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Process Yield, Proximate And Chemical Composition And Antioxidant Activity Of Astaxanthin From *Solenocera Crassicornis*

Venkatesh Kuncham and Kandra Prameela*

Department of Biotechnology, GITAM Institute of Technology, GITAM (Deemed to be University), Visakhapatnam - 530045, Andhra Pradesh, India

Abstract: Aquaculture is the major source of several organisms in Indian resources. Coastal aquaculture is rich in natural resources with several species. Shrimp is one of the major aquacultural food and is processed by separating meat from head and carapace. The head and carapace is treated as shrimp waste. Generation of huge amounts of waste and dumping into the environment is a real nuisance to the public since putrification starts immediately after dumping of the waste. Shrimp waste is rich of natural valuable compounds. *Solenocera crassicornis* is a very rare shrimp found at a 40m of sea depth . In the present study we attempted to know the process yield, proximate and chemical composition and antioxidant activity of astaxanthin extracted from *S.crassicornis*. The frozen shrimps were thawed, peeled manually by separating meat from head and carapace (waste). The waste was homogenized in a laboratory mixer. The material was vacuum packed in polyethylene bags and kept at -20°C until further use. The total antioxidant activity of extracted astaxanthin from *S. crassicornis* was determined by using FRAP method where the reducing property of ferric ions with extracted astaxanthin was determined by taking different concentrations (10 to 100 µg/ml). High amount of waste in the form of head (75%) and carapace (82%) was observed when compared to the meat. Astaxanthin content is high in head (73.5%) and carapace (65.2%) compared to the meat. The scavenging activity has shown higher DPPH activity similar to Quercetin (80%). The IC 50 values of 265 µg/ml was observed for astaxanthin extracted from *S. crassicornis.* Low chitin (0.9%) content and high protein (15.5%) content was observed in the meat part. These results indicate that significant (*P=0.05)* difference was found between different body components of *S.crassicornis.*

Keywords: *S. Crassicornis*, Process yield, chemical composition, antioxidant, Astaxanthin

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***Corresponding Author**

Kandra Prameela , Department of Biotechnology, GITAM Institute of Technology, GITAM (Deemed to be University), Visakhapatnam - 530045, Andhra Pradesh, India

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1. INTRODUCTION

The Indian aquatic food sector comprises nearly 20% of crustaceans like shrimp, prawns, lobsters, crab, krill. . In Indian water, nearly 85 shrimp species exist, however, few species have commercial importance in the national and international markets. The species that have commercial importance belong to the marine water shrimp Panaeid group, deep sea shrimp belong to *solenocera* group (*Solenoceracrassicornis*). The *penaeus* group shrimps were cultured in marine water and commonly *P. monodon* is called tiger shrimp and *P. vannamei* as white shrimp. According to MPEDA statistics in the EEZ (Indian Exclusive Economic Zone), estimated fishery potential for Panaeid shrimp is 1,78,000 tonnes. Penaeid shrimps *Penaeusmonodon* and *Penaeus vannamei* are two major marine species being cultivated in India. However, there seems an increasing shift in marine shrimp farming from *Penaeus monodon* to *Penaeus vannamei* in coastal districts of Andhra Pradesh, during 2010-15, *as P. vannamei* are more resistant to diseases, tolerant to high stocking densities and have shown high growth rate in low saline waters [1]. The production of *P. vannamei* for the year 2010-11 was 80,717 tonnes [2], whereas, by the end of the year 2014, production surged to as high as 375,000 tonnes, owing to 92% export growth record in quantity terms. *P. vannamei* was originally from the pacific coast of Mexico and was introduced to Asia in the year 1996. *P. vannamei* can grow at temperatures that are less than 20°C throughout the year in some areas and are known to live in tropical marine habitats. It is observed that *P. monodon* is highly prone to stress-induced diseases compared to *P. vannamei*, thus affecting shrimp farming. However, the stress conditions are actually leading to increased production of reactive oxygen species. Shrimp (*P. vannamei*) fed with dietary astaxanthin have shown good growth, a better survival rate in low salinity conditions [3] and also shown good pigmentation in their exoskeletons [4]. Deep sea shrimp species *Solenocera* (50 to 70m depth) which are also an important shrimp, harvested from the coastal regions of India. The annual estimated availability of these deep sea shrimp varieties is 3000 tonnes. Deep sea shrimp species are seasonal and are available in the market for few months only. However, the processing of these crustaceans involves the separation of meat from the head and exoskeleton. There are only a few reports on process yield and proximate composition of some species of shrimps[5,6]. However, there is a lack of information on process yield and proximate composition of deep sea shrimp species *Solenocera*. Therefore, the current study is aimed to know the process yield , proximate and chemical composition and antioxidant activity of astaxanthin (carotenoids) from *S. crassicornis*.

