Comparison of proximate and fatty acid composition of shell wastes of marine edible shrimps, *Heterocarpus gibbosus* (Bate, 1888) and *Aristeus alcocki* (Ramadan, 1938)

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THE THESIS SUBMITTED TO THE

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CONTENTS

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

1. Introduction

Marine foods are of great importance for their excellent nutritional value. Biochemical assays and nutrients play a vital role in physical growth, development, maintenance of normal body function of physical activity and health. The knowledge of the biochemical composition of any edible organism is extremely important since the nutritive value is reflected in biochemical contents.

In animals, the nutrients are known to vary with season, size of the animals, stage of maturity, temperature and availability of food etc. Protein is essential for the sustenance of life and accordingly exists in the largest quantity of all nutrients as a component of the human body (Okuzumi and Fujii, 2000). Seafood products have attracted considerable attention as important protein sources of nutrients in the human diet.

In this connection, shrimp are small in size, they collectively represent the biggest and the most valuable seafood commodity traded worldwide. Over the last two decades the worldwide production of shrimp has increased exponentially and account for 16% of global seafood exports (FAO, 2014). Shrimp is an extremely good source of protein and very low fat and calories, making it a healthy food choice for consumers. Shrimp is basically composed of water, lipid, and protein, which create the nutritional value, functional aspects, and sensory characteristics of the flesh (Gokoglu and Yerlikaya, 2015).

In addition, shrimp consists of highly unsaturated fatty acid (FAs), such as eicosapentaenoic (C20:5n-3, EPA) and docosahexaenoic (C20:6n-3, DHA) acids, which are essential in the human diet (Dincer, 2014). Fat and essential polyunsaturated fatty acids (PUFA) contribute to shrimp' dietary quality and sensory values. The consumption of W-3 polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid

(EPA,C20: W-3) Hexadecanoic acid, and metyl ester has both anti-atherogenic and antithrombotic effects as well as an important role in the control of hypertension, preventing cardiac arrhythmias, reducing the risk of coronary heart diseases, diabetes and cancer. Health problems such as asthma, arthritis, multiple sclerosis, headaches and some kidney disease may also be controlled or alleviated by ω-3 fatty acids (Schmidt, 2003; Mahaffey, 2004). PUFAs play an essential role in the development of the nervous (brain), photoreception (vision) and reproductive systems (Horrocks, and Yeo 1999).

PUFA are not only important for maintenance of the membranes of cells but also important for the formation of prostaglandins in body which regulate inflammation and blood clotting. These fats are also needed to absorb fat-soluble vitamins such as A, D, E and K from food; and it also regulate cholesterol metabolism in body (Jabeen and Chaudhry, 2011). PUFA particularly the n-3 and n-6 PUFA have been considered an essential fatty acid. This EFA has been proved the healing effects and preventive effects on cardiovascular diseases, neurodevelopment in newborn babies, cancer and fat glycerin control on human kinsella *et al*. (1990). The PUFA composition level may differ among the fish species, little awareness has been compensated to the PUFA composition level of different species when selecting species for human diets.

The study concentrates on proximate composition of shrimp shell waste. Proximate composition in non-edible part determination involves analysis of moisture, protein, carbohydrates, ash and lipid contents. Shells have also been ground up for use in potions and for various medicinal uses throughout history. Shrimp processing industry waste represent a serious problem, mainly because sea food industries process and package the harvested products. During the processing, only the meat is taken,

while the head and shells of shells are discarded as waste (Guilherme *et al*., 2007). This results in generation of large amount of shell waste globally. Sachindra *et al*., 2006 reported that the yield of waste (head and carapace) from processing of the deep sea shrimps, *A.alcocki* and *S. indica* ranged from 62.6-65.6 %. A quick and effective solution to this is recycling of shell wastes and extraction of commercially viable substances such as chitin that can further be deacetylated to form chitosan which has a wide range of uses (Ravi Kumar, 2000).

In the present study, proximate composition of shell waste from two selected marine species namely *Heterocarpus gibbosus* and *Aristeus alcocki* were examined. These deep sea species are commercially important and commonly available along the south east coast of India. *H. gibbosus* and *A. alcocki* containing the high level of protein, carbohydrates, moisture and also contain the essential fatty acids. The *H. gibbosus*s species was first identified by Bate, 1888. *H. gibbosus* family pandalidae is a taxon of caridean shrimp. These species are commonly called pandalid shrimp. It is otherwise called Hump-back nylon shrimp. The *A. alcocki* species was first identified by Ramadan, 1938. It is Aristeide family. *A. alcocki* commonly called Red shrimp. They are edible and have high economic value.

With global population expansion, the demand for high quality protein especially from aquatic sources is rising dramatically. An increased demand for aquaculture production is clearly needed to meet this demand in the third millennium.

Accordingly to the report of FAO, the world aquaculture production is likely to increase by 2.69 times by 2025. A recent report of consultative

group for International Agricultural Research stated that within the next fifty years fish farming and searching might provide nearly 40%.

The proportion of fish eating in India increased from 27.2% in 1987-88 to 39.7% in 1996-97, assuming that this proportion would increase at least to 50%. The total fish eating population in India by 2020 will be around 700 million.

Increased demand for aquaculture production means increased pressure for development of more efficient production system. Major improvements have already been achieved through enhanced management, nutrition disease diagnostic and genetic improvement of production trials.

In fishery industry in the world all over is undergoing a crisis due to two simultaneous trends. The first is an increase in the seafood consumption caused by the increase in the world population and the increase in per capita consumption. The second is the dwindle supply from natural ocean fisheries caused by over fishing. Over fishing not only reduce the amount of marketable fish in sea, but also winders the sea's ability to replenish its stocks. The combination of these two trends is causing a steady increase of seafood prices and this situation is expected to continue indefinitely.

The present investigation provides adequate information on the formulation of fish feed by compounding shrimp shell wastes growth promoter (containing nutritional value) to feed the fish. Hence in aquaculture practices it is preferable to use shrimps shell wastes growth promoter with fish feed for the enhancement of growth, better feed efficiency and biochemical constituents of edible fishes.

Aquaculture is the fastest expanding food producing sector in the world, growing at a rate of almost 12% p.a. since 1984, with production

almost trebling from 13 to 36 million tons in the last 10 years (Tacon, 1999). This expansion will have to continue well into the next century if production is to keep pace with the increase in demand solely due to the increase in population. However, much of the increase in aquaculture production has been brought about through the adoption of intensive farming practices using formulated feeds.

Aqua feeds accounts for about 40 to 60% of the total cost of aquaculture operations. It is therefore necessary to look for non-traditional low cost feed stuffs to decrease the overall cost of fish production. To reduce feed cost, it is imperative to find out cheaper source of protein for aquaculture to formulate economic diet with unconventional feed like shell waste.

Shrimp waste is an excellent source of protein (50–65%, dry weight basis), nutritive components and enzymes (Fanimo *et al*. 2000 and Heu et al. 2003). Therefore, using of such shrimp wastes has drawn much interest from researchers in recent years. The biochemical improvement of shrimp waste in the feed processing plant for fish is of economic and environmental benefits.

Bearing all these in mind, a present investigation was focused to study the proximate composition (such as protein, carbohydrate, moisture, lipids and ash) and fatty acid composition of shell wastes of shrimp *H. gibbpsus* and *A. alcocki.* These unused wastes will pollute the environment and is hazardous to human population. To overcome these problems, these shell wastes can be utilized for the preparation of poultry feed, fish feed and also used as a fertilizer in the agricultural farm.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The available literature pertaining to the present study entitled "Comparison of proximate and fatty acid composition of shell wastes of marine edible shrimps, *Heterocarpus gibbosus* (Bate, 1888) and *Aristeus alcocki* (Ramadan, 1938)" was reviewed and presented in this chapter.

2.1. Utilization of shrimp shell waste

In India, more than 60.000 tones of prawn head and shell waste are available every year for disposal from processing industry (Mathew *et al*., 1989). At aquaculture is focus as answer to the growing demand for food. As production in this industry gains momentum, protein emerges as the most important and expensive input component. The shellfish processing industry in India generates 8.5 million tons of shell waste per year (FAO, 1998).

At present, waste finds very little practical application and is categorized as a major environmental contaminant. Hence proper technology has to be developed for the utilization of the waste for the production of materials which are beneficial to mankind (Nair *et al.,* 2002). Thus the generation of the raw material will lead to a profitable income without causing environmental pollution. About 35-45% by weight of shrimp raw material is discarded as waste when processed into headless shell- on products. Only 40% of the shrimp is edible and remaining 60% is discarded as shrimp shell waste (Sindhu, 2010).

Shrimp basically contain the good source of protein, lipids carbohydrates. It also contains the minerals and vitamins. Effective utilization of shrimp shell waste will enhance its status as a biomedical research material, for development of natural medicine without side effects

(Sindhu, 2010). Shrimp is control the hypertension, heart disease, diabetes preventing cardiac arrhythmias, headaches and cancer.

2.2. Proximate composition of shrimp shell wastes

Mandeville *et al*., 2009 observed the proximate analysis, isolation and identification of amino acids and sugars from raw and cooked commercial shrimp waste. Crustacean waste generated from the fishing industry represents approximately 70% of the total landings. This abundant waste may pose an environmental hazard due to the ease of deterioration of the fish tissue in the landfill sites; disposing of the waste, however, can be achieved at considerable cost to the industry; alternatively, the waste can be utilized by extracting useful components and incorporating them into desirable seafood products. In order to achieve a better management of shellfish waste, the composition of the raw and cooked waste should be determined and economically viable methodologies should be developed, to extract the important components. Proximate analysis of the commercial shrimp waste indicated the presence of 94.6 % protein and 4.2% fat on dry basis. The HPLC analysis indicated the presence of 17 amino acids (Asp, Glu, Ser, Thr, Arg, Gly, Ala, Pro, Val, Met, Leu, Ile, Phe, Cys, Lys, His and Tyr, proline being the most abundant) and 7 sugars (ribose, xylose, fructose, mannose, glucose, glucosamine and galactosamlne; ribose being the most abundant). The changes in the concentration of the amino acids and sugars after the heat treatment are explained based on their interaction through the Maillard reaction and by the thermal hydrolysis of proteins and polysaccharides found in the tissue.

