

## Comparison of Antioxidant Activity of Chitosan Extracted Via Different Extraction Methods

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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<sup>1</sup>. 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<sup>2</sup>.

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<sup>3-6</sup>. They are found in various

natural recourses and can prevent from a lot disease in human<sup>7-14</sup>.

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<sup>15</sup>. In our previous study, we indicated that the antibacterial activity of chitosan depends on the methods involved in preparation of chitosan<sup>16</sup>. 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

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

°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 purpose, the residue is placed in 50% NaOH and boiled at 100 °C for 2 h<sup>2</sup>.

#### Determination of chitosan yield

The chitosan obtained was assessed according to the previous method<sup>17</sup>. 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 NaNO<sub>2</sub> 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<sup>18</sup>. 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<sup>19</sup>. 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 discoloration of the samples was calculated according to following formulas:

$$\% \text{ discoloration} = [1 - (\text{A sample}/\text{A control})] \times 100$$

#### Assay of chitosan power on the oxidative stability in sunflower oil

The extract, copper sulfate (CuSo<sub>4</sub>), and sunflower oil

(without antioxidant) were mixed and shaken for 3 hours in room temperature. The capacity of the chitosan to inhibit lipid peroxidation against CuSo<sub>4</sub> was assayed. BHT used as positive control. The formation of thiobarbituric acid in samples was assessed according to an original method<sup>20</sup>. 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  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

## 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<sup>21</sup>. 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  $2.4 \pm 0.59$  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<sup>22</sup>. The antioxidants are vital nutrient for healthy<sup>23-27</sup>. They have an important role on the prevention oxidative damages<sup>28-31</sup>. 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 ( $\mu\text{mol/ml}$ )
Sunflower oil + Chitosan extracted by trypsin	$2.4 \pm 0.59$
Sunflower oil + Chitosan extracted by TCA	$5.1 \pm 1.1$
Sunflower oil + Chitosan extracted by alkaline treatment	$4.9 \pm 1.1$
Sunflower oil + 2%BHT	$3.9 \pm 0.05$

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<sup>2</sup>. 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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### REFERENCE

- Pagnoncelli M, Araújo N, Silva N, Assis C, Rodrigues S, Macedo G. Chitosanase Production by *Paenibacillus ehimensis* and its application for Chitosan Hydrolysis. *Brazilian Archives of biology and Technology*. 2010; 53: 1461-1468.
- Sadighara P, Tohidi Moghadam H, Eskandari S, Salehi A. Optimization of extraction of chitosan and carotenoids from shrimp waste. *International Journal of Fisheries and Aquatic studies*. 2015; 2: 51-54.
- Khodadadi S, Rafieian-Kopaei M. Herbs, health and hazards; a nephrology viewpoint on current concepts and new trends. *Annals of Research in Antioxidants*. 2016; 1(1):e05.
- Rafieian-Kopaei M, Baradaran A. Plants antioxidants: From laboratory to clinic. *Journal of Nephropathology*. 2013; 2(2): 152-153.
- Nasri H. Herbal drugs and new concepts on its use. *Journal of Preventive Epidemiology*. 2016; 1(1):e01.
- Nasri H, Baradaran A, Shirzad H, Rafieian-Kopaei M. New concepts in nutraceuticals as alternative for pharmaceuticals. *International Journal of Preventive Medicine*. 2014; 5:1487-99.
- Samarghandian S, Asadi-Samani M, Farkhondeh T, Bahmani M. Assessment the effect of saffron ethanolic extract (*Crocus sativus* L.) on oxidative damages in aged male rat liver. *Der Pharmacia Letter*. 2016;8(3):283-90.
- Asadi-Samani M, Kooti W, Aslani E, Shirzad H. A systematic review of Iran's medicinal plants with anticancer effects. *Journal of Evidence-based Complementary & Alternative Medicine*. 2016; 21(2):143-53.
- Mahmoudian Sani M, Asadi-Samani M, Rouhi-Boroujeni H, Banitalebi-Dehkordi M. Phytopharmacology and phytotherapy of regulatory T cells: A new approach to treat multiple sclerosis. *Der Pharmacia Lettre*. 2016;8(3):215-20.
- Jivad N, Bahmani M, Asadi-Samani M. A review of the most important medicinal plants effective on wound healing on ethnobotany evidence of Iran. *Der Pharmacia Lettre*. 2016;8(2):353-7.
- Rouhi-Boroujeni H, Asadi-Samani M, Moradi MT. A review of the medicinal plants effective on headache based on the ethnobotanical documents of Iran. *Der Pharmacia Lettre*. 2016;8(3):37-42.
- Hajian S. Positive effect of antioxidants on immune system. *Immunopathology Persa*. 2015;1(1):e02.
- Baradaran A. Herbal antioxidant to ameliorate vascular biology. *Angiologica Persica Acta*. 2017;2(1):e01.
- Rafieian-Kopaei M. Medicinal plants for renal injury prevention. *Journal of Renal Injury Prevention*. 2013 Jun 1; 2(2):63-5.
- Talon E, Trifkovic KT, Nedovic VA, Bugarski BM, Vargas M, Chiralt A, González-Martínez C. Antioxidant edible films based on chitosan and starch containing polyphenols from thyme extracts. *Carbohydrate Polymers*. 2017; 157: 1153-1161.
- Sadighara P, Erfanmanesh A, Hagi E, Nikaein D, Mohajerfar T, Tohidmoghadam T, Bahmani M, Abaszadeh A, Rafieian-Kopaei M. The antibacterial activity of chitosan between different extraction methods. *Journal of Chemical and Pharmaceutical Sciences*. 2016; 9: 1325-1328.
- Badawy M. A new rapid and sensitive spectrophotometric method for determination of a biopolymer chitosan. *International Journal of Carbohydrate Chemistry*. 2012; 2012: 1-7.
- Abou Shoer M. A Simple Colorimetric Method for the Evaluation of Chitosan. *American Journal of Analytical Chemistry*. 2010; 2: 91-94.
- Upadhyay R, Chaurasia JK, Tiwari KN, Singh K. Antioxidant property of aerial parts and root of *phyllanthus fraternus* Webster, an important medicinal plant. *The Scientific World Journal*. 2014; 2014: 1-7.
- Sicinska P, Bukowska B, Michalowicz J, Duda W. Damage of cell membrane and ant-oxidative system in human erythrocytes incubated with microcystin-LR in vitro. *Toxicon*. 2006; 47: 387-397.
- Zhang J, Zhang H, Wang L, Guo X, Wang X, Yao H. Antioxidant activities of the rice endosperm protein hydrolysate: identification of the active peptide. *European Food Research and Technology*. 2009; 229: 709-719.

