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Characterization and antioxidant properties of alcoholic extracts from gamma irradiated κ-carrageenan

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HIGHLIGHTS

- Alcoholic extracts from irradiated κ-carrageenan (solid and 1% w/v) were obtained.
- Extracts consisted of low molecular weight oligomers with Mw from 2300 to 5000 Da.
- Structural analysis showed C=O and C=C functional groups in the extracts.
- Fraction with Mw < 2000 Da exhibited higher antioxidant potential.

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ABSTRACT

Different extracts from unirradiated and gamma irradiated κ-carrageenan (solid and 1% w/v aqueous solution) were obtained with isopropyl alcohol (IPA) at concentrations of 40%, 60% and 80% v/v at room temperature. Physical and chemical properties of the different IPA extracts were analyzed by GPC, UV, and FT-IR. The extracts consisted of low molecular weight fragments with an average molecular weight (Mw) ranging from 2300 Da to 5000 Da. UV analyses of extracts from irradiated carrageenan showed varying maximum absorptions in the range of 265–280 nm. FT-IR spectra of all extracts from irradiated carrageenan showed all the important functional groups of carrageenan in the fingerprint region (4000–600 cm^{-1}) and additional carbonyl C=O and C=C double bond peaks. Antioxidant properties of the different extracts were investigated using reducing power assay. The reducing power of extracts from the irradiated solution follows the order of 80% > 60% > 40% while no trend was observed for all extracts from irradiated solid κ-carrageenan.

1. Introduction

Carrageenans are hydrophilic polymers that comprise the main structural polysaccharides of numerous species of seaweed e.g., Eucheuma, Chondrus, Gigartina, Fucellaria. There are three types of carrageenan iota (ι-), kappa (κ-) and lambda (λ)-carrageenan. The most widely available and utilized is the κ-carrageenan. It is composed of D-galactose units linked alternately with β(1,3)-D-galactose-4-sulfate and α(1-4)-3,6-anhydro-D-galactose. The idealized chemical structure of κ-carrageenan is shown in Fig. 1.

Carrageenans or their sulfated galactan oligomers prepared by chemical and enzymatic depolymerization have been reported to possess valuable bioactivities including antitumor, immunomodulatory, antioxidant and antiviral activities (Zhou et al., 2006; Yuan et al., 2006; Yuan et al., 2005; Wang et al., 2011). Radiation-degraded polyssacharides including alginate, carrageenan and chitosan by gamma rays or electron beam irradiation were likewise reported to induce various kinds of bioactivities such as growth promotion of plants, suppression of heavy metal stress on plants and anti-microbiological activities (Kume et al., 2002; Hien et al. 2000; Hai et al., 2003; Relleve et al., 2005). The technological and economical effectiveness of radiation processing in producing low molecular weight oligomers from its corresponding polysaccharides has been proven in the past years. Advantages of radiation technology include no remains of degradation-resistant fractions, chemicals are not introduced, reaction proceeds at room temperature and can be terminated easily.

Upon controlled irradiation, carrageenans in solid and gel states or in aqueous solution can be depolymerized to form shorter fragments. We have reported that when solutions of the irradiated κ-carrageenan and ι-carrageenan (both irradiated at 100 kGy in solid state) are mixed with the growth medium for rice seedlings and bok-choi under hydroponics conditions, stimulation of growth is observed (Relleve et al., 2000). Oligomer derived from irradiated κ-carrageenan with Mw of 10000 Da (obtained from 1%

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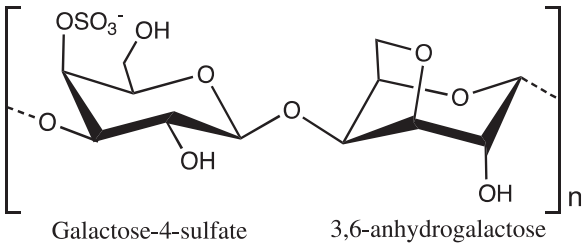

Fig. 1. Idealized chemical structure of κ-carrageenan.

Table 1
Yield of alcoholic extraction.

Carrageenan sample	% IPA (v/v)	%Yield
0 kGy	40%	3
	60%	4
	80%	1
Solid-100 kGy	40%	4
	60%	4
	80%	1
1% w/v-30 kGy	40%	49
	60%	14
	80%	11

irradiated at 30 kGy) has been shown to have optimum growth promoting effect in potato tissue culture bioassay (Relleve et al., 2005). Recently, foliar application of irradiated carrageenan solid at 250 kGy improved growth, yield and quality attributes of essential oil bearing plant, mint (Naeem et al., 2012). Antioxidant activities of irradiated κ-carrageenan in solution to doses 10–50 kGy have been shown to be increasing with radiation dose using various antioxidant assays (Abad et al., 2013).

