# Comparative Evaluation of Antioxidant Potentials of Chitosan Extracts Obtained via Diverse Extraction Techniques

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ABSTRACT

Chitosan is a natural polysaccharide that has antioxidant properties. It can consider as an antioxidant agent in food supplements. In this study the effect of extraction methods on the antioxidant activity of chitosan was investigated. Three methods including enzymatic, acid and alkaline methods were applied for isolating the chitosan from shrimp waste. The content of extracted chitosan was measured and its antioxidant activity was investigated by employing various analyses such as assay of their power on the oxidative stability in sunflower oil and DPPH. Inhibition of lipid per-oxidation was evaluated by thiobarbituric acid reactive substances assay. The extracted chitosan by enzymatic method had best antioxidant activities. Furthermore, the results demonstrated that good yields can be obtained via enzymatic treatment. According to the results, the content of chitosan influences on antioxidant activity. The chitosan extracted by enzymatic method can be a source of antioxidant agents in manufacturing food and supplement.

**Keywords**: Shrimp Waste, Extraction methods, Chitosan, Antioxidant Activity.

## INTRODUCTION

About 50 percent of total body weight of shrimp is waste which is produced as a byproduct of the shrimp industry. This waste can be used as a source for the extraction of chitosan. Chitosan, a D-glucosamine polymer, is a totally or partially deacetylated derivative of chitin. Chitin can be extracted from shrimp or crab shells and is second most abundant polysaccharides in the nature[1]. Chitosan as a natural polysaccharide which has antioxidant properties can be considered in the supplements. Several deproteinization treatments such as acidic, alkaline and enzymatic method were previously used, involving various reaction conditions. Chitosan extracted by these methods have some differences in the degree of deacetylation and its content[2].

The oxidative reactions of food lead undesirable features such as discoloration and rancidity. These reactions decrease nutritional value and quality of food. The food industry has to use antioxidant agents. An antioxidant is defined as any substance that significantly delays or inhibits oxidation. There are concerns about safety of synthetic antioxidants. Currently, synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) are used under strict regulations because of their toxic effects. More attention is being paid to natural antioxidants[3-6]. They are found in various

Antioxidant activity is one of the attractive features of chitosan. There is interest in using biodegradable active material in food packaging such as chitosan. This polysaccharide has a great potential for using in food industry[15]. In our previous study, we indicated that the antibacterial activity of chitosan depends on the methods involved in preparation of chitosan[16]. This paper has been designed with the objective of evaluating the effect of extraction method on the antioxidant activity of chitosan.

## MATERIALS AND 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 powdered.

*The experimental design*

Five grams of sample was placed in a test tube and dissolved in 1 N HCL for 24h at room temperature for demineralisation treatment. In deproteinization step, three methods were used. The residue was placed in 1 N NaOH, Trypsin and Trichloroacetic acid (TCA). In these step complex protein-carotenoids was separated. In enzymatic method; 10% of trypsin was added to waste. pH was adjusted to 8 for enzyme activity and heated at 37 °C for 4 h. Then, the hydrolysate was centrifuged and the supernatant was used for determination of total carotenoids. The remaining of this process is chitin. The chitin obtained from this process should change to chitosan. Chitin changes to chitosan in deacetylation process. In this process, the acetyl groups were removed from the chitin. For this

Table 1: Level of total chitosan at different procedures.

| Extraction method | Chitosan Content (mg/ml) |
|---|---|
| Chitosan extracted by Trypsin | 2.33±1.13 |
| Chitosan extracted by TCA | 1.85±2.1 |
| Chitosan extracted by alkaline treatment | 1.05±0.007 |

Table 2: Antioxidant activity of chitosan at different procedures.

| The kind of extraction method | DPPH (% IP) |
|---|---|
| Chitosan extracted by trypsin | 45.43±0.9 |
| Chitosan extracted by TCA | 14.69±0.01 |
| Chitosan extracted by alkaline treatment | 14.69±1.1 |

purpose, the residue is placed in 50% NaOH and boiled at 100 °C for 2 h[2].

*Determination of chitosan yield*

The chitosan obtained was assessed according to the previous method[17]. Briefly, a stock solution was prepared by dissolving 100 mg of chitosan in 100 ml of %1 aqueous acetic acid. The solutions are made from this stock solution. The solutions of chitosan were mixed with 0.5M NaNO2 reagent and the mixture was heated at 80C for 30 min. pH was adjusted to 8. Then, thiobarbituric acid was added and heated at 80C for 10 min in water bath. The absorbance of the supernatant was measured at 555 nm.

