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# Comparison of gamma ray and electron beam irradiation on extraction yield, morphological and antioxidant properties of polysaccharides from tamarind seed

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#### ABSTRACT

Tamarind (*Tamarindus indica L*) seed polysaccharide (TSP) is of great important due to its various biological activities. The present investigation was carried out to compare extraction yield, morphological characteristics, average molecular weights and antioxidant activities of TSP from gamma- and electron beam (EB)-irradiated tamarind kernel powder. The tamarind kernel powder was irradiated with 0, 5 and 10 kGy by gamma ray (GR) and electron beam, respectively. The extraction yield of TSP was increased significantly by EB and GR irradiation, but there was no significant difference between irradiation types. Morphological studies by scanning electron microscope showed that TSP from GR-irradiated tamarind seed had a fibrous structure, different from that of EB irradiated with a particle structures. The average molecular weight of TSP was decreased by the irradiation, and EB treatment degraded more severely than GR. Superoxide radical scavenging ability and total antioxidant capacity of EB-treated TSP showed higher than those of GR-treated TSP.

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#### 1. Introduction

Tamarind seed polysaccharide (TSP) from *Tamarindus indica L*. is a cell wall polysaccharide consisting of a (1-4)- $\beta$ -D-glucan backbone chain which has (1-6)- $\alpha$ -D-xylose branches that are partially substituted by (1-2)- $\beta$ -D-galactoxylose. TSP, so called xyloglucan, is of great importance due to its unique rheological properties in pharmaceutical, medical and cosmetic industries. (Miyazaki et al., 1998; Burgalassi et al., 1996). Various biological activities, such as immunomodulatory (Sreelekha et al., 1993), hypolipidemic (Yamatoya et al., 1997) and antiviral effects (Mastromarino et al., 1997) have been reported for the tamarind seed polysaccharides.

Many functional properties of TSP depend on the molecular weight of the product used. Even though the tamarind seed polysaccharides were used as thickening and stabilizing agents based on their large molecular weight, recent study suggested that polysaccharides of low molecular mass have selected advantages over the high molecular mass candidates due to their improved diffusion into biological tissues, especially in the field of medical treatment (Vodeničarová et al., 2006). However, by

lowering molecular weight of polysaccharides, they would likely be depleted of some important properties and it does not compromise the rheological and thermal properties of the polymers. Shortening of the polysaccharide macromolecular chains can be achieved by various methods such as ultrasound, microwave heating and ionizing irradiation. Among these, an ionizing radiation was found to be effective in degradation of hyaluronic acid (Kim et al., 2008), β-glucan (Byun et al., 2008) and chitosan (Choi et al., 2002) by the energy generated from different sources such as a cobalt-60 and an electron accelerator. These two energies have been known to show a similar effect on materials, but they have differences regarding a penetration and the method of their use. Compared to other degradation methods, ionizing radiation is free of initiators and side products which results in chemically pure degradation product. Therefore, this technology is simpler and more environmental friendly than the current conventional ones. A significant advantage in the case of ionizing irradiation is a final biological sterilization of irradiated materials that can be easily used for manufacturing biomedical products. Also, it has been reported that the antioxidant parameters of polysaccharides were increased by the irradiation (Byun et al., 2008; Kim et al., 2008).

In this study, crude tamarind seed powder was irradiated with 0, 5 and 10 kGy by gamma ray (GR) and electron beam (EB), respectively and the extraction yield (%), morphological changes,

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molecular weight distribution and antioxidant changes of TSP were compared.

#### 2. Materials and methods

#### 2.1. Gamma irradiation

Tamarind kernel powder was irradiated in a cobalt-60 gamma irradiator (AECL, IR-79, Nordion, Ottawa, Canada). The source strength was approximately 11.1 PBq with a dose rate of 10 kGy/h and the actual doses were within 2% of the target dose. The absorbed dose was monitored with both free-radical and ceric/cerous dosimeters.

#### 2.2. Electron beam irradiation

ELV4-electron accelerator (energy 10 MeV, beam power 570 kW) was used for the electron beam irradiation of the tamarind kernel powder. The beam current was 1 mA. Irradiation was performed in the presence of air and the