RESEARCH ANTIOXIDANT ACTIVITY OF CHITOOLIGOSACCHRIDE BY UV-VIS ABSORPTION SPECTROCOPY

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ABSTRACT

Chitosan with 80% degree of deacetylation was hydrolyzed by cellulase of *Trichoderma viride* to prepare chitooligosaccharides (COSs) by the fractionation of the COSs with ultrafiltration membrane. The antioxidant activities of the COSs were clarified in this study by reducing power and free radical scavenging ability assay by UV-VIS absorption spectrum. The results show that the COS 1 (10,000-5,000 Da), COS 2 (5,000-3,000 Da), COS 3 (3,000-1,000 Da) and COS 4 (less than1,000 Da) segments have antioxidant properties.The antioxidant activitives of the COSs increased with the increment of concentration, and they also depended on molecular weight.

Keywords: Antioxidant; Chitooligosaccharide; DPPH; Reducing power; UV-VIS.

1. Introduction

Chitosan is a derivative of chitin, which is a naturally abundant mucopolysaccharide and distributes in the shell of crustaceans, in the cuticle of insects, and also in the cell wall of some fungi and microorganisms. The molecular structure of chitosan consists two units of N-acetylglucosamine and glucosamine. Chitosan has attracted much attention during past decades because of a lot of their bio-activity as anti-bacterial, anti-fungi, anti-inflammation. However, chitosan shows its bio-activity only in acidic medium and it is not soluble in water. These reasons caused the limitation of chitosan applications in fields. Therefore, converting chitosan into chitooligosaccharides (COSs) with hight soluble degree in water have been concerned by many researchers. COSs have importantly biological properties in medicinal and pharmaceutical applications such as antioxidative, anti-bacterial, immuno-stimulant, adipogenesis inhibitory and anticancer activities (Kim and Rajapakse, 2005; Jeon and Kim, 2000).

Free radicals cause the oxidation that reduces the quality of food products. Chemical synthetic antioxidants have free radical scavenging ability. Howeve, the use of synthetic antioxidants in food products must to conform strict food safety regulations due to their potential health hazards. COSs with free radical scavenging ability are a natural compound in the prevention of oxidation. However, free radical scavenging ability of COSs depends on their molecular weights (Park and Kim, 2003). Therefore, in this study, the free radical scavenging activity of COSs were investigated to clarify this problem.

2. Materials and Methods

2.1. Materials

Chitosan (degree of deacetylation more than 80%, Viet Nam). Cellulase (Fungal cellulase, 4000 UI/g, *Trichoderma viride*), India). Lactic acid (India), $NaHCO_3$ (India),

[DPPH] 2,2-Diphenyl-1-picrylhydrazyl (Sigma), [BHT] butylated hydroxytoluene (India), Ascorbic acid (India), [TCA] trichloroacetic acid (India), [$K_3Fe(CN)_6$] potassium ferricyanide (India), Phosphate buffer (India), Ethanol (China), [$FeCl_3$] ferric chloric (India), Sodium hydroxyl (China).

Instrument: Spectrophotometer of model 6600 UV-VIS (WTW-Germany).

2.2. Methods

2.2.1. Preparation of COSs

COSs solution was created from our previous research (Bui V. H. et al., 2017) Briefly, 0.8% chitosan solution was prepared in 0.8% lactic acid solution. A 0.8% chitosan solution was hydrolyzed by cellulase with optimum parameters as 50 ^oC, pH of 5.5, 7 UI cellulase and hydrolysis time of 180 minutes. COSs after hydrolysis were filtered though ultrafiltration membrane with molecular weight cut offs (MWCO) of 10, 5, 3, and 1 kDa to fractionate COSs into four kinds. COS 1 are oligosaccharides passing through the 10 kDa membrane but not through the 5 kDa membrane. COS 2 are oligosaccharides passing through the 5 kDa membrane but not through the 3 kDa membrane. COS 3 are oligosaccharides passing through the 3 kDa membrane but not through the 1 kDa membrane. COS 4 are oligosaccharides passing through the 1 kDa membrane. COSs fractionation were carried out spray drying (SD-06AG, LabPlant, UK) to create COSs powder (Park and Kim, 2003).