2. MATERIALS AND METHODS

Deep sea species *Solenocera crassicornis* was collected from harbor Visakhapatnam and sample was identified by Dr. Nenavath Rajendra Naik, Scientist, CIFT (Central Institute of Fisheries Technology), Visakhpatnam and transported to the laboratory under frozen conditions and kept at -20°C till further use.. All chemicals and reagents used for this study were of analytical grade.

2.1 *Preparation and Processing of S. crassicornis*

The frozen shrimps were thawed at room temperature. Shrimps were peeled manually (hand) by separating meat from head and carapace. The separated head and carapace was considered as shrimp waste. The material was homogenized in a laboratory mixer and the material was vacuum packed in polyethylene bags and kept at -20°C until further use.

2.2 *Proximate composition of S. crassicornis waste*

The mass and length of the *S. crassicornis* was analyzed by weighing and measuring individually. Peeled samples were weighed to calculate process yield. The moisture content was measured by drying the samples in an oven at 105°C [7]. Crude ash content was determined by incineration of shrimp waste in a muffle furnace at 550°C [8]. Total fat by soxhlet extraction, chitin was estimated as per the method of Spinelli et al., 1974 [9]. The total protein content was measured by micro-Kjeldahl method [10] and total carbohydrate content was measured by using Anthrone method [11].

2.3 *Determination of total carotenoids (Astaxanthin)*

Total carotenoid content was determined by using spectrophotometric method from shrimp waste [12]. To prepare a standard graph for astaxanthin, different concentrations of working standards were prepared from the stock solution of astaxanthin (1%) and solutions were diluted with solvent hexane to 10ml. Using hexane as blank the absorbance was detected and measured at 472nm. The experiment was repeated thrice, a standard graph for astaxanthin was constructed.

2.4 *Antioxidant activity of astaxanthin*

Antioxidant activity of astaxanthin was determined by spectrophotometric methods using double beam spectrophotometer (200-830nm. Different concentrations of methanol dissolved astaxanthin were prepared and were used for assay of antioxidant activity)[13].

2.5 *Estimation of ferric reducing activity of Astaxanthin*

The antioxidant activity of extracted astaxanthin from *S. crassicornis* was determined by using FRAP method where the reducing property of ferric ions with extracted astaxanthin was determined by taking different concentrations (10 to 100 µg/ml). 1ml of extracted astaxanthin extract was placed in 10mL volumetric flask and 2.5mL of potassium buffer (0.2M) and 1% potassium ferricyanide (0.4mL) was added [13].

2.6 *Determination of radical 1,1- diphenyl-2-picrylhydrazyl (DPPH) scavenging activity*

Scavenging activity against DPPH was measured for astaxanthin. The reaction mixture was prepared by taking 3.0ml of a methanol solution of DPPH with a concentration of 0.1mM and 1ml of astaxanthin was added and incubated at 37^0C and the procedure was followed with gallic acid as standard. The absorbance was measured at 517 nm using UV visible spectrophotometer by taking control where the astaxanthin extract was replaced by methanol. Quercetin was used as standard. From the below equation the percentage of inhibition was calculated.

chprameela5@gmail.com*

$$\text{Percentage of inhibition} = \frac{A_0 - A}{A} \times 100$$

Where A_0 and A were absorbance of control and sample,

3. STATISTICAL ANALYSIS

The experiments were carried with three replicates. The results were analysed and significant differences were represented by ANOVA and means separation was carried using STATISTICA software (2006).

