Muscle and exoskeleton extracts analysis of both fresh and marine crustaceans *Procambarus clarkii* and *Erugosquilla massavensis*

Salwa A. H. Hamdi

Department of Zoology, Faculty of Science, Cairo University, Cairo, Egypt. E-mail: salwa_abdelhamid@hotmail.com.

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Both fresh and marine edible crustaceans *Procambarus clarkii* and *Erugosquilla massavensis*, respectively, are now important components of our local aquatic fauna in Egypt that have a small yet growing economic importance in our markets. So, the purpose of the present work was, therefore, to assess the protein, amino acids, fatty acids, minerals, vitamins, protein electrophoresis and dendrogram analysis of their extracts for the first time in Egypt which may be in the future play an important role in some pharmaceutical industries and may be used as a specific health foods (functional supplement). In the present study, nutritional analysis of muscle and exoskeleton extracts of *P. clarkii* and *E. massavensis* indicated the presence of a high amount of protein, 9 essential amino acids and 9 non essential amino acids including taurine which has a very scientifical action and benefit for men. Also, the results showed the presence of 9 unsaturated fatty acids which included essential fatty acids linoleic acid (Omega 6) and alpha linolenic acid (Omega 3) which have many functions for man (food supplement) in addition to 4 saturated fatty acids. The minerals recorded were Cu, Zn, Mn, Fe, Mg, Ca, K and P and the vitamins were Vitamins A, D, E, B₁, B₂, B₃, B₆ and B₁₂. From the electrophoretic analysis of protein, the dendrogram analysis showed the highest similarity between the muscle and exoskeleton extracts of *P. clarkii*.

Key words: *Procambarus clarkii*, *Erugosquilla massavensis*, extracts, total protein, amino acids, fatty acids, minerals, vitamins, protein electrophoresis, dendrogram analysis.

INTRODUCTION

A widespread practice among human inadvertent destructive activities is the introduction of exotic animals and plants into many areas of the world. Also the migration included in these transfers is species of freshwater crawfish and marine mantis shrimp *Erugosquilla massavensis*. There are other 400 species of freshwater crawfishes within the families Astacidae, Cambaridae and Parastacidae (Huner and Lindqvist, 1991). The crawfish of our concern is *Procambarus clarkii* (Crustacean: Cambaridae) which had been accidentally introduced to the Egyptian Nile water via a private fish farm during the early 1980's (Ibrahim et al., 1995). The range of the crawfish populations has clearly expanded northwards since the middle of 1980's until Damietta and Rashid and Southward up to Aswan and also in some of the newly inhabited areas in Egypt; reaching to some ditches in Sinai desert through the irrigation system (Ibrahim and Khalil, 2009).

Also, the mantis shrimp *E. massavensis* is a potentially important Egyptian constituent of the fishery for economic crustaceans both in the area of the Suez Canal and in the Mediterranean Sea at Port Said displacing and dominating the local species *Squilla mantis*. Since landings of these mantis shrimps (Kocatas and Katagan, 1995; Sallam et al., 2006) are incorporated with those of the penaeid prawns, the nature of its fishery is therefore described as a part of the overall fishery of commercial crustaceans.

Both fresh and marine edible crustaceans, *P. clarkii* and *E. massavensis*, respectively, are now important components of our local aquatic fauna that have a small yet growing economic importance in our markets. So, the extracts of both crustaceans can be beneficial as nutraceutical and function of foods and may be used in some pharmaceutical industries if we can use it in the treatment of some diseases as reported by Fahmy et al. (2009) and Fahmy and Hamdi (2011).

It is interesting to note that the majority of marine and
freshwater organisms have attracted much attention as potential sources of drugs, recently, that are mainly found in invertebrates such as sponges, tunicates, molluscs and coelenterates. Some species produce very active biochemical compounds which have pharmaceutical value as anticancer drugs and antibiotics (Alonzo et al., 2003). Other species have shown clinical antitumor activity in refractory soft tissue sarcoma and ovarian cancer (D’Incalci et al., 2004). Several reports have described aspects of the crustaceans, particularly the edible species that have been intensely investigated and used as model organisms in a number of studies on biochemical composition and nutritive quality (Rosa and Nunes, 2002; Hamdi and Zaghoul, 2006; Hamdi and Abd El-Monem, 2006; Ibrahim and Khalil, 2009).

