacterial load have been carried out on shell fishes for many years. Ozone and chlorine are the chemical agents used to inactivate, reduce, and destroy all harmful microorganisms were studied by Salmon and Legall (1936), Hong and Rapson (1968), Haraguchi et al.,(1969), Kinman (1972), Brungs (1973), Blogoslawski et al., (1975), Honn and Charvin (1976), Yang and Chen (1979), Sproul et al., (1985), Glaze (1986), Sheldon and Brown (1986), Katz (1986), Korich et al., (1990), Mitstuda et al., (1990), Restaino et al., (1995), Richardson et al., (1996), Zhang and Farber (1996), Collins (1997), Graham (1997), Beuchat et al., (1998), Khadre et al., (2001), Garcia et al., (2003), Kuangii et al., (2007) and Joel Leusink (2011)


Though a plenty of works have been carried out in different species of crabs in various parts of the world, there is paucity information (Sivakumar and Krishnakumar, 2011) on edible newly moulted soft mud crabs in India. To promote the market value by assessing the consumption qualities, it is necessary to analyse the primary metabolites,
aminoacids composition, microbial load and pH in the body tissues of mud crabs as these parameters echo the quality of its meat. Hence, the present study is focused on consumption assessment qualities of hard and soft shell crabs, adopting different kinds of treatment methods and to evaluate of organoleptic attributes of the newly moulted mud crab *S. serrata* to prove its palatability.
1.3 DESCRIPTION OF THE STUDY AREA

Andrapradesh State has nine coastal districts covering 974 km of coastline from Srikakulam to Nellore, having rich marine resources. Kakinada is the Southeastern Indian city situated in Andrapradesh State, located at Long 82°13’E to 82.22°E and Lat 16°56’N to 16.93°N. The city has roughly a north-south orientation and is confined to a long narrow strip parallel to the sea coast. It has an average elevation of 2 meters (6 ft) so that many areas of the city are below at sea level. The average width of the city is around 6 km but its length is around 15 km. The Kakinada bay is mainly due to the estuary of the Godavari River. A small sandy formation of 16 kilometers in the Bay of Bengal protects Kakinada from strong cyclones and tidal waves. It is called as Hope Island and has rich biodiversity with turtles and fishes. Mud crabs landing is more common in the Kakinada coasts. The mud crab fishery in the Coringa mangroves near Kakinada supports some 5000 fisherman families (Venkataraju et al., 2005). The study area for the mud crab collection is shown in the map 1. The present study was carried out from the period of March 2010 to February 2011 in Kakinada coast, Andhra Pradesh State.
1.4. Systematic Position of *Scylla serrata* (Forskal, 1775)

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1.5. DESCRIPTION OF THE STUDY ANIMAL

*S. serrata* (Plate 1) is commonly known as mud crab, mangrove crab or green crab belonging to the phylum Arthropoda, class Crustacea. It is native to Indo – Pacific region and distributed throughout the Indo – Pacific region mainly within tropical latitudes. In India, it is distributed along Puducherry, Vizagapatnam, Port Blair, Karwar, Gulf of Mannar and Kochi. It is widely distributed in brackish water, mangrove marshes and river mouths exhibiting euryhaline and eurythermal i.e it can tolerate wide changes of water salinities and temperature ranging from 5 to 33 ppt and 18°C to 32°C respectively. When salinity decreases below 7 ppt, they often dig holes to survive in adverse environmental conditions. Feeding rate decreases when water temperature drops below 18°C. Crabs survive in holes when water temperature drops as low as 12°C. As water temperature continues to drop to 7°C, they stop feeding and become dormant. Generally this species lives in holes excavated in muddy or sandy muddy bottoms. It has smooth, broad and deep mottled green to very dark brown carapace in which nine broad similar sized teeth projecting obliquely outwards on both sides of eyes, also irregular small whitish spots are found on the carapace and on the 5th pair of legs, are the distinctive features of this species. Hind legs are flattened to ease swimming. It has very robust claws to crush the shells while feeding.

