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QUALITATIVE AND QUANTITATIVE ANALYSIS OF THE SHELL WASTES OF A FRESH WATER CRAB, *OZIOTELPHUSA SENEX SENEX* AND ITS BIOCHEMICAL PROPERTIES

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ABSTRACT

Enormous amount of shell wastes are generated due to industrial processing of crabs and prawns. Natural means of shell degradation becomes slower due to large quantities of shell waste dumped in the lands or burning them cause major environmental issues. So it is essential and necessary to reduce, recycle and reuse these shell waste. The present study was conducted using the crab shell powder of two different sizes (0.2 mm and 0.4 mm) to verify the minerals, protein, lipid, chitin, chitosan, moisture and carotenoid content of male and female freshwater crab, *Oziotelphusa senex senex* by traditional chemical method slightly modified. The minerals and lipids was recorded to be high in female crab shell waste than that of male crab shell waste, whereas the proteins, chitin and chitosan was recorded to be highest in male crab shell waste than that of female crab shell waste of both the particle size. The moisture and carotenoid content was almost same for of all particle size of male and female crab shell powder. The statistical analysis (t-test) showed a significant difference between male and female crab shell waste. Some bioactive compounds present in this crude shell waste of a fresh water crab, *Oziotelphusa senex senex* had an antioxidant activity. The results of the present study confirmed that the crude male shell waste had highest antioxidant activity.

Key Words:- Chitin, Chitosan, Qualitative, Quantitative, Carotenoid, Antioxidant, *Oziotelphusa senex senex*.

INTRODUCTION

Environmental pollution is an innate outcome of anthropogenic activities that has a potential threat to human health to a greater height (Fereidoun *et.al.*, 2007). Industrialization and agriculture contributes extensive and various types of waste products, out of which industries contributes more waste that pollutes the environment (Sabahi *et al.,* 2009). The industries generate about 960 million tons wastes each year are in the form of organic chemicals, inorganic chemicals, primary iron and steel, plastics and resin manufacturing, stone, clay, glass and concrete, pulp and paper, food and kindred products (Gopakumar, 2002). Food processing industry generates huge amount of the waste that are biodegradable. These bio-wastes obtained from the sea food industries are either disposed in the open land or dumped in large quantities or incinerated (Arvanitoyannis *et al.*, 2007). All these process are of environmental concern because biodegradation becomes slower or it is cost effective. So it is essential to reduce, recycle and reuse these bio-wastes which increase the economy of the nation using clean technologies (Nair, 2004). Since the shell waste becomes a greater threat to the environment, it is of great interest to explore all essential components and isolate them and reuse it.

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Hence the present study was carried out on the following aspects:

1. To analyze the components present in the male and female crab shell waste of the fresh water crab, *Oziotelphusa senex senex* qualitatively and quantitatively.
2. To determine the antioxidant activity (DPPH Assay) of the male and female crab shell wastes.

MATERIALS AND METHODS

The shell wastes of male and female crab, *Oziotelphusa senex senex* were collected separately and washed thoroughly with dechlorinated tap water to remove other impurities. The shells were then dried in the oven at 70ºC for a period of 24 hours. The shells were then ground finely using a mixer grinder and the sieve size of 0.2 mm and 0.4 mm were used to obtain the respective shell powder sizes.

Qualitative analysis

The shell waste of the male and female crabs were taken separately for the qualitative analysis of minerals such as Carbonate, Sulphate, Phosphate and Calcium by standard protocol (AOAC, 1990), Proteins (Gornall *et.al.*, 1949), Lipids (Cheng *et.al.*, 2011), Chitin and Chitosan (Richard, 1951) and Carotenoid (Lopez-Cervantes, *et.al.*, 2006).

Quantitative analysis

The shell wastes of the male and female crabs were taken separately for the quantitative analysis using a standard protocol. The male and female crab shell wastes of different particle size were taken separately to determine the total minerals by gravimetrical method (AOAC, 1990). The estimation of total proteins for the male and female crab shell was determined by spectroscopic methods (Marion, 1976). The total lipid was analyzed spectroscopically by sulfo-phospho-vanillin (SPV) method using cholesterol as standard (Cheng *et.al.*, 2011). The chitin and chitosan present in male and female crab shell of various particle sizes were carried out using a standard protocol (traditional method) with slight modification (No *et.al.*, 1995).

The moisture content of the male and female crab shell powder of different size was determined by the gravimetric method. The moisture content was determined measuring the sample after and before drying (Black, 1965). The carotenoid present in them were estimated following a standard method using acetone (Lopez-Cervantes, *et.al.*, 2006). To determine the optimum percentage of total minerals, protein, lipid, chitin, chitosan, moisture and carotenoid of male and female crab shell waste, sets of experiments were conducted with 10 trials. The mean, standard deviation and t-test were carried out for statistical analysis.

