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Effect of gamma irradiation on the structure of fucoidan

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HIGHLIGHTS

- Fucoidan was degraded by gamma-irradiation.
- Structural changes after irradiation were characterized by GPC, UV, and FTIR.
- The polydispersity decreased by radiation degradation.
- Sulfate content was not changed by gamma irradiation.

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ABSTRACT

The change of molecular structure of fucoidan by gamma irradiation was analyzed by spectral and chemical methods. Fucoidan samples with different molecular weights of 85, 30, 15, and 7 kDa were prepared by radiation degradation of 217 kDa fucoidan. In the molecular weight analysis, the polydispersity decreased by gamma radiation because of further degradation of higher weight molecules. Ultraviolet absorption and Fourier-transform infrared spectroscopy analyses were carried out to define the changes of the functional groups in fucoidan by gamma irradiation. Carboxyl groups and carbon double bonds increased by gamma irradiation; however, sulfate content remained unchanged. The granular fissures were observed from scanning electron microscopy in gamma-irradiated fucoidan.

1. Introduction

Fucoidan is a homo- and heteropolysaccharide containing substantial numbers of fucose and sulfate groups. Galactose, mannose, xylose, and rhamnose moieties have also been found in various fucoidans. This molecule is extracted mainly from brown algae. Fucoidan has a variety of biological activities, including anticoagulant, antibacterial, antivirulent, anti-inflammatory, and antioxidant activities (Li et al., 2008).

The biological activities of fucoidan depend on several structural parameters, such as sugar type and fucose linkage, the content of the sulfate group, and the molecular weight of the polysaccharides. Several reports have indicated that low molecular weight fucoidan shows higher antioxidative and anticoagulation activities (Wang et al., 2010a, 2010b). In addition, low molecular weight fucoidan promotes revascularization of hindlimb ischemia in rats (Luyt et al., 2003), boosts osteoblast proliferation for bone regeneration (Igondjo Tchen Changotades et al., 2008), and enhances human endothelial cell formation (Lake et al., 2006).

Low molecular weight fucoidan can be prepared by acidic, enzymatic, and radical methods. In the acidic method, higher temperature or acidity leads to lower molecular weight products, but the content of sulfate group necessary for many of the bioactivities also decreases (Pomin et al., 2005). Fucoidanase isolated from bacteria and the digestive glands of marine invertebrates has also been studied (Holtkamp et al., 2009). However, because of low enzyme activity and the different molecular structures of fucoidan from different sources, the commercial utilization of these enzymes remains infeasible.

The radical method utilizes hydrogen peroxide to generate hydroxyl, superoxide, and hydroperoxyl radicals (Hou et al., 2012). These radicals degrade polysaccharides by attacking and breaking glycosidic linkages. But, the radical method using hydrogen peroxide has some problems including a long processing period, additional neutralization and purification steps, and difficulties adjusting the molecular weight. Recently, several studies on the radical degradation of polysaccharides by gamma irradiation

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have been published (Byun et al., 2008; Choi et al., 2009, 2010 and 2011).

Gamma irradiation has been used as a sanitary decontamination treatment for food and medical devices. The free radicals generated by gamma irradiation can be used to degrade polysaccharides. In some case, degradation by gamma irradiation enhances antioxidant activities of polysaccharides (Choi et al., 2011; Choi and Kim, 2013). Choi and Kim (2013) reported that the low molecular weight fucoidan degraded by gamma irradiation has increased antioxidant activities. But, the molecular structure of low molecular fucoidan degraded by gamma irradiation has not been reported. The molecular structure will be the key to define the reason for the enhanced biological activities of low molecular weight fucoidan.

Therefore, the present study was conducted to characterize the molecular structure of low molecular weight fucoidan prepared by gamma irradiation using spectroscopic and chemical analytical methods.

2. Materials and methods

2.1. Preparation of low molecular fucoidan by gamma irradiation

Fucoidan, with molecular weight of 217 kDa and originating from *Fucus vesiculosus*, was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Low molecular weight fucoidan was prepared by gamma irradiation following the method of Choi and Kim (2013). The average molecular weight of prepared fucoidan samples was 85, 30, 15, and 7 kDa. The applied doses were 8 kGy, 10.5 kGy, 30 kGy, and 100 kGy. The fucoidan was dissolved in water and gamma-irradiated in a ^{60}cobalt gamma-irradiator (IR-221, Nordion International Ltd., Ontario, Canada) with a strength of 11.1 pBq at 22 ± 2 °C at a dose rate of 10 kGy/h.

