Astaxanthin Extraction of Vanname Shrimp (*Litopenaeus vanname*) Using Palm Oil

R. Karnila, B. Hasan, M. Ilza, T. Leksono, M. A. Ahmad

1Lecturer at Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science Universitas Riau
2Student at Department of Fisheries Product Technology, Faculty of Fisheries and Marine Science Universitas Riau

*Corresponding author: karnilarahman@gmail.com

Abstract. Vanname shrimp is an important economic commodity that has carapace waste which is rich in the pigment astaxanthin. This study aims to determine (1) the chemical composition (proximate) of vanname shrimp carapace flour (2) the astaxanthin content of vanname shrimp carapace flour with different extraction times (3) Randemen of astaxanthin extract of vanname shrimp carapace flour. This research consisted of two stages, namely (1) Preparation and analysis of the chemical composition of vanname shrimp carapace flour (2) Extraction and analysis of the astaxanthin content of vanname shrimp carapace with different extraction times. Experimental research method with completely randomized design (CRD). The different extraction time treatments consisted of three tare, namely 120, 180, and 240 minutes. The data obtained were analyzed using Analysis of Variance (ANOVA). The results showed that carapace flour had a chemical composition of moisture content of 10.28%, ash content of 33.73% db, protein content of 38.82%db, fat content of 1.56%db, crude fiber of 25.9%db. Meanwhile, the highest astaxanthin content from this study was 5.0909 mg / g (240 minutes), the resulting levels were 2.6351 mg / g (120 minutes), 3.2569 mg / g (180 minutes) and 5.0909 mg / g (240 minutes), respectively. The yield of astaxanthin extract produced were 50.95% (120 minutes), 65.36% (180 minutes), and 78.92% (240 minutes), respectively. The highest yield of astaxanthin extract in the study was in the 240 minute treatment of 78.92%.

I. Introduction

One of the shrimp commodities that have high economic value and become an export commodity to various countries is vanname shrimp (*Litopenaeus vanname*). Utilization of vanname shrimp usually produces a relatively large amount of by-product in the form of waste, ranging from 30-75% of the weight of the shrimp. One form of the by-product is carapace, which is still limited as a source of minerals, chitin and chitosan.

The results of research by [1] indicate that shrimp carapace contains carotenoid pigments, especially astaxanthin. This astaxanthin pigment has high antioxidant power compared to other well-known antioxidants such as vitamin E and β-carotene. Utilization of shrimp carapace as a source of astaxanthin can not only provide added value but can also reduce environmental impact. In addition, astaxanthin can also be used as a functional food, as a coloring agent for crustaceans and salmon, as cosmetics and used in the food industry as well as a source of antioxidants [2].

Recently, several methods have been developed to extract astaxanthin including using organic solvents [3, 15], with the help of proteolytic enzymes [4], using Super Critical Carbon Dioxide [5], and using oil [6, 14].

The method of astaxanthin extraction using vegetable oil is almost the same as the extraction method using organic solvents. However, the extraction process using vegetable oil is easier and less dangerous so that it can be applied directly to feed and food products [7].

The vegetable oil that is affordable and easy to find in Indonesia is palm oil. Indonesia is the largest producer of palm oil in the world, so the availability of palm oil is high.
The success of the astaxanthin extraction method using crude palm oil which has undergone a process is degumming influenced by temperature and time, especially to obtain carotenoids from caroteno-proteins. The heating process at different temperatures will affect the caroteno-protein releasing free carotenoids which will bind to the fatty acids contained on palm oil. The results showed that the optimal temperature for extracting astaxanthin from shrimp carapace using vegetable oil was 70°C [8].

Besides that, the extraction time also affects the formation of carotenoids. Increasing the extraction time can increase the amount of astaxanthin extracted from the shrimp carapace. A short extraction time will only extract astaxanthin from the surface of the shrimp shell particles, but for astaxanthin that comes from the inside of the shrimp shell particles it takes a longer time to be extracted out. Therefore it is necessary to conduct research to determine the appropriate extraction time using palm oil as the extracting material.