4. RESULTS AND DISCUSSION

4.1 *Process yield*

Process yield and proximate composition of different varieties of shrimps is one of the criteria in shrimp processing industries as the individual species varies in its body parts and their composition. The results of process yield of different body parts of *S.crassicornis*was shown in Figure 1.

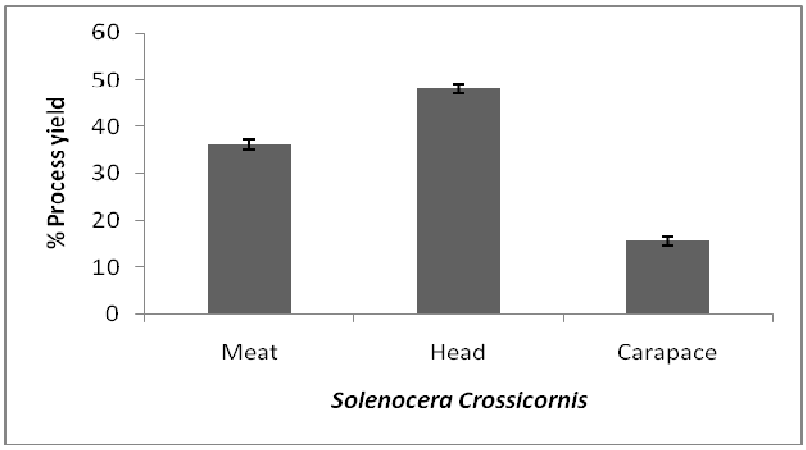

Fig 1. Process yield of body components of *S. crassicornis*

From the results, the process yield of *S. crassicornis* has high head content (48.2±1.20 %) compared to meat (36.3±0.73 %) and carapace (15.5±0.90 %). Highly significant difference was noticed between body components of *S. crassicornis*. From the results, it is clear that the highest quantity of head and carapace was observed in deep sea shrimp in the form of waste compared to meat.

4.2 *Proximate and chemical composition of S. crassicornis*

Analysis of proximate and chemical composition is critical in shrimp processing industries since the nutritional and storage stability depends on proximate and chemical composition of shrimps. The results of moisture content in body components of *S. crassicornis* are presented in Figure 2.

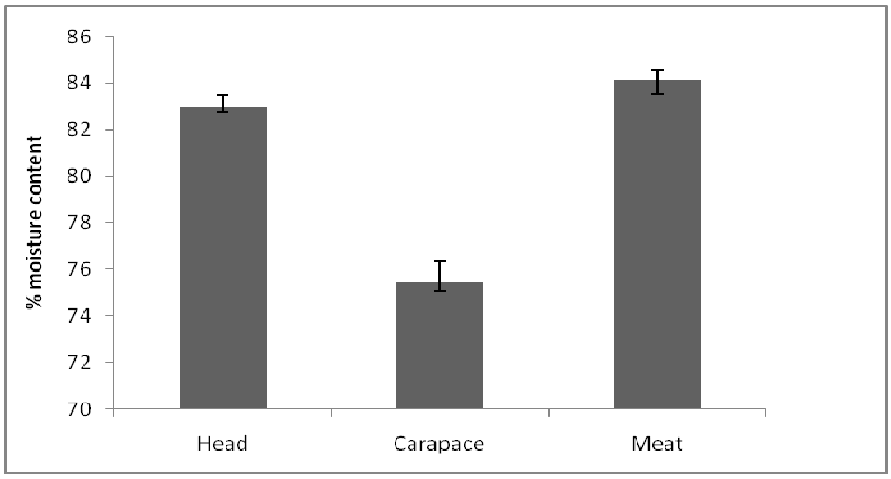

Fig 2. Total moisture content of body components of *S. crassicornis*

From Figure 2 it is clear that moisture content in head and carapace ranges from 75 to 82% and in meat was 84%. A significant difference ($p \leq 0.001$) was observed in different body parts of *S. crassicornis*. The results of ash content (Table 1) were noted in carapace 6.4% compared to head 3.2% and meat 0.8 %. A highly significant *(P≤0.001)* difference was observed in ash content between different body components. Similarly, the results of total protein, fat, and chitin were reported in Table 1. Different body components have different ranges of protein content. High protein content was

observed in meat (15.5 %) than the head (8. 9%) and carapace (8.6%). There was a significant difference *(P≤0.001)* in protein content between different body components.