DPPH activity assay

The DPPH free radical scavenging activity was measured for both male and female shell waste using standard method (Blois, 1958). During the assay, two ml of $6x10^{-5}$ M methanolic solution of DPPH were added to 50 µl of the samples. The mixture was incubated in dark place for 15 min at 25°C, after which the absorbance was recorded at 515 nm using UV spectrophotometer. The percentage of DPPH free radical scavenging activity was calculated using the formula:

% Scavenging Activity = $[(A_0-A_1/A_0)] \times 100$,

Where, A_0 is the absorbance of Blank and A_1 is the absorbance of Sample.

RESULT AND DISCUSSION

The qualitative analysis was recorded for both the male and female crab shells separately (Table 1). Almost all the parameters showed positive result for both male and female crab shells except sulphate. The parameters mentioned in qualitative analysis were quantified in both male and female crabs separately (Table 2). The quantitative analysis of minerals and lipids was recorded to be high in female crab shell than that of male crab shell, where as the proteins, chitin and chitosan was recorded to be highest in male crab shell than that of female crab shell waste of both particle size. The moisture and carotenoid content was almost same for both male and female crab shell powder of all sizes. The results of the antioxidant study showed 49.76 % and 43.32 % of free radical scavenging activity in male and female crab shell wastes respectively (Table 3).The statistical analysis (t-test) showed a significant difference in minerals and lipids between female and male crabs. The protein, chitin and chitosan of male crab shell powder of different sizes were significantly different from the female crab shell powder. The crude extract of the male shell waste had a significant difference in the antioxidant activity than that of female.

In general, minerals were found to be 30 to 50% in crustacean shell wastes, which are either in the form of calcium carbonate or phosphate (Johnson *et.al.*, 1978). To maintain the biological role of nerve transmission, muscle contraction, glandular secretion as well as mediating vascular contraction and vasodilatation calcium is highly essential (Straub, 2007). According to the present report the freshwater crab, *Oziotelphusa senex senex* shell waste that are free from heavy metal pollution can be considered as an appropriate dietary source of calcium.

Table 1. Qualitative analysis of fresh water crab, *Oziotelphusa senex senex* shell.

S.No.	Parameters	Male	Female
1.	Carbonate	+	+
2.	Sulphate	-	-
3.	Phosphate	+	+
4.	Calcium	+	+
5.	Proteins	+	+
6.	Lipids	+	+
7.	Chitin	+	+
8.	Chitosan	+	+
9.	Carotenoid	+	+

Table 2. Quantitative analysis of fresh water crab, *Oziotelphusa senex senex* shell.

S.No.	Parameters	Crab Shell Powder			
		Particle Size – 0.4 mm		Particle Size – 0.2 mm	
		Male	Female	Male	Female
1.	Minerals %	47.25±0.73	51.27±0.29*	45.52±0.26	49.14±0.38*
2.	Protein %	14.75±0.67*	12.09±0.69	14.17±0.62*	12.19±0.57
3.	Lipids %	1.78±0.23	2.32±0.58*	1.44±0.34	2.37±0.37*
4.	Chitin %	33.77±0.35*	32.12±0.33	36.27±0.41*	34.14±0.25
5.	Chitosan %	24.99±0.53*	21.64±0.86	28.75±0.44*	23.57±0.29
6.	Moisture %	28.67±0.49	28.44±0.49	28.67±0.49	28.44±0.49
7.	Carotenoid %	2.47±0.19	2.20±0.14	2.60±0.22	2.16±0.17

Each value represents the percentage Mean ± S.D on dry weight basis. *indicates the significant difference ($p \leq 0.05$) between male and female crab shell waste.

Table 3. Antioxidant Activity of fresh water crab shell *Oziotelphusa senex* shell.

S. No.	Sample	Mean Optical Density	DPPH %
1.	Male Crab Shell Waste	0.317 ± 0.002	49.76 ± 0.93*
2.	Female Crab Shell Waste	0.364 ± 0.005	43.32 ± 1.09
3.	Reagent (Blank)	0.631 ± 0.002	0

*indicates the significant difference ($p \leq 0.05$) between male and female crab shell waste.

DISCUSSION AND CONCLUSION

The lipids were found to be low but still it has essential fatty acid which has been reported in other crustaceans and plants that exhibited anti-inflammatory activity in mice (Fernandes *et al.*, 2007). The protein content was higher in the male crab shell waste than that of female. In comparison to the mud crab *Scylla serrata* the protein content was found to be slightly lower (Sujeetha *et al.*, 2015). The moisture content in the crab shell waste tends to promote microbial contamination and chemical degradation that is very much essential for the composting (Hussain *et al.*, 2009). The chitin content extracted from the fresh water crab shell waste was similar to the results obtained in shrimp waste (Ornum, 1992) and crawfish shell waste (Johnson *et al.*, 1978). According to species and seasons these proportion varies (Green *et al.*, 1984). The result of the present study showed that crab shell waste components such as chitosan, carotenoid and protein had proved itself to be as a good antioxidant in which the male crab shell waste was proved to be better than the female. A similar work of antioxidant activity was reported in marine crab *Scylla serrata* (Sujeetha *et al.*, 2015).

