

# Amino acid composition in the muscles of male and female commercially important crustaceans from Egyptian and Saudi Arabia coasts

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**Abstract:** Seafood products have attracted considerable attention as important sources of nutrients in the human diet. Apart from their delicacy, crustaceans species such as shrimps and crabs consist of protein and amino acids. The present study was conducted to evaluate flesh of both sexes of mantis shrimp, shrimp and crab species with the emphasis on protein and amino acids composition. The results showed that significant differences in protein and amino acid contents were found between sex of each species and among studied crustacean species. Furthermore, the highest protein percentage was recorded in edible muscles of males mantis shrimp (*Erugosquilla. Massavensis*) followed by crab species (*Portunus pelagicus*) and shrimp species (*Peaneus semisulcatus*, *Metapenaeus monoceros* and *Peaneus indicus*). Sixteen amino acids have been determined in edible portions of studied crustaceans, among these, nine essential amino acids were histidine, arginine, methionine, leucine isoleucine, lysine, threonine, valine and phenylalanine and seven non- essential amino acids were alanine, glycine, proline, tyrosine, serine, aspartic acid and glutamic acid. The quantities of amino acids vary considerably within and between species as well as between sexes. The edible muscles of studied crustaceans are enriched in amino acids that in turn open the door to carry out further studies on the mode of action, characterization of the active components and the antioxidant properties of marine crustaceans which may be used in the future as a specific health foods (functional supplements).

**Keywords:** Protein, Amino Acid Analysis, Muscles, Crustaceans Species

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## 1. Introduction

Seafood products have attracted considerable attention as important sources in the human diet. Apart from their delicacy, crustaceans species such as shrimps, crabs and lobster consist of amino acids, protein and other useful nutrients [1, 2]. The nutritive values of edible marine organisms depend upon their biochemical composition, such as protein, amino acids, lipid, fatty acids, carbohydrate, vitamins and minerals. 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 [3, 4]. Due to, 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 [5]. Proteins do play both structural and functional role of cellular level. Being an integral part of the cell membrane, intracellular and extra cellular

passages are linked through it [6]. Any sort of cellular metabolism occurring in body involves one or many different proteins. The proteins are among the most abundant biological macromolecules and are extremely versatile in their function and interaction during metabolism of proteins, amino acids, enzymes and co-enzymes [7]. Biological value of protein is obviously reflected upon its amino acids concentration. Amino acids are the building blocks of proteins and serve as body builders. The role played by amino acids in isosmotic intracellular regulation has been illustrated in several investigators [8, 9], and it can be an important source of energy producing compounds [10, 11]. In addition to amino acids play important roles in physiological functions such as osmoregulation and buffer capacity in the tissues of aquatic animals [12] and some amino acids are involved in neurotransmission [13]. In

general, the crustaceans have a balanced distribution of all essential amino acids required for an adult per day. There are 20 amino acids found in crustaceans proteins. Some of these are listed as essential amino acids (EAAS), *i.e.* arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine because these are not synthesized in the body. The essential amino acids are required for maintenance of life, growth, synthesis of vitamins and reproduction. The lowest level of any one of these essential amino acids in a protein source, which limits the utilization of that protein, makes it the "First limiting amino acid" [13]. While the non-essential amino acids were alanine, cysteine, proline, aspartic acid, glutamic acid, glutamine, glycine, serine and tyrosine. The nonessential amino acids (NEAAS) play a more important part than the essential ones in the regulation of cellular osmotic pressure [4, 14]. However, the importance of estimation of amino acid contents in marine organisms, the literature is scanty regarding the amino acids composition of crustaceans species except a few reports [1, 4, 12, 15-22]. Therefore, in the present study, an attempt has been made to evaluate the analysis of amino acids in edible muscles of both sexes of crustaceans species (mantis shrimps, shrimps and crabs) which may in the future play an important role in some pharmaceutical industries and may be used as a specific health foods (functional supplements). Additionally, to assess and differentiate their nutritional quality.