This study aims: 1) to determine the chemical composition (proximate) of vanname shrimp carapace flour, 2) the astaxanthin content of vanname shrimp carapace flour with different extraction times, and 3) Rendemen of astaxanthin extract of vanname shrimp carapace flour.

2. Research Methods
2.1 Materials and Instruments
The main material used in this research is vanname shrimp carapace which is obtained from traditional markets in Pekanbaru. Other ingredients are n-hexan, asta force and palm oil. The tools used include knives, cutting boards, blenders, analytical scales, filter paper, waterbath, centrifuge, and UV-Vis spectrophotometry.

2.2 Research Stages
The method of this study was experimental method into two steps, they were 1) Preparation and analysis of the chemical composition of vanname shrimp carapace flour and 2) Extraction and analysis of the astaxanthin content of vanname shrimp carapace with different extraction times (120, 180, and 240 minutes). The research design used a completely randomized design (CRD).

2.2.1 Preparation and analysis chemical composition (proximate) of carapace flour vanname shrimp.
Making vanname shrimp carapace flour used the [9] method with a few modifications. The vanname shrimp carapace is washed using clean water and sun-dried to dry. Furthermore, the size reduction process is carried out using a dry blender with a particle size of 80-100 mesh. Then the chemical composition (proximate) measurements are carried out including: protein, fat, ash, water, and carbohydrates. Protein analysis used the Kjeldahl method, fat using the Soxhlet method, ash [10], moisture, and Crude Fiber (by difference).

2.2.2 Extraction and analysis of the astaxanthin content of vanname shrimp carapace with different extraction times. Extraction of astaxanthin (Modified by [8]).
Astaxanthin extraction used the [8] with slight modifications. A sample of 50g of vanname shrimp carapace was mixed with palm oil in a ratio (1: 2 w / v), heated using a waterbath at a temperature of 70°C with different lengths of time (120 minutes, 180 minutes, and 240 minutes). Furthermore, it is filtered and the filtrate is agitated using a centrifuge at a speed of 4500 rpm for 10 minutes. The agitation process will produce two phases of the filtrate, namely the orange pigmented oil phase and the shrimp carapace phase. The orange pigmented oil phase was taken to identify the amount of astaxanthin and its yield.

2.3 Data Analysis
The collected data from each treatment were analyzed using analysis of variance (ANOVA).
3. Results and Discussion

3.1 Chemical composition (proximate) of vanname shrimp carapace flour

The results of the chemical composition analysis (proximate) of vanname shrimp carapace flour are presented in Table 1.

Table 1. The average chemical composition (proximate) of vanname shrimp carapace flour

| Parameter      | Amount (%) |
|----------------|------------|
| Moisture       | 10.28      |
| Ash (%db)      | 33.72      |
| Protein (%db)  | 38.82      |
| Fat (%db)      | 1.56       |
| Crude fiber (%db) | 25.90     |

The results showed that the ash content in vanname shrimp carapace flour was quite high, namely 33.72%db, this was due to the fact that most carapace contains quite high minerals. Protein content is only 38.82% db, fat 1.56% db. The protein contained in vanname shrimp is quite high which consists of amino acids. This condition shows that vanname shrimp has good nutritional value as food. Protein in the body can be in the form of food reserves, building blocks and regulatory substances [11]. Amino acids are useful in protein synthesis and muscle building. Protein cycles occur in cells, tissues or other body parts that involve the digestive tract [12].

Crude fiber 25.90% db, and moisture content 10.28%. In general, the chemical composition of vanname shrimp carapace is influenced by two factors, namely endogenous and exogenous factors. Endogenous factors that affect the chemical content of shrimp include genetic factors, gender, size, level and maturity of the gonads. While the exogenous factors that influence are salinity, temperature, season, availability of food and habitat.