Oksuh *et al*., 2009 reported on proximate, fatty acids (FA) and element compositions of two shrimp species, deep seawater rose shrimp *(parapenaeus longirostris)* and red shrimp *(Plesionika martia),* were

determined. Amount of lipids in *P. longirostris* and *P. martia* was found as 1.1 and 2.61% respectively. Proportion of lipids in both shrimps was lower than that of marine fish. Surprisingly, Fatty acid profile of these two shrimp species can be comparable with that of marine fish. The amounts of PUFA's in both shrimp species were found higher than those of SFA and MUFA. Level of DHA in *P.longirostris* was significantly ($p<0.05$) higher than of *P. martia*. In addition, major macro elements found in both of the shrimp species were Ca,K,Na,P and Mg. Zinc and iron were the major micro elements followed by Cu and Mn. Heavy metals such as, Cd and Cr, were below the safe limits.

Dinakaran *et al*., 2009 studied the proximate composition and fatty acids in different size groups and sexes of *Macrobrachium idae*. The protein content was higher in younger ones than in adults. The total value of saturated fatty acids was maximum in females than in males. Among various saturated fatty acids, the amount of oleic acid was higher in both sexes and the total amount of monounsaturated fatty acid showed a maximum in males rather than females. The total amount of polyunsaturated fatty acids of *M. idea* was minimum than monounsaturated fatty acids and saturated fatty acids. The present study was clearly indicated that the nutritive value of *M. idea* could be used as food and perhaps as a candidate species in future for culture.

Bhavan *et al*., 2010 investigated the proximate composition and biochemical constituents in the muscle of adult male and female prawns of *Macrobrachium rosenbergii* collected from two different natural culture sites. The proportion of total protein, amino acids, lipid, fatty acids, carbohydrate and RNA were found to be higher in female prawns than in the males. In contrast, the proportions of moisture and ash contents were higher in male prawns when compared with females. The level of DNA was

found to be unchanged in both male and female prawns. HPTLC analysis of amino acids revealed higher levels of essential amino acids, such as phenylalanine, leucine, tyrosine, isoleucine, tryptophan, methionine, valine, threonine, arginine, histidine, lysine in female prawns when compared to the male prawns. Similarly, GC analysis of fatty acids showed both polyunsaturated and saturated fatty acids levels were found to be higher in female prawns when compared to the males. The variation in muscle constituents between male and female prawns reflects the differences in sex development and their energy requirements for body maintenance during the adult stage.

Sánchez-Camargo *et al*., 2011 investigated the proximate composition and extraction of carotenoids and lipids from Brazilian red spotted shrimp waste (*Farfantepenaeus paulensis*). The shrimp waste was found to have high protein and ash contents, but low lipid content. The fatty acid compositions showed that the lipids had a high content of unsaturated fatty acids, mainly EPA and DHA. The results showed that the mixture of 60% (v/v) n-hexane:isopropyl alcohol gave the highest (53 mg/kg waste) carotenoid extraction yield as compared to acetone, super-critical fluid extraction SC-CO_2 and SC-CO_2 + ethanol. The SC-CO_2 showed the lowest extraction yield of astaxanthin, but the addition of the entrainer (10% w/w) produced an important effect, increasing the astaxanthin extraction to values of 57.9%, similar to extraction with acetone (63.3%).

Ali Aberoumand, 2011 conducted the study to compare the proximate composition and energetic value of three selected marine fishes and prawn. Three fish species; Skip Jack Tuna, Yellow fin Tuna and Long tail Tuna were found to contain significantly lower moisture and lower fat contents. Prawn contained the highest protein compared to other studied fishes. The highest fat content among the three studied fish species was in

Yellow fin Tuna fish at 6.89+2.76% while the fat content of prawn was 1.06%. Yellow fin Tuna fish contained the highest energetic value of 33.6kcal/g of all the samples.

Rangappa *et al*., 2012 investigated the proximate composition of freshwater prawn *Macrobrachium malcomsonii* and *Macrobrachium rosenbergii*. The present study indicates the proximate composition including protein, carbohydrates, lipid, amino acid and fatty acids of two species clearly demonstrated that *M. rosenbergii* was possessing relatively high amounts of organic constituents and highly nutritious when compared to *M. malcomsonii*.

Ehigiator *et al*., 2012 observed the proximate composition of three body parts of two freshwater prawns' species from Ovia River, Edo State, Nigeria. The proximate composition of the whole prawn, exoskeleton and edible portion of *Macrobrachium vollenhovenii* and *M. macrobrachion* were determined. There was no significant difference ($P>0.05$) in all the parameters analyzed for the two species except in the moisture content of the exoskeleton and the edible portion in the two species that showed a significant difference ($P<0.05$). Proximate composition results showed that these prawns can serve as an alternative source of high quality protein, energy and mineral supply for human consumption and for feed formulation for animals.

Ekpenyong *et al*., 2013 observed the variation in the proximate, energy and mineral compositions of different body parts of *Macrobrachium macrobranchion* from the Great Kwa River, a major tributary of Cross River estuary in Cross River State, Nigeria. Results showed that the flesh had significantly higher ($p < 0.05$) levels of protein, fat and moisture (22.32, 7.70 and 58.40%), than the other body parts which were analyzed. High protein was observed in the head (20.11%) and appendages (19.28%), while the exoskeleton recorded the least protein content (14.02%). The

flesh had the least ($p < 0.05$) crude fiber (0.03%) and carbohydrate (7.22%) contents and conversely had the least energy value (187.50 kcal/g) among the body parts. Ash content was significantly higher ($p < 0.05$) in the exoskeleton (7.14%), the appendages (7.01%) and the head (6.05%) than in the flesh (4.30%). The concentration of iron was generally low among the body parts; however, it was highest ($p < 0.05$) in the head. The usual practice of retaining the flesh and discarding the “hard” parts (head, exoskeleton and appendages) of prawn during food preparation should be discouraged as this may promote wastage of important nutrients.

Siva Reddy *et al*., 2013 observed the proximate composition of the prawn, *Macrobrachium rosenbergii* from Andhra Pradesh Coast, India. Ten different samples were selected from both the sexes for the experimentation. Protein, moisture, fats and ash contents in female and male *Macrobrachium rosenbergii were* measured as 25.92±0.40, 75.96±0.51, 5.01±0.42, 1.84±0.05 and 23.14±0.47, 74.16±0.75, 3.35±0.61, 1.52±0.09 respectively. There was significant difference between males and females in case of proteins, moisture, fat and ash.

Gunalan *et al*., 2013 studied the biochemical composition in *Litopenaeus vannamei.* In the present study, protein, carbohydrate, lipid, moisture and ash contents were 35.69, 3.20, 19, 76.2 and 1.2% respectively. Calcium content was maximum (154.5 mg) followed by sodium (67.7 mg) and potassium (56.7 mg). Manganese was reported to be minimum (0.898 mg). Copper and chromium are observed in trace. Totally 18 amino acids were detected, among these, argentine, histamine, isoleucine, leucine, methionine, phenylalanine, tryptophan, lysine and valine are essential amino acids and alanine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine and tyrosine are non-essential amino acids. In individual essential amino acids, valine (23.72%)

was maximum, followed by lysine (13.42%) and methionine (13.06%). Histidine was minimum (1.08%). Glycine (9.8%), cystine (5.56%) and proline (4.26%) contributed as major non-essential amino acids. Ten individual fatty acids were identified, which includes both unsaturated and saturated fatty acids. Three saturated fatty acids (SFA) were recorded (Palmitic acid, Margaric acid and Stearic acid). The polyunsaturated fatty acids (PUFA) were the most dominant common fatty acids (38.5%) with the higher levels of linoleic acid (16.3%) and alpha-linolenic acid (11.2%). Oleic acid is the only monounsaturated fatty acid (MUFA) contributed 12.48% of total fatty acids. The Omega – 6 and omega - 3 fatty acids accounted for 16.3 and 35.4% of the total PUFA (51.7%). The results revealed that the, *L. vannamei* species can be considered as a good source of fatty acid as well as protein rich species.

Puga-lopez, 2013 observed the physicochemical, proximate composition, microbiological and sensory analysis of farmed and wild harvested white shrimp *Litopenaeus vannamei* (boone, 1931) tissues. Both, the farmed and wild white shrimp muscle, supplied a good source of protein and polyunsaturated fatty acids. The physicochemical composition, microbiological and sensory properties could be associated to their origin and handling. The wild shrimp tended to have a better proximate composition than the farmed shrimp, due to the availability of a greater diet variety in their environment.

The comparative proximate, amino and fatty acid composition in *Penaeus monodon (Fabricius, 1798), Fenneropenaeus indicus (H. Milne Edwards, 1837)* and *Aristeus virilis (Bate, 1881)* collected from the Nagapattinam landing centre (south eastcoast of India). The shrimps showed a significant ($p<0.05$) result and varying concentration of protein, lipid, carbohydrate and moisture. Higher concentration of protein was found in *A. virilis* (17.25) followed by *F. indicus* and *P. monodon*. The

essential amino acids that cannot be synthesized in human body are prevailing more in *A. virilis* than the other two shrimps. The fatty acids including saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and poly- unsaturated fatty acids (PUFAs) were analyzed. The shrimps have higher concentrations of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) and their ratios were estimated. *A. virilis* has (47.1% and 23.6% of EPA and DHA, *A. virlis* had 47% of EPA and 23.6% DHA than other two shrimps (Karuppasamy *et al*., 2014).