## 2. Material and Methods

### 2.1. Collection of Samples

The study was carried out on the marine crustaceans (mantis shrimps, shrimps and crabs) and aquaculture shrimps that were collected separately from different sites as follows:

1. Males and females marine mantis shrimp *Erugosquilla. massavensis* were collected from the Suez Canal of Suez, Egypt.
2. Red Sea strain crustaceans included : male and female shrimps *Peaneus semisulcatus* and male and female crabs *Portunus pelagicus* that were obtained from local fishermen at Jeddah, Saudi Arabia.
3. Arabian gulf strain crustaceans : both sexes of *Metapenaeus monoceros* (shrimps) and *Portunus pelagicus* (crabs) were collected from local fishermen at Dammam, Saudi Arabia.
4. Cultured *Peaneus indicus* samples of both sexes were collected from fisherman from Saudi Arabia.

An attempt was made to collect consistent size ranges. Samples were washed with deionized water to remove any adhering contamination and drained using filter paper. Samples were put in crushed ice in insulated containers and brought to the laboratory for preservation prior to analysis. The male and female samples were segregated and were wrapped in aluminum foil and frozen.

### 2.2. Separation of Muscle away from Exoskeleton

Fresh whole bodies of all samples of studied crustaceans species were stored at  $-20^{\circ}\text{C}$  to facilitate peeling process after thawing when needed as most crustaceans. After defrosting, the samples were separated into the exoskeleton and the endoskeleton (*i.e.* edible muscles).

### 2.3. Biochemical Analysis

1. Powered samples of muscles were analyzed for total proteins by [23]. The total protein was estimated as per the Folin-Ciocalteu method with bovine serum albumin (BSA) as standard. 1g of wet muscle tissue was homogenized in Homogenizer with 10 ml of 0.1 M Phosphate buffer. Take 1 ml of tissue homogenate, 1 ml of 0.1 N NaOH and keep it for 30 minutes, at room temperature, now add 8ml of distilled water and centrifuge at 4000 rpm for 30 minutes. Take only 0.1 ml supernatant and add 0.9 ml of distilled water to make volume 1 ml. Add 5 ml of alkaline reagent (2 g  $\text{Na}_2\text{CO}_3$  in 0.1 N NaOH: 4% Na-K tartarate 2%  $\text{CuSO}_4$ , 200:1:1) leave it for 30 minutes at room temperature. Add 0.5 ml of Folin phenol reagent; leave it for 40 to 45 minutes at room temperature. The color intensity was measured at 750 nm against reagent blank.

2. Amino acids measured by high performance liquid chromatography (HPLC); Beckman 6300 amino acid analyzer [24]. The profile of amino acids was done following high performance thin layer chromatographic (HPTLC), the muscles were dried ( $80^{\circ}\text{C}$  for 3 hrs.), digested with 6 M aqueous hydrochloric acid and dried under vacuum. The powdered sample was dissolved in distilled water and 5  $\mu\text{l}$  of sample was loaded on 8 mm Hase. The plate was sprayed with ninhydrin reagent prepared in propan-2-ol and dried. The developed plate was documented using photo-documentation chamber (CAMAG-REPROSTAR 3) at UV 254 nm and UV366 nm lights, the plate was scanned at 500 nm. The peak area of the sample was compared with standard amino-acids and quantified.

### 2.4. Statistical Analysis

The obtained data were used for descriptive statistical analysis consisting of means  $\pm$  standard error of five separated determinations. In order to test the significance of the differences among the mean values of the present studied species one-way ANOVA test were applied. Means with the same letter for each parameter are not significantly different, otherwise they do ( $P < 0.05$ ). SPSS, for Windows (Version 15.0) was used for statistical analysis.

## 3. Results

### 3.1. Total Protein Analysis

Table 1 shows the protein contents of edible portion of both sexes of studied crustaceans species. The protein content of edible muscles of all studied species was significantly different ( $P < 0.000$ ) from each other. The

highest protein percentage was recorded in edible muscles of males *E. massavensis* (48.85%) followed by crab species *P. pelagicus* from Red Sea and Arabian gulf (44.28%). While, females *E. massavensis* had the lowest protein

percentage (35.14%). Moreover, it was observed that the protein content in edible portions of different species of studied shrimps had more or less the same value.

