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NOTE

Extraction of shrimp waste pigments by enzymatic and alkaline treatment: evaluation by inhibition of lipid peroxidation

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Abstract In recent years, interest has grown in the potential to utilize more natural materials in the food industry. Shrimp waste is an important natural resource with functional properties and no known side effects. The major components of shrimp waste are protein, chitin, minerals and carotenoids. In the present study, the extraction of carotenoids was performed with two methods, the use of proteolytic enzymes and extraction by alkaline and enzyme treatment, and the total amount of carotenoids present in the waste was determined. Furthermore, the effectiveness of the method was evaluated through the inhibition of lipid peroxidation. Inhibition of lipid peroxidation was effectively done with carotenoid extracted by trypsin and alkaline treatment.

Keywords Shrimp waste · Carotenoid · Extraction methods

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Introduction

About 50 % of a shrimp's total body weight is comprised of waste products. Since these wastes are environmental contaminants, their utilization in innovative ways can prevent environmental degradation. Shrimp waste is a good natural source of raw materials such as protein, chitin, minerals, carotenoids (mostly astaxanthin and its esters), and shrimp flavor components [1, 2].

Astaxanthin, a keto oxycarotenoid, has been found widely throughout nature in many plants, algae, micro-organisms and animals. The main carotenoid found in shrimp, astaxanthin's antioxidant activity is reported to be higher than that of α-carotene, β-carotene, and lutein, and is higher than tocopherol against certain reactive oxygen species [3]. The occurrence of astaxanthin in shrimp is mainly due to the absorption of pigments from their diet, and it has been shown to be an effective pigment when incorporated into feeds for Salmonidae and crustaceans. Therefore, the extraction of shrimp wastes can be used as a source of coloring and flavoring agent in marine products [4]. Moreover, this carotenoid could also be used as a natural additive in the food industries. Because of concern over the safety of synthetic antioxidants in food industries, the search for natural antioxidants to replace the synthetics has been of great interest tothe food industry. The extracted carotenoids would also be a cheaper resource than synthetic carotenoids [1].

There are different techniques for the extraction of carotenoids such as fermentation, using enzymes and organic solvents. However, the use of organic solvents is not a safe extraction method, and there is a need to develop a more suitable method [2]. The aim of this study was to compare the bioactivity of carotenoids in shrimp wastes based on their extraction using the inhibition of lipid peroxidation method.

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Method

The test materials

The shrimp wastes, *Penaeus semisulcatus*, were collected from the processing plants. Then, the wastes were air-dried in the shade and turned into powder.

Extraction of carotenoids by trypsin

0.5 g of sample was placed in a test tube and dissolved in 20 mL of deionized water. Separation of carotenoids was done with trypsin. Five percent of trypsin was added to the waste and the hydrolysate was heated at 37 °C for 120 min. Then, the hydrolysate was centrifuged and the supernatant was used for experiments.

Extraction of carotenoids by trypsin and alkaline treatment

Approximately 0.5 g of samples were solved in 20 mL of the sodium hydroxide (1 N) for 48 h. Then the pH was adjusted back to 8 for enzyme activity. Five percent of trypsin was added to the waste and the solution was heated at 37 °C for 120 min. The solution was centrifuged and the supernatant was used for experiments.

Determination of total carotenoids

The total amount of carotenoids were determined by β 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 [5].

Inhibition of lipid peroxidation

The formation of thiobarbituric acid was measured for lipid peroxidation according to the previous method [6]. Inhibition of lipid peroxidation was evaluated by oxidation inhibition of lipids in egg yolk. Briefly, 1 mL of egg yolk was exposed to the hydrolysate samples and $CuSo_4$. $CuSo_4$ induced lipid peroxidation. The solution mixed with 20 % trichloroacetic acid. The samples were centrifuged. Thiobarbituric acid was added to the supernatant and the samples were heated. The absorbance of the supernatant was measured at 532 nm. Butylated hydroxytoluene (BHT) 0.02 and 0.01 % were selected as the positive controls. All of experiments were performed in triplicate.

Results and discussion

Determination of total carotenoids

The concentration of carotenoid pigment in the extracts was calculated using the standard curve obtained by commercial β carotene.

$$y = 3240.7x + 0.1129,\ R^2 = 0.9974$$

The majority of the pigments were removed through the extraction method using trypsin and alkaline treatment. The carotenoid pigment content in this group was 0.36 ± 0.02 (compared with the results of extraction of carotenoids using trypsin, 0.02 ± 0.007).

Inhibition of lipid peroxidation by carotenoids extracted by enzymatic hydrolysis

This study was used to survey the influence of extraction methods on the bioactivity of shrimp waste. The results of experiments are shown in Table 1. The results of this step in the present investigation indicated a low inhibition as compared with other groups. The inhibition of lipid peroxidation was significantly different betweenthe groups of carotenoids extracted using trypsin with BHT (0.01 %), BHT (0.02 %), and trypsin and alkaline ($P < 0.05$).

Inhibition of lipid peroxidation by carotenoids extracted with trypsin and alkaline treatment

The highest inhibition of lipid peroxidation was obtained when carotenoids were extracted using trypsin and alkaline treatment (Table 1). This inhibition was significantly greater than in the trypsin group. Therefore, this inhibition could be predicted based on the level of carotenoid content. Furthermore, the proteins denatured in alkaline conditions and were deposited at the bottom of the test tube. Therefore, the carotenoids separated from the protein complex.

Table 1 Level of lipid peroxidation

Component	Level of lipid peroxidation (absorbance unit)
Carotenoids extracted by trypsin	0.205 ± 0.057
Carotenoids extracted by trypsin and alkaline treatment	0.04 ± 0.005
BHT (0.02 %)	0.02 ± 0.01
BHT (0.01 %)	0.05 ± 0.001
Control (with no carotenoids extracted and BHT)	1.69 ± 0.21

The values of antioxidant activity were significantly different in carotenoids extracted with trypsin compared to alkaline treatment plus trypsin ($P < 0.05$)

Collectively, this study showed that trypsin plus alkaline extraction was effective as compared with trypsin. The extraction of pigment from the shrimp waste could be efficiently and economically achieved by this mixed method.

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