2.2. Molecular weight distribution

The molecular weight distribution of the fucoidan samples was measured by gel permeation chromatography using the following system (Choi et al., 2011): A separation module (Waters 2690, Waters Co., Milford, MA, USA), refractive index detector (Waters 2410, Waters Co.), Empower software (System Software, Emp7ower option GPC, Waters Co.), and PL aquagel-OH -60, -40, and -30 columns (300×7.5 mm^2, 8 μm, Polymer Laboratories Ltd., Shropshire, UK) were used in the analysis. The mobile phase was 0.1 M sodium nitrate at a flow rate of 1 mL/min, and analyses were performed at 40 °C. Injection volume was 200 μL, and calibration was carried out using pullulan as the standard (Showa Denko K. K., Tokyo, Japan). Polydispersity was determined by the ratio of weight average molecular weight to number average molecular weight.

2.3. Ultraviolet (UV) absorption

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

2.4. Fourier-transform infrared (FT-IR) spectroscopy

FT-IR spectra were acquired using a Bruker Spectrometer VERTEX 70 (Billerica, MA, USA) at a wavelength of 455–3996 cm^{-1}. Samples were prepared as a thin film of fucoidan mixed with KBr at a polymer/KBr (w/w) ratio of 1:100. The spectra obtained were the result of 24 scans at a spectrophotometer resolution of 8 cm^{-1}.

2.5. Determination of sulfate contents

The sulfate content in fucoidan was determined following the method of Farndale et al. (1986). The sample solution (10 mg/mL fucoidan, 0.1 mL) was added to 0.2 mL of 1,9-dimethyl-methylene blue (DMMB) reagent (16 mg DMMB, 3.04 g glycine, and 2.37 g sodium chloride per 1 L). The absorbance of the reaction mixture was measured after thorough mixing at 525 nm.

2.6. Scanning electron microscopy (SEM)

The microstructural changes in the fucoidan samples were observed by scanning electron microscopy (JEOL, Tokyo, Japan) using the method of Rayas-Duarte and Rupnow (1993). Samples were fixed on a cylindrical microscope stub covered with a carbon strip and coated with a thin layer of gold, followed by observation. A 100× magnification was used.

3. Results and discussion

3.1. Molecular weight distribution

The original molecular weight of fucoidan was about 217 kDa. Low molecular weight fucoidan samples with average molecular weights of 85, 30, 15, and 7 kDa were prepared by gamma irradiation. Choi and Kim (2013) reported that the average molecular weight of fucoidan decreased following gamma irradiation depending on the absorbed dose. The molecular weight distribution of each fucoidan sample was measured by GPC analysis. Fig. 1 shows the polydispersity of fucoidan samples with different molecular weights. The polydispersity of high molecular weight fucoidan (217 kDa) was 2.22. However, the polydispersity decreased to 1.46 in fucoidan with a molecular weight of 85 kDa and further decreased to 1.2 in fucoidan with a molecular weight of 30 kDa. Polydispersity tended to decrease further in 7 kDa fucoidan, but the difference was not significant ($p > 0.05$). A similar result has been observed in low molecular weight laminarin samples prepared by gamma irradiation (Choi et al., 2011). Polysaccharides with a higher molecular weight are more severely degraded by gamma irradiation than those with a lower molecular weight (Choi et al., 2008, 2011). Therefore, polydispersity decreased during gamma irradiation. In addition, the extent of degradation of polysaccharides is also dependent on molecular weight. The extent of decrease in molecular weight is more

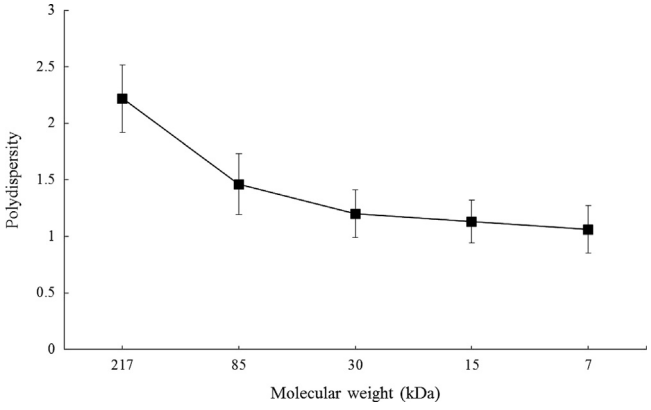

Fig. 1. The polydispersity of different molecular weight fucoidan samples prepared by gamma irradiation.

significant in high molecular weight carboxymethylcellulose compared to that in low molecular weight carboxymethylcellulose at the same absorbed dose of gamma irradiation (Choi et al., 2008, 2009). The molecular weight of laminarin in solution irradiated by gamma rays also decreased, but the extent of degradation was not severe compared with that of fucoidan, because the molecular weight of laminarin was relatively lower than that of fucoidan (Choi et al., 2011, 2013).

