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Short communication

Antioxidant activities of fucoidan degraded by gamma irradiation and acidic hydrolysis

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HIGHLIGHTS

- Antioxidant activity of fucoidan degraded by gamma-irradiation was analyzed.
- Degree of change of antioxidant activity was dependent on the measuring method.
- Acid hydrolyzed fucoidan was compared to gamma-irradiated for its antioxidant activity.

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ABSTRACT

Low molecular weight fucoidan, prepared by radical degradation using gamma ray was investigated for its antioxidant activities with different assay methods. As the molecular weight of fucoidan decreased with a higher absorbed dose, ferric-reducing antioxidant power values increased, but β-carotene bleaching inhibition did not change significantly. The antioxidant activity of acid-degraded fucoidan was also examined to investigate the effect of different degradation methods. At the same molecular weight, fucoidan degraded by gamma irradiation showed higher 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity than that observed with the acidic method. This result reveals that in addition to molecular weight, the degradation method affects the antioxidant activity of fucoidan.

1. Introduction

Fucoidan is a heteropolysaccharide containing a substantial number of fucose units and sulfate groups (Li et al., 2008). This molecule is extracted mainly from brown algae such as *Fucus vesiculosus* and *Laminaria japonica*. Fucoidan has a wide variety of biological activities, including anticoagulant and antithrombotic, antivirus, antitumor, and antioxidant effects (Li et al., 2008). Among them, the antioxidant activity of fucoidan has become an increasingly popular subject of intensive investigation because of increasing demands in the food and pharmaceutical industries (Pihlanto et al., 2008). Significant environmental pollution in modern society has led to rising levels of free radicals in the human body, resulting in accelerated aging as well as causing cancer and cardiovascular diseases (Getoff, 2007). Because antioxidants prevent the formation of or scavenge free radicals, the use of antioxidants in one's daily diet has gained interest.

Several studies have investigated the antioxidant activity of fucoidan. Rupérez et al. (2002) reported the potential antioxidant capacity of sulfated polysaccharides from *F. vesiculosus*, and another study evaluated the in vitro antioxidant activities of sulfated polysaccharides from different seaweeds (de Souza et al., 2007). The antioxidant activity of fucoidan depends on several structural parameters including molecular weight. Low molecular weight fucoidan has shown improved biological activities, including higher anticoagulation activity (Wang et al., 2010), the ability to promote revascularization in the treatment of hindlimb ischemia (Luyt et al., 2003), and enhanced formation of human endothelial cells (Chabut et al., 2003). Choi and Kim (2013) reported that low molecular weight fucoidan prepared by radical degradation using gamma ray is highly toxic to cancer cells. There are several reports on the degradation of polysaccharides by ionizing irradiation (Byun et al., 2008; Choi et al., 2011). Jo and Choi (2014) degraded fucoidan in hydrogen peroxide solution by ultrasonic or electron-beam irradiation. Radical degradation using ionizing irradiation has several advantages over enzymatic and acidic methods such as its short processing time, no required acid or enzyme, no required purification after degradation, and possible degradation of the

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powdered form (Choi et al., 2010). However, until now, there has been no systematic study on the change in the antioxidant activity of fucoidan degraded by irradiation. Furthermore, because the change in activity is dependent on the assay method, different methods should be carried out and compared to investigate their effects on antioxidant activity.

Therefore, in this study, the antioxidant activities of fucoidan molecules with different molecular weights prepared by gamma irradiation were measured. Moreover, because the molecular structure of low molecular weight polysaccharide is dependent on the degradation methods, the antioxidant activities of fucoidan prepared by gamma irradiation and acidic heating were compared.

2. Materials and methods

2.1. Preparation of low molecular weight fucoidan

Fucoidan, with a molecular weight of 217 kDa and originating from *F.vesiculosus*, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Low molecular weight fucoidan was prepared by radical degradation using gamma irradiation following a previously reported method (Choi et al., 2014). The average molecular weights of the prepared fucoidan samples were 85, 30, 15, and 7 kDa at the doses of 8, 10.5, 30, and 100 kGy, respectively.

To degrade fucoidan by acidic heating, the native fucoidan was hydrolyzed with 0.01 N HCl. Acidic degradation was terminated by neutralizing the reaction with NaOH solution (Yang et al., 2008). To investigate the effect of degradation method on the antioxidant activity of fucoidan with the same molecular weight, degraded fucoidan was collected by fractionation using a Millipore Ultrafiltration system with 10 kDa and 50 kDa molecular weight cut-off membranes (Millipore, Billerica, MA, USA).

The sulfate content in fucoidan was determined by the method using 1,9-dimethyl-methylene blue (Farndale et al., 1986).