Studies cited above used different irradiation conditions but all have shown that degraded carrageenan to be bioactive. These suggested that whatever is the irradiation condition, a certain fraction is responsible for the enhanced bioactivity. To completely degrade carrageenan into di-tetra, hexa, octa-mer and so on, high irradiation dose is required which may alter the structural arrangement of the carrageenan. Dynamic light scattering study showed that irradiation of carrageenan in solid state above 200 kGy may have destroyed the pyranose structure (Abad et al., 2004). Therefore, in order to obtain fraction with Mw < 10000 Da, it has to be fractionated out from carrageenan partially depolymerized (in solid or solution) at a radiation dose which retain the structural integrity of oligomer. It is our aim to obtain these substances in good yield from irradiated carrageenan by simple extraction procedure based on solubility of carrageenan in different isopropanol/water mixtures and to initially test the bioactivity through antioxidant assay. For comparative study, extraction of unirradiated carrageenan was also undertaken. A series of carrageenan oligomer with different molecular weights were isolated and their chemical and physical properties will be presented.

Many researchers have found different types of antioxidants in various kinds of higher plants. The activity of antioxidants has been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging (Diplock, 1997; Yildirim et al., 2000). Correlation of the size and structure of the fractions with antioxidant activity will be discussed in this paper.

2. Materials and methods

2.1. Materials

Refined K^+ type κ-carrageenan sample was obtained from Shemberg Corporation, Philippines. Carrageenan was used without further purification for all radiation works. All reagents used were of analytical grade and ultrapure water was used in all preparations of samples and analyses.

2.2. Gamma irradiation of carrageenan

Carrageenan was irradiated in powder at 100 kGy and 1% w/v solution at 30 kGy in aluminum foil pouch using Co-60 irradiator of the Philippine Nuclear Research Institute with a dose rate of 10 kGy/hr. All irradiations were carried out in air and at ambient temperature. After irradiation, the 1% w/v solution was neutralized and lyophilized.

2.3. Alcoholic extraction of unirradiated and irradiated κ-carrageenan

The following carrageenan samples were extracted with 40% v/v, 60% v/v and 80% v/v isopropyl alcohol solution: (a) unirradiated κ-carrageenan powder; (b) κ-carrageenan powder irradiated at 100 kGy; (c) lyophilized 1% w/v solution irradiated at 30 kGy. Extraction was done by pouring 200 ml of different concentration of isopropyl alcohol solution into each bottle containing 20 g powder of samples a–c. The mixture was stirred at room temperature for 8 h. Supernatant was then filtered with 0.45 μm nylon membrane filter and dried using vacuum concentrator. The dried extracts obtained were stored at 4 °C for further analysis.

2.4. Characterization of extracts

2.4.1. Gel permeation chromatography (GPC)

GPC studies were done using the method in our previous works (Relleve et al., 2005, Abad et al., 2009). GPC was performed on a Shimadzu Prominence chromatograph equipped with DGU-20A degasser, LC-20AD pump, autosampler SIL-20AHT, controller CBM-20A, CTO-20A column oven, RID-10A refractive index detector, and Tosoh TSK gel guard column PWXL and four TSK gel serially connected analytical columns (G6000 PWXL, G4000 PWXL, G3000 PWXL and G2500 PWXL). Elution was carried out using 0.1 M $NaNO_3$ (to suppress electrostatic effects) as the mobile phase at a flow rate of 0.5 ml/min. The temperatures of the column and detector were both maintained at 40 °C. A calibration curve was constructed using polyethylene oxide (PEO) and polyethylene glycol (PEG) as standards. One hundred microliter of carrageenan samples and standards (1 mg/ml) were injected. Average molecular weights were obtained using Shimadzu Labsolutions/LCsolution GPC software. All molecular masses reported in this work are based on PEO/PEG standards and are not absolute. The UV absorbances of all extracts were also monitored by GPC using UV–Vis UV detector at wavelengths 265 nm and 276 nm. The parameters used were similar to the method described previously.

2.4.2. UV–Vis Analyses

The UV spectra were obtained using Shimadzu UV–Vis 1800 spectrophotometer in the range of 200–400 nm. Concentration of sample used was 1.5 mg/ml.

2.4.3. FT-IR spectral Analyses

Analysis by infrared spectrometry was carried out using a Shimadzu FT-IR Spectrometer IR-Prestige 21 in the range from 4000–600 cm^{-1} with resolution of 4 cm^{-1}. Dried extracts were