3.2. Spectral analysis

The UV spectra of fucoidan samples with different molecular weights were analyzed after preparation by gamma irradiation. The height of the peaks at 265 nm and 280 nm in low molecular weight fucoidan increased. The structural changes corresponding to a UV peak at ~280 nm may have been due to formation of carboxyl groups in fucoidan (Ulanski and Rosiak, 1992). The low molecular weight fucoidan solution also showed decreased pH, which may also have been due to the formation of carboxylic groups. The increase in the absorbance at 265 nm was assigned to double bonds of polysaccharides formed after main chain scission and/or the hydrogen abstraction reaction by gamma irradiation for degradation (Nagasawa et al., 2000).

FT-IR spectral analysis was carried out to define the structural changes in more detail. Fig. 2 shows the FT-IR spectra in the spectral range between 3996 and 455 cm^{-1} for different molecular weight fucoidans. Noteworthy absorptions were observed at 3458, 2930, 1736, 1639, 1251, 1056, 829 and 583 cm^{-1} in the infrared region of the spectra corresponding to functional groups. The absorbance band at 3458 is due to a hydroxyl group (−OH) (Yang et al., 2008; Chandía and Matsuhiro, 2008). The band at 1056 cm^{-1} may also be due to C–O stretching vibrations. Several smaller bands and shoulders at 2864–2991 cm^{-1} were assigned to CH stretching in the pyranoid ring and the C-6 groups of fucose and galactose units (Kim et al., 2010). The overall spectral pattern did not change in the low molecular size of fucoidan.

However, a difference was detected in the height and shape of certain absorption bands at 1736 and 1639 cm^{-1}, which were associated with the formation of an O-acetyl group and a carbonyl group, respectively. These changes in spectra also confirmed that a carboxyl group was formed and that COO– was interrupted during irradiation degradation of fucoidan. Huang et al. (2007) also reported the formation of carboxyl or carbonyl groups in carboxymethylated chitosan following gamma irradiation. The changes are due to breakage of the glycosidic bond of the polysaccharide (Rayas-Duarte and Rupnow, 1993; Choi et al., 2011).

Sulfate groups at the equatorial C-2 and C-3 positions produced a small shoulder of absorption at 820 cm^{-1} and there was such a shoulder in the fucoidan samples at 829 cm^{-1}. According to numerous IR analyses (Qiu et al., 2006; Mahner et al., 2001; Yang et al., 2003; Lijour et al., 1994; Rupérez, 2002), sulfate groups found in fucoidan are bound at the equatorial C-2/3 position detected at 820 cm^{-1} or at the axial C-4 position detected at 840 cm^{-1}. The sulfate group at C-4 position was not detected in our fucoidan sample. The peak at 1251 cm^{-1} indicated the presence of an S=O stretching vibration of a sulfate group. The peaks at 820 cm^{-1} and 1259 cm^{-1} are reduced when fucoidan is desulfated, but, we found no difference in sulfate group peaks among the different sized fucoidan samples. This means that low molecular weight fucoidan prepared by gamma irradiation maintained their sulfate groups. The asymmetric deformation of O–S–O group absorption at 583 also confirmed the presence of a significant number of sulfate groups (Chandía and Matsuhiro, 2008).

3.3. Sulfate content

The peaks in the FT-IR spectra responsible for the sulfate groups were presented in low molecular weight fucoidan. But, to define exactly, sulfate content was quantitatively measured in the different molecular weight fucoidan molecules prepared by gamma irradiation. Initially sulfate content was 821 g/mL in a 0.1% fucoidan solution with a molecular weight of 217 kDa, and was approximately the same in the low molecular weight fucoidan samples. The sulfate contents of