22. Korhonen H, Pihlanto A. Bioactive peptides: Production and functionality. *International Dairy Journal*. 2006; 16:945-960.
23. Heidarian E, Rafieian-Kopaei M. Protective effect of artichoke (*Cynara scolymus*) leaf extract against lead toxicity in rat. *Pharmaceutical Biology*. 2013;51(9):1104-9.
24. Pourianezhad F, Tahmasebi S, Abdusi V, Nikfar S, Mirhoseini M. Review on feverfew, a valuable medicinal plant. *Journal of Herbmed Pharmacology*. 2016;5(2):45-49.
25. Shirzad H, Taji F, Rafieian-Kopaei M. Correlation between antioxidant activity of garlic extracts and WEHI-164 fibrosarcoma tumor growth in BALB/c mice. *Journal of Medicinal Food*. 2011; 14(9):969-74.
26. Ghatreh-Samani M, Esmaili N, Soleimani M, Asadi-Samani M, Ghatreh-Samani K, Shirzad H. Oxidative stress and age-related changes in T cells: is thalassemia a model of accelerated immune system aging? *Central European Journal of Immunology*. 2016;41(1):116-24.
27. Bahmani M, Shirzad H, Rafieian S, Rafieian-Kopaei M. *Silybum marianum*: Beyond Hepatoprotection. *J Evid Based Complementary Altern Med*. 2015, 20(4) 292-301.
28. Baharvand-Ahmadi B, Bahmani M, Tajeddini P, Naghdi N, Rafieian-Kopaei M. An ethno-medicinal study of medicinal plants used for the treatment of diabetes. *Journal of Nephropathology*. 2016; 5(1):44-50.
29. Madihi Y, Merrikhi A, Baradaran A, Rafieian-kopaei M, Shahinfard N, Ansari R, Shirzad H, Mesripour A. Impact of sumac on postprandial high-fat oxidative stress. *Pakistan Journal of Medical Sciences*. 2013; 29 (1): 340-345.
30. Rafieian-Kopaei M, Baradaran A, Rafieian M. Oxidative stress and the paradoxical effects of antioxidants. *Journal of Research in Medical Sciences*. 2013; 18(7): 628.
31. Nasri H, Rafieian-Kopaei M. Protective effects of herbal antioxidants on diabetic kidney disease. *Journal of Research in Medical Sciences*. 2014;19(1):82-3.