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The Comparative Survey between Extraction Methods for Determination of Bioactivity Level in Shrimp Wastes of *Penaeus semisulcatus*

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Abstract: In the last years, there has been growing interest in finding natural material for treatment and prevention of disease. Shrimp wastes are important natural sources with medical properties without side effects. They have potentially valuable components with different bioactivity. Several extraction methods can be used for obtaining biomaterial of shrimp wastes. In this study, the antioxidant and anti-inflammatory activity, soluble protein and carotenoids of shrimp wastes were examined in different extraction methods. These extractions included the use of ethanol, chloroform, sodium hydroxide and acetic acid. Our results indicated ethanolic extraction is suitable for antioxidants and soluble protein. The fat-phase of chloroform is an excellent of the total carotenoids, while anti-inflammatory activity in its water-phase is higher than other extractions.

Key words: Shrimp Waste · Bioactivity · Extraction Methods

INTRODUCTION

About 50% of shrimp total body weight is waste. These wastes are water and environmental contaminants. Contaminated water is not suitable for aquaculture [1]. Therefore, utilization of these wastes can prevent environmental contamination. There are many products that utilize organic compounds from shrimp waste [2]. Shrimp wastes are good natural source of raw materials such as protein, chitin, minerals, carotenoids, mostly astaxanthin and its esters and shrimp flavor components [3-6].

Astaxanthin, the main carotenoid found in shrimp, 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. Moreover, astaxanthin also has other important applications in the functional food and cosmetic industries [7].

The components in extract from the cephalothoraxes of shrimp are shown to be responsible for the radical scavenging properties and antioxidant activity [8]. They contain natural antioxidants, mainly phenolic compounds [9]. Therefore, this waste is an excellent source of antioxidant. Moreover, shrimp wastes can be applied as such for animal feeding because of its protein sources. The extracted protein would be a cheaper alternative for animal rations.

There are different techniques for extraction with using organic/inorganic acids, polar and non polar solvents. The level of biomaterial component depends on the processing of extraction. The aim of this study was to compare the bioactivity of shrimp wastes based on their extraction techniques. This experiment can be an effective preliminary screening tool for using an excellent extraction method in extracting biomaterial. In present study, antioxidant and anti-inflammatory activity, total carotenoid and total protein of shrimp wastes were analyzed in different extraction methods.

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MATERIALS AND METHODS

The Test Materials: The shrimp wastes, *Penaeus semisulcatus*, were collected from the processing plants. Then, the wastes were air dried in the shade and powdered. The extraction was prepared in four different methods. Approximately 0.5 g samples were solved in 25 ml of ethanol (96%), chloroform (50%), sodium hydroxide (50%) and acetic acid (50%). The chloroform extraction separated in two phases, upper layer (water-soluble phase) was saved for analyzing. The extracts was filtered and transferred to vials and kept at 4°C.

Chemicals: Neocuproine from Sigma Co and $CuCl_2$, NH_4Ac, Bovine Serum Albumin (BSA) were purchased from Merk Co.

Analysis of Antioxidant Activity: The CUPRAC assay was applied for analyzing total antioxidants capacity according to the previous method [10]. The extractions were incubated with 10^{-2} M $CuCl_2$ + 7.5×10^{-3} Neocuproine +1 M NH_4Ac. The absorbance of the supernatant was measured at 450 nm. Ascorbic acid was determined as the positive control.

Analysis of Anti-inflammatory Activity: The anti-inflammatory activity surveyed by inhibition Bovine Serum Albumin (BSA) denaturation. A solution of 2.5%w/v of BSA was prepared. The extractions were incubated with 2 ml of BSA. The test tubes were heated at 61°C for 10 min. The absorbance of the solutions was measured at 660 nm [11].

Estimation of Total Carotenoids: The absorbance of extraction was measured in a 1-cm-path-length quartz cuvette at 444 nm. The fat-soluble phase of chloroform was also done.

Measurement of Protein Content: The amount of protein in extractions was measured by Bradford method. Bovine serum albumin was determined as the standard.

RESULTS

All of experiments were performed in triplicate. The results of experiments are shown in Tables (1), (2) and (3). The antioxidant activity was analyzed with respect to optical density (OD) measured at particular wavelength with spectrophotometer. All of the extraction showed high level of activity more than vitamin C (0.005%) as positive control. The strong antioxidant activity was observed for ethanolic extraction. Moreover, this extraction indicated high level of total protein (Table1, 2).