Reddy *et al*., 2014 investigated the study on the nutritional status of the fresh water prawn *Macrobrachium rosenbergii* from Nellore Coast, India. Fifteen different samples were selected randomly for the estimation of the proximate composition. The average values of the proteins, carbohydrates, lipids, ash and moisture as in cultured and frozen prawns were recorded as 74.24 ± 0.49, 5.50 ± 0.34, 9.09 ± 0.09, 9.71 ± 0.19, 77.14 ± 0.19 and 60.55 ± 0.35, 8,23 ± 0.18, 7.98 ± 0.13, 21.61 ± 0.42, 74.93 ± 0.23, respectively. The higher amounts of proteins, lipids were identified in cultured prawn. As the prawn contains greater amount of proteins would be helpful to minimize the protein demand in the country.

Dincer *et al*., 2014 have studied proximate composition and fatty acid profiles of male and female jinga shrimps (*Metapenaeu saffinis*, H. Milne Edwards, 1837).The compositions of both sexes confirmed that the jinga shrimp is a healthy food source due to its balanced nutrients, with efficient levels of protein and mineral contents. In fatty acid composition, the saturated fatty acid fraction was dominant, followed by polyunsaturated fatty acid and monounsaturated fatty acid. The atherogenic and thrombogenic index values were found to be higher in the jinga shrimp compared to other food sources, such as lamb, pork, and chicken.

Padidela *et al*., 2015 conducted the experiment to assess the proximate, amino acid, fatty acid and minerals of bivalve *Parreysia*

cylindrica from waddepally and Kaleshwaram Lake. The total protein content was found to be varied between 55.67% and 40.21%. The carbohydrate concentrations were ranged from 5.21 to 16.67% and lipid were ranged from 6.63 to 2.11% respectively at the Station I and II. The total essential amino acids were ranged from 14.28% to 0.33% and non-essential amino acids were 7.35 to 1.15% at Station I and II. Vitamin A, vitamin D, vitamin E, and K were 15.40IU, 300IU, 2.18 mg/g and 0.72 mg/g from the Station I and 9.200IU, 200IU, 2.08 mg/g and 0.19 mg/g found from Station II. Totally, 4 macro minerals and 2 trace minerals were detected from both the stations.

Moronkola *et al*., 2015 studied the proximate and mineral contents of crab (*Callinectes amnicola*) from the shore of Ojo river, Lagos, Nigeria. The crude protein ranged between 19.2-28.3 g/100 g and the study showed that the crab tissue are rich in protein (28.00 ± 0.071 %) while the walking legs are (19. 233 ± 0.066%) and the crunchy (19.820 ±0.069%) respectively. Crude fiber and crude fat were found the range from 0.02 to 11.70 % and 0.6-1.020 %. The crude fiber is higher in the walking legs with a value of 11.070 ± 0.037 % when compared to tissue and the crunchy part with a value of 0.023 ± 0.071% and 6.680 ± 0.074% respectively. The moisture content ranged from 67.37 ± 0.226% to 70.046 ± 0.049% in all the parts analyzed. Ash content recorded an average value of 1.040 ± 0.017%, 1.300 ± 0.001 % and 1.041 ± 0.002% in crunchy, walking legs and tissue respectively. Therefore, the crab *Callinectes amnicola* could be a balanced human diet and could be employed as an alternative dietary supplement used as protein.

Prasuna Devi *et al*., 2015 studied the proximate composition of male, female and berried females of freshwater prawns *Macrobrachium rosenbergii*. Due to edible nature of crustaceans including prawns

constitute one of the major sources of nutritive materials for humans and form one of the key points of food chain cycle.

Eswar *et al*., 2016 conducted an experiment to investigate the biochemical composition and preliminary qualitative analysis of marine clam *Gafrarium divaricatum* (Gmelin) from Mumbai, West Coast of India. The results of biochemical analysis showed very high protein content (26.32%), Carbohydrate (11.23%) and lipid (1.29%). The protein consists of ten essential and nine non essential amino acids which are as follows (Lysine 14.36%, Histidine 9.02, Methionine 8.92 %) and (Alanine 5.94%, Aspartic acid 4.98, Asparagine 3.79, Tyrosine 3.52 and Proline 3.21%) are the predominant essential amino and non essential amino acids. *G. divaricatum* consists of six different fatty acids out of which two are saturated fatty acids (SFA), one monounsaturated fatty acids (MUFA) and three polyunsaturated fatty acids (PUFA). SFA, MUFA and PUFA content was 27.18, 11.02, 12.47, 17.96, 11.38 and 11.38% respectively, and fat content (1.29 gm/100gm). In addition it contains Vitamins such as Vitamin A (112.3 IU), Vitamin C (24.11 mg/g), D (13.96 IU), B12 (1.98µg/g.), E(1.14 mg/g), K (0.59 mg/g) and B6 (0.31mg/g). This result revealed that the clam species is a good alternative food source to fish and can be very well exploited after its toxicity evaluation.

2.3. Fatty acid composition of shrimp shell wastes

Guipu Li *et al* ., 2011 analyzed the lipid content and fatty acid composition in the edible meat of twenty-nine species of wild and cultured freshwater and marine fish and shrimps were investigated. Both the lipid content and fatty acid composition of the species were specified due to their unique food habits and trophic levels. Most of the marine fishes demonstrated higher lipid content than the freshwater fish, whereas shrimps had the lowest lipid content. All the marine fish and shrimps had

much higher total n-3 PUFA than n-6 PUFA, while most of the freshwater fish and shrimps demonstrated much lower total n-3 PUFA than n-6 PUFA. This might be the biggest difference in fatty acid composition between marine and freshwater species. The cultured freshwater fish demonstrated higher percentages of total PUFA, total n-3 PUFA, and EPA + DHA than the wild freshwater fish.

Yerlikaya *et al*., 2013 have studied the fatty acid profiles of different shrimp species caught from deep water and shallow water. The shrimp species investigated in the study *were Aristeus antennatus, Aristeomorpha foliacea, Plesionica martia, Parapenaeus longirostris,* and *Plesionica edwardsi from deep water;* and *Metapenaeus monoceros, Penaeus semisulcatus, Penaeus kerathurus,* and *Penaeus japonicus* from shallow water. Fatty acid composition of shrimps varied and the main fatty acids were C18:1n9, C16:0, C25:6n3, C22:5n3, and C18:0. Saturated, monounsaturated, and polyunsaturated fatty acid (PUFA) contents of *P. longirostris, P. edwardsi,* and *M. monoceros* were markedly different, respectively. The ratio of n6/n3 of the edible tissue of M. monoceros was found to be 0.795, whereas this value was 0.152 in *A. foliacea*. Among the species studied, the highest docosahexaenoic acid + eicosapentaenoic acid value was found for *P. kerathurus.* The levels of PUFAs of shallow water shrimps ranging from 33.44 to 42.77% and found to be higher than those of deep water shrimps (ranging from 29.68 to 33.95%). Marine animals in the upper water layers gain nutrition through phytoplankton which provides n-3 PUFA depending on solar energy. Shallow water shrimp species provide a satisfying amount of PUFA.

Jaime lópez-cervantes *et al*., 2013 observed the fatty acid composition and total lipid content in protein hydrolysates of shrimp heads. In this research, fermented shrimp heads, three consistencies of hydrolyzed protein were analyzed: liquid, paste, and powder. The lipid

content ranging from 4.33 to7.75% for the hydrolyzed liquid, 6.32 to 9.51 % for the concentrate in paste form and 24.28 to 36.78% for the hydrolyzate powder. Gas chromatography was employed in the determination of the fatty acid composition (%). The fatty acid content was found high in the liquid hydrolyzate was eicosapentanoeic acid (C20:5n3), while the paste and the powder hydrolyzate registered hexadecanoic acid (C16:00). The paste and the powder rhydrolyzates, contained a higher concentration of saturated fatty acid than unsaturated. Thus the research complements the characterization of the protein hydrolyzate obtained from the fermentation of shrimp heads.

Eskandari *et al*., 2014 observed the fatty acid profile in Persian Gulf shrimp, *Metapenaeus affinis* that is one of the edible and well-known shrimps and has suitable amount of fatty acids specific polyunsaturated fatty acids (PUFA). It has been reported that, a high dietary consumption of marine n-3 fatty acids may prevent the development of atherosclerosis and thrombosis. The fatty acids profiles were analyses in the male and female shrimps. The maximum amount of saturated fatty acids (SFA) was 35.88 percent of total fatty acids in Bandar Abbas (St. A) samples. Highest monounsaturated fatty acids (19.59%) in station C and uppermost of PUFA was in Bushehr samples (47.2 %). MUFA hadn't significantly different ($p>0.05$) and finally PUFA differed statistically only between station A and B. ω3 and ω9 in station A also had statistically differ with other stations and demonstrate that ω3 lower but ω9 higher than other stations. Difference in percentage of fatty acids among stations may consequence of consuming different nutrients by each group of shrimp.

Emami *et al*., 2014 studied the fatty acid and amino acid composition of marine (*Penaeus semisulcatus*) and Farmed (*Penaeus vannamei*) shrimp species from Bushehr, Iran. The results indicated that *Penaeus vannamei* shrimp has higher volume of MUFA, PUFA, SFA, Omega 3, and

Omega 6 than the marine shrimp *Penaeus semisulcatus*. *P. semisulcatus*has had higher amount of amino acids compared with *P. vannamei*. Both shrimps have equal level of cholesterol.