2.2. Ferric-reducing antioxidant power (FRAP) values

The FRAP assay was performed as described by Benzie and Strain (1996). The FRAP reagent contained 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine, Sigma) solution in 40 mM HCl, 2.5 mL of 20 mM $FeSO_4$, and 25 mL of 0.3 M acetate buffer (pH 3.6). The FRAP reagent was freshly prepared and pre-warmed to 37 °C. The fucoidan solution was mixed with 90 μL of distilled water and 0.9 mL of FRAP reagent. After 10 min of incubation at 37 °C, the absorbance was measured at 593 nm with a UV/Vis spectrophotometer (UV-1601PC, Shimadzu, Tokyo, Japan). Standards of known Fe^{2+} concentrations ($FeSO_4 \cdot 7H_2O$) were run at 20–1000 μM.

2.3. β-Carotene bleaching assay with linoleic acid

The β-carotene bleaching assay was carried out using the method described by Mahinda and Fereidoon (1999). The β-carotene solution was prepared by dissolving 1 mg of β-carotene in 5 mL of chloroform. To 1 mL of this solution, 40 μL of linoleic acid and 400 μL of Tween 80 were added. After the chloroform was removed under nitrogen gas (ultra-pure, 99.999%), 100 mL of distilled water was added while slowly shaking the flask. 5 mL of this emulsion was mixed with 200 μL of fucoidan, and the absorbance was measured at 470 nm. Distilled water (200 μL) was used as a control. The experimental and control samples were kept at 50 °C, and the absorbance readings were recorded 1 h later. The antioxidant index was calculated using the following equation:

$$\text{Antioxidant index (\%)} = \frac{100 \times \text{Absorbance of } \beta\text{–carotene solution after 1 h of assay}}{\text{Initial absorbance of } \beta - \text{carotene solution}}$$

2.4. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

Free radical scavenging activity was estimated according to the method of Blois (1958). Fucoidan samples (1 mL) were added to 1 mL of 0.2 mM DPPH radical solution (Sigma), and 1 mL of 70% ethanol solution was used as the blank. The mixtures were shaken and left to stand for 30 min at room temperature, and then measured at 517 nm with a spectrophotometer (UV-1601PC). The scavenging activity of the DPPH radicals was calculated as a percentage using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left[1 - \left(A^{\text{sample}}/A^{\text{blank}}\right)\right] \times 100$$

where A^{sample} and A^{blank} are the sample and blank absorbance values, respectively.

2.5. Statistical analysis

All experiments were performed three times. One-way analysis of variance was performed using SPSS software (Nie et al., 1970) and Duncan's multiple range test was used to compare the differences among the mean values. A value of $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. FRAP values

The FRAP assay treats the antioxidants contained in samples as reductants in a redox-linked colorimetric reaction, and the value reflects the reducing power of the antioxidant. Because this procedure is relatively simple and easy to standardize, it was used to analyze the antioxidant activity of low molecular weight fucoidan prepared by the radical method using gamma irradiation. As shown in Fig. 1, the FRAP values of fucoidan increased as the molecular weight of fucoidan decreased. The FRAP values of fucoidan with molecular weights of 217, 85, 30, 15, and 7 kDa were observed to be 0.137, 0.534, 0.945, 1.253, and 1.754 mM, respectively. These results suggest that fucoidan with lower molecular weights prepared by radical degradation with gamma irradiation may have higher antioxidant activity. It was reported that the

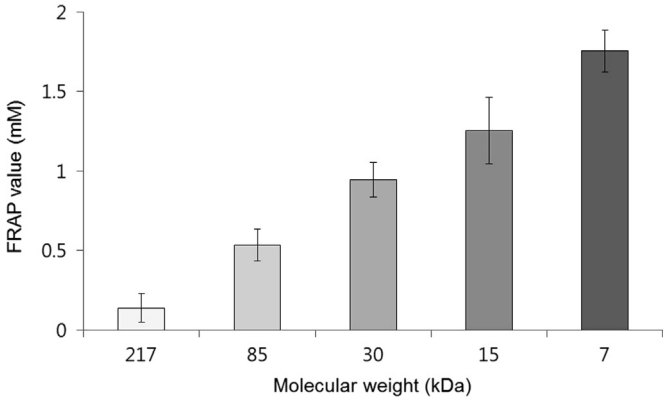

Fig. 1. Ferric-reducing antioxidant power (FRAP) value (mM) of fucoidan molecules with different molecular weights degraded by gamma irradiation.

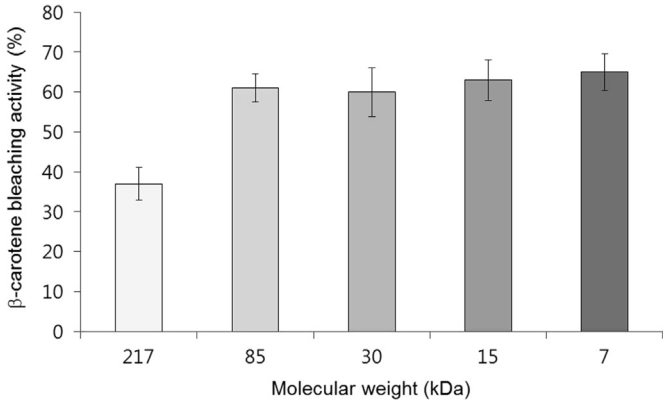

Fig. 2. β-Carotene bleaching inhibition assay of fucoidan molecules with different molecular weights degraded by gamma irradiation.