Table 1. Proximate and chemical composition of different body components of *S.crassicornis.*

Description	Meat	Head	Carapace
Ash [a]	$0.80\pm0.2^{x,1}$	$3.82\pm0.20^{y,1}$	$6.4\pm0.43^{z,1}$
Total protein [a]	$15.5\pm0.3^{x,2}$	$8.9\pm0.5^{y,2}$	$8.6\pm0.1^{z,2}$
Fat content [a]	$2.2\pm0.01^{x,1}$	$3.1\pm0.1^{y,3}$	$0.3\pm0.2^{z,3}$
Chitin [a]	$0.90\pm0.2^{x,1}$	$4.2\pm0.20^{y,1}$	$7.4\pm0.43^{z,2}$

Different superscripts in individual columns (x,y,z) and in rows indicate (1,2,3,4) significant difference (p≤0.05)

From the results, it is observed that the fat content is high in the head (3.1%) than meat (2.2%) and carapace (0.3%). A Significant difference was noted in body components of different species of shrimp waste. Chitin content in the head of *S. crassicornis* ranges from 0.9% to 7.4% in different body components. The High content of chitin (7.4%) was observed in carapace compared to head (4.2%) and meat (0.9%). The results of astaxanthin (carotenoid) content were presented in Figure 3.

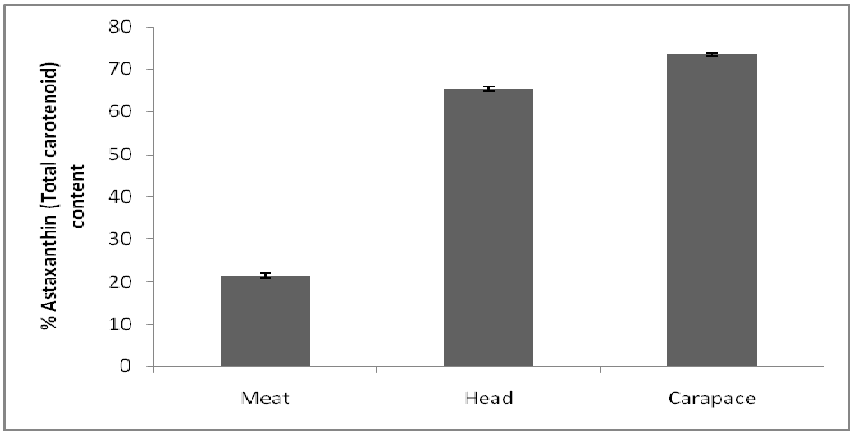

Fig 3. Total carotenoid (Astaxanthin) content of different body components of *S. crassicornis*

Astaxanthin content is high in carapace (73.5%) than the head (65.2 %) and meat (21.3%). A significant difference (*P ≤ 0.001)* was noted in between different species and between different body components of *S. crassicornis.*

4.3 *Antioxidant activity of astaxanthin*

Several oxidants are in the form of reactive oxygen species which are generally produced continuously in tissues and cause several degenerative diseases. The result of total antioxidant activity extracted astaxanthin from *S. crassicornis* was presented Figure 4.