3.2 Astaxanthin content of vanname shrimp carapace flour

Measurement of astaxanthin levels was carried out to determine the levels of astaxanthin extracted from vanname shrimp carapace using palm oil solvent with a UV-Vis spectrophotometer. Measurements were made with a wavelength of 482 nm [8]. Based on the results of research with different extraction times, the average astaxanthin content was obtained as shown in Figure 1 below:

Figure 1. The Average Astaxanthin Content Of Vanname Shrimp Carapace

Based on Figure 1, the analysis of variance (appendix 4) shows that the extraction time has a significant effect on the astaxanthin levels obtained, which is based on the analysis of variance.
(ANOVA) shows that $F_{\text{calculated}} (25.42 > F_{\text{table}} (5.14)$ in the confidence level is 99%, then $H_0$ is rejected and to see which treatment is different, it is continued with the honest real difference test (BNJ). The results of the further test of the astaxanthin levels in the extract showed that the treatment time of 120 minutes was not significantly different from the treatment of 180 minutes, but very different. The highest level of astaxanthin was obtained in the treatment duration of 240 minutes at 5.09 mg/g.

The difference in extraction time had an effect on the resulting astaxanthin content, because the longer the extraction time would affect the process of removing astaxanthin from the shrimp carapace. A brief extraction will only extract astaxanthin from the surface of the shrimp shell particles while the long extraction time can extract astaxanthin from the inside of the shrimp shell particles [8].

Based on Ashahedi's research (2010), the use of extraction time for 4 hours produces carotenoids (Astaxanthin) with high yields of 3.4747 μg / g, this is because the extraction process for 4 hours provides sufficient time for the solvent to penetrate the cell wall and attract out carotenoids (Astaxanthin), resulting in high yield carotenoids (Astaxanthin).

3.3 The yield of astaxanthin extraction

The yield is the ratio of the amount (quantity) of extract produced from the extraction. The yield calculation uses percent (%) units. The yield is obtained by calculating the total flour produced, then dividing the weight of fresh sea cucumber meat used multiplied by one hundred percent. The higher the yield value produced will be economically beneficial and indicates the value of the extract produced is more and more [13]. The average yield of Astaxanthin extract can be seen in Figure 2.

![Figure 2](image-url)  
**Figure 2.** The average yield of astaxanthin extract from carapace vanname shrimp

Based on the analysis of variance, it shows that the astaxanthin extract from the vanname shrimp carapace produced by the extraction time has a significant effect on the amount produced, where $F_{\text{count}} (3050.99 > F_{\text{table}} (10, 92)$ at the 99% confidence level, $H_0$ is rejected, then a further test is carried out with a further test of honest real difference (BNJ). The results of further test yield astaxanthin extract showed that the difference in each treatment had a significant effect on the extraction time (120 minutes, 180 minutes and 240 minutes). The highest yield was obtained in the treatment of extraction time of 240 minutes of 78.92.

The increased extraction time results in an increase in the yield value, as well as the increased extraction time will increase the ability of solvent penetration in the raw material. The solubility of the components in the material goes slowly with increasing time however, after reaching the optimal time the number of components extracted from the raw material has decreased. This is due to the limited number of components contained in the material and the solvent used has a limited ability to dissolve the existing material so that even though the extraction time is increased, the solute inside is not there.
4. Conclusions
Based on the results of the research that has been done, the following conclusions can be drawn.
1. Shrimp carapace flour has nutritional content, namely water content of 10.46% (bb), ash content of 32.32% (bk), protein 38.38% (bk), fat 1.95% (wk) and carbohydrates 27, 35(by different).
2. The highest concentration of astaxanthin extract from vanname shrimp carapace (L.vannamei) using palm oil was found at 240 minutes (5.0909 mg / g) and the lowest was at 120 minutes (2.6351 mg / g).
3. The highest yield of extract results was found in the treatment of 240 minutes amounting to 78.9233% and the lowest was in the 120th minute 50.9533%.