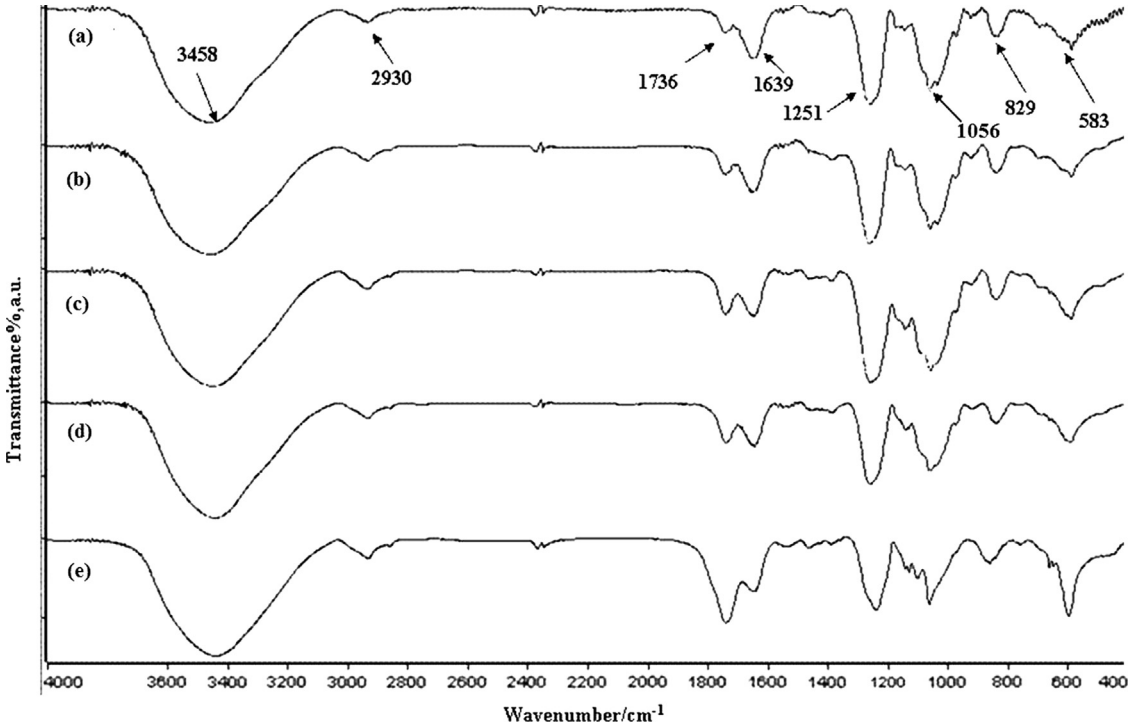

Fig. 2. Fourier transform-infrared spectra of different molecular weight fucoidan samples: (a) 217 kDa fucoidan, (b) 85 kDa, (c) 30 kDa, (d) 15 kDa, and (e) 7 kDa.

fucoidan samples with molecular weights of 85 kDa and 30 kDa were about 803 μg/mL and 786 μg/mL, respectively ($p > 0.05$). Sulfate content decreased to 760 μg/mL in 7 kDa molecular weight fucoidan; however, no difference in sulfate content from that of high molecular weight fucoidan (217 kDa) was observed ($p > 0.05$) (Fig. 3). When fucoidan was degraded by the acidic method, the decrease of sulfate content was reported. Yang et al. (2008) prepared low molecular weight fucoidan by heating in a microwave with acid, but the degraded fucoidan had few sulfate groups resulting in low anticancer activity. There was also a report that a 2-sulfate ester of the first fucose unit was selectively removed and then the glycosidic linkage between the nonsulfated fucose residue and the subsequent 4-sulafated was preferentially cleaved by acid hydrolysis (Pomin et al., 2005). However, our results indicate that preparing low molecular weight fucoidan by irradiation does not cause significant changes in sulfate content.

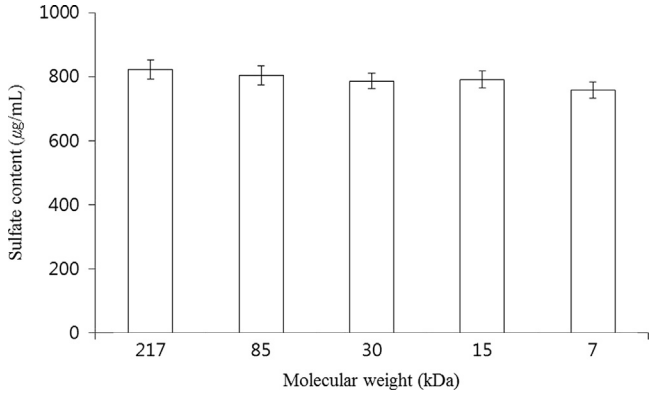

Fig. 3. Sulfate concentrations in different molecular weight fucoidan molecules. No differences were observed between the sulfate content in native fucoidan and the low molecular weight fucoidan samples ($p > 0.05$).