**Table 1.** Protein content (%) in edible muscles of male and female crustaceans species.

Species	Region	Protein %	
		Males	Females
<i>E. massavensis</i>	Suez	48.85±0.32 <sup>a</sup>	35.14±0.08 <sup>e</sup>
<i>P. semisulcatus</i>	Red Sea	41.45±1.99 <sup>d</sup>	41.84±1.22 <sup>d</sup>
<i>M. monoceros</i>	Arabian gulf	40.53±0.89 <sup>d</sup>	41.88±0.64 <sup>cbd</sup>
<i>P. indicus</i>	Aquarium	42.88±0.64 <sup>bcd</sup>	40.68±1.32 <sup>cd</sup>
<i>P. pelagicus</i>	Red Sea	44.06±0.88 <sup>bc</sup>	45.19±1.03 <sup>b</sup>
<i>P. pelagicus</i>	Arabian gulf	44.28±0.98 <sup>b</sup>	44.88±2.31 <sup>b</sup>
ANOVA test		F=8.854 P< 0.000	

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

### 3.2. Amino Acids Analysis

Analysis of muscles of male and female species of mantis shrimps, shrimps and crabs species indicate the presence of 9 essential amino acids (EAAS) (histidine, arginine, methionine, leucine isoleucine, lysine, threonine, valine and phenylalanine) (Figure 1 & 2). Moreover, the present data indicate that the concentration of essential amino acids varied among species as well as between sexes of each species. Statistically significant variations (P < 0.000) in the content of essential amino acids in edible portion were observed among studied samples. The mean value of histidine ranged from 1.05 to 2.01 mg/ 100g, for arginine were 1.54 to 5.63 mg/ 100g. The range of methionine content was 1.20 to 1.87 mg/ 100g. Furthermore, the average content of leucine was 1.44 to 2.81 mg/ 100g, while for isoleucine was 1.42 to 1.91 mg/ 100g and 1.11 to 1.88 mg/ 100g for lysine. The contents of threonine, valine and phenylalanine were ranged from 0.523 to 2.64 mg/ 100g, from 1.64 to 2.90 1.64 and from 1.81 to 2.95 respectively. The present results showed that males *E. massavensis* had the highest average content of all essential amino acids except for methionine in comparison with other crustaceans species (Figure 1). The maximum level of methionine was recorded in edible muscles of males *P. pelagicus* from Red Sea. On the other hand, females *E. massavensis* had the lowest concentrations of all essential amino acids except for threonine and valine (Figure 2), whereas male *P. semisulcatus* from Red Sea and male *P. pelagicus* from Arabian gulf had the lowest content of threonine and valine respectively. In the present study, the arrangement of EAAS was recorded according to the following order; in males *E. massavensis* : arginine >

phenylalanine > valine > leucine > threonine > histidine > isoleucine > lysine > methionine. While their females showed this order: phenylalanine > valine > arginine > leucine > threonine > isoleucine > lysine > methionine > histidine. Furthermore, in shrimps species these following arrangements were detected: phenylalanine > valine & arginine > leucine > lysine > isoleucine > methionine > histidine > threonine and arginine > valine > phenylalanine > leucine > isoleucine > threonine > methionine > lysine > histidine in males and females *P. semisulcatus* respectively, whereas, in males *M. monoceros* : arginine > leucine & phenylalanine > valine > threonine > isoleucine > methionine > lysine > histidine and in their females: arginine > phenylalanine > leucine > valine > isoleucine > threonine > lysine > methionine > histidine. Moreover, in aquaculture shrimps this order was observed: arginine > phenylalanine > leucine > valine > isoleucine > threonine > lysine > histidine > methionine and arginine > leucine phenylalanine > valine > isoleucine > threonine > methionine > lysine > histidine in males and females respectively. Furthermore, in crab species these orders were recorded; in males Red strain: arginine > leucine > valine > threonine > phenylalanine > methionine > isoleucine > lysine > histidine and in females: arginine > leucine > phenylalanine > valine > threonine > lysine > methionine > isoleucine > histidine. In Arabian gulf crab strain, EAAS was recorded according to the following order: arginine > leucine > histidine > phenylalanine > threonine > valine > lysine > isoleucine > methionine and arginine > leucine > phenylalanine > histidine > threonine > valine > lysine > isoleucine > methionine in males and females respectively.