Suggestion
The suggestion that can be given to the results of this study is that it is necessary to test the antioxidant activity of astaxanthin extract using palm oil.

References
[1]  Cahu, T.B. S.D. Santos, A Mendes, C. R. Cordula, S.F. Chavante, L. B. Carvallo Jr, and R. S. Bezerra. 2012. Recovery of protein, chitin, carotenoids and glycosaminogycans from Pacific white shrimp (Litopenaeus vannamei) Processing waste.Process Biochemistry, 47(4):570-577.
[2]  Handayani, A. D. N. Indraswati, and S. Ismadji. 2008. Extraction of astaxanthin from giant tiger (Penaus Monodon) shrimp waste using palm oil: studies of extraction kinetics and thermodynamic. Biosource Technology, 99 (10): 4414-4419.
[3]  Britton, G., Liansen-Jensen, S. and Pfander, H. 1995.Isolation and analysis. Carotenoids 1A: 203-206, ed. Bir-khauser, Basel. Switzerland
[4]  Lee SH, Roh SK, Park KH, Yoon KR. 1999. Efective extraction of Astaxanthin pigment from shrimp using proteolityc enzymes. Biotech Bio Eng. 4: 199-204.
[5]  Meyers, S.P., and Bligh, D. 1981. Characterization of astaxanthin pigments from heat processed crawfish waste. J. Agric. Food Chem, 3:505–508.
[6]  Chen, H. M. and S. P. Meyers. 1982. Extraction of astaxanthin pigment from crawfish waste using a soy oil Proccess. Journal of Food Science, 47(3): 892-896.
[7]  Sachindra, N. M. N. Bhaskar, G.S. Siddegowda, A.D. Sathisha, P.V.Suresh. 2007. Recovery of carotenoids from ensilaged shrip waste. Biosource Technology, 98(8): 1642-1646.
[8]  Handayani, A. D. 2007. Ekstraksi Karotenoid dari limbah kulit udang.Skripsi.Universsitas Katolik Widya Mandala.Surabaya.76 hal.
[9]  Istifa, R. 2010. Recovery dan Karakteristik kalsium dari limbah demineralisasi Kulit Udang Jerbung (Penaeus Merguinensis)[Skripsi]. Jurusan Teknologi Hasil Perairan, Fakultas Perikanan dan Ilmu Kelautan, Insitut Pertanian Bogor.
[10] AOAC,2005. Official Methods of analysys. Asosation of Official Analytical Chemists 18th Edition,USA:AOAC International.
[11] Karnila, R. Made Astawan, Sukarno, dan Tutik Wresdiyati. 2011a. Karakteristik Konsentrat Protein Teripang Pasir (Holothuria scabra J.) Dengan Bahan Pengekstrak Aseton. Jurnal Perikanan dan Kelautan 16(1): 90-102.
[12] Karnila R. 2012. Daya hipoglikemik hidrolisat,konsentrat,isolat protein teripang pasir (Holothuria scabra J) pada tikus percoabaan[disertasi]. Bogor: Institut Pertanian Bogor
[13] ____________ 2011b. Analisis Kandungan Nutrisi Daging Dan Tepung Teripang Pasir (Holothuria scabra J.) Segar. Berkala Perikanan Terubuk, 39(2):51 – 60. Fakultas Perikanan dan Ilmu Kelautan, Universitas Riau
[14] Sachindra, N.M. N. Bhaskar, and N. S. Mahendrakar. 2005. Process optimization for extraction of carotenoids from shrimp waste with vegetable oils. Biosource Technology, 96 (10): 1195-1200.

[15] Passos, R. L. Beirao, A. Palavia, R. Mendes, B. Nobre, dan L. Gouvela. 2006. Astaxanthin from the yeast phaffia rhodozyma Supercritical Carbon Dioxide and Organic Solvent Extraction. Journal of Food Technology, 4 (1): 59-63