No one studied the muscle and carapace extracts components of both fresh and edible crustaceans, P. clarkii and E. massavenses, respectively, as well as their protein, amino acids, fatty acids, minerals, vitamins, electrophoretic pattern of protein and dendrogram analysis, which are done in the present study.

Amino acids are useful components in a variety of metabolism. Even though, some roles can be highlighted as a function of an amino acid, it is important to be aware that they are part of complex pathways and biological systems. The function and use of an amino acid is dependent on other amino acids, mineral elements, and fatty acids and has indirect effects that are manifested in myriad metabolisms.

Fatty acids are one of the defining constituents of lipids and are in large part responsible for the distinctive physical and metabolic properties of the latter where they are of course of vital importance. However, it has become evident that there are a number of more dynamic functions of fatty acids, which are attracting great interest.

Minerals are particularly significant in the different biological functions. Copper (Cu) and iron (Fe) are oligo-elements which play vital roles in the enzymatic and respiratory processes of crustaceans. Zinc (Zn) is one of the important essential metals, a constituent of more than 90 enzymes and proteins which regulates the activities of many other enzymes (Bruland and Franks, 1983). Manganese (Mn) is needed for growth and good health in humans; magnesium (Mg) is an essential mineral for cell function and it occupies a key role in all reactions with phosphorus (P). Calcium (Ca) is considered that most important of the principal mineral element (macronutrients) which constitutes 60 to 80% of all the inorganic material in the human body. Potassium (K) regulates the electrolyte and acid-alkali balances, the conductive capacity of the nerves, muscle contractions and the production of adrenaline and amino acids. Vitamins have a nutritional value and play important roles in cell metabolism. Vitamin A plays an important role as a growth factor, maintaining integrity of epithelial cells and it is an essential component of the visual pigment found in the rods of the eye. Vitamin D plays a central role in calcium and phosphate metabolism. Vitamin E plays an important role as antioxidant. The B vitamins often work together to deliver a number of health benefits to the body.

So, the purpose of the present work was, therefore, to assess the protein, amino acid, fatty acid, minerals, vitamins, protein electrophoresis and dendrogram analysis of muscle and exoskeleton extracts of both P. clarkii and E. massavensis, respectively, which may in the future play an important role in some pharmaceutical industries and may be used as a specific health foods (functional supplements).

MATERIALS AND METHODS

Collection of samples

The study was carried out on the freshwater crawfish P. clarkii and the marine mantis shrimp E. massavensis that were collected separately from different sites as follows:

1. Crawfish samples were collected from the River Nile, Cairo sector using 0.7 cm diagonal net mesh size.
2. Mantis shrimp samples were obtained from the Suez Canal of Ismailia.

Separation of muscle away from exoskeleton

Fresh whole bodies of crawfish and mantis shrimp samples (about 5.0 kg of each) were stored at -20°C to facilitate peeling process after thawing when needed as most crustaceans.

Preparation of muscle and exoskeleton product extracts

After peeling, each muscle of P. clarkii and E. massavensis were coarsely chopped and homogenized with distilled water by a mixer. The homogenates of each crustacean were extracted with water for 3 h. After filtration, the filtrate obtained was then concentrated and lyophilized to a brownish residue using LABCONCO lyophilizer, shell freeze system, USA. The crustacean extracts were stored in dry place avoiding water vapor until used.

Exoskeleton of each crustacean was extracted with boiling water for 15 min, then removed and put in oven at 220°C for one hour and allowed to cool to room temperature. After cooling, the dried waste products were powdered by a mixer.

Extracts analyses

Powder extracts of both muscles and exoskeleton from each studied crustacean species were analyzed as follows:

1. Total protein analysis: Total protein was determined using the semimicrokjeldahl method (Josylin, 1950).
2. Amino acids measured by high performance liquid chromatography (HPLC); Beckman 6300 amino acid analyzer (Gaithersburg, 2000).
3. Taurine and amino acids analysis: Taurine was measured by high performance liquid chromatography (HPLC) using precolumn derivatization formation with fluorescamine (Sakai and Nagaswa, 1992).
4. Fatty acids analysis: Fatty acids composition was determined by...
Table 1. Total proteins and essential amino acids analysis of muscle and exoskeleton extracts of both fresh and marine crustaceans, *P. clarkii* and *E. massavensis*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Muscle extract of</th>
<th>Exoskeleton extract of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. clarkii</em></td>
<td><em>E. massavensis</em></td>
</tr>
<tr>
<td></td>
<td><em>E. massavensis</em></td>
<td><em>P. clarkii</em></td>
</tr>
<tr>
<td></td>
<td><em>E. massavensis</em></td>
<td></td>
</tr>
<tr>
<td>Total protein (mg/100 g)</td>
<td>43.24± 0.71bc</td>
<td>48.11 ± 1.71abc</td>
</tr>
<tr>
<td></td>
<td>39.8 ± 1.78c</td>
<td>50.24 ± 1.06d</td>
</tr>
<tr>
<td>Essential amino acids (mg/100 g)</td>
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<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>288.29 ± 1.41a</td>
<td>160.53 ± 2.12d</td>
</tr>
<tr>
<td></td>
<td>257.73 ± 0.71c</td>
<td>277.25 ± 2.12b</td>
</tr>
<tr>
<td>Histidine</td>
<td>146.04 ± 0.71c</td>
<td>201.29 ± 1.78ab</td>
</tr>
<tr>
<td></td>
<td>130.56 ± 3.54d</td>
<td>210.76 ± 2.83e</td>
</tr>
<tr>
<td>Lysine</td>
<td>178.51 ± 2.42a</td>
<td>126.64 ± 1.56c</td>
</tr>
<tr>
<td></td>
<td>105.21 ± 2.83d</td>
<td>167.10 ± 1.58b</td>
</tr>
<tr>
<td>Threonine</td>
<td>416.64 ± 2.83b</td>
<td>261.17 ± 1.41d</td>
</tr>
<tr>
<td></td>
<td>372.48 ± 2.12c</td>
<td>451.07 ± 2.12d</td>
</tr>
<tr>
<td>Methionine</td>
<td>167.43 ± 0.21b</td>
<td>133.01 ± 0.07c</td>
</tr>
<tr>
<td></td>
<td>132.16 ± 1.05d</td>
<td>229.73 ± 0.35e</td>
</tr>
<tr>
<td>Leucine</td>
<td>189.68 ± 0.35c</td>
<td>161.64 ± 0.21d</td>
</tr>
<tr>
<td></td>
<td>169.57 ± 0.28c</td>
<td>279.17 ± 0.71f</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>392.81 ± 2.12d</td>
<td>227.47 ± 1.06c</td>
</tr>
<tr>
<td></td>
<td>351.17 ± 1.41b</td>
<td>392.86 ± 0.28d</td>
</tr>
<tr>
<td>Valine</td>
<td>229.87 ± 0.41b</td>
<td>176.83 ± 2.12d</td>
</tr>
<tr>
<td></td>
<td>205.50 ± 3.54c</td>
<td>305.41 ± 1.78e</td>
</tr>
<tr>
<td>Phenyl alanine</td>
<td>244.72 ± 0.14c</td>
<td>182.16 ± 1.56d</td>
</tr>
<tr>
<td></td>
<td>218.77 ± 2.41c</td>
<td>314.52 ± 1.06d</td>
</tr>
</tbody>
</table>

Data are expressed as means± S.E. of five separated determinations; means with the same letter for each parameters are not significantly different, otherwise they are (P<0.05).

RESULTS

Total protein, taurine and amino acids analysis

Analysis of muscle and carapace extracts of both *P. clarkii* and *E. massavensis* indicate the presence of high level concentrations of total protein (ranged from 43.24 to 50.24 g/100 g and 9 essential amino acids (Arginine, Histidine, lysine, threonine, methionine, leucine, isoleucine, valine and phenylalanine (Table 1). The highest average concentrations of total protein and essential amino acids were observed in the carapace extract of *E. massavensis* by Duncan analysis.

The results obtained from this study indicated the presence of 10 non-essential amino acids (Tyrosine, alanine, praline, serine, glycine, aspartic acid, glutamic acid, glutamine, cystein and taurine) (Table 2). The highest average concentration of taurine was recorded in the carapace extract of *P. clarkii* (201.26 mg/100 g). Furthermore, the highest average concentrations of sulphur containing amino acids Methionine and cysteine (229.73 and 284.83 mg/100 g) was recorded in the carapace extract of *E. massavensis* (Tables 1 and 2).