Table 1: Level of total antioxidant activity

The extraction method	Total antioxidant
Ethanol	0.213±0.03
Chloroform Water-soluble	0.115±0.01
Acetic acid	0.123±0.03
Sodium hydroxide	0.177±0.001
Ascorbic acid (as positive control)	0.02±0.001

The values of antioxidant activity were significant difference in extract of Ethanol. Ascorbic acid is considered as control ($P<0.05$).

Table 2: Level of total protein and anti- inflammatory activity

The extraction method	Total protein (PPM)	The anti-inflammatory activity
Ethanol	8.75±0.51	0.332±0.22
Acetic Acid	6.8±1.5	0.045±0.004
Sodium hydroxide	7.15±0.02	0.196±0.009
Chloroform Water-soluble	4.54±0.19	0.003±0.001

Each value represents the mean ±SD per group. The values of total protein were significant difference in extract of ethanolic extraction.

Table 3: Level of total carotenoids was done in chloroform extraction in fat-soluble phase and water-soluble phase

The extraction method		Total carotenoids
Ethanol		0.361±0.034
Acetic Acid		0.316±0.002
Sodium hydroxide		0.529±0.002
Chloroform	Water-soluble	Fat-soluble
	0.232±0.051	0.774±0.012

The value of total carotenoids was significant difference in fat-soluble phase of chloroform ($P<0.05$).

Significant results were observed from anti-inflammatory activity assay for water-soluble phase of chloroform. Oppositely, its fat-soluble phase of chloroform exhibited high level of carotenoids (Table 2, 3).

DISCUSSION

This study was used to survey the influence of different extraction method on bioactivity of shrimp waste. The wastes of shrimp have complex mixtures biomaterials. Therefore, using suitable method should be considered in separating of specific component.

Results of *in vitro* antioxidant activity in present investigation indicated the high antioxidant potential of shrimp waste as compare with vitamin C. This difference in ethanolic extraction was high and this extraction has significantly been shown to exhibit a high antioxidant activity. The previous studies, the similar results had been approved and the ethanol extract from shrimp had considerable antioxidant activity. In other survey, polar solvents were also considered a good media for

antioxidant activity whereas non polar solvent were not recommended [12]. It is possible that free amino acids and peptides due to hydrolase's protein in ethanolic extraction have been found to exhibit antioxidant activity.

Carotenoids are a group of fat-soluble pigments distributed widely in nature in many plants, algae, micro-organisms and animals. Among aquatic animals, carotenoids are responsible for the color of many important fish and shellfish. The occurrence of carotenoids in shrimp is mainly due to the absorption of pigments from the diet. The role of carotenoids as a source of pigment and in immune defense system of cultures aquatic species has been established [13]. Lipids are reservoir of caratonoids, therefore the level of carotenoids in polar solvent will be low. In this experiment (Table 3), carotenoids in water-soluble phase of chloroform extraction was low. Oppositely, fat-soluble phase of chloroform extraction was rich in carotenoids and the highest carotenoid was obtained in this phase. The extracted carotenoids would be a cheaper alternative than synthetic carotenoids in aquaculture feed formulations [5].

The role of shrimp wastes as a source of protein in animal feeding has been approved [14]. In this study was demonstrated that ethanol extract from shrimp waste has considerable protein content. The amount of protein was followed by sodium hydroxide extraction (Table 2). Therefore, ethanolic and alkaline extraction of shrimp waste could be a potential source in animal nutrition.

The inflammation response is typified by redness, swelling, heat, pain and loss of function. This process involves in pathogenesis of some disease such as rheumatoid arthritis, atherosclerosis and asthma. The inflammatory activity is a complex process, involving a variety of proteins. The water soluble phase of chloroform was shown to possess strong inflammatory properties. It is possible that the water-soluble peptides and proteins are responsible for this activity.

Collectively, this study provides a useful extraction method for separating a specific bioactive component of shrimp wastes. We found that the nutritive and medical value bioactive components of shrimp wastes could be depended to their extraction method. The antioxidant activity and soluble protein content in ethanolic extraction were high. Therefore, each extract is suitable for isolation a specific active agent.

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