*S. serrata* is principally a carnivore, preying on small invertebrates such as, polychaetes, crustaceans, molluscs, trash fish and on small quantities of detritus and plant materials. Usually, the mud crabs reach the reproductive stage when its shell width becomes greater than 7.8cm and its body weight over 100 g. Female being normally a little larger than males, shell width over 8.5 cm and body weight over 130 g. Spawning takes place at sea in inshore water and the female may lay about 2 million eggs.
After hatching, the juveniles migrate into river mouth where moulting occurs. Crabs moult thirteen times during their life span, six times during the larval stage, six times during the grow-out stage and once during reproduction. Normally, moulting process depends on body size and environmental factors chiefly, when water temperature is at least 15°C. When crabs moult they breathe rapidly, their oxygen consumption being higher. Newly moulted crabs lose their swimming ability and sink to the bottom of the water column. It takes 2 to 3 hours for soft-shell individuals to regain their swimming ability. Hardening of the shell lasts for six to seven hours, 3 to 4 days being needed to complete this process. With each moult, shell width, shell length and body weight generally increases by 28%, 30% and 41% respectively.
1.6 SCOPE OF THE PRESENT STUDY

Providing quality food for ever increasing human population is highly imperative for survival, growth, development, reproduction and maintaining good health throughout the life. Also the developing countries are more serious as there is widespread protein malnutrition. To meet the protein requirements, many countries have taken steps to increase the supply of food particularly from non-conventional resources of marine ecosystem. Marine invertebrates, fish and other marine lives are widely used as food and feed supplements throughout the world for its rich sources of protein. Generally fish and shell fish meat is considered to be highly nutritious (Adeyeye, 2008 and Onyia, 2010).

Crab is one of the most important renewable resources of protein rich marine organism. The nutritional quality of the crabs are very favourable than that of muscle meat of mutton, chicken, duck and fish. While most of the animal protein need is provided from land animals, now-a-days the tendency of benefiting from seafood for protein supply is increasing rapidly worldwide (Jhaveri et al., 1984; Ackman and Mcleod., 1988). Mathai and Devi (1993) reported that the meat of crab contains high nutrients including protein, fat, vitamins, carbohydrates, minerals, GAG – glycosaminoglycans, collagen, elastin etc. Crabs are highly nutritious and healthy owing to its contents like essential amino acids, proteins, unsaturated fatty acids and minerals (Adeyeye 2002; Celik et al., 2004; Naczk et al., 2004; Kuley et al., 2007; Kucukgulmez and Celik 2008 and Rameshkumar et al., 2009). The increased demand of crab meat and soft shelled crab in foreign countries envisage the need for further investigations on the improving the quality of soft shelled crab. The estimation of proximate composition is often necessary to ensure that they meet the dietary requirements and commercial specifications. Hence, a study is necessary to analyze the total carbohydrates, protein,
lipid and aminoacids in the muscle of crab *S. serrata* is necessary, as these components emulate the quality of food products.

Bacteria play an important role in minimizing the shelf life of products and to also reveals the inferior quality of food products. These bacteria are present in ubiquitously in the marine environment, can come from many sources like air, water or soil and may naturally associate with the crab or from the contaminated environments. The intensity of total bacteria indicates the freshness, texture, flavour of the crabs, as well as their potential shelf life. The various types of bacteria in the crab can give information about the products to be processed while marketing. The enormous bacterial count shows the level of contamination of the product which hastening processing, storage and marketing. The Total Plate Count gives an overall representation of quality of the product. The presence of Coliforms and *Vibrio* sp., in certain numbers may indicate the potential presence of pathogens in food. So screening of bacterial load including Coliforms and *Vibrio* sp., along with pH of the crab meat is a suitable solution for quality assessment of the mud crab.

Food processing is an art of science in manufacturing good products, which should be safe, nutritious, healthy, economy and novelty. For this, food industry meets the challenges to fulfil the needs and demands of new consumer products in the challenging world market. Though the conventional techniques are not as good as modern techniques, food industry has to seek new, rapid new technologies to identify and control possible food hazards, thereby increasing consumer acceptance. Hence, the present study is designed to find a simple and cost effective technology from the existing techniques to improve the quality of palatable mud crab *S. serrata.*
While marketing, screening of food is essential to find the consumption level. The quality of food is determined by certain attributes such as colour, flavour, appearance etc., which mends the price at market. Though, a lot of tools have been used to measure the quality of products, sensory evaluation method gives the accurate at commercial level.
1.7. THESIS WORK PLAN

The present study is planned and carried out with the following objectives:

- To assess the consumption qualities such as biochemical components, total bacterial load, pathogenic bacteria (Coliforms and Vibrio), and pH of the wild/hard and soft/newly moulted mud crab *S. serrata* from the study area.
- To fix the best suitable method from the available methods such as treating the soft shell crab in chlorine (100 ppm), ozone; and combined use ozone and chlorine.
- To analyze the quality of soft shell crab treated in the selected best suitable method (combined ozone and chlorine) by examining the bacterial load, pathogenic bacteria, biochemical components and pH.
- To highlight the significance of the selected method for the quality improvement of the soft shell mud crab.
- To evaluate the overall acceptability of the improved quality product and to examine organoleptic attributes such as colour, appearance, flavour, taste and texture by applying 1-9 point Hedonic Rating Scale.