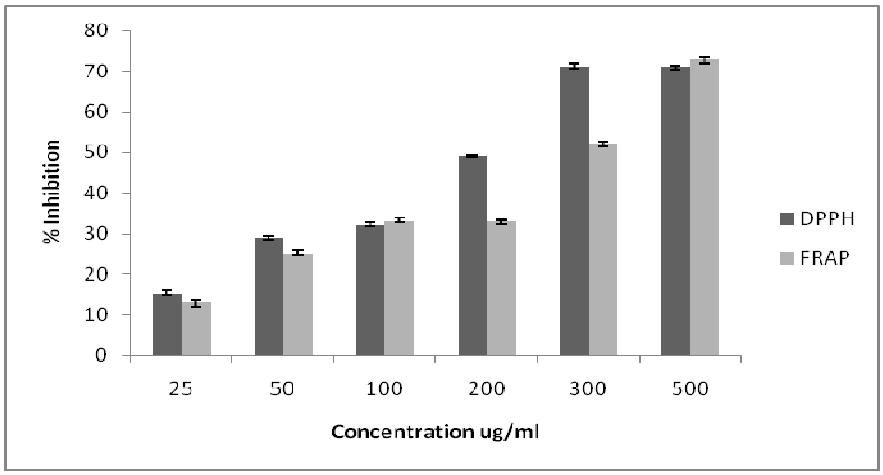

Fig 4. Total antioxidant (FRAP) and DPPH scavenging activity of astaxanthin extracted from *S. crassicornis*

The total antioxidant activity was compared by taking gallic acid as a standard. However, FRAP is reduced by the compounds that can donate hydrogen atoms to free radicals. Similarly, the scavenging activity has shown higher DPPH activity similar to Quercetin (80%). The IC 50 values of 265 µg/ml was observed for astaxanthin extracted from *S. crassicornis.* Analysis of the proximate and chemical composition is critical in shrimp processing industries since

the nutritional and storage stability depends on proximate and chemical composition of shrimps. One of the primary analysis of *S. crassicornis is* process yield. Gopakumar in 1993[5] has reported that the waste from Indian shrimp processing industries of Indian species was 40 to 50%. Another researcher reported that depending on varieties of species the generation of waste in the form of head and carapace varies from 40 to 80% [14]. The present results were similar to the previous reports in the literature since the difference in process yield is not only depends on species but also on the type of processing methodology [14]. One of the primary analyses of crustaceans is moisture content. Determination of moisture content is critical as it determines the stability of residue. Similar results were reported by Sachindra*et al.,* 2005 [15]. They observed the difference in the moisture content of marine, deep sea shrimp and fresh water prawn varieties. The present results will support the reported results [15]. However, results in variation of moisture and ash content of different body components were reported in marine *peanaeus*, fresh-water *macrobrachium* from Nigerian waters [16]. Few shrimp species have shown similar results in the proximate composition of different body components of shrimp [17]. Process yield is an important part of the shrimp processing industries. Ariyani and Buckle in 1991 [14] reported the moisture content to be 75 to 77%, ash content to be 4.5 to 6.9 %, 14.2 to 15.2 % of protein and 1 to 1.5 % of fat in shrimp species *Metapenaeus endeavor*. Similarly, Jeong*et al.,* in 1999 [18] reported protein content of Korean water shrimps ranges from 12.74 to 20.80%. The results of the present study and reported literature suggested that the proximate and chemical composition of different shrimps and their body components varies according to the size and species. Similar results in chitin content were reported in *metapenaeus spp*. 2.6 to 3.6% [14,15]. In general, the chitin content of crustaceans ranges from 20 to 50% [19]. High carotenoid (astaxanthin) was noted in carapace and head compared to meat. The Moderate content of astaxanthin was reported in penaeus species from tropical waters [20]. In 1999 Okada [21] *et al.,* 1994 reported 23 to 33µg/g of astaxanthin from exoskeleton of Indo pacific tiger prawn (*P. monodon*). Similarly, 30.9 to 35µg/g of astaxanthin were reported in *Pandalus borealis* from Canadian waters [22]. Among ROS hydroxyl radicals are the most reactive with shortest half life compared to other free radicals and Free radicals and formation of ROS due to several metabolic reactions in the body is a major cause and play a major role in causing several diseases. These free radicals are neutralized by natural antioxidants. Over production of free radicals or decrease in removal of reactive oxygen species results in oxidative stress and creates complications in the maintenance of proper health results in damage to tissues or organs. Even though, several studies have shown the antioxidant activity of astaxanthin from different sources. One of the studies with salmon has synergistic effect with vitamin E in *in-vitro* stimulation of lipid peroxidation [23]. Another study reported that antioxidant activity (IC_{50}) values were found to be 8.0 ug/ml with *Haematococcus pluvialis* astaxanthin esters [24]. In this study, similar antioxidant activity was noted with less ng/ml of astaxanthin. The increased antioxidant activity is due to high content of polyunsaturated fatty acids from marine shell waste compared to freshwater shrimp waste [25]. As there is increase in PUFA mono and di-esters of astaxanthin from different sources may increase the antioxidant activity due to synergistic effect of extract. Therefore, astaxanthin from natural sources which is having mono and di esters have more biological activity than synthetic astaxanthin.