Gómez-Estaca *et al*., 2016 observed the characterization and storage stability of astaxanthin esters, fatty acid profile and α-tocopherol of lipid extract from shrimp (*L. vannamei*) waste with potential applications as food ingredient. In this work a lipid extract from shrimp waste was obtained and characterized. The most abundant fatty acids found were C16:0, C18:2n6c, C18:1n9c, C22:6n3, and C20:5n3. The extract contained all-transastaxanthin, two cis-astaxanthin isomers, 5 astaxanthin monoesters, and 10 astaxanthin diesters(7 ± 1 mg astaxanthin/g). C22:6n3 and C20:5n3 were the most frequent fatty acids in the esterifies forms. Appreciable amounts of a-tocopherol and cholesterol were also found as 126 ± 11 mg/g and 65 ± 1 mg/g, respectively. Little lipid oxidation was observed after 120 days of storage at room temperature, revealed by a slight reduction of x-3 fatty acids, but neither accumulation of TBARS nor formation of oxidized cholesterol forms was found. This is attributed to the antioxidant effect of astaxanthin and a-tocopherol, as their concentrations decreased as storage continued. The lipid extract obtained has interesting applications as food ingredient, owing to the coloring capacity and the presence of healthy components.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Sample collection and preparation

Marine shrimps were collected from Mandapam landing centre, Southeast coast of India during November, 2016. They were transferred to the laboratory in ice boxes. Further species were taxonomically identified as *Aristeus alcocki* and *Heterocarpus gibbosus*. The study focused on the biochemical composition of the non-edible part (shell waste). Hence exoskeletons (carapace and body shells) were peeled, separated, oven dried at 95-105° C and ground into fine powder and stored until use. All the solvents used in the present study were of analytical grade.

3.2 Proximate composition

The moisture content (%) was estimated by subtracting the dry weight of the sample from the wet weight (APHA, 2005). Total protein (mg/g) content of the samples was estimated using bovine serum albumin as a standard (Lowry *et al*., 1951). Carbohydrate (mg/g) content was estimated using the methodology of Dubois *et al*., 1956 with glucose as a standard. Lipid (%) content was accessed by adopting Folch *et al*., 1957 method. To determine the ash content (APHA, 2005), samples were incinerated in muffle furnace at 600°C for 4 hours and. All the parameters were determined (in triplicates) on dry weight basis.

1. Estimation of Total Protein (Lowry *et al.,* 1951)

Principle

Protein reacts with folin ciocalteus reagent to give a coloured complex. The colour formed is due to the reaction of alkaline copper with the protein at the reduction of phosphomolybdate by tyrosine and tryptophan present in the protein. The intensity of the colour depends upon the amount of these aromatic acids present and thus vary for different proteins.

Reagents

1) **80% ethanol:** 80 ml of ethyl alcohol was dissolved in 20 ml distilled water.
2) **NaOH (0.1N):** 400 mg of NaOH was dissolved in 100 ml of distilled water.
3) **NaOH (1N):** 4 g of NaOH was dissolved in 100 ml of distilled water.
4) **Solution A:** 2 g of sodium carbonate was dissolved in 100 ml of 0.1N NaOH.
5) **Solution B:** Solution B was prepared by dissolving 500 mg of copper sulphate in 1% sodium potassium tartarate (1 g of sodium potassium tartarate in 100 ml of D. H_2O).
6) **Solution C:** Solution C was prepared by mixing 50 ml of solution A with 1 ml of solution B.
7) **Folin ciocalteus reagent:** Folin ciocalteus reagent was prepared by mixing 1 ml of folin ciocalteus reagent with 1 ml of distilled water.
8) **Blank:** 5 ml of solution C, 0.5 ml of 1N NaOH and 0.5 ml of Folin ciocalteus reagent served as the blank solution.

9) **Standard:** Bovine serum albumin (BSA) at the concentration of mg/ml and different dilutions from this stock solution served as the standard.

Procedure

A known amount of tissue / feed sample was taken and it was homogenized well using 2 ml of 80% ethanol. Then it was centrifuged at 5000 rpm at 4°C for 15 min. The precipitate was dissolved in 1N NaOH and made upto 5 ml. From this, 0.5 ml was taken and then 5 ml of the solution C was added and incubated for 20 min. Finally 0.5 ml of Folin ciocalteus reagent was added and the intensity of the colour developed was read at 660 nm in a Spectrophotometer.

Calculation

$$\text{Protein present in the sample (\%)} = \frac{\dfrac{\text{OD of the sample}}{\text{OD of the standard}} \times \text{Conc. of the standard (mg)}}{\text{Weight of the sample (mg)}} \times 100$$

2. Estimation of Carbohydrate (Roe *et al.,* 1955)

Principle

Sulphuric acid hydrolyzes the di and oligosaccharides into monosaccharides and converts the monosaccharides into furfural or furfural derivatives, which react with anthrone and produces a complex coloured product.

Reagents

1) **80% ethanol:** 80 ml of ethanol was dissolved in 20 ml of distilled water.
2) **Anthrone reagent:** 200 mg of anthrone powder was dissolved in 50 ml cold concentrated sulphuric acid. To this, 0.5 ml of thiourea was added to stabilize the colour.
3) **Standard:** 100 mg of D-glucose was dissolved in 100 ml of saturated benzoic acid and different dilutions from this stock solution served as a standard.

Procedure

A known amount of tissue / feed sample was taken and it was homogenized well using 2 ml of 80% ethanol. Then it was centrifuged at 5000 rpm for 15 min. at 4°C. To the clear supernatant (0.5 ml), 4 ml of anthrone reagent was added and the test tubes were kept in a boiling water bath for 15 minutes. The test tubes were taken out and kept in a dark room for 10 min. and finally the colour developed was measured at 620 nm in a spectrophotometer.

Calculation

$$\text{Carbohydrate present in the sample (\%)} = \frac{\dfrac{\text{OD of the sample}}{\text{OD of the standard}} \times \text{Conc. of the standard (mg)}}{\text{Weight of the sample (mg)}} \times 100$$

3. Estimation of Lipid (Folch *et al.,* 1957)

Principle

The quantitative determination of lipid by sulphophosphovanillin method depends on the reaction of lipids extracted from the sample using chloroform – methanol, with sulphuric acid, phosphoric acid and vanillin to give a red complex.

Reagents

1) **Chloroform methanol (2:1):** This reagent was prepared by mixing 200 ml of chloroform and 100 ml of methanol.
2) **Sodium chloride (0.9%):** 900 mg of NaCl was dissolved in 100 ml distilled water.
3) **Sulphophosphovanillin reagent:** 800 ml of Orthophosphoric acid was added to 200 ml of distilled water. To this, 2 g of vanillin powder was added and mixed well.
4) **Standard:** 10 mg of olive oil was dissolved in 10 ml chloroform methanol mixture (2:1) and different dilutions from this stock solution served as the standard.
5) **Blank:** Vanillin reagent was used as a blank solution.

Procedure

A known amount of tissue / feed sample was taken and homogenized well with 4 ml of chloroform methanol mixture. After mixing well, 0.2 ml of 0.9% sodium chloride was added and the mixture was kept undisturbed overnight. The lower layer of lipid was collected carefully and dried in a vaccum dessicator. The dried lipid content was dissolved in concentrated sulphuric acid (0.5 ml) and kept in a boiling water bath for 10 min. From the lipid sample, 0.2 ml was taken in a test tube and 5 ml of sulphophosphovanillin reagent was added, shaken well and kept

undisturbed for 30 minutes. The intensity of red colour was measured at 520 nm in a spectrophotometer.

Calculation

Lipid present in the

Sample (%) = Final weight – Initial weight

4. Estimation of Ash and Moisture (APHA, 2005)

Principle

The sample was allowed to dry by kept in desiccator. The difference between the wet weight of the tissue and its dry weight give the amount of water present in the fresh tissue. On heating the dry material to higher temperature all the organic constituents were burnt leaving only the inorganic constituents in the form as ash.

Procedure

Known amount of wet sample was taken individually on previously weighed concave glass and they were kept in a desiccator, maintaining 0.5% relative humidity. Dry the tissues in the desiccator till they reached a constant weight. Then the dried materials were transferred individually in silica crucible and kept in a muffle furnace and heated at 550-600°C for 4 h. Finally the ash formed was weighed.

Calculation

$$\text{Moisture} = \frac{\text{Wet weight (g)} - \text{Dry weight (g)}}{\text{Wet weight (g)}} \times 100$$

$$\text{Ash} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample taken (g)}} \times 100$$

3.3. Fatty acid composition (GCMS)

Total lipid was extracted from (5g) sample using the chloroform: methanol (2:1, v/v; containing BHT 0.1mg/100g) method (Folch *et al*., 1957). The lipids were trans- esterifies using 2ml of methanol and 3ml of freshly prepared 5% methanolicH_2SO_4 were added. After mixing, the contents were heated for 2h in a water bath at 70°C. Finally 1ml of hexane was added after cooling the sample.

The Fatty Acid Methyl Ester (FAME) analysis was performed by the Clarus 680 GC (Perkin Elmer) equipped with a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250µm df) and Helium was used as the carrier gas with a constant flow of 1.1 ml/min. The conditions used for GC analysis was the injector temperature maintained at 300ºC during the run period and1µL of extract sample injected into the instrument oven temperature was kept constant at 100ºC for 2 min and was then increased to 250ºC at a rate of 10ºC/min and to 280ºC at 30ºC/min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 ev, a scan time 0.2 sec and scan interval of 0.1 sec. The compounds were identified by comparison with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

3.4 Statistical analysis

Results were expressed as Mean ± SD. The data obtained were analyzed for significant differences ($P < 0.05$) by one way Analysis of Variance (ANOVA) and mean separation were accomplished by Duncan's Multiple Range Test (DMRT) using STATISTICA software (Statsoft Inc. 1999).