Fatty acids analysis

Analysis of 9 unsaturated fatty acids (oleic acid, palmitoleic acid, myristoleic acid, linoleic acid, arachidonic acid, docosahexaenoic acid, Eicosapentaenoic acid, linolenic acid and Erucid acid) of muscle and carapace extracts of *P. clarkii* and *E. massavensis* showed that the highest average concentrations were found in the carapace extract of *E. massavensis* (Table 3).

The aforementioned unsaturated fatty acids analysed include the essential fatty acids linoleic acid (omega 6) and alpha-linolenic acid (omega 3) of muscle and carapace extracts of *P. clarkii* and *E. massavensis*. Also, four saturated fatty acids were analysed (Lauric acid, Myristic acid, palmitic acid and stearic acid). The results obtained from this study showed the highest average concentrations present in the carapace extract of *E. massavensis* (Table 4).

Mineral analysis

Analysis of 8 elements (Cu, Zn, Mn, Fe, Mg, Ca, K and P) present in muscle and carapace extracts of both *P. clarkii* and *E. massavensis* were measured using Perkin Elmer Atomic Absorption Spectrometer (800) with flow injection analysis system (FIAS) (Larsen and Sandstrom, 1993).

6. Vitamin analysis: All vitamins were analyzed using HPLC, the Varian 940-LC (Brubacher et al., 1985).

Electrophoretic separation of proteins

Polyacrylamide gel electrophoresis (SDS-PAGE) electrophoresis was carried out using silver stain protocol (Laemmli, 1970).

Dendrogram analysis

Dendrogram was constructed to reveal the relationship between the two different species of the studied crustaceans including their muscles and carapace using gel Pro analyzer ver. 4.5 Cypermedica, USA.

Statistical analysis

Data are expressed as means±S.E. of five separated determinations. Means with the same letter for each parameters are not significantly different, otherwise they do (P<0.05). SPSS, for Windows (Version 15.0) was used for statistical analysis.
Table 2. Non-essential amino acids analysis of muscle and exoskeleton extracts of both fresh and marine crustaceans *P. clarkii* and *E. massavensis*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Muscle extract of</th>
<th>Exoskeleton extract of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. clarkii</em></td>
<td><em>E. massavensis</em></td>
</tr>
<tr>
<td>Non-essential amino acids (mg/100 g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td>350.71 ± 3.54b</td>
<td>217.19 ± 2.12d</td>
</tr>
<tr>
<td>Alanine</td>
<td>333.81 ± 1.41b</td>
<td>216.19 ± 0.71f</td>
</tr>
<tr>
<td>Proline</td>
<td>402.63 ± 3.53b</td>
<td>260.11 ± 1.06d</td>
</tr>
<tr>
<td>Serine</td>
<td>146.87 ± 2.83b</td>
<td>178.20 ± 2.83c</td>
</tr>
<tr>
<td>Glycine</td>
<td>206.72 ± 2.12b</td>
<td>217.44 ± 1.41a</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>218.65 ± 1.56a</td>
<td>133.49 ± 0.71d</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>110.29 ± 0.71c</td>
<td>220.46 ± 2.12a</td>
</tr>
<tr>
<td>Glutamine</td>
<td>323.51 ± 0.71d</td>
<td>730.14 ± 1.41a</td>
</tr>
<tr>
<td>Cysteine</td>
<td>259.62 ± 1.06b</td>
<td>164.92 ± 0.04c</td>
</tr>
<tr>
<td>Taurine</td>
<td>126.49 ± 0.71c</td>
<td>133.05 ± 1.41c</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E. of five separated determinations; means with the same letter for each parameter are not significantly different, otherwise they are (P<0.05).

Table 3. Unsaturated fatty acids analysis of muscle and exoskeleton extracts of both fresh and marine crustaceans, *P. clarkii* and *E. massavensis*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Muscle extract of</th>
<th>Exoskeleton extract of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. clarkii</em></td>
<td><em>E. massavensis</em></td>
</tr>
<tr>
<td>Unsaturated fatty acids (mg/100 g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oleic acid</td>
<td>5.12 ± 0.71a</td>
<td>4.86 ± 1.41a</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>8.59 ± 2.12a</td>
<td>8.18 ± 2.83a</td>
</tr>
<tr>
<td>Myristoleic acid</td>
<td>1.28 ± 0.35a</td>
<td>1.22 ± 0.14a</td>
</tr>
<tr>
<td>Linoleic acid (Omega 6)</td>
<td>67.74 ± 0.73a</td>
<td>64.46 ± 2.12a</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>48.58 ± 1.41bc</td>
<td>47.46 ± 0.71c</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>1.36 ± 0.35b</td>
<td>1.29 ± 0.21b</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>0.77 ± 0.14a</td>
<td>0.73 ± 0.07a</td>
</tr>
<tr>
<td>Linolenic acid (Omega 3)</td>
<td>0.51 ± 0.14a</td>
<td>0.49 ± 0.07a</td>
</tr>
<tr>
<td>Erucic acid</td>
<td>0.28 ± 0.07a</td>
<td>0.26 ± 0.07a</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E. of five separated determinations; means with the same letter for each parameters are not significantly different, otherwise they do (P<0.05).

