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Application of gamma irradiation for the enhanced physiological properties of polysaccharides from seaweeds

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ABSTRACT

Polysaccharides from seaweeds, fucoidan and laminarin, were irradiated with gamma rays, and their structural changes and anti-oxidative activities were investigated. The gamma irradiation decreased the average molecular weights of polysaccharides, and UV spectra of irradiated polysaccharides showed increases in the numbers of carboxyl and carbonyl groups and double bonds. DPPH radical scavenging ability and reducing power of the gamma irradiated polysaccharides were significantly higher than those non-irradiated.

1. Introduction

Edible seaweeds are rich sources of active compounds, such as polysaccharides and polyphenolic compounds. Indeed, they contain large amounts (about 40% of the dry weight) of polysaccharides (Devillé et al., 2007). Polysaccharides from some seaweeds have been reported to possess the biological activities of potential medicinal values and are also considered as dietary fibers (Smit, 2004). Among the polysaccharides, fucoidan and laminarin contents were up to 50% of defatted alga dry weight and were well characterized for their structure and activities (Zvyagintseva et al., 1999).

Fucoidans are the family of sulfated homo- and heteropolysaccharides composed mainly of α(1–2)- and/or α(1–2)-linked L-fucose residues. They can also contain residues of galactose, mannose, xylose and glucuronic acids. Fucoidans from different sources are well known to vary considerably in structure and pharmacological activities (Zvyagintseva et al., 1999).

Laminarin is the main storage polysaccharide of *Laminaria* spp (up to 36% of the dry weight). It is a short polymer of about 20–25 glucose residues linked by β(1–3) bonds with some β(1–6) bonds that lead to a ramification of the molecule (Devillé et al., 2007).

Because the high molecular weight of polysaccharides caused some problems such as high viscosity and low permeability into cell, several degradation methods with acid, enzyme and mechanical shear have been reported (Hasegawa et al., 1993; Ilyina et al., 2000). Recently, it has been reported that the gamma irradiation led to the degradation of polysaccharides by the cleavage of the glycosidic bonds (Byun et al., 2008; Charlesby, 1981; Cho et al., 2003; Kim et al., 2008). The basic advantages of the degradation of polymers by radiation include the ability to promote changes reproducibly and quantitatively, without the introduction of chemical reagents and without the need for special equipments/setup (Charlesby, 1981). Therefore, this technology is simpler and more environmentally friendly than other conventional methods.

Hence, the aim of this study is to apply radiation technology to polysaccharides from seaweeds, fucoidan and laminarin, so that their molecular weights can be reduced. Also, we investigated the changes of structure and the antioxidant activities of polysaccharides by gamma irradiation.

2. Materials and methods

2.1. Sample preparation

Fucoidan and laminarin were purchased from Sigma Chemical Co., St. Louis, MO. Samples were dissolved to a concentration of 10 mg/mL (w/v) in double distilled water (DDW) for a gamma irradiation. When the polysaccharide samples were analyzed for

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reducing power, the solutions with a concentration of 5 mg/mL (w/v) were prepared for the irradiation.

2.2. Gamma irradiation

The fucoidan and laminarin solutions were irradiated in a cobalt-60 gamma-irradiator (IR-221, Nordion International Ltd., Ontario, Canada) equipped with a 11.1 PBq strength at 15 ± 0.5 °C and operated at a dose rate of 10 kGy/h. The applied dose levels were 10, 30 and 50 kGy, respectively. Dosimetry was performed with 5 mm-diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and after irradiation process the samples were stored at 4 °C for further experiments.

2.3. Measuring the molecular weight by gel permeation chromatography

Gel permeation chromatography (GPC) was conducted to monitor the changes of the molecular weight distribution of the polysaccharides in the solution by the gamma irradiation. GPC was performed by Waters system (Milford, MA) equipped with separation module (Waters 2690), refractive index detector (RI, Waters 2410) and PLaquagel-60 column, 40 column, 30 column (300×7.5 mm, 8 μm, Polymer laboratories, Ltd., UK). The mobile phase was 0.1 M sodium nitrate at the flow rate of 1 mL/min, and the column was operated at 40 °C. The injection volume was 200 μL and the calibration was carried out using pullulan standard (Showa Denko, Tokyo, Japan). The weight average molecular weight was calculated using Empower software (System Software, Empower option GPC, Waters Co.).

2.4. UV absorption

Irradiated polysaccharide solutions were measured at 25 °C by using a spectrophotometer (UV-1601PC, Shimadzu, Tokyo, Japan). The UV spectra were recorded between 200–400 nm. The polysaccharide concentration of aqueous solution used for the spectroscopy was 0.02% (w/v).

