EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 6
197-200
Year: 2015

ANTIOXIDANT PROPERTY OF THE PIGMENT EXTRACTED FROM THE EDIBLE CRUSTACEAN SHELL WASTES

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Article Received on 04/09/2015 **Article Revised on 25/09/2015** **Article Accepted on 16/10/2015**

ABSTRACT
Due to the increased consumption of crustacean cuisines, the shell waste also increases. During recent days these shell wastes are used for chitosan extraction, which has variety of biological potentials. The crustacean shell contains pigments which also has biological significance (Chien., 1996). In this current research an attempt has been undertaken to extract the pigment from the crustacean shell wastes, to characterise and explore their biological potentials. The antioxidant property of the extracted pigment was compared to the standard Ascorbic acid by using 2, 2-Diphenyl, 1-Picryl Hydrazyl (DPPH) as the oxygen scavenging radical.

KEYWORDS: Astaxanthin, UV-Spectroscopy, Raman Spectroscopy, Antioxidant assay.

INTRODUCTION
For shrimp processing and freezing removal of head and body carapace is a normal process. It is estimated that that the generation of waste byproducts in the form of head and body carapace from the Indian sea food industry is around 100,000 tonnes. Through various types of research it has proved that these byproducts are good source of proteins (35 to 40 % of dry body weight), Chitin (10 to 15% dry body weight), minerals and natural carotenoids. At present these byproducts are used in small quantities to prepare as aquaculture and poultry feeds and for the production of chitin/chitosan as they have biomedical importance. A considerable quantity of these byproducts is being wasted resulting not only into loss of many valuable components but also results in environmental pollution (Gopakumar, 1993). Crustaceans such as shrimps, prawns, lobsters, krills and crabs contain astaxanthin as their carapace chromophores. The crustacean shell has pigments which is important for camouflage and to attract the opposite sex for reproduction (Torrison *et al*; 1989).

Among the groups of carotenoids, Astaxanthin is a proud member. Crustaceans and other aquatic animals are unable to produce astaxanthin by de novo, only plants and protists are capable of synthesis carotenoids. Therefore aquatic fauna accumulates these pigments through their diet by consuming phytoplanktons, which naturally synthesize or by consuming zooplanktons which has already accumulated astaxanthin (Steven *et al*., 1948). Carotenoid in crustaceans binds with proteins called as carotenoproteins and carotenolipoproteins. Astaxanthin appears as a red pigment, but when complexed with various crustacean proteins, the light absorbance shifts and causes crustaceans to range in colour from green, yellow, blue to brown (Britton *et al*., 1981).The pigment also plays a vital role in the generation of innate immune response. Crustacean waste could be the cheapest raw materials for carotenoid source (Hanif *et al*., 2012).

Many of the Crustacea, especially the smaller and more delicate forms, vary in colour according to the intensity of the illumination and according to their bioniche, to be red in darkness and yellow in light. This particular change is associated with change in the shape and colour of the contractile chromatophores. The colour-change is exceedingly difficult to understand unless there is an intimate relation between the red and yellow pigments (Newbigin, 1897).Astaxanthin is present in crustacean carapace and also in their flesh. The majority of carotenoids present in wild *P.mondon* are astaxanthin, astaxanthin esters as mono or di-ester and small amounts of β-carotene.

The antioxidant capacity of these chromophores is used in the treatment of several diseases including cancer, coronary heart diseases, inflammatory disorders, neurological degeneration and aging (Wollgast *et al*.,2000). It is now evident that the antioxidant potential

of carotenoids can significantly reduce the level free radicals and the oxidative load to help the body to maintain a healthy state. It has antioxidant activity 6000 times more than vitamin C. Carotenoids, especially astaxanthin, protect cells against oxidation by 1) quenching singlet oxygen and dissipating the energy as heat and 2) scavenging free radicals to prevent and terminate chain reactions (Bennedsen *et al*., 1999, Liu *et al*., 2003 and Ranga *et al*., 2010).