Table 4. Saturated fatty acids analysis of muscle and exoskeleton extracts of both fresh and marine crustaceans, *P. clarkii* and *E. massavensis*.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Muscle extract of</th>
<th>Exoskeleton extract of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. clarkii</em></td>
<td><em>E. massavensis</em></td>
</tr>
<tr>
<td>Saturated fatty acids (mg/100 g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lauric acid</td>
<td>1.27 ± 0.36b</td>
<td>1.20 ± 0.23b</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>2.23 ± 0.71a</td>
<td>2.14 ± 0.35a</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>0.49 ± 0.14a</td>
<td>0.47 ± 0.07a</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>1.29 ± 0.35a</td>
<td>1.23 ± 0.28a</td>
</tr>
</tbody>
</table>

Data are expressed as means ± S.E. of five separated determinations; means with the same letter for each parameter are not significantly different, otherwise they do (P<0.05).
and *E. massavensis* (Table 5) showed the highest average concentration of all minerals measured in the carapace extracts of *E. massavensis*.

### Vitamin analysis

A wide variability of vitamins were analysed in the muscle and carapace extracts of both *P. clarkii* and *E. massavensis* (Table 6). Vitamins B₁, B₂, B₃, B₅, B₆ and B₁₂ record the highest average concentrations. Among the studied vitamins (Vitamin A, D and E), extracts of both *P. clarkii* and *E. massavensis* were found in the muscle and carapace.

### SDS-gel electrophoresis

The electrophoretic analysis of muscle and carapace extract proteins of both fresh and marine crustaceans *P. clarkii* and *E. massavensis* showed 16, 16, 19 and 16 bands around molecular weight 15.27 to 181.74, 15.62 to 120.63, 14.44 to 242.75 KD, respectively (Table 7 and Figure 1).

### Dendrogram analysis

The dendrogram analysis showed the similarity between the aforementioned three examined extracts of the muscle and carapace extracts of *P. clarkii* with 82.35%. However, the recorded similarity between them and muscle extract of *E. massavensis* was 76.02%, while 70.91% similarity was recorded between them and the carapace extract of *E. massavensis* depending on the simple band match. According to the density and position of different bands in the electrophoretic analysis of the muscle, the carapace extract proteins of both *P. clarkii* and *E. massavensis* were recorded (Figure 2).

### DISCUSSION

Products from fresh and marine sources have recently
Table 7. SDS-PAGE gel electrophoresis of muscle and exoskeleton extracts of both fresh and marine crustaceans, *P. clarkii* and *E. massavensis*.

<table>
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<tr>
<th>Lane</th>
<th>Mol.wet (KD)</th>
<th>Amount (%)</th>
<th>Mol. wt. (KD)</th>
<th>Amount (%)</th>
<th>Mol.wet (KD)</th>
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<tr>
<td></td>
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<td><em>P. clarkii</em></td>
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<td><em>E. massavensis</em></td>
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<td><em>P. clarkii</em></td>
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<tr>
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<td>181.47</td>
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M: Protein marker; Mol. Wt.: Molecular weight in KD.

**Figure 1.** Variations in electrophoretic pattern of muscle and exoskeleton extract proteins of both fresh and marine crustaceans *P. clarkii* and *E. massavensis*. M: Protein marker; A: Muscle extract protein of *P. clarkii*; B: Muscle extract protein of *E. massavensis*; C: Exoskeleton extract protein of *P. clarkii*; D: Exoskeleton extract protein of *E. massavensis*. 
become attractive as nutraceutical and functional foods and as a source material for the development of drugs and specific health foods (functional supplements). Supplements derived from marine foods have been shown to have various functions in animal and clinical experiments. For example, proteins and its functional peptides, saturated and unsaturated fatty acids, minerals and vitamin K are being increasingly used to treat and prevent a wide variety of lifestyle-related diseases and to improve the quality of life. In this study, the extracts of both fresh and marine crustaceans *P. clarkii* and *E. massavensis*, respectively, was analysed to open the door for production of a new products of nutraceutical and healthy food or production of a source material of drugs from unusable fresh and marine sources already present in our waters in Egypt.