2.2.2. DPPH radical scavenging ability assay

The DPPH radical scavenging ability of COSs was measured using the modified method of Blois (1958) (Blois, 1958) described by Matute (2013). Briefly, 1.5 mL of DPPH solution (40 µg/mL, dissolved in ethanol of 99%) was mixed with 1 mL of COSs (different concentration from 1 to 5 mg/mL, dissolved in water). Mixture was vortexed in 15 s and incubated at room temperature for 30 min. The absorbance of mixture was measured at a wavelength of 517 nm. Butylated hydroxytoluene and ascorbic acid were used as positive control sample. The DPPH activity was calculated as an inhibition percentage based on the following equation:

DPPH free radical scavenging activity (%) = $(1 - Abs_{sample}/Abs_{control})*100$. (Eq 1)

where Abs_{sample} and $Abs_{control}$ are the absorbance of the sample and control, respectively.

EC50/IC 50 value is defined as the amount of antioxidant necessary to decrease the absorbance of DPPH by 50% of the initial absorbance.

2.2.3. Reducing power assay

The reducing power of the COSs was determined by the method of Oyaizu (1986) and described by Yen and Chen (1995). Briefly, samples were prepared in different concentrations (1 mL, 1 to 5 mg/mL) and mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50 ^oC for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added to the mixture and then centrifuged for 10 min at 3000 rpm. An aliquot from upper layer (2.5 mL) was diluted with 2.5 mL distilled water and 0.5 mL of 0.1% $FeCl_3$. The absorbance was measured at a wavelength of 700 nm. Butylated hydroxytoluene and ascorbic acid were used as positive control sample. Reducing power was calculated based on the following equation:

Reducing power = Abs1 − Abs2. (Eq 2) where Abs1 is the absorbance of sample and Abs2 is the absorbance of the sample under identical conditions as Abs1 with water instead of $FeCl_3$ solution only. A higher absorbance indicates a higher reducing power.

2.2.4. Statistical Analysis

Data were expressed as the mean ± standard deviation (SD) of triplicates. The least significant difference (LSD), Duncan's

multiple range test, and one-way analysis of variance (ANOVA) were used for multiple comparisons by Statgraphic centurion. The difference was considered to be statistically significant if $p < 0.05$.

3. Results and Discussion

3.1. Preparation of COSs

A powder of COSs were successfully prepared using an UF membrane with four different membranes as the COS 1 (10,000-5,000 Da), COS 2 (5,000-3,000 Da), COS 3 (3,000-1,000 Da) and COS 4 (less than1,000 Da). The COSs were determined antioxidant activity through the DPPH free radical scavenging ability and reducing power by UV- Vis spectrophotometry.

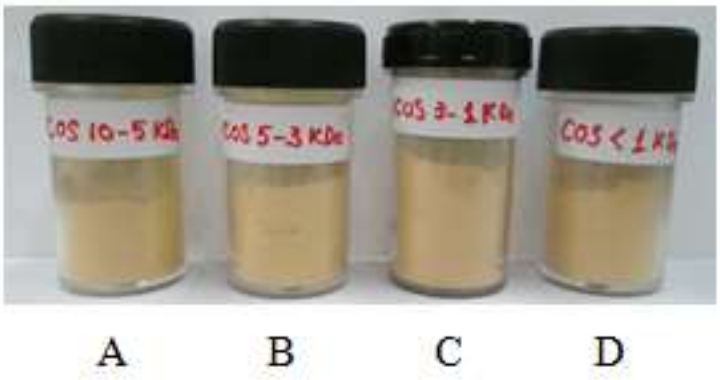

Figure 1. Powder of COSs

(A) COS 1, oligosaccharides that are passed through the 10 kDa membrane but not through the 5 kDa membrane; (B) COS 2, oligosaccharides that are passed through the 5 kDa membrane but not through the 3 kDa membrane; (C) COS 3, oligosaccharides that are passed through the 3 kDa membrane but not through the 1 kDa membrane; (D) COS 4, oligosaccharides that are passed through the 1 kDa membrane. Fractions of COSs were carred out spray drying to created COSs powder