FRAP value of laminarin, another polysaccharide from seaweed, increased with degradation by gamma irradiation (Choi et al., 2012). In a previous study, similar results were reported in which the DPPH radical scavenging activity increased in fucoidan degraded by gamma irradiation (Choi et al., 2009).

3.2. β-Carotene bleaching inhibition activity

The result of the β-carotene bleaching assay using fucoidan molecules with different molecular weights is shown in Fig. 2. β-Carotene bleaching inhibition activity of fucoidan with 217 kDa was 37%, and the bleaching inhibition activities were increased in fucoidan molecules with lower weights. However, the difference in activity among the molecules with different molecular weights was not significant. The inhibition activities of fucoidan were 60%–67% regardless of molecular weight.

The β-carotene bleaching assay is used to measure the decolorization of a β-carotene solution using the lipid peroxyl radical ($LOO^{\cdot}$). The difference in the degree of change in the antioxidant activities of the different sized fucoidan molecules may have been caused by the different radicals used in each assay. Similarly, Choi et al. (2012) showed that the DPPH radical scavenging ability and the reducing power of low molecular weight laminarin were higher compared to high molecular weight molecules, but no differences among laminarin molecules with different molecular weights were observed in the β-carotene bleaching assay with linoleic acid. However, when chitosan was degraded by irradiation with gamma ray, the inhibition activity of linoleic acid peroxidation, the reducing power, and the superoxide scavenging activity were all enhanced by low molecular weight chitosan (Feng et al., 2008). From these results, the antioxidant activity of irradiated fucoidan was shown to have increased depending on the absorbed dose, but the changes were different among the assay methods. It was reported that fucoidan degraded by gamma irradiation had an increased number of carbonyl groups (Choi et al., 2014). The carbonyl group was reported to play an important role in radical scavenging steps. Therefore, it was concluded that gamma irradiation caused a higher concentration of carbonyl groups in fucoidan during degradation, resulting in an increase in antioxidant activity. To confirm the effect of gamma irradiation on the antioxidant activity and to investigate the characteristics of radiation degradation, low molecular weight fucoidan samples prepared by other methods were compared.

3.3. Effect of degradation method on antioxidant activity

High molecular weight polysaccharides can be degraded by various methods including radical degradation, acidic degradation, and enzyme digestion. The molecular structure of low molecular weight polysaccharides obtained after degradation could differ depending on the degradation method, as it causes differences in biological activity. Choi et al. (2010) reported that hyaluronic acid powder degraded by different methods had different molecular structures and showed varying degrees of antioxidant activity. In this study, the DPPH radical scavenging activities of low molecular weight fucoidan degraded by different methods were compared. The 30 kDa fucoidan samples were prepared by radical degradation and acidic heating. The polydispersity of fucoidan decreased at lower molecular weights in the case of radical degradation with gamma ray. However, the molecular weight of fucoidan was more widely distributed after acidic heating (data not shown). Therefore, 30 kDa fucoidan was collected by fractionation with 10 kDa and 50 kDa membranes after degradation by both methods. The DPPH radical scavenging activities of 30 kDa fucoidan are shown in Table 1. The DPPH radical scavenging activity of acid-degraded fucoidan was 47%, which was relatively low compared with that of radical-degraded fucoidan of the same size. The carbonyl group generated was shown not to be significantly different between gamma-irradiation and acidic heating methods from ultraviolet spectroscopy. However, the sulfate content of 30 kDa fucoidan prepared by acidic heating was lower than that by radical degradation (Table 1). Acid hydrolysis tended to selectively cause desulfation at the second position of the first fucose unit (Pomin et al., 2005). In contrast, sulfate content was maintained during radical degradation with gamma ray (Choi et al., 2014). A positive correlation was observed between fucoidan sulfate content and antioxidant activity (de Souza et al., 2007). Therefore, the method of degradation can change the molecular structure and, furthermore, affect the antioxidant activity of fucoidan.

Table 1
DPPH radical scavenging activity of low molecular weight fucoidan (30 kDa) prepared by radical degradation and acidic heating.

Sample	DPPH radical scavenging activity (%)	Sulfate content (μg/mL)
Fucoidan prepared by radical degradation	78% ± 5.1	790
Fucoidan prepared by acidic heating	47% ± 8.9	440

4. Conclusions

There was investigated the effect of gamma irradiation on the antioxidant activity of fucoidan. Low molecular weight fucoidan prepared by radical degradation with gamma ray showed an increase in antioxidant activity, but the degree of change was dependent on the assay method. Furthermore, different degradation methods affected the antioxidant activities of fucoidan molecules at the same molecular weight. These results suggest that molecular weight and degradation method are two parameters that are related to the biological activities of fucoidan.

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