Fig.1: Shell waste of *Aristeus alcocki*

Fig.2: Shell waste of *Heterocarpus gibbosus*

Fig.3: Powdered shell waste of *Aristeus alcocki*

Fig.4: Powdered shell waste of *Heterocarpus gibbosus*

RESULTS AND DISCUSSION

4. RESULT AND DISCUSSION

4.1. Proximate composition of shrimp shell waste in *H. gibbosus* and *A. alcocki*

In general the proximate composition is well known as proportion composition of basic elements such as protein, carbohydrates, lipids, ash and moisture. The present study focused on the proximate composition which includes the analysis on moisture, protein, carbohydrate, lipid and ash in *H. gibbosus* and *A. alcocki*.

3.1.1. Moisture (%)

In the present study, the shrimp shell waste of the *H. gibbosus* and *A. alcocki* the moisture content of about 41% and 51%. *H. gibbosus* shows the high moisture content when compared to the *Aristeus alcocki* (Table 1). Moisture content of fresh shrimp is generally reported as 75 to 80% (Yanar and Celik, 2006; Sambhu and Jayaprakash, 1994).

Moisture is required for normal functioning of many biological molecules. It is present in two forms, bound to the protein and in the free form (Stanchena, 2013). These forms have well defined for the biological roles. There exists an inverse relationship between the moisture and lipid content. The percentage of water is a good indicator of its relative content of energy, proteins and lipids (Olagunju *et al.,* 2012).

3.1.2. Protein (mg/g)

The proximate study of two marine shrimp shell wastes, *H. gibbosus* and *A. alcocki* showed significance for protein ($P < 0.05$). Table 1 shows the proximate content of the species, *H. gibbosus* and *A. alcocki*. The results revealed higher protein content 3.12mg/g in shell waste of *A. alcocki* and 2.4mg/g in *H. gibbosus* shell waste (Fig. 5).

Protein plays a very important role in the maintenance of blood glucose level (Jrueger *et al.* 1968). Proteins serve as building blocks for cellular and organic structure. Proteins form components of catalysts called enzymes which are essential for the metabolic reactions.

Protein is essential sustenance of life and exists in largest quantity of nutrients as a component of the human body. Protein was found as the major constituent in the shrimp for the growth and maintenance of body tissues. It is very important to provide all essential amino acids to human in a appropriate amount for optional protein synthesis (Elshehawy *et al*., 2016). The quantity of protein in shrimp is largely influenced by the extent of fat and water content (Geiger and Bergastrom, 1962). The high protein content in the lowest size groups may be attributed to increased protein synthesis during the active growth phase as it has been observed elsewhere in shrimp and mantis shrimps (Achuthan kutty and Parulekar, 1984; Ajit kumar, 1990; Tanuja, 1996). Protein is one of the most prominent biochemical compounds of crustaceans and its quantity in this class of organisms is largely influenced by the extent of fat and water contents (Dinakaran and Soundarapandian, 2009).

Protein is important component of every cell in the body. It also an important repair tissues and building black bones, skin, cartilage, muscles, and blood. It has been reported that protein content of shrimp ranged between 17 and 21% depending on shrimp species (Sriket *et al*., 2007 and Yanar and Celik, 2006). Garg *et al*. (1977) reported that the protein content in *Squilla* was varied from 70.09 to 75.46% and in Jawla prawn from 61.93 to 72.64%. The result was in accordance with Abdel - salam, 2013, who reported that the carapace of females had the highest mean value of protein than male crustaceans.

4.1.3. Carbohydrates (mg/g)

The shrimp shell wastes contains low level of carbohydrate when compared to protein. Table 1 shows the carbohydrates content of the species, *H. gibbosus* and A*. alcocki*. *H. gibbosus* contain 1 mg/g of carbohydrates whereas *A. alcocki* contain 1.29 mg/g of carbohydrates. The shell wastes contained the high value of carbohydrates in *A. alcocki* when compared with *H. gibbosus.* The low carbohydrate content recorded in this study agrees with Okuzumi and Fujii, 2000 which stated that carbohydrates constitute only a minor percentage of total biochemical composition. Thus carbohydrates are considered to be the first among the organic nutrients to be utilized to generate required energy (Health, 1987). They are best source of energy producers of body through metabolism.

Carbohydrates supply the major portion of the daily requirements. The major function of carbohydrate is to serve as a fuel and get oxidized to provide energy for metabolic process. Carbohydrates are utilized by the cells mainly in the form of glucose. A major part of dietary glucose is converted to glycogen for storage.

The carbohydrate metabolism is distributed when the animals are subjected to stress (Mcleay and Brown, 1975). Under stressful situations fish elicit neuro endocurie response which in turn affect carbohydrate metabolism (Mazeaud *et al.,* 1977 and Subramanyam, 1984).

Carbohydrates exhibited an inverse relationship with protein. No distinguished trend in carbohydrates fluctuation was noticed among the size groups of many shrimp (Achuthan kutty and Parulekar, 1984; Ajit kumar, 1990).

4.1.4. Lipids (mg/g)

Lipids are highly efficient as sources of energy and they contain more than twice the energy of carbohydrates and proteins (Okuzumi and fujii *et al*., 2000). Table 1 shows the lipids content of the species, *H. gibbosus* and *A. alcocki*. *H. gibbosus* contain 2.76 mg/g of lipids content whereas *A. alcocki* contain 3.66 mg/g of lipids. It is subjected to periodic fluctuations influenced by environmental variables like temperature (Johnstene, 1917 and Dinakaran *et al*., 2009). But this does not affect the lipid composition of muscle tissue to any great extent (Nargis, 2006). From the study, proximate parameters were generally more concentrated on shell waste.

The greater the protein and lipid content represents higher energy density (Dempson *et al.,* 2004). In general, the lipids act as major food reserves along with potentials and are known to play an important role not only in the production of energy at cellular level and also play a vital role in the maintenance of structural integrity as the cellular and sub cellular membranes.

The lipid also acts as vehicles for the transport of lipid soluble vitamins such as A, D, E and K. several researchers reported the inverse relationship between lipids and proteins (Ravichandran, 2000, Sriaman, 1978, Pillay and Nair, 1973; Nair and Prabu, 1990 and George and Patel, 1956).The highest mean value of lipid was recorded in the shells of male crustaceans than females (Abdel-Salam, 2013).

Generally, the muscle of the prawn contains lower quantity of lipid (Bhavan *et al*. 2008; Bhavan, 2009).The inverse relationship between lipids and protein was earlier reported by Nair and Prabhu (1990) and Ravichandran (2000). The percentage of water is a good indicator of its relative content of energy, proteins and lipids (Olagunju *et al.,* 2012).

4.1.5. Ash (%)

Ash is the measure of the mineral content of a food item, this results indicates that the Shrimp shell wastes are good sources of minerals such as calcium, potassium, zinc, iron and magnesium. Table 1 shows the ash content of the species, *H. gibbosus* and *A. alcocki.* The *H. gibbosus* shrimp shell wastes contain 20 % whereas, *A. alcocki*, contain 16 % of ash content.

In this study, protein was greater followed by lipid, moisture, ash and carbohydrate. The higher value of proximate composition was reported in the shell waste of the *H. gibbosus* and *A. alcocki*. Accordingly, protein components of muscle tissue varied with the change of season (Nargis, 2006). Protein is essential for normal function, growth and maintenance of body tissues. Its content is considered to be an important tool for the evaluation of physiological standards (Diana, 1982). Biochemical components (Protein, lipid, carbohydrate, etc) are involved in energy metabolism and are regulated by various factors, such as sex, reproductive cycle, capture period, food availability, hydrologic level etc (Tzeng *et al*., 2001; Oliveira *et al*., 2003; May-Ku *et al*., 2006 and Nargis, 2006).

4.2. Fatty acid composition (g/100g) of *Heterocarpus gibbosus* and *Aristeus alcocki* shrimp shell wastes

Fatty acids profiles are obtained from the shell wastes of two species namely *H. gibbosus* (Fig.11) and *A. alcocki* (Fig.12). These shell wastes contained high level of fatty acids and given in Table 3. *H. gibbosus* contains fifteen fatty acids such as Methanesulfonyl chloride (0.29%), Benzoyl isothiocyanate (8.29%), Tetradecanoic acid Ethyl ester (0.77%), Ethyl 13-methyl-tetradecanoate (1.13%), Hexadecanoic acid, methyl ester (1.13%), Hexadecanoic acid ethyl ester (27.61%), Heptadecanoic acid, ethyl ester (0.88%) Octadecanoic acid, ethyl ester (3.27%), Ethyl Oleate (0.42%), Eicosanoic acid, ethyl ester (0.37%), Tetradecanoic Acid (2.42%), Pentadecanoic Acid (3.49%), Hexacosanoic acid, methyl ester (3.13%), Docosanoic acid ethyl ester (0.30%), n-Hexadecanoic acid (45.71%).