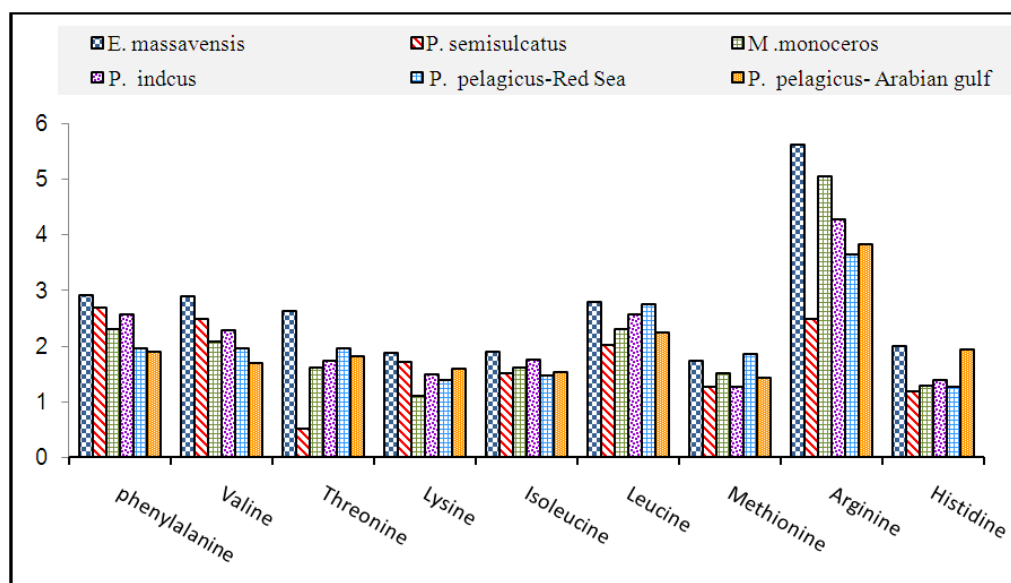


Figure 1. Essential amino acid contents (mg/100g) in edible muscles of male crustaceans species.

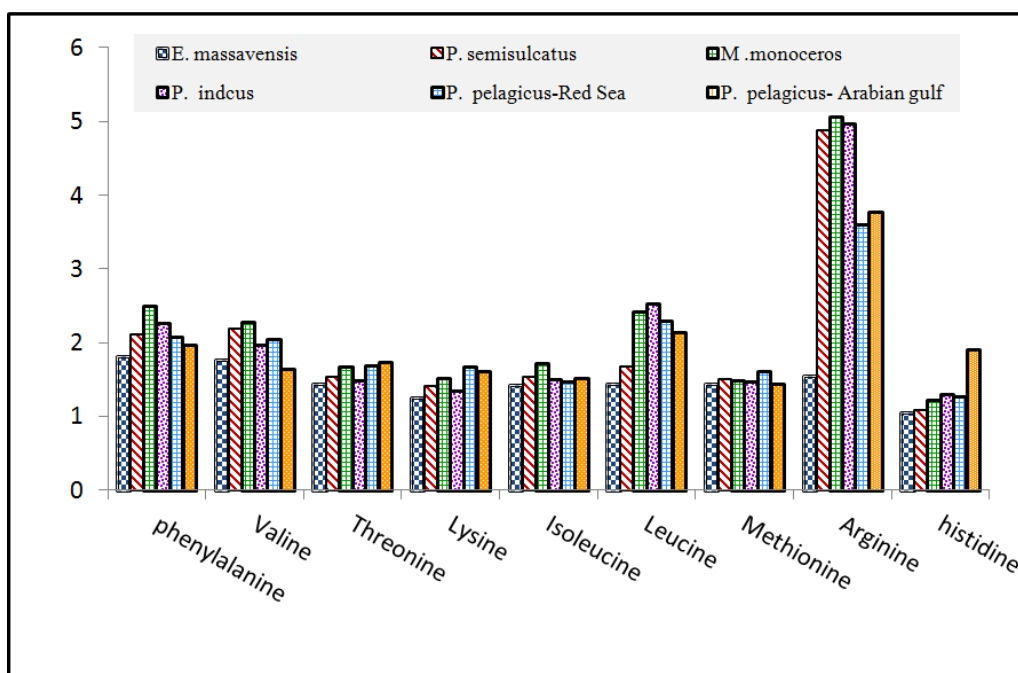


Figure 2. Essential amino acid contents (mg/100g) in edible muscles of female crustaceans species

