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The Comparison Survey Antioxidant Power and Content of Extracted Astaxanthin from Shrimp Waste with Acid, Alkaline and Enzymatic Methods

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Abstract: Shrimp waste is one of the important sources of natural carotenoids. In This study describes pigments and carotenoid extraction from shrimp wastes under different methods. And, evaluated protective effects of carotenoids on lipid peroxidation of sunflower oil. Level of total carotenoids by alkaline extraction method was significantly ($P < 0.05$) higher than other methods. Also, the highest inhibition of lipid peroxidation was obtained in alkaline extraction method. These results demonstrated that good yields and antioxidant activity can be obtained alkaline treatment.

Key words: Astaxanthin · Shrimp Waste · Extraction Methods

INTRODUCTION

Global production of fish and shrimp increased significantly in the recent years. Much of the increase in later years is attributable to shrimp farming. Asia plays a leading role in shrimp farming, accounting for almost 80% of world shrimp production [1-3]. According to the Iranian Fisheries Organization, the shrimp farming was about 13,000 tons in the year 2013-14 in Iran.

About 48-56% of shrimp total body weight is waste [4]. Shrimp waste is the main byproduct in fishery industries [3]. Use of shrimp wastes has been of interest to researchers for two reasons: The first these wastes are environmental contaminants. Second these are the important sources of natural carotenoids [5]. Therefore reuse of this waste will perform the prevention of environmental pollution and can be used to produce useful compounds.

The major carotenoids in the wastes of shrimps are astaxanthin, Zeaxanthin, lutein and beta-carotene [4]. Astaxanthin, the main carotenoid found in shrimp, it is responsible for the color and antioxidant activity [1]. Its antioxidant activity was reported to be higher than that of carotene and lutein and is higher than tocopherol against certain reactive oxygen species [3].

Lipid oxidation are major deterioration causes of the quality of seafood and can decreases consumer acceptability of foods by causing undesirable changes in flavour, texture, appearance and nutritional quality [6].

Since the synthetic antioxidants have possible health effects and toxicity, searching natural preservatives for replacing has been attracted by food industries [7].

Carotenoids are one of major component of the crustaceans waste [8]. One of the important characteristics of carotenoids is their ability to act as antioxidants. Astaxanthin (3, 30-dihydroxy-b, b-carotene-4, 40-dione) is one of the most important pigments that its antioxidant properties have been reported in many studies. Owing to its outstanding antioxidant activity and attractive pink colour can be used as a colourant and antioxidant in food and medicine[9, 10]

Carotenoid has been extracted various method. The recovery and extraction of these valuable components from the shrimp waste would improve the economics of the shrimp farming process [4]. As well as the use of carotenoids as antioxidant in the food industry is affordable.

The present study was undertaken to find out the antioxidant activity in the composition of carotenoid pigments during Acidic, alkaline and enzymatic techniques extraction from shrimp wastes.

Incidentally, carotenoid ability to prevent the oxidation of sunflower oil was studied.

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MATERIALS AND METHODS

In this study, first the carotenoid extraction during enzymatic, alkaline and acidic methods, then its antioxidant effects on sunflower oil was investigated by spectrophotometry.

Collection of Samples: The shrimp wastes were obtained from the processing plants. Then, the wastes were air dried in the shade and turned into powder and stored at -20°C until use.

Fractionation of Carotenoids: At first, the samples dissolved in 0.1 N HCL for 24h at room temperature for demineralization treatment. In order to extract carotenoids,three methods were used.

Alcalase Deproteinization: Approximately 0.5 g of samples were solved in 20 mL of the sodium hydroxide (1 N) at 90°C for 2h. Then the pH being maintained at 8.5 by the addition of 1 N NaOH. The insoluble fraction was centrifuged and the supernatant was used for experiments [11, 12].

Enzymatic Deproteinization: In this method 10% of trypsin was added to samples. pH was adjusted to 8 for enzyme activity and heated at 37 °C for 4 h. In this step, the carotenoid-protein compounds are removed from wastes. Then, the solution was centrifuged and the supernatant was used for determination of total carotenoids [1, 3].

Acidic Deproteinization: In this method the wastes were solved in 20% Trichloroacetic acid (TCA (for 24h.