*Qualification determination of degree of deacetylation of chitosan yield*

Degree of deacetylation is one of the main parameters of chitosan. This parameter was assayed according to published method[18]. Chitosan (0.1 grams) was dissolved in 10 mL of %1 aqueous acetic acid. The solutions of chitosan were mixed with 200 ml of bromocresol. The free amino group has reactive binding site for bromocresol. The samples were measured at the 589 nm. *Radical DPPH Scavenging Activity*

Antioxidant activity was assayed according to the previous method[19]. A dose of 3.8 cc ethanol solution of DPPH radical (final concentration was 0.1 mM) and 0.2 cc extract (1% extract) were mixed (A sample). The respective extraction solvent was used as negative control (A control). The samples were shaken for 1 min and kept at room temperature in the dark for 30 min. Then, absorbance of them was read at 517 nm against ethanol blank. The percent of DPPH discolouration of the samples was calculated according to following formulas: % discolouration = [1 – (A sample/A control)] × 100 *Assay of chitosan power on the oxidative stability in sunflower oil*

The extract, copper sulfate (CuSo4), and sunflower oil (without antioxidant) were mixed and shaked for 3 hours in room temperature. The capacity of the chitosan to inhibit lipid peroxidation against CuSo4 was assayed. BHT used as positive control. The formation of thiobarbituric acid in samples was assessed according to an original method[20]. Briefly, the samples were mixed with 20% trichloroacetic acid and the mixture was centrifuged. Then, thiobarbituric acid was added to the supernatant and heated. The absorbance of the supernatant was measured at 532 nm. The values were expressed in nmoles malodialdehyde, using a molar extinction coefficient of $1.56 \times 10^5$ $M^{-1}$ $cm^{-1}$.

## RESULTS

Levels of chitosan and their antioxidant activity in different exaction methods are shown in tables 1, 2 and 3 (Table 1). In DPPH assay, antioxidant activity as percentage of inhibition (%IP) was calculated (Table 2). The *p* value between enzymatic group and other groups was significant ($p \leq 0.05$).

The level of lipid oxidation was significantly different between the enzymatic group compared to BHT ($p$ = 0.002) and the enzymatic group compared to alkaline ($p$ = 0.01) and TCA ($p$ = 0.007) (Table 3).

## DISCUSSION

This study evaluated the antioxidant activity of extracted chitosans through different extraction methods. Our results showed that there is a significant different in antioxidant activity of chitosans extracted by different extraction methods.

In a study, chitosan has been shown the beneficial effects on relieving the oxidative stress[21]. In the present study, the antioxidant activity was calculated. With the aim of evaluating the capacity of the chitosans to inhibit lipid peroxidation, an assay in sunflower oil oxidation system was performed in comparison with BHT as positive control. Chitosan extracted by enzymatic method significantly decreased MDA level in oil. The inhibition level of lipid peroxidation by chitosan extracted by enzymatic possessing was 2.4± 0.59 that it was lower than BHT. Actually our study indicated that chitosan extracted by enzymatic possessing has good antioxidant activity against lipid peroxidation. Peptides are remained from shrimp waste protein during extraction. Bioactive peptides are specific protein fragments that have effects on body function and health. They affect the cardiovascular, digestive, immune and nervous system[22]. The antioxidants are vital nutrient for healthy[23-27]. They have an important role on the prevention oxidative damages[28-31]. DPPH is widely used to assay the free radical scavenging effect of antioxidant. In present study, we also evaluate antioxidant activity of chitosan by DPPH assay. The results obtained from DPPH assay suggest that chitosan extracted by enzymatic method possess high radical scavenging ability. Actually, these results confirmed the antioxidant power of chitosan extracted by enzymatic method. The potential of chitosan extracted by enzymatic as antioxidant agents may be due Table 3: The inhibition of lipid peroxidation by chitosan in sunflower oil.

| Component | Level of lipid peroxidation (µmol/ml ) |
|---|---|
| Sunflower oil + Chitosan extracted by trypsin | 2.4± 0.59 |
| Sunflower oil + Chitosan extracted by TCA | 5.1± 1.1 |
| Sunflower oil + Chitosan extracted by alkaline treatment | 4.9± 1.1 |
| Sunflower oil + 2%BHT | 3.9± 0.05 |

to the presence of peptides result of enzyme reaction. The level of chitosan was significantly different in enzymatic method compared with other methods. It means that the content of chitosan can influence on the activity power. Furthermore, our previous study has found that chitosan extracted by enzymatic method has good solubility in water[2]. Therefore, it can have more biological activities.

## CONCLUSION

Collectively, the data obtained from this study exhibited good antioxidant potency in enzymatic method among the

three methods used for extraction of chitosan from shrimp waste.

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