A. alcocki contained twenty one fatty acids (Table 4) such as Butanedioic acid, diethyl ester (0.23%), Diethyl adipate (0.20%), Tetradecanoic Acid, Ethyl Ester (1.87%), Ethyl 13-Methyl-Tetradecanoate (1.53%), Diethyl Azelate (0.28%) Hexadecanoic Acid, Methyl Ester (1.16%), Hexadecanoic Acid, Ethyl Ester (29.79%), Ethyl 15-Methyl-Hexadecanoate (0.67%), Ethyl 15-Methyl-Hexadecanoate (0.43%), Ethyl 3,7,11,15-Tetramethylhexadecanoate (0.45%), Heptadecanoic Acid, Ethyl Ester (1.45%), Methyl Stearate (0.34%), Octadecanoic Acid, Ethyl Ester (10.37%), 9-Octadecenoic Acid (Z)-, Ethyl Ester (1.67%), Nonadecanoic Acid, Ethyl Ester (0.24%), Eicosanoic Acid, Ethyl Ester (0.41%), Tetradecanoic Acid (1.58%), Pentadecanoic Acid (1.21%), N-Hexadecanoic Acid (35.45%), Heptadecanoic Acid (0.65%), Octadecanoic Acid (10.02%).

In the present study *Aristeus alcocki* showed large number of fatty acids when compared to *Heterocarpus gibbosus.* n- Hexadecanoic acid was present in large percentage in both the samples. In this, PUFA (poly unsaturated fatty acid) contents are generally higher than the SFA (saturated fatty acid). A minimum value of PUFA/SFA ratio recommended as 0.45 (HMSO, 1994) is used to prevent cardiovascular diseases. An increase in n-3/n-6 rate is essential in the human diet to aid prevention of coronary heart diseases by reducing plasma lipids and to reduce cancer risk (Kinsella *et al*., 1990).

Tetradecanoic acid, ethyl ester (1.87) showed the higher percentage in *A. alcocki* when compared to *H. gibbosus.* Higher level of Pentadecanoic acid(1.21), Tetradecanoic acid (1.58) and Hexacosanoic acid, methyl ester (1.93) were found in *H. gibbosus* than *A. alcocki* shell waste.

The lipids of marine shrimp are characterized by their high ratio of polyunsaturated fatty acid, such as the nutritionally important EPA (eicosapentaenoic) and DHA (docosahexaenoic acid), which are highly susceptible to autoxidation because of their degree of unsaturation (Gunstone and Norris, 1983). Biological value is an index of nutritional evaluation of oil or fat calculating as a ratio between total unsaturated fatty acids and total saturated fatty acids. The increase of biological value, the high nutritional value of fat or oil occurred (Shady *et al*., 2016). EPA can prevent blood clotting.

These fatty acids also reduce the pain and swelling. Eswar *et al*., 2014 observed the fatty acid composition on puffer fish. Consumption of lipids emulsions rich in omega 6 PUFAs according to Tvrzicka *et al*., 2011 leads to increased cholesterol synthesis and increased activity of LDL-receptors. The omega-3 fatty acids have anti-inflammatory and anti-

coagulant properties as well as many other important health benefits (Bell and Sargent, 2003).

EPA and DHA may have individual potential roles in the function of the human organs, since EPA enriched supplements significantly improved psychological distress and depressive symptoms during menopausal transitions and have been suggested as an effective anti - cachexia anti inflammatory agent (Lucas, 2009). The results are in good correspondence with those reported on *Penaeus monodon* fed the lupine meal (Sudaryono *et al*., 1999). Digestibility of fatty acid is known to be influenced by a number of incorporation in dietary fat and by other constituent's fatty acids and their melting points (Lin *et al*., 2006). High levels of digestibility were observed for fatty acids 16:1n7, 18:2n6, 18:3n3, EPA and PUFA. The highest fatty acids digestibilities for *P.monodon* were observed such as HUFA, EPA and DHA (Glencross *et al*., 2002).

The ARA found in *F. merguiensis* and *F. penicillatus* in the present study is higher than reported in the same species by Nisa and Asadaullah, 2006. Findings of C18:1 n-9 and C18:1 n-7 of two shrimp species in this study were similar to previously reported findings of white and black tiger shrimp (Lin *et al*., 2003; Sriket *et al*., 2007). In addition, DHA and EPA, belonging to n-3 fatty acids family are considered as essential (Feliz *et al*., 2002). The arachidonic acid (n-6) is a precursor of prostaglandin hormone, which is essential for reproduction and vitellogenesis (Bell and Sergent, 2003; Tamaru *et al*., 1997). Krysnoweld and Murphy, 1987 reported that similar fatty acid composition result on Jinga shrimp.

Both the species contain high value of polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA). PUFA is important for the maintenance of membrane cells and also for the formation of prostaglandins in body which regulate inflammation and blood clotting. The EPA fatty acid can be considered as the most important for continuation of

the efficient functioning of the brain and body at the cellular level. However, the *A. alcocki* had greater quantity of these fatty acids than H*. gibbosus* which indicates their nutritional value.

Aquaculture industries can takes steps in the manufacture of fish feed by using the wastes for the enhancement of growth rate of edible fishes. Women entrepreneurs can be involved in this project to ensure their economy.

Fish farmers and their families can involve in the preparation of nutritionally rich, balanced fish feeds using the selected low cost, locally available wastes and medicinal herbs.

Table 1: Proximate composition in *Heterocarpus gibbosus* and *Aristeus alcocki*

S. No	Proximate composition	Shell waste	
		Heterocarpus gibbosus	*Aristeus alcocki*
1	Moisture (%)	41.49 ± 0.55	50.92 ± 0.66
2	Protein (mg/g)	2.14 ± 1.00	3.12 ± 0.57
3	Carbohydrates (mg/g)	1.00 ± 0.13	1.29 ± 0.41
4	Lipids (mg/g)	2.76 ± 0.23	3.66 ± 0.32
5	Ash (%)	16.10 ± 1.37	19.56 ± 1.27

Table 2: Fatty acid composition (g/100g) of *Heterocarpus gibbosus*

Peak	Retention Time	Area%	Fatty acid profile
1	5.871	0.29	Methanesulfonyl Chloride
2	7.990	8.29	Benzoyl Isothiocyanate
3	29.272	0.77	Tetradecanoic Acid, Ethyl Ester
4	31.691	1.13	Ethyl 13-Methyl-Tetradecanoate
5	33.44	1.93	Hexadecanoic Acid, Methyl Ester
6	34.055	27.61	Hexadecanoic Acid, Ethyl Ester
7	36.241	0.88	Heptadecanoic Acid, Ethyl Ester
8	38.391	3.27	Octadecanoic Acid, Ethyl Ester
9	38.760	0.42	Ethyl Oleate
10	42.461	0.37	Eicosanoic Acid, Ethyl Ester
11	42.932	2.42	Tetradecanoic Acid
12	45.434	3.49	Pentadecanoic Acid
13	45.595	3.13	Hexacosanoic Acid, Methyl Ester

14	47.694	0.30	Docosanoic Acid, Ethyl Ester
15	48.699	45.71	N-Hexadecanoic Acid

Table 3: Fatty acid composition (g/100g) of *Aristeus Alcocki*

Peak	Retention Time	Area%	Fatty acid profile
1	19.538	0.23	Butanedioic Acid, Diethyl Ester
2	25.383	0.20	Diethyl Adipate
3	29.262	1.87	Tetradecanoic Acid, Ethyl Ester
4	31.677	1.53	Ethyl 13-Methyl-Tetradecanoate
5	32.909	0.28	Diethyl Azelate
6	33.129	1.16	Hexadecanoic Acid, Methyl Ester
7	34.065	29.79	Hexadecanoic Acid, Ethyl Ester
8	35.178	0.67	Ethyl 15-Methyl-Hexadecanoate
9	35.502	0.43	Ethyl 15-Methyl-Hexadecanoate
10	36.085	0.45	Ethyl 3,7,11,15Tetramethylhexadecanoate
11	36.225	1.45	Heptadecanoic Acid, Ethyl Ester
12	37.601	0.34	Methyl Stearate
13	38.391	10.37	Octadecanoic Acid, Ethyl Ester
14	38.746	1.67	9-Octadecenoic Acid (Z)-, Ethyl Ester
15	40.432	0.24	Nonadecanoic Acid, Ethyl Ester
16	42.432	0.41	Eicosanoic Acid, Ethyl Ester
17	42.877	1.58	Tetradecanoic Acid
18	45.359	1.21	Pentadecanoic Acid
19	48.627	35.45	N-Hexadecanoic Acid
20	52.793	0.65	Heptadecanoic Acid
21	58.437	10.02	Octadecanoic Acid