Determination of Total Carotenoids: The total amount of carotenoids were determined by b carotene standard curve and by spectrophotometric method at 470 nm. The total carotenoid content of the samples was calculated on the basis of the standard curve of β carotene [13].

Inhibition of Lipid Peroxidation: Inhibition of lipid peroxidation was evaluated by oxidation inhibition of sunflower oil. At first, 15 tubes were selected and in 5 rows were named (A-B-C-D-E). Then 1 ml of sunflower oil, 1 ml lecithin and 0.2 CuSo4 added to all tubes. And were shaken for 24 hours. The solutions mixed with 20 % trichloroacetic acid. The samples were centrifuged. Thiobarbituric acid was added to the supernatant and the samples were heated at 90°C for 15 min. The absorbance of the supernatants was measured at 532 nm.All of experiments were performed in triplicate [13].

Statistical Analysis: The results were evaluated by the analysis of variance and the differences between the means by Tukey's test using the SPSS Statistics ver23.

RESULTS AND DISCUSSION

Table 1 shows the amount of carotenoids extracted from shrimp wastes by Acidic, alkaline and enzymatic methods. The concentration of carotenoid pigment in the extracts was calculated using the standard curve obtained by commercial β carotene. $Y= 6.3994x + 0.0096$, $R^2= 0.9998$

Amount of total carotenoid by alkaline extraction method was significantly ($P < 0.05$) higher than other methods. Accordingly, it can be introduced as an applicable method for carotenoid extraction. Alkaline extraction of shrimp waste could be a potential source for pigments. The recovery of carotenoids extraction from the waste would improve the economics of the shrimp processing plant. The use of organic solvents are not safe extraction methods Sachindra *et al.* [4]. Our study showed that alkaline extraction was more effective as compared with other methods and the extraction of pigment from the shrimp waste could be efficiently and economically achieved by this method Jeddi *et al.* [8].

The protective effects of extracted carotenoids from shrimp wastes on lipid peroxidation of sunflower oil is displayed in Table 2. The highest inhibition of lipid peroxidation was obtained when carotenoids were extracted using alkaline treatment. The high inhibition of lipid peroxidation in alkaline treatment method could be due to carotenoid content and its alkaline conditions [14]. Therefore, the carotenoids separated from the protein complex. Results similar to our data have also been reported. Studies on Extraction of shrimp waste pigments by enzymatic and alkaline treatment demonstrated that the carotenoids extracted from wastes inhibited lipid peroxidation through enzymatic hydrolysis [5, 12]. Therefore, in alkaline condition enzymatic hydrolysis done much better.

Among the carotenoids, astaxanthin as an antioxidant is more effective than β-carotene and other carotenoids to destroy free radicals.Astaxanthin extraction techniques from crustacean wastes have include fermentation, enzymes, organic solvents (Such as acetone, methanol, alcohol), hydrochloric acid and edible oils [15].

Table 1: Level of total carotenoid

Extraction Method	Carotenoid Content (ppm)
Carotenoid extracted by alkaline treatment	0.07±0.001
Carotenoid extracted by enzyme	0.028±0.001
Carotenoid extracted by acid	0.005±0.002

Values are presented as Mean ± SD

Table 2: Level of lipid peroxidation

Samples	Level of lipid peroxidation (µM MDA/g sample)
Control (with no carotenoids extracted and synthetic carotenoids)	8.46±0.6[a,b]
Synthetic carotenoids + Sunflower oil	3.9±0.4[a]
Acidic carotenoid + Sunflower oil	6.9±0.2
Alkaline carotenoid + Sunflower oil	5.06±0.3[b]
Enzymatic carotenoid + Sunflower oil	5.51±0.19

Values are presented as Mean ± SD

[a,b]Means within a column with differ significantly ($P<0.05$)

In our study, alkaline extraction method was more effective than other methods to achieve carotenoids from shrimp waste.

CONCLUSION

Shrimp wastes are an important source of carotenoids, particularly that of astaxanthin and its esters. The crude carotenoids extract obtained by enzymatic, acidic and alkaline extraction of shrimp processing discards, and its fractions were evaluated for their antioxidant activity. The results revealed the strong antioxidant activity of the carotenoids obtained from alkaline extraction method. The antioxidant activity of shrimp carotenoid extract could be used for natural antioxidant in food and biomedical applications.

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