5. CONCLUSION

Shrimp process yield is economically important to the processing industries in India and other countries. The process yield is not only significant in calculating weight loss on shelling procedures but also is significant in the generation of the enormous amount of residue as waste, which contains valuable products. Depending on the type of shelling, the process yield differs. Deep sea shrimp *S. crassicornis* have noted with high waste quantity in head and carapace (75% and 82%) and in meat was 84%. The Meat contained more protein (15.5 %) than the head (8. 9%) and carapace (8.6%). Chitin is high in carapace compared to head and meat. Deep sea shrimp *S. crassicornis* yielded high total astaxanthin content in head and carapace compared meat. High total antioxidant activity was observed in *S. crassicornis.* These results indicated that different species have from Indian waters differed in its proximate and chemical composition and significant differences were found between different body components and antioxidant activities. Further studies are needed on the stability of astaxanthin to use as colorants for commercial purposes in oil-based foods such as meat soups, cheese, ice cream, and butter.

6. AUTHORS CONTRIBUTION STATEMENT

Mr. Venkatesh Kuncham conceptualized and gathered the data with regards to this work. Dr. Kandra Prameela analyzed these data and necessary inputs were given towards the designing of the manuscript.

7. FUNDING ACKNOWLEDGEMENTS

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8. ACKNOWLEDGEMENTS

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9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. Briggs JM, Knapp AK, Blair JM, Heisler JL, Hoch GA, et al. An ecosystem in transition, woody plant expansion into Mesic grassland. Bio Science. 2005;55(3):243–54. DOI: 10.1641/0006-3568(2005)055[0243:AEITCA]2.0.CO;2
2. SEAI. 35th Annual Report of Seafood Exporters Association of India. 2011-12;1-15. Available from: http://seai.in/seaidatafiles/uploads/2013/01/35th-Annual-Report-.pdf
3. Vernon-Carter EJ, Ponce-Palafox JT, Pedroza-Islas R. Pigmentation of Pacific white shrimp (*Penaeus vannamei*) using Aztec marigold (*Tagetes erecta*) extracts as the carotenoid source. Arch Latinoam