Furthermore, the recorded results indicate the presence of 7 non-essential amino acids (NEAAS) represented by (alanine, glycine, proline, tyrosine, serine, aspartic acid and glutamic acid, (Figure 3 & 4). Similarly, as in essential amino acids significant differences in the concentrations of non-essential amino acids were observed between sex of each species and among studied species. The mean values of non-essential acids were ranged as the following: 1.52 to 2.20 mg/100g for alanine; 2.00 to 3.20 mg/100g for glycine; 1.98 to 3.61 mg/100g for proline, 1.60 to 2.44 mg/100g for tyrosine; 1.78 to 2.50 mg/100g for serine; 2.40 to 4.60 mg/100g for aspartic acid and 3.61 to 5.93 mg/100g for glutamic acid. According to the present data in Figure 2, males *E*

*massavensis* had the highest average levels of alanine, glycine, proline and serine. Additionally, tyrosine showed the maximum content in muscles of females *P. indicus*. While, edible portion of male *P. semisulcatus* had the highest concentration of aspartic acid comparable to all studied crustaceans species, moreover, their females had the highest level of glutamic acid. On the other hand, as shown in Figure 3, females *P. pelagicus* from Arabian gulf had the lowest average concentrations of alanine, glycine, proline and tyrosine in their edible muscles. Furthermore, the lowest contents of serine, and glutamic acid were detected in females *E. massavensis*, whereas females *P. pelagicus* from Red Sea had the minimum concentration of aspartic acid.

Furthermore, NEAAS content in muscles of crustacean species exhibited the following arrangement : glutamic acid > aspartic acid > proline,> serine > tyrosine > alanine in all studied crustaceans species except for females of *E. massavensis*, *P. semisulcatus* and *P. indicus*, where these order were observed glutamic acid > aspartic acid > proline,> alanine > glycine > tyrosine > serine , glutamic acid > aspartic acid > glycine > tyrosine > serine > alanine and glutamic acid > proline,> aspartic acid > glycine > serine > alanine > tyrosine respectively.

Figure 5 summarize total amino acid which ranged from 30.15 mg /100g to 46.95 mg /100g. Whereas, the maximum value of total amino acid was detected in males *E. massavensis* and minimum content was observed in females *E. massavensis*. Moreover, from the recorded data it was observed that average total amino acid in males of *E*

*massavensis*, *P. indicus* and *P. pelagicus* from the studied regions were higher than their females. Conversely, males shrimps of *P. semisulcatus* and *M. monoceros* had lower mean values of total amino acids as compared with their females. Additionally, it was observed that all studied samples of shrimps had higher total amino acid content than crab species. Regarding with the levels of EAAS and NEAAS in edible muscles of studied crustaceans organisms, the present data declare that the contents of NEAAS showed an increase than EAAS in all studied species except for males *E. massavensis* and both sexes of *P. pelagicus* from Arabian gulf. The ratio of EAAS to NEASS ranged from 0.70 to 1.09 where the lowest value was observed in male shrimps *P. semisulcatus*, while the highest value was recorded in males *E. massavensis*.