MATERIALS AND METHODOLOGY

Extraction of pigment

Six grams of each crustacean shell wastes of Banana prawn (*Fenneropeneaus. merguiensis*), Tiger prawn (*Peanaus monodon*), Rice field crab (*Oziotelphusa senex senex*), Three spotted crab (*Portunus sanguniolentus*), Sand lobster (*Theneaus unimaculatus*) was procured from local fish market in Kovalam, Chennai and was washed under running tap water and was completely dried in hot air oven at 60℃ for 2hrs.Then the shells were ground with hand blender until fine powder was obtained. 3gms of the fine powder was weighed and added to 10ml dark brown bottle and 5 ml of acetone was added and the test tube was incubated at dark condition for two days. By centrifugation at 2000 rpm the pellet and the supernatant was separated.

Pigment characterisation by UV Spectroscopy

The pigment was characterised by UV Spectroscopy. The spectrum scan was performed from 470 to 485nm and the peak was obtained at 480 nm, which confirms the presence of carotenoid. The concentration of the pigment was calculated by using the formula given below (Uma Nath *et al*.,2012).

$$\text{Where,AST } (\mu g/g) = \frac{AxDx10^6}{100xGx\ dxE^{1\%\text{-}1cm}}$$

Where: AST is concentration of astaxanthin, A is Absorbance, D is volume of the hexane extract (2ml), G is the weight of the sample in grams→3 gms, d is width= 1 cm, E = 2100

Characterisation of pigment by thin layer chromatography

The stationary phase is silica gel and the mobile phase is acetone (6ml): butanol (3ml): isopropanol (1ml): water (few drops). 10µl of pigments that were extracted from the various crustacean shell wastes were spotted on to the silica gel and after drying at room temperature for 5 minutes, the plate were transferred into TLC chamber, to which 5 ml mobile phase has been already added and the chamber has been saturated. Once the mobile phase has reached ¾ of the stationary phase and the mobile phase front was marked. The plates were placed in a dark chamber and left for air drying for 15 minutes and then the yellow coloured spots were marked and the R_f values was calculated and tabulated, by using the below mentioned formula,

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

Raman spectroscopic analysis

The specific advantage of Raman spectroscopy is that spectra can frequently be obtained from chromophores and the strong resonance enhancement allows one to observe selectively vibrational modes of a chromophore without interference of the nonresonant scattering of a complex material like biological material. Astaxanthin gives rise to a unique Raman spectrum that is characteristic of its molecular structure (Chiasa Uragami *et al*., 2012).Astaxanthin shows three strong Raman lines at 1508, 1145, and 993 cm–1. These three lines are ascribable to the C=C stretching, C-C stretching, and C-CH_3 in-plane rocking vibrational modes, respectively.

DPPH antioxidant assay

The effect of the antioxidant activity of the extracted astaxanthin was determined according to Sanchez-Moreno *et al*., 1998. The absorbance was recorded at λ = 515 nm. The antiradical value represents the percentage of radical scavenging ability according to the following formula

% scavenging effect =100- [(Abc – Abbs)x100 / Abc]
Where

Abbs – sample absorbance,
Abc – control sample absorbance.
In this current experiment Vitamin C was used as the standard.

RESULTS

The highest concentration of astaxanthin was extracted by acetone as solvent from Banana shrimp shell waste (2.15µg/ml). The extracted pigment was confirmed by UV-Spectroscopic analysis as astaxanthin, since the peak absorption was obtained at 480nm.The extracted pigment was eluted and analysed by thin layered chromatography. As the extracted pigment has a specific colour, Raman spectroscopic analysis records the pattern of bond stretching for these chromophores are unique. Thus in this current investigation the extracted pigment was analysed by Bruker FT-Raman spectroscopic analysis and the three bonding stretching was unique for C-C double bond, C-C single bond and C-CH_3 bonds, which are unique for astaxanthin as 1508, 11445,993 cm^{-1}respectively. Among all astaxanthin extracted from *F. merguiensis* shell waste is able to control the oxidation of DPPH than the standard Vitamin C (Table.3).

Table.1: Weight of Dry Crustacean Shell Waste.