3.2. DPPH radical scavenging ability

The DPPH radical scavenging ability of COSs was shown in Table 1. Results showed that COSs had DPPH radical scavenging ability. At the same concentration, the DPPH radical scavenging ability of COS 3 is the highest. The COSs activity increased from COS 1 to COS 3 and depended on the increment of their concentration from 1 mg/mL to 5 mg/mL. The DPPH activity of the COS 3 is hightest with 76.61 ± 1.64% at 5 mg/mL. This result is lower than the research of Park (2003) with 96.79% at 5 mg/mL. This result showed that the free radical scavenging ability is enhanced with increased concentration and reduced molecular weight. A value of IC 50 of COS 3 and COS 4 is 3 mg/mL.

Table 1

DPPH radical scavenging ability of COSs (%)

Concentration mg/mL	COS 1	COS 2	COS 3	COS 4
1	17.61 ± 2.98^{d}	23.99 ± 1.79^{c}	31.74 ± 1.39^{a}	28.12 ± 3.29^{b}
2	31.82 ± 2.31^{d}	36.93 ± 1.09^{c}	48.43 ± 3.33^{a}	45.21 ± 2.38^{b}
3	44.44 ± 0.68^{d}	49.80 ± 2.30^{c}	56.09 ± 2.65^{a}	53.60 ± 2.07^{b}
4	53.79 ± 1.88^{d}	58.39 ± 0.86^{c}	65.87 ± 0.45^{a}	62.18 ± 2.80^{b}
5	61.36 ± 1.42^{d}	65.28 ± 2.08^{c}	76.61 ± 1.64^{a}	71.82 ± 1.16^{b}

Note: Values represent the mean ± SD, n = 3, abcd are mean value of rows, statistically significant defference with p < 0.05. COS 1, oligosaccharides that are passed through the 10 kDa membrane but not through the 5 kDa membrane; COS 2, oligosaccharides that are passed through the 5 kDa membrane but not through the 3 kDa membrane; COS 3, oligosaccharides that are passed through the 3 kDa membrane but not through the 1 kDa membrane; and COS 4, oligosaccharides that are passed through the 1 kDa membrane.

Table 2

DPPH radical scavenging ability of ascorbic acid and BHT (%)

Concentration µg/mL	Ascorbic acid	BHT
1000	97.18 ± 1.67	79.56 ± 1.06
500	94.23 ± 1.34	76.81 ± 1.10
250	89.03 ± 1.42	72.98 ± 1.07
100	85.09 ± 1.24	69.56 ± 1.05
50	76.78 ± 1.29	65.34 ± 1.39
25	**62.45 ± 1.02**	**56.23 ± 1.33**
12.5	46.35 ± 1.05	40.12 ± 1.22

Note: Values represent the mean ± SD, n = 3

3.3. Reducing power

The COSs powder was dissolved in water into different concentrations of 1 mg/mL to 5 mg/mL and determined reducing power showed in Table 3. The reducing ability of the COS 4 is hightest with absorbance increased from 0.120 ± 0.009 Abs to 0.432 ± 0.014 Abs with increasing concentration from 1 mg/mL to 5 mg/mL. The absorbance was recorded at 5 mg/mL of COS 1, COS 2 and COS 3 with reducing power 0.297 ± 0.007 Abs, 0.309 ± 0.004 Abs and 0.423 ± 0.020 Abs, respectively. The reducing power results of COSs showed that their reducing ability depended on their molecular weight and concentration. However, this activity is still lower than the reducing ability of ascorbic acid and BHT (shown Table 4).