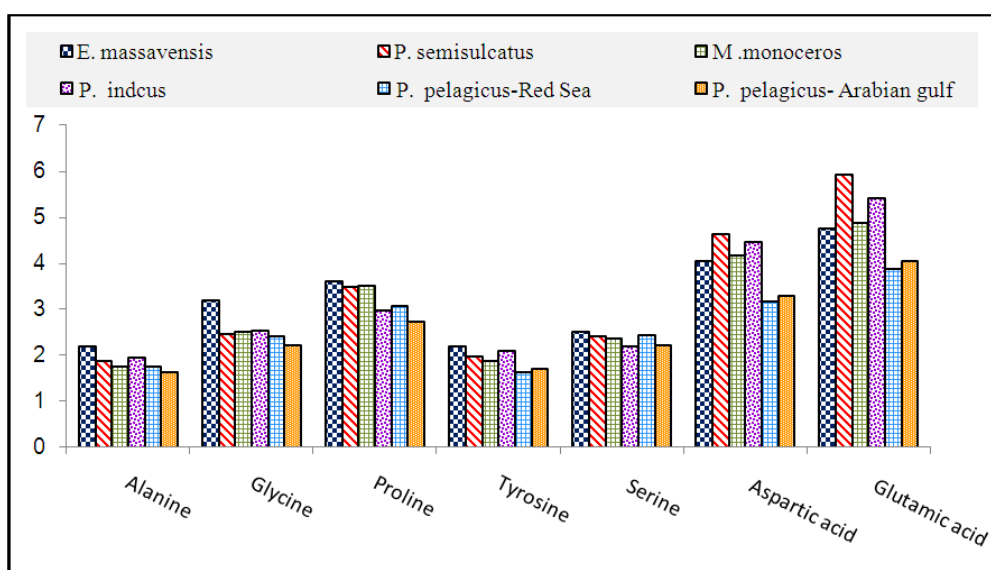


Figure 3. Non-essential amino acid contents (mg/ 100g) in edible muscles of male crustaceans species.

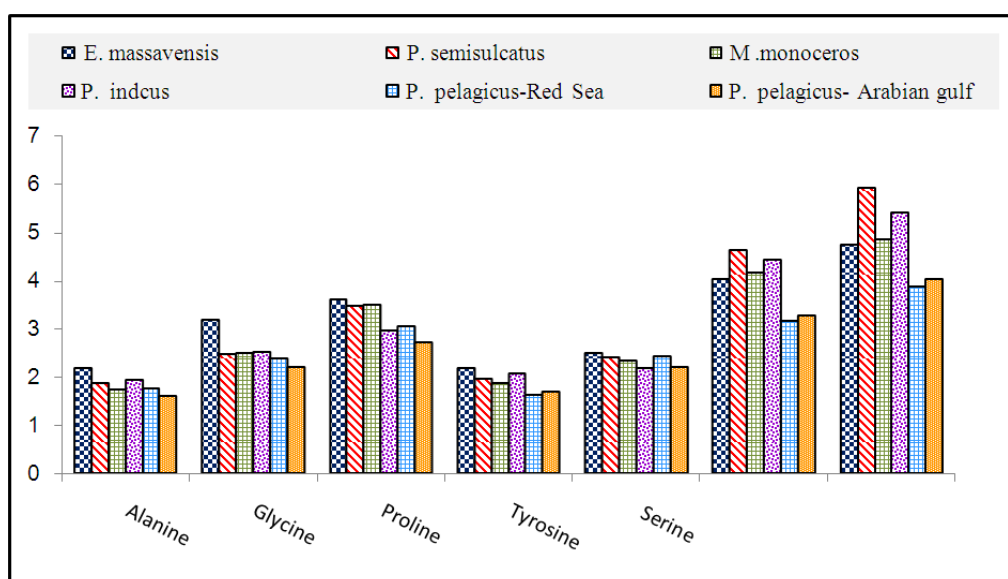


Figure 4. Non-essential amino acid contents (mg/ 100g) in edible muscles of female crustaceans species.