2.5. DPPH radical scavenging activity

A sample of 1 mL was mixed with 1 mL of 0.2 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Co.) solution in methanol (Blois, 1958). The concentration of samples in the assay mixture was appropriately diluted to meet the calibration range of spectrophotometer. The mixture was shaken and left to stand for 30 min at room temperature and then measured at 517 nm with a spectrophotometer (UV-1601PC, Shimadzu, Japan) and then compared with a blank control containing a polysaccharide solution and pure methanol instead of DPPH. A blind control containing DPPH and distilled water instead of a polysaccharide solution was also assayed. The following equation was used for the calculation of the DPPH radical scavenging activity:

$$\text{scavenging activity } (\%) = (1 - A_{517}^{\text{sample}}/A_{517}^{\text{blind}}) \times 100$$

where the scavenging activity refers to the free-radical scavenging percentages, A^{sample} refers to the absorbance of a sample and A^{blind} refers to the absorbance of the blind control.

2.6. Reducing power

The reducing power of polysaccharides was determined according to the method of Oyaizu (1986). A polysaccharide solution of 1 mL was mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide ($K_3Fe(CN_6)$). The concentration of samples in the assay mixture was appropriately diluted to meet the calibration range of spectrophotometer. The reaction mixtures were incubated in a temperature-controlled water bath at 50 °C for 20 min, followed by the addition of 2.5 mL of 10% trichloroacetic acid. The mixtures were then centrifuged at $750 \times g$ using a centrifuge (VS-5500, Vision scientific Co. Ltd., Seoul, Republic of Korea) for 5 min at 25 °C. The supernatant obtained (5 mL) was treated with 5 mL of distilled water and 1 mL of 1% ferric chloride. The absorbance of the reaction mixture was measured at 700 nm. An increase in absorbance was used as a measure of the reducing power.

3. Results and discussion

3.1. Gel permeation chromatography

The modification of the weight average molecular weight of polysaccharides by gamma irradiation is shown in Fig. 1(a) and (b). The average molecular weight of fucoidan in the solution has been found to be 217 kDa in the non-irradiated sample, whereas a considerable decrease in the average molecular weight was observed in the gamma-irradiated fucoidan samples; in addition, the average molecular weights of the samples irradiated at doses of 10, 30 and 50 kGy were about 37, 15 and 10 kDa, respectively. The changes that occurred after irradiation were due to the breakage of the glycosidic bond of the polysaccharide (Sokhey and Hanna, 1993; Simic, 1983). When the laminarin solution was irradiated by the gamma ray, the molecular weight was also

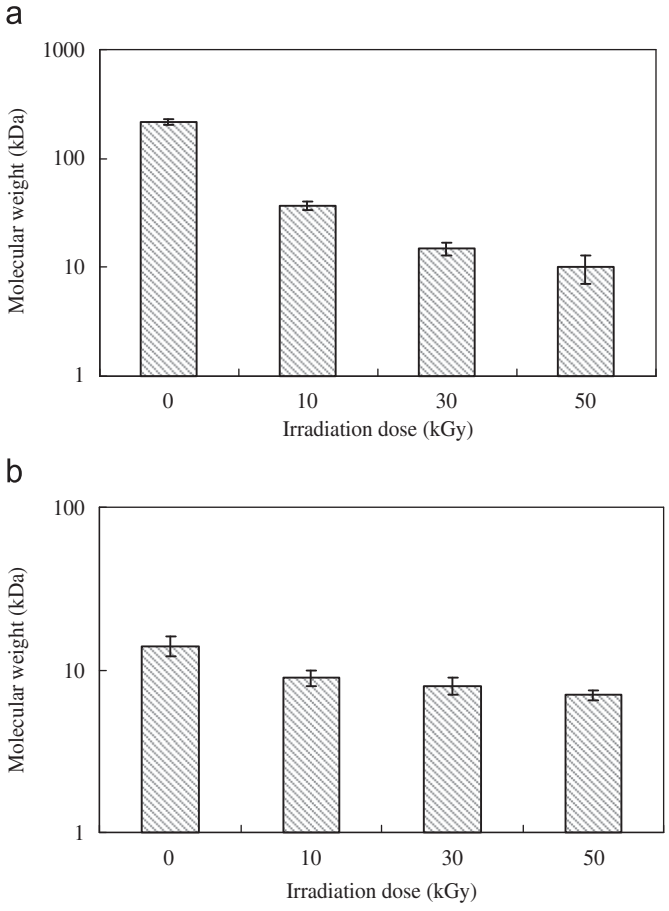

Fig. 1. The weight average molecular weight of polysaccharides with different doses of gamma irradiation: (a) fucoidan and (b) laminarin.