Those edible crustaceans were chosen according to their invading power and spreading activity in our fresh water (River Nile) for *P. clarkii* and marine (canals citry; Ismailia, El-Suez and Port Said) and in the eastern Mediterranean at Port Said City for *E. massavensis*. The analysis included were the protein, amino acids (essential and non essential), fatty acids (Saturated and unsaturated), minerals, vitamins and the electrophoretic pattern of protein with dendrogram analysis.

Many works were done on the flesh and carapace of *P. clarkii* and *E. massavensis* (Gradwell et al., 1998; Mona et al., 2000; Ibrahim and Khalil, 2009); on the other hand, studies on their extracts were very limited in Egypt (Fahmy et al., 2009; Fahmy and Hamdi, 2011a, b).

Nutritional analysis of muscle and carapace extracts of *P. clarkii* and *E. massavensis* indicated the presence of high amount of protein, 9 essential amino acids and 9 non essential amino acids including taurine which has a very scientifical action and benefits to man.

Taurine was known to have antioxidant activity. Li et al. (2009) studied its renoprotective effect and found that taurine protected kidney from oxidative injury through mitochondrial-linked pathway in rat model. Also taurine has wide applications and good used in anti-inflammation, ease pain, lower blood sugar, liver protection, benefit gall bladder, prevent arrhythmia, treat rheumatoid arthritis and eye inflammation. It was found that oyster *Ostrea gigas* contains highest taurine in all water products, thus brings obvious and fascinating applicable values.

In the present study, protein analysis also indicated the presence of large quantities of sulfur-containing amino acids, such as methionine, and cystine with stimulative activities on enzymes and liver function. Essential amino acids supplements were reported to increase bone strength, trabecular architecture, and cortical thickness (Yin et al., 2005).

In the present study, the analysis indicated the presence of 9 unsaturated fatty acids including eicosapentanoic acid (EPA) and 4 saturated fatty acids which are being increasingly used to treat and prevent a wide variety of lifestyle related diseases and to improve the quality of life.

Also, the unsaturated fatty acids analysed in the present study indicated the presence of essential fatty acids linoleic acid (Omega 6) and alpha-linolenic acid (Omega 3) which have many functions for man (food supplement); reduce inflammation throughout the body, keep blood from clotting excessively, maintain the fluidity of the cell membranes, lower the amount of lipids (fats such as cholesterol and triglycerides) circulating in the blood stream, decrease platelet aggregation; prevent excessive blood clotting, inhibit thickening of the arteries by decreasing endothelial cells' production of a platelet-derived growth factor (the lining of the arteries is composed of endothelial cells), reduce the production of messenger chemicals called cytokines, which are involved in the inflammatory response associated with atherosclerosis, reduce the risk of becoming obese and improve the body's ability to respond to insulin by stimulating the secretion of leptin, a hormone that helps regulate food intake, body weight and metabolism, and is

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*Figure 2. Dendrogram analysis of SDS-PAGE gel electrophoresis of muscle and exoskeleton extract proteins of both fresh and marine crustaceans P. clarkii and E. massavensis.*
expressed primarily by adipocytes (fat cells) and help prevent cancer cell growth. A body of epidemiologic, clinical and experimental evidence suggests that fatty acids have a modulatory effect on bone metabolism in animals and humans.