3.4. Scanning electron microscopy

Micrographs of different molecular weight fucoidan granules were obtained by SEM (Fig. 4). Granular fissures or splitting of low molecular weight fucoidan was observed, particularly in the 7 kDa fucoidan sample irradiated at 100 kGy. The fissures are caused by general modifications of the compound. Some researchers have reported that the shape of the polysaccharide and starch granules is deformed by gamma irradiation (Byun et al., 2008; Abu et al., 2006). A similar result was reported for gamma irradiated chitosan samples. When chitosan is irradiated with gamma rays, it undergoes degradation resulting in the breaking of polymer chains, and irradiated chitosan shows smaller structures as depicted in SEM images (Ulanski and Rosiak, 1992). von Sonntag (1980) reported that β-cleavage of the radical can lead to opening of the anhydroglucose ring or breaking off of the glucoside bond.

4. Conclusion

In this study, the molecular structure of low molecular weight fucoidan prepared by gamma irradiation was analyzed using spectral and chemical methods. The UV and FT-IR spectral analyses revealed that carboxyl groups were newly formed during degradation by gamma irradiation. The sulfate groups in fucoidan, which plays an important role in its activities, were unchanged and sulfate content was maintained at a constant level based on FT-IR spectral and chemical analyses. These results suggest that radical degradation with gamma irradiation could be used as a promising method to prepare low molecular weight fucoidan.

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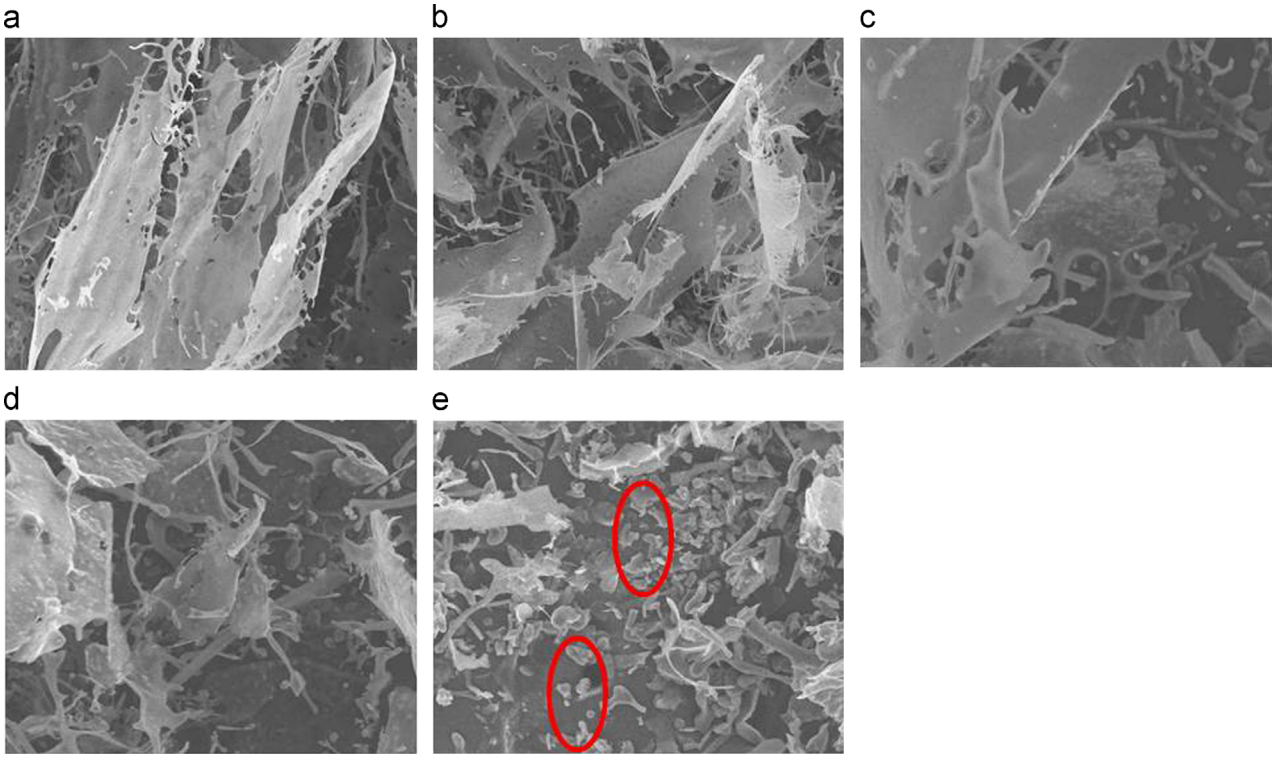

Fig. 4. Scanning electron microscopy images of different molecular weight fucoidan samples: (a) 217 kDa fucoidan, (b) 85 kDa, (c) 30 kDa, (d) 15 kDa, and (e) 7 kDa.

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