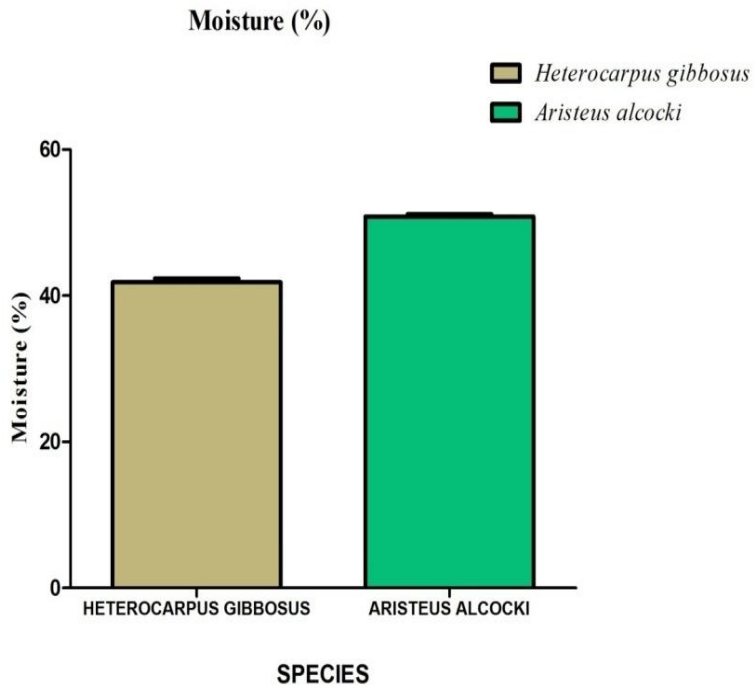

Fig.5: Moisture content of shell wastes from *H. gibbosus* and *A. alcocki*

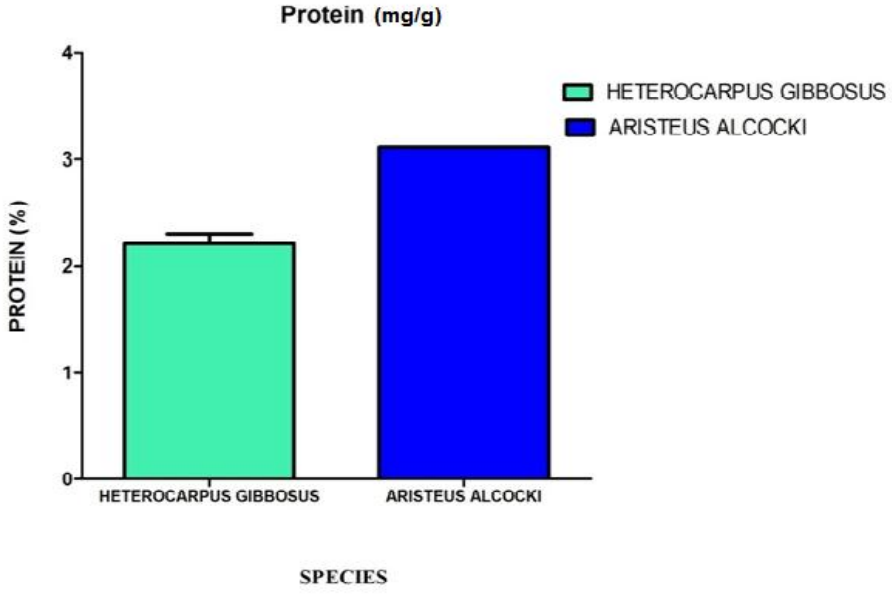

Fig.6 Protein content of shell wastes from *H. gibbosus* and *A. alcocki*

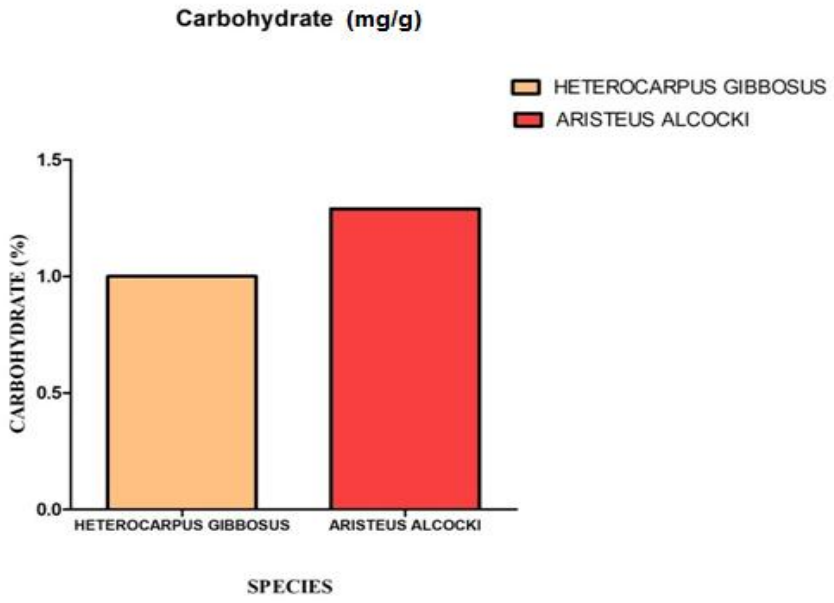

Fig.7: Carbohydrate content of shell wastes from *H. gibbosus* and *A. alcocki*

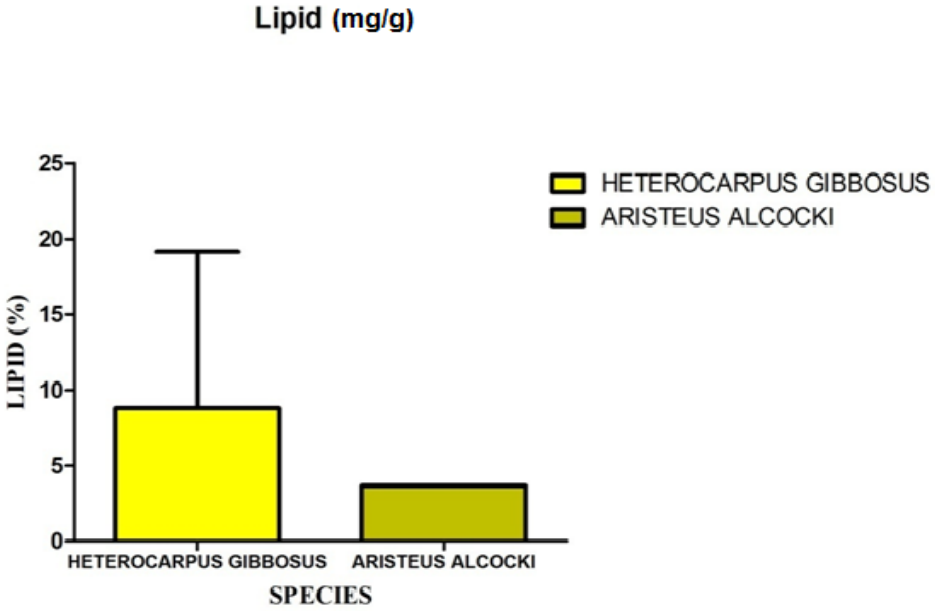

Fig.8: Lipid content of shell wastes from *H. gibbosus* and *A. alcocki*

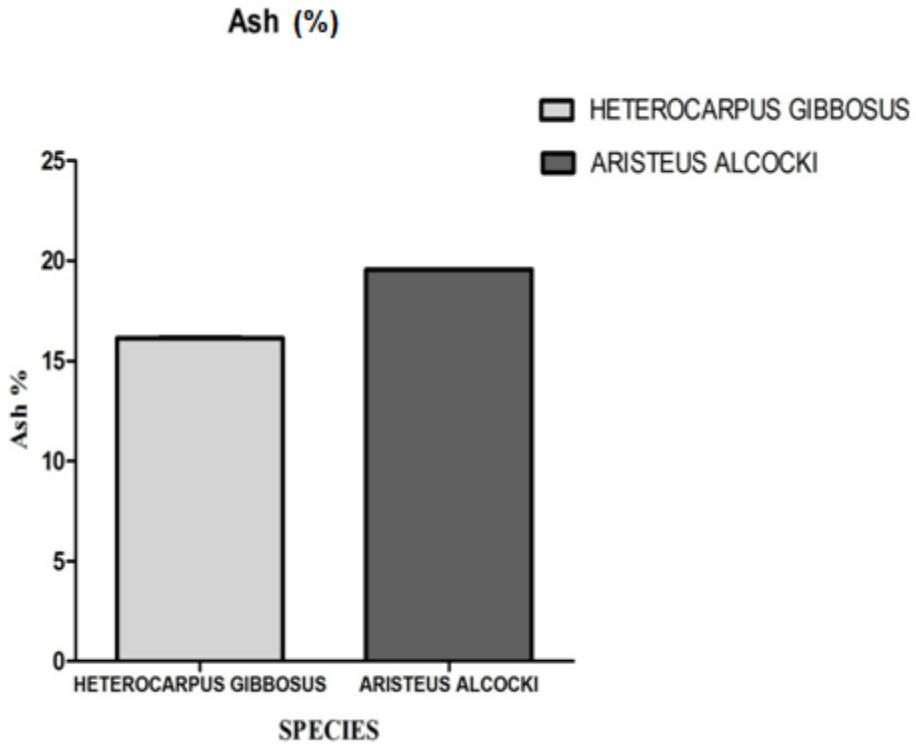

Fig.9: Ash content of shell wastes from *H. gibbosus* and *A. alcocki*

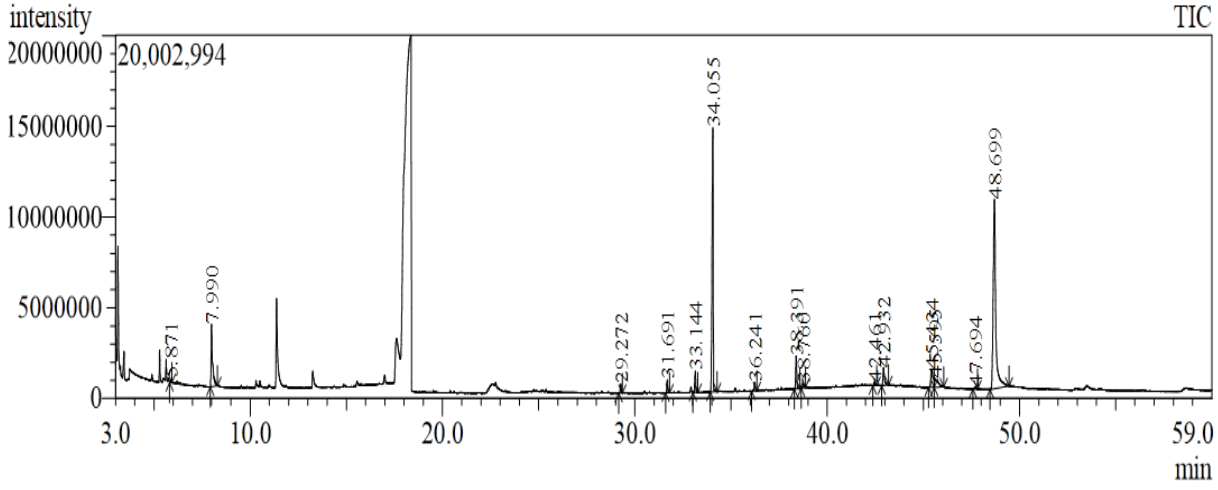

Fig.10: GC of shell wastes from *H. gibbosus*

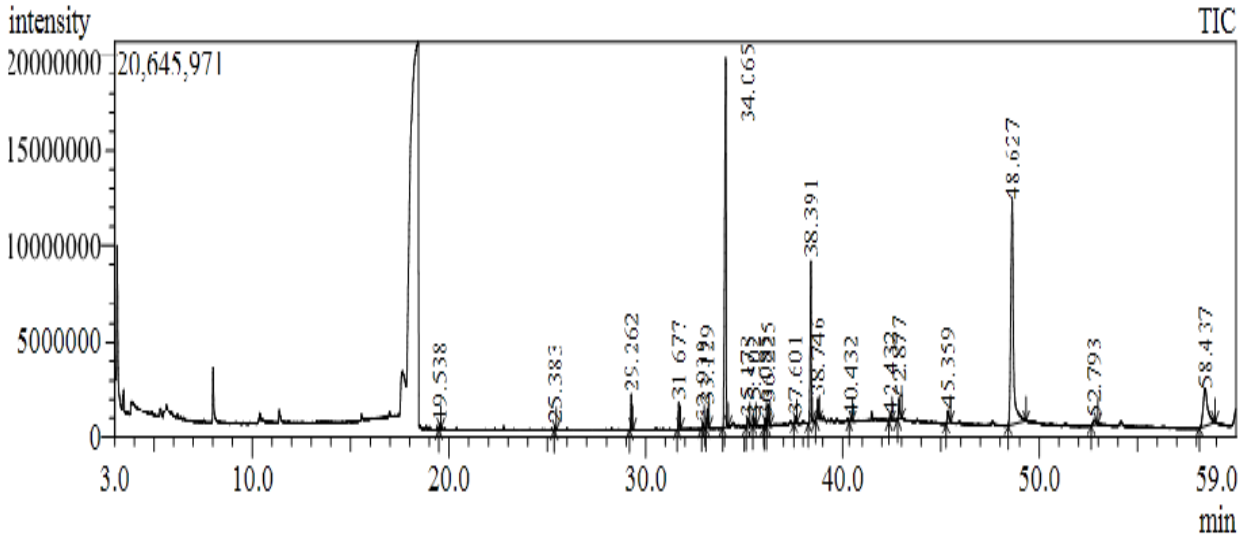

Fig.11: GC of shell wastes from *A. alcocki*

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

Biochemical assays and nutrients play a vital role in physical growth, development, maintenance of normal body function of physical activity and health. The knowledge of the biochemical composition of any edible organism is extremely important since the nutritive value is reflected in biochemical contents.

In animals, they are known to vary with season, size of the animals, stage of maturity, temperature and availability of food etc. Protein is essential for the sustenance of life and accordingly exists in the largest quantity of all nutrients as a component of the human body.

Sea foods are excellent sources of high quality proteins which are superior to those in meat and poultry. Shrimp meat is an excellent source of protein and it is one of the most popular species as it is a part of almost every nation's traditional meal rich in protein and minerals. Seafood products have attracted considerable attention as an important source of nutrients in the human diet.

Shellfish contains potent source of nutrient for the maintenance and growth of human body. Shrimp is an extremely good source of protein and very low fat and calories, making it a healthy food choice for consumers.

Sea food industries process and package the harvested products. During the processing, only the meat is taken, while the head and shells of shell fish are discarded as waste. This results in generation of large amount of shell waste globally.

Shrimp processing waste is the largest industrial waste in the country causing diverse environmental problems. Shrimp is basically composed of water, lipid, and protein, which create the nutritional value, functional aspects, and sensory characteristics of the flesh.

Edible shrimps have become an economically important species and the study focused on the species of genus *Heterocarpus* and *Aristeus* (*H. gibbosus and A. alcocki*). From the present study, protein content in the shells of *A. alcocki* was higher as 3.12 mg/g when comparing with *H. gibbosus* (2.14 mg/g) which reveals the species to be a nutritional source.

Carbohydrates constitute only a minor percentage of total biochemical composition. Carbohydrates in fishery products contain no dietary fiber but only glucides, the majority of which consist of glycogen. Carbohydrate content was 1.29 mg/g in *A. alcocki* and 1 mg/g in *H. gibbosus*. Carbohydrates play important roles in production of nucleic acids, as intermediate in production of energy.

Lipids are highly efficient as sources of energy and they contain more than twice the energy of carbohydrates and proteins. The present study showed excellent lipid content in *A. alcocki* shell waste (3.66 %) than in the *H. gibbosus* (2.76 %).

Moisture was more concentrated in the shell waste of *H. gibbosus* (51 %) than *A. alcocki* (41.49 %). Ash is the measure of the mineral content of a food item, this results indicates that the Shrimp shell wastes are good sources of minerals such as calcium, potassium, zinc, iron and magnesium. The shrimp shell wastes contained the ash content of 20% in *A. alcocki and 41 % in H. gibbosus*.

Biological value is an index of nutritional evaluation of oil or fat calculating as a ratio between total unsaturated fatty acids and total saturated fatty acids.