Table 3

Reducing power of COSs by absorbance value (Abs)

Concentration mg/mL	COS 1	COS 2	COS 3	COS 4
1	0.092 ± 0.007^{d}	0.107 ± 0.004^{c}	0.112 ± 0.010^{b}	0.120 ± 0.009^{a}
2	0.144 ± 0.011^{d}	0.153 ± 0.006^{c}	0.166 ± 0.002^{b}	0.218 ± 0.03^{a}
3	0.190 ± 0.006^{d}	0.209 ± 0.009^{c}	0220 ± 0.011^{b}	0.272 ± 0.032^{a}
4	0.234 ± 0.009^{d}	0.246 ± 0.006^{c}	0.332 ± 0.015^{b}	0.346 ± 0.006^{a}
5	0.297 ± 0.007^{d}	0.309 ± 0.004^{c}	0.423 ± 0.020^{b}	0.432 ± 0.014^{a}

Note: Values represent the mean ± SD, n = 3, abcd are mean value of row, statistically significant defference with p < 0.05. COS 1, oligosaccharides that are passed through the 10 kDa membrane but not through the 5 kDa membrane; COS 2, oligosaccharides that are passed through the 5 kDa membrane but not through the 3 kDa membrane; COS 3, oligosaccharides that are passed through the 3 kDa membrane but not through the 1 kDa membrane; and COS 4, oligosaccharides that are passed through the 1 kDa membrane.

Table 4

Reducing power of ascorbic acid and BHT by absorbance value (Abs)

Concentration µg/mL	Ascorbic acid	BHT
1000	2.528 ± 0.018	1.977 ± 0.095
500	2.449 ± 0.122	1.808 ± 0.087
250	2.207 ± 0.034	1.262 ± 0.085
100	0.764 ± 0.025	0.718 ± 0.070
50	0.418 ± 0,024	0.329 ± 0.052

Note: Values represent the mean ± SD, n = 3

4. Conclusion

Results of this research showed that the COSs (less than 10 kDa) had antioxidant ability. The antioxidant activity depended on their molecular weight and concentration. Because COS 3 and COS 4 activities are highest, the production of COSs powder should focus on molecular weight less than 3000 Da. These results suggest that COSs have to application research in food products to evaluate potentially applicative ability as natural antioxidant instead of synthetis antioxidant. Although the results of DPPH and reducing power actibity of COSs are still lower than BHT and ascorbic acid, they have the potential to be used as antioxidants instead of synthetic antioxidants■

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References

Bui V. H., Dao A. Q., Nguyen T. N. P., Vo D. N., Tran T. K. Q., and Ngo D. N. (2017). Research of chitosan hydrolysis by cellulase to produce chitooligosaccharide. *Journal of Science Technology & Food*, *12*(1), 11.

Blois, M. S. (1958). Antioxidant determination by the use of a stable free radical. *Nature, 181,* 1199.

Jeon, Y. J. and Kim, S. K. (2000). Continuous production of chitooligosaccharides using a dual reactor system. *Process Biochemistry*, *35*, 623.

Kim, S. K. and Rajapakse, N. (2005). Enzymatic production and biological activities of chitosan oligosaccharides (COS): A review. *Carbohydrate Polymers*, 62, 357.

Matute, A. I. R., Cardelle-Cobas, A., García-Bermejo A. B., Montilla, A., Olano A, and Corzo, N. (2013). Synthesis, characterization and functional properties of galactosylated derivatives of chitosan through amide formation. *Food Hydrocolloids*, *33*, 245.

Oyaizu, M. (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, *44*, 307.

Park, P. J, Je, J. Y. and Kim, S. K. (2003). Free radical scavenging activity of chitooligosaccharides by electron spin resonance spectrometry. *Journal of Agricultural and Food Chemistry, 51*, 4624.

Yen, G. C. and Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimut agenicity. *J. Agric. Food Chem, 43*, 27.