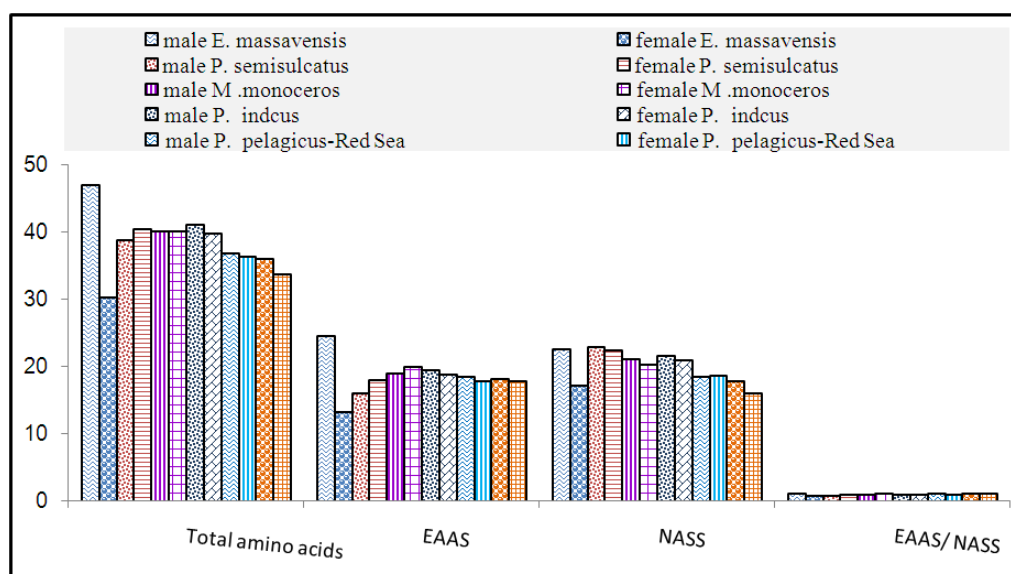


Figure 5. summarize total amino acids, the ratio of essential amino acids (EAAS) to non-essential amino acids (NAAS) in studied crustaceans species.

## 4. Discussion

The recorded data of the present study indicated the presence of high protein concentration in edible muscles of males mantis shrimps followed by both sexes of crab species then both sexes of shrimps, whereas the lowest level recorded in females mantis shrimps. The high protein concentration in crustaceans species can be attributed to their omnivorous feeding habit [25] and also may be due to the fact that it is the main component of the contractile elements of the striated muscles [26, 27]. Furthermore, the elevation of protein percentage in the edible muscles in studied crustaceans samples also indicates their high nutritive quality. In the present study, males *E. massavensis* had the highest contents of protein in their edible portion compared to their females. This confirms the values reported by [28] for *E. massavensis* which collected from the Egyptian Mediterranean, coast off Port Said. In this respect, some males of decapod crustaceans grow faster than their females [26], so the high protein content in the muscles of males indicates a faster growth rate as compared to the females [27]. The elevation of protein percentage in edible muscles of *E. massavensis* ranked this species as protein rich edible crustaceans, therefore, this species can stand as a source of cheap animal protein for Egyptian people. This agrees with the previous findings [26- 30].

Amino acids are the building blocks of proteins and serve as body builders. They are utilized to form various cell structures, of which they are key components and they serve source of energy [10- 11, 31]. In the present study, sixteen amino acids have been determined in edible portions of studied crustaceans species. Among these, nine EAAS were histidine, arginine, methionine, leucine isoleucine, lysine, threonine, valine and phenylalanine and seven NEAAS were alanine, glycine, proline, tyrosine, serine, aspartic acid and glutamic acid. In comparison with the other previous studies, such as in aquaculture prawn *Macrobrachium rosenbergii*.

nineteen amino acids were detected, among these eleven are essential and, eight are non-essential amino acids [19], while nine essential amino acids and nine non-essential were detected in edible muscles of *Procambarus clarkii* and *E. massavensis* [20]. Furthermore, in flesh of shrimps *Litopenaeus vannamei*, eighteen amino acids were detected, among these nine were EAAS and nine were NEAAS [22]. In amino acids analysis of edible muscles of fresh water prawn *Macrobrachium rosenbergii* and marine shrimps *Penaeus semisulcatus*, sixteen amino acids were detected; nine were EAAS and seven were NEAAS [1]. Thirteen amino acids were reported in *Macrobrachium vollohovenii* African river prawn and *Tympanotonus fuscatus* (tropical periwinkle) had eight EAAS and five NEAAS [32]. Regarding, crab species, [12] recorded seven EAAS and seven NEAAS in muscles of ghost crabs *Ocypode platytarsis*, Similarly seven EAAS and seven NEAAS in muscles of soft crabs *Portunus sanguinolentus* were observed. While in edible muscles of hard crabs of the same species eight EAAS and eight NEAAS were detected by [15]. The nutritive value of any animal is decided by the presence of EAAS [15]. In this sense, total EAAS had the highest mean value in males mantis shrimps. So males *E. massavensis* are declared superior over other studied crustaceans in terms of nutritive value. Furthermore, the present data show that males of mantis shrimps, shrimps and crabs species had higher total EAAS than their females except for *P. semisulcatus* and *M. monoceros*. The variations in EAAS proportions from male to female crustaceans were reported by [12, 14, 19, 31]. This suggests sex differences and physiology mechanism.