Synthetic antioxidants are used in active food packing because of their high stability, low cost and efficiency, there are significant concerns related to their toxicological aspects. Synthetic antioxidants are used in food industries under strict regulation that caused potential damage to health by such compounds (Chan *et al.*, 2007). Therefore, a preliminary research has been conducted to check the natural antioxidants alternative to synthetic antioxidants. Hence the present preliminary study of the fresh water crab shell waste gives us a better understanding of how to reduce, recycle and reuse these materials. Moreover it makes an ordinary person to adapt

the skill of extracting the important components from these waste adapting clean technologies making the world free from pollution.

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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

REFERENCES

AOAC. Official Methods of Analysis, 15th edition, Assoc. Official Analytical Chemist, Washington, D.C, 1990.

Arvanitoyannis IS and Kassavetti A. Fish Industry waste: treatments, environmental impacts, current and potential uses. *Int. J. Food Sci. Tech*, 2007, 1-17.

Black CA. "Methods of Soil Analysis: Part I Physical and mineralogical properties". American Society of Agronomy, Madison, Wisconsin, USA, 1965.

Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*, 29, 1958, 1199 1200.

Chan EWC, Lim YY, Omar M. Antioxidant and antibacterial activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular Malaysia. *Food Chem*. 104, 2007, 1586-1593.

Cheng YS, Zheng Y and Vander Gheynst JS. Rapid quantitative analysis of lipids using a colorimetric method in a microplate format. *Lipids*, 46(1), 2011, 95-103.

Fereidoun H, Nourddin MS, Rreza NA, Mohsen A, Ahmad R and Pouria H. The effect of long-term exposure to particulate pollution on the lung function of Teheranian and Zanjanian Students. *Pakistan Journal of Physiology*, 3(2), 2007, 1-5.

Fernandes ES, Passos GF, Medeiros R, Cunha FMD, Ferreira J, Campos MM, Pianowski LF, Calixto JB. Anti-inflammatory effects of compounds alpha-humulene and (−) trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *Eur. J. Pharmacol*, 569, 2007, 228–236.

Green JH and Kramer A. Food Processing Waste Management. p. 214. AVI publishing Co., Westport, CT, 1984.

Gopakumar K. Textbook of fish processing technology, Indian council of agricultural research, New Delhi, 2002, 467-483.

Gornall AG, Bardawill CJ and David MM. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem*, 177, 1949. 751-66.

Hussain K, Ismail Z, Sadikun A and Ibrahim P. Proximate and qualitative analysis of different parts of *Piper sarmentosum*, and quantification of total amides in various extracts. *Phcog. Res*, 1, 2009, 113–119.

Johnson EL and Peniston QP. The production of chitin and chitosan. In Proceedings of the First International Conference on Chitin/Chitosan; Muzzarelli, R.A.A. and Pariser. E.R. (Eds.), 1978, 80-87.

Lopez-Cervantes J, Sanchez-Machado DI, Gutierrez-Coronado MA and Ríos- Vazquez NJ. Quantification of astaxanthin in shrimp waste hydrolysate by HPLC. *Biomedical chromatography*, 20(10), 2006, 981–984.

Marion M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 1976, 248-254.

Nair KGR, Mathew PT, Joseph A, Martin Xavier and Anoop Krishna. Live stock feed from cephalopods processing waste In: Report of National Agricultural Technology project on Development of value added products and byproducts from low cost fish and processing waste from fish and shell fish, Central Institute of Fisheries Technology, Cochin, 2004.

No HK, Meyers SP. Preparation and characterization of chitin and chitosan-A Review. Journal of Aquatic Food Product Technology, 4(2), 1995, 27-52.

Ornum JV. Shrimp waste, Info-fish International, 6/92, 1992, 48-51.

Richards A Glenn. The integument of arthropods. Univ. Minnesota Press, Minneapolis, 1951.

Sabahi EA, Rahim SA, Zuhairi WYW, Nozaily FA, Alshaebi F. The Characteristics of leachate and groundwater pollution at municipal solid waste landfill of Ibb City, Yemen. *American Journal of Environmental Sciences*, 5(3), 2009, 256-266.

Straub DA. Calcium supplementation in clinical practice: A review of forms, Doses and indications. *Nutr. Clin. Pract*, 22, 2007, 286–296.

Sujeetha M, Sharmila S, Jayanthi J and Ragunathan MG. Quantitative and Qualitative analysis of chitin and chitosan from the shell of the mud crab, *Scylla serrata* (Forskal, 1775). *International Journal of Pharmacy & Therapeutics*, 6(2), 2015, 69-72.

Sujeetha M, Sharmila S, Jayanthi J and Ragunathan MG. Antioxidant property of some extract derived from the mud crab, *Scylla serrata*. *International Journal of Phytopharmacology*, 6(2), 2015, 111-113.