H. gibbosus contains fifteen fatty acids such as Methanesulfonyl chloride (0.29%), Benzoyl isothiocyanate (8.29%), Tetradecanoic acid Ethyl ester (0.77%), Ethyl 13-methyl-tetradecanoate (1.13%), Hexadecanoic acid, methyl ester (1.13%), Hexadecanoic acid ethyl ester (27.61%), Heptadecanoic acid, ethyl ester (0.88%) Octadecanoic acid,

ethyl ester (3.27%), Ethyl Oleate (0.42%), Eicosanoic acid, ethyl ester (0.37%), Tetradecanoic Acid (2.42%), Pentadecanoic Acid (3.49%), Hexacosanoic acid, methyl ester (3.13%), Docosanoic acid ethyl ester (0.30%) and n-Hexadecanoic acid (45.71%).

A. alcocki (Table 4) contained twenty one fatty acids such as Butanedioic acid, diethyl ester (0.23%), Diethyl adipate (0.20%), Tetradecanoic Acid, Ethyl Ester (1.87%), Ethyl 13-Methyl-Tetradecanoate (1.53%), Diethyl Azelate (0.28%) Hexadecanoic Acid, Methyl Ester (1.16%), Hexadecanoic Acid, Ethyl Ester (29.79%), Ethyl 15-Methyl-Hexadecanoate (0.67%), Ethyl 15-Methyl-Hexadecanoate (0.43%), Ethyl 3,7,11,15-Tetramethylhexadecanoate (0.45%), Heptadecanoic Acid, Ethyl Ester (1.45%), Methyl Stearate (0.34%), Octadecanoic Acid, Ethyl Ester (10.37%), 9-Octadecenoic Acid (Z)-, Ethyl Ester (1.67%), Nonadecanoic Acid, Ethyl Ester (0.24%), Eicosanoic Acid, Ethyl Ester (0.41%), Tetradecanoic Acid (1.58%), Pentadecanoic Acid (1.21%), N-Hexadecanoic Acid (35.45%), Heptadecanoic Acid (0.65%) and Octadecanoic Acid (10.02%).

In the present study *A. alcocki* showed large number of fatty acids when compared to *H. gibbosus.* n- Hexadecanoic acid was present in large percentage in both the samples. In this, PUFA (poly unsaturated fatty acid) contents are generally higher than the SFA (saturated fatty acid).

A. alcocki showed large number of fatty acids when compared to *H. gibbosus.* n- Hexadecanoic acid was present in large percentage in both the samples. In this PUFA (poly unsaturated fatty acid) contents are generally higher than the SFA (saturated fatty acid).

Both the species contain high value of polyunsaturated fatty acid (PUFA) and monounsaturated fatty acid (MUFA). High value of fatty acid was observed in both species shell wastes. Thus, it is very useful that

details on the sampling procedures and methods of analysis used in this study will be able to provide sufficient information for any comparative purpose in the future.

The present study revealed that the *H. gibbosus* and *A. alcocki* shell wastes are having high saturated fatty acid and protein content which contribute to a good nutritional status. Thus the proximate and fatty acid values obtained from this study would be very useful to help the consumers in choosing shell wastes from shrimp based on their nutritional values besides providing a food composition database.

Good nutrition in animal production system is essential to produce economically, a healthy, high quality product. In fish farming, nutrition is critical because feed represents 40 – 50 per cent of the production costs. Aquaculture is the farming and husbandry of economically important aquatic animals and plants under controlled conditions. Aquaculture could help integrated rural development by generating employment opportunities for many unemployed and underemployed people in the rural areas of developing countries (Reddy and Rao, 1999). Aquaculture industry expands to satisfy increasing demand for affordable, safe and high quality fish and sea food products.

The protein component generally represents the largest portion of the total cost of a diet, but protein ingredients are not necessarily the most expensive feedstuffs. The use of protein as a dietary source of energy is undesirable because of the high cost of protein relative to the cost of non protein energy (Watanabe, 2002).

The nutrient rich shrimp waste can be recommended for the preparation of fish feed, poultry feed and also utilized as a fertilizer in the agricultural farm.

Finally, if scientific opinion joined with the knowledge of fish farmers and industry, the supply of protein rich food to the burgeoning population as well as the economic efficiency of the country will certainly improve a lot.

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6.BIBLIOGRAPHY

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