Classen et al. (1995) reported that dietary intake of linolenic acid and EPA influenced osteoblast and osteoclast activity in rats. Kruger and Horrobin (1997) suggested animals deficient in essential fatty acids develop severe osteoporosis, coupled with increased renal and arterial calcification. Yin et al. (2005) suggests that freshwater soft-shell turtle (SST) *Trionyx sinensis* would be useful for the prevention and treatment of bone loss and bone fracture. These observations suggest a close link between fatty acids and bone metabolism. Supplements derived from fresh and marine foods have been shown to have various functions in animal and clinical experiments. The extracts of the freshwater oyster (Ostrea gigas) were found to reduce the levels of serum free fatty acids, triglycerides, lipid peroxide and liver cholesterol in the peroxidized oil-treated rats (Kimura et al., 1998). Also, Chijimatsu et al. (2008) proved that the extract of freshwater clam *Corbicula fluminea* reduced the serum and hepatic cholesterol levels in rats fed on a high cholesterol diet. On the other hand, oyster has been widely used all over the world as a health food source. In ancient Chinese medicine, oysters were used as a remedy for fatigue (as a tonic), and as a beauty treatment for skin. Also Kimura et al. (1998) proved that oysters could be beneficial as a healthy food source in the prevention of hyperlipidemia. Also, a freshwater soft-shell turtle (*SST*) widely cultured in Southern China, Japan, Koran, and Southeast Asia (Ernst et al., 1994). The turtle is considered to be aneublem and has been used in medicinal cuisine and as an energetizer in tonics in China, Japan, and Korea since ancient times (Alderton, 1998). Analysis of (SST) *T. sinensis* indicates that it is a good source of protein, collagen, minerals including calcium and phosphorus, 20 amino acids, 11 unsaturated fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and vitamins A, B₁, B₂ and D (Yin et al., 2005).

Koyama et al. (2006) suggested that the marine edible thorny oyster *Spondylus varius* (Mizuirishoujou) exerts a protective effect against liver injury. These results reveal that this edible oyster may be suitable as a source of functional food for the prevention of liver diseases.

The fresh water and seafood processing industry is not well known in our country, Egypt, but it is one of the major food processing industries in India. In the years 2003 to 2004, 129,785 ton of frozen shrimps were produced (MPEDA, 2004). Processing of shrimps invariably generates solid waste (body carapace). The waste generation from processing of Indian shrimps ranges from 48 to 56% of the total weight depending on the species (Sachindra et al., 2006).

In the present study, carapace analysis of fresh and marine crustaceans *P. clarkii* and *E. massavensis*, respectively have shown the presence of high amount of protein, amino acids (9 essential and 9 non essential), fatty acids (9 unsaturated and 4 saturated), minerals (Cu, Zn, Mn, Fe, Mg, Ca, K and P) and vitamins.

Sachindra (2006) stated that the major components (dry weight basis) of shrimp waste are protein (35 to 50%), chitin (15 to 25%), minerals (10 to 15%) and carotenoids. A small quality of this waste is used in the dry form as an ingredient in animal feed and for the production of chitin/chitosan. However, large quantities of this byproduct are being wasted, resulting not only in the loss of valuable components but also in environmental pollution (Sachindra et al., 2006).

Beaney et al. (2005) reported that the most abundant minerals in prawn shell were Ca, Mg, Na, Sr, K and Fe in that order and that calcium was by far the most abundant (about 17 times more than magnesium). Mona et al. (2000) reported that the amount of calcium present in the shells was 6 and 23 times higher than the amounts of phosphorus and magnesium, respectively.

In the present study, the muscle and carapace extracts of *P. clarkii* and *E. massavensis* were recorded the highest amount of P (phosphorus) followed by Ca (calcium) and K (potassium) than other minerals analyzed (Mg, Fe, Mn, Zn, Cu) (Table 5). Also, Vitamin B₉ was recorded the highest amount values measured followed by Vitamin E and then Vitamin D than other vitamins analysed; (Vitamins B₁₂, B₂, B₆, B₁, B₁ and A). Also analysis of *T. sinensis* indicates that it is a good source of minerals (Calcium and Phosphorus) and Vitamins A, B₁, B₂ and D (Yin et al., 2005). Regarding the applications of these very important rich extracts component in Egypt, Fahmy et al. (2009), Fahmy and Hamdi (2011a, b) throw the light for the first time in Egypt on their antioxidant effects as a curative effect of both extracts on kidney and liver dysfunction. However, the solubilized protein wastes of *P. clarkii* (Peptones) have an application, among others, in the formulation, among others, in the formulation of culture media, in the biosynthesis of pharmaceutical and food products, in clinical or dietetic nutrition, as additives in cosmetics and as components of organic fertilizers (Pedro, 2009).

Further studies on the mode of action and characterization of the active components in *P. Clarksii* and *E. massavensis* extracts must be done in progress.

REFERENCES


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