Nutr. 1996;46(3):243-6. Available from: https://www.ncbi.nlm.nih.gov/pubmed/9429630
4. RenataTonhosolo, Fabio L D'Alexandrie, Veridiana V de Rosso, Marcos L Gazarini, Miriam Y Matsumura, et al. Carotenoid Biosynthesis in Intraerythrocytic Stages of Plasmodium falciparum. J Biol Chem. 2009;284(15):9974-85. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2665121/
5. Gopakumar K. Shrimp and crab shell utilization. Fishing Chimes. 1993;13(1):67-9.
6. Sachindra NM, Bhaskar N, Mahendrakar NS. Recovery of carotenoids from shrimp waste in organic solvents. Waste Manag. 2006;26(10):1092-8. Available from: https://www.ncbi.nlm.nih.gov/pubmed/16129592
7. AOAC. Official methods of analysis, 15th ed. Association of official analytical chemists, Washington. DC, USA.1995. Available from: https://law.resource.org/pub/us/cfr/ibr/002/aoac.methods.1.1990.pdf
8. AOAC Official Methods of Analysis, 18th ed. Association of Official Analytical Chemists. Gaithersburgs, MD 2006. Available from: https://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=1387682
9. Spinelli J, Lehman L, Wieg D. Composition, processing, and utilization of red crab (Pleuroncodesplanipes) as an aquacultural feed ingredient. J Fish Res Board Can. 1974;31(6):1025-9. DOI: 10.1139/f74-115
10. AOAC, Carotenes and Xanthophylls in Dried Plant Materials and Mixed Feeds: Spectrophotometric Method. AOAC Official Methods. 1974;970:64. Available from: http://www.sciepub.com/reference/185680
11. Morris DL. Quantitative determination of carbohydrate with Dreywood's anthrone reagent. Science. 1948;107(2775):254-5. DOI: 10.1126/science.107.2775.254.
12. Holanda HD, Netto FM. Recovery of components from shrimp (Xiphopenaeuskroyeri) processing waste by enzymatic hydrolysis. J Food Sci. 2006;71(5):298-303.
13. Lim YY, Murtijaya J. Antioxidant properties of Phyllanthusamarus extracts as affected by different drying methods. Food science and Technology. 2007;40:1664-9. Available from: https://www.researchgate.net/publication/223644056_Antioxidant_properties_of_Phyllanthus_amarus_extracts_as_affected_by_different_drying_methods
14. Ariyani F, Buckle KA. Ensilaging of prawn heads. Asean Food J. 1991;6:58-63. Available from: http://shodhganga.inflibnet.ac.in/jspui/bitstream/10603/126971/17/17_bibliography.pdf
15. Sachindra NM, Bhaskar N, Mahendrakar NS. Carotenoids in different body components of Indian Shrimps. J Sci Food Agric. 2005;85:167-72. Available from: http://lib3.dss.go.th/fulltext/Journal/J.Sci.Food%20and%20Agri/2005v85/no.1/2005v85no1p167-172.pdf
16. Balogun AM, Akegbejo-Samsons Y. Waste yield, proximate and mineral composition of shrimp resources of Nigeria's coastal waters. Bioresour Technol. 1992;40(2):157-61. DOI: 10.1016/0960-8524(92)90202-9
17. King I, Childs T, Dorsett C, Ostrander JG, Monsen ER. Shellfish: proximate composition, minerals, fatty acids, and sterols. J Am Diet Assoc. 1990;90(5):677–85. Available from: https://europepmc.org/article/med/2335682
18. Jeong BY, Choi BD, Moon SK, Lee JS, Jeong WG, Kim PH. Proximate composition and sterol contents of 35 species of marine invertebrates. Korean Journal of Fisheries and Aquatic Sciences. 1999;32:192– 197. Available from: http://www.koreascience.or.kr/article/JAKO199923607619163.page
19. Ornum JV. Shrimp waste- must it be wasted. Info Fish. 1992;6(92):48-52.
20. Lambertsen G, Braekkan OR. Methods of analysis of astaxanthin and its occurrence in some marine products. J Sci Food Agric. 1971;22:99–101. DOI: 10.1002/jsfa.2740220215
21. Okada S, Nur-E-Borhan SA, Yamaguchi K. Carotenoid composition in the exoskeleton of commercial black tiger prawns. Fish Sciences. 1994;60(2):213–5. DOI: 10.2331/fishsci.60.213
22. Guillou A, Khalil M, Bambounou L. Effects of silage preservation on astaxanthin forms and fatty acid profiles of processed shrimp (Pandalus borealis) waste. Aquaculture. 1995;130(4):351-60. DOI: 10.1016/0044-8486(94)00324-H
23. Bell JG, McEvoy J, Tocher, DR, Sargent JR. Depletion of alpha-tocopherol and astaxanthin in Atlantic salmon (Salmosalar) affects auto oxidative defense and fatty acid metabolism. J Nutr. 2000;130(7):1800-8. DOI: https://doi.org/10.1093/jn/130.7.1800
24. Kamath BS, Srikanta BM, Dharmesh SM, Sarada R, Ravishankar GA. Ulcer preventive and antioxidant properties of astaxanthin from Haematococcus pluvialis. Eur J Pharmacol. 2008;590(1-3):387-95. DOI: 10.1016/j.ejphar.2008.06.042
25. Albalat A, Nadler LE, Foo N, Dick JR, Watts AJ, Philp H, Neil DM, Monroig O. Lipid composition of oil extracted from wasted Norway lobster (*Nephrops norvegicus*) heads and comparison with oil extracted from Antarctic krill (*Euphasia superba*) Mar Drugs. 2016;14(12):219. DOI: 10.3390/md14120219.