In the present study, the most abundant amino acid in all studied species was arginine except for female *E. massavensis* and male *P. semisulcatus*. This amino acid found in higher amount because it is the important substance for the regulation of osmotic pressure in crustacean muscles [12, 31]. Moreover, the presence of high content of valine in



edible muscles of crustaceans organisms was recorded in the present study. Valine is involved in many metabolic pathways and is considered indispensable for protein synthesis and optimal growth [33]. Additionally, in the present work, the elevation of histidine content was recorded in males *E. massavensis*, both sexes of *P. indicus* and both sexes of crab species. Histidine is indispensable amino acid involved in many metabolic functions including the production of histamines, which take part in allergic and inflammatory reactions. It plays a very important role in maintaining the osmoregulatory process and is related to energy production or is used in other metabolic pathways during certain emergencies harsh conditions [34]. Regarding, NEAAS in the present work, the edible portions of studied crustaceans are enriched in glutamic acid, and aspartic acid. Glutamic acid, and glycine are amino acids component of glutathione; that has been to be an important cellular protectant against reactive oxygen metabolites in several cells by serving as a substrate for glutathion peroxidase [29]. Therefore, the marine crustaceans can be used as antioxidant agents that play a critical role in body protection by scavenging active oxygen and free radicals and neutralizing lipid peroxides as reported by [35-36]. In recent years many studies have shown that many traditional natural products have a wide range of physiological, biochemical and pharmacological effects due to the properties of their constituents [37]. In particular, they contain a variety of substances having antioxidant activity including thiol-containing amino acids, in this respect, it was reported that, administration of such amino acids like methionine [38] replete the levels of antioxidants and minimizes oxidative stress. Therefore, there is need for a natural product that protects the body but cost-effective, safe and without side effects. So, the present study threw the light to open the door to study the antioxidant properties of marine crustaceans.

In the present work, the quantities of amino acids vary considerably within and between species as well as between sexes. The different amino acids in flesh of crustaceans species might be associated with the varying tastes as well as textural properties of meat of the crustaceans species [32]. in this respect, according to [39] glycine, alanine, serine and threonine give tasty sweet, while arginine, leucine, valine, methionine, phenylalanine and histidine give bitter taste. The ratios of EAAS to NEAAS in the crustaceans species in the present study ranged from 0.70 to 1.12. [40] explained that EAAS/ NEAAS ratio of many fish species is 0.70 on average, whereas the ratio was reported to be 0.59 in crab *Portunus trituberculatus* and squid *Doryteuthis bleekeri*. The ratios of EAAS to NEAAS in *M. vollenhovenii* and *T. fuscatus* were 1.05 to 1.09 respectively [32]. While, in giant fresh prawn *M. rosenbergii* and green tiger shrimp *P. semisulcatus* were 0.65 and 0.61 respectively [1]. Thus, the different amino acids might be associated with varying tastes as well as textural properties of meat of studied species [1].

## 5. Conclusion

It is the first attempt to provide holistic amino acids analysis in edible muscles of commercially important crustaceans species which may in the future used as a specific health foods (functional supplements) and may play an important role in some pharmaceutical industries. The marine crustaceans were selected on the basis of their economic importance, abundance and use pattern in the study areas. The present study indicated the presence of high protein concentration in edible muscles of males mantis shrimps followed by both sexes of crab species then both sexes of shrimps. The elevation of protein percentage in the edible muscles in studied crustaceans samples also indicates their high nutritive quality. Furthermore, sixteen amino acids edible portions, have been determined in edible portions of studied crustaceans species. Among these, nine essential amino acids and seven non-essential amino acids were detected. The amino acids contents vary considerably within and between species as well as between sexes. The edible muscles of studied crustaceans are enriched in amino acids that in turn open the door to carry out further studies on the mode of action, characterization of the active components and the antioxidant properties of marine crustaceans which may in the future play an important role in some pharmaceutical industries and may be used as a specific health foods (functional supplements).

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