S.No.	Samples	Weight of Shell Waste (gms)
1	*F. merguiensis*	12
2	*O.senex senex*	15
3	*P.indicus*	14
4	*P. sanguniolentus*	14
5.	*T. unimaclatus*	13

Table.2: Concentration of Astaxanthin Extracted From Each Of Crustacean Shell Waste

S.No.	Sample	Absorbance	Concentration µg/g
1.	*F. merguiensis*	0.68	2.15
2.	*O.senex senex*	0.05	0.15
3.	*P.indicus*	0.23	0.73
4.	*P. sanguniolentus*	0.34	0.56
5.	*T unimaclatus*	0.18	0.57

Table. 3: Tlc Analysis Of The Acetone Extract

S.No.	Sample	Solvent front, (cm)	Solute front, (cm)	R_f Value
1	*F. merguiensis*	5.5	2.5	0.42
2	*O.senex senex*	5.5	2.5	0.42
3.	*P.indicus*	5.5	2.0	0.44
4.	*P. sanguniolentus*	5.4	2.3	0.42
5.	*T. unimaculatus*	5.2	2.7	0.64

Raman Spectroscopic Analysis

D:\RAMAN DATA\EXTERNAL\EXT 2015\MARCH EX 2015\ 12MAR15S1\ *F. merguiensis* Laser 100 mW (srl=350) 19/03/2015

Wavenumber cm^{-1}: 4000, 3500, 3000, 2500, 2000, 1500, 1000, 500.

Raman Intensity: 0.02, 0.04, 0.06, 0.08, 0.10, 0.12.

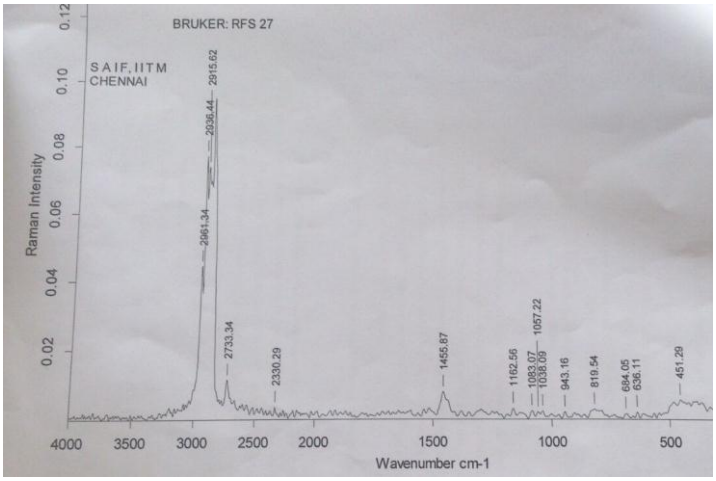

Table.4: DPPH – Antioxidant Activity

S.No.	Sample	Absorbance. 520nm	% of antioxidation
1.	$Standard_1$	0.10	
2.	$Standard_2$	0.12	
3.	$Standard_3$	0.15	
4.	$Standard_4$	0.18	
5.	$Standard_5$	0.22	
6.	*F. merguiensis*	0.24	-98%
7.	*O.senex senex* sample	0.17	-15%
8.	*P.indicus* sample	0.20	9%
9.	*P. sanguniolentus* sample	0.20	9%
10.	*T. unimaculatus* sample	0.15	00%

DISCUSSION

The concentration of total carotenoid content in crustaceans was found to vary in depending on species (Lambertson and Brakken., 1971). There are only minimal reports available on carotenoid content in crustaceans from tropical waters. Okada *et al*.,1994 analysed tiger prawn (*P.mondon*) of Indo-Pacific region and reported that the total carotenoid content of the exoskeleton ranges from 23-331µg/g and the from shrimp *Pandalus borealis* from Canadian waters, the total carotenoid content estimated as 30.9 to 35.8 µg/g (Guillou *et a*l ., 1995) and the total carotenoid content from Nowergian shrimp (*Phasiphaea sp*) extracted was estimated as 19.9 µg/g (Lambertsen and Braekkan., 1971). In this current study the astaxanthin from various crustacean shell wastes of *F. merguiensis*, *P. sanguniolentus*, *P.indicus*, *O.senex senex* and *T.unimaculatus* were extracted and the concentration of astaxanthin was determined by UV spectrum analysis (Table.1).

The type of pigment extracted from the five edible crustacean shell wastes by TLC and based upon the R_f value and presences of a single band in the chromatogram, confirms the pigment as astaxanthin. The characterisation of the extracted pigment by Raman spectroscopic analysis, which exhibited that the pigment extracted from the Banana shrimp carapace is astaxanthin. Raman spectroscopic analysis is strictly based upon the bond stretching phenomenon, the spectrum peaks are authentic for chromophore analysis. According to Eugenio *et al*., 2012, Vitamin C exhibited the highest antioxidant activity of DPPH. In this current research, results presented a significantly higher ability to scavenge the DPPH radical by Banana shrimp extracted astaxanthin when compared to the other shell extracts and standard Vitamin C.

ACKNOWLEDGEMENT

The authors would like to thank SAIF IIT-M for helping us with the Raman spectroscopic analysis of the extracted Astaxanthin.

REFERENCES

1. Bennedsen, M.; Wang, X.; Willen, R.; Wadstrom, T.; Andersen, L.P. Treatment of H. pylori infected mice with antioxidant astaxanthin reduces gastric inflammation, bacterial load and modulates cytokine release by splenocytes. Immunol. Lett. 1999; 70: 185–189.
2. Britton G; Armitt G, M; LauS,Y,M; Patel A,K and Shone C,C. Carotenoproteins, in carotenoid chemistry and biochemistry, Pergamon Press, Oxford, 1981; 237-251.
3. Chiasa Uragami, Eiji Yamashita, Andrew Gall, Bruno Robert and Hideki Hashimoto. Application of resonance Raman microscopy to in vivo carotenoid., 2012; 59(1): 53–56.
4. Eugenio José Garcia; Tatiane Luizacadorin Oldoni; Severino Matiasdealencar Alencar; Alessandra Reis; Alessandro Loguercio ;Rosa Helena Miranda Grande. Antioxidant Activity by DPPH Assay of Potential Solutions to be Applied on Bleached Teeth. Braz Dent J. 2012; 23(1): 22-27.
5. Gopakumar, K. Shrimp and crab shell utilization . Fishing chimes, 2012; 13:67-69.
6. Guillou,A and Khalil,M and Adambounou, L. Effects of silage preservation of astaxanthin forms and fatty acid profile of processed shrimp (Pandalus borealis) waste. Aquaculture,1995; 130: 351-360.
7. Hanif Houshmand Kouchi ,Bahareh Shabanpour , Marzieh Moosavi-Nasab.(2012). Extraction of carotenoids from crustacean waste with vegetable oils. The 11th International and the 4th National Congress on Recycling of Organic Waste in Agriculture 26 – 27 April 2012 in Isfahan, Iran.
8. Okada, S; Nur-E-Brohan, S, A and Yamaguchi,K. Carotenoid composition in the exoskeleton of commercial tiger prawns.Fish. Sci, 1994; 60: 213-215.
9. Lambertsen, B and Braekken, O, R. Methods of analysis of astaxanthin and its occurrence in some marine products. J.Sci food agri, 1971; 22: 99-101.
10. Liu,B,H and Lee, Y,K. Effect of total secondary carotenoids extracts from Chlorococcum sp. on Helicobacter pylori infected BALB/c mice. Int.Immunopharmacol. 2003; 3: 979-986.
11. Ranga Rao, A; Harshvardhan Reddy, A and Aradhya, S, M. Antibacterial properties of Spirulina platensis, Haematococcus pluvialis, Botryococcusbraunii micro algal extracts. Curr. Trends Biotechnol. Pharm. 2010; 4: 809–819.
12. Torrison, O, J; Hardy,R,W and Shearer, K,D. Pigmentation of salmonoids-carotenoids deposition and metabolism. Rev. Aquatic Sci, 1989; 1: 209-227.
13. Steven, D, M. Studies on animal carotenoids. I. Carotenoids of the brown trout (Salmotrutta Linn.) J.Exp. Biol. 1948; 25: 369.
14. Sanchez-Moreno, C; Larrauri, J,A and Saura-Calixto, F. A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric. 1998; 76: 270-276.
15. Newbigin, Pigments of crustacean, 21(4-5), J. Physiology: 237-257.
16. Wollgast, J and Anklam, E. Review on polyphenols in Theobroma cacao: changes in composition during the manufacture of chocolate and methodology for identification and quantification. Food Research International, 2000; 33: 423–447.
17. Uma Nath Ushakumari and Ravi Ramanujan. Astaxanthin from shrimp shell waste. Int J. Pharmaceutical Chemistry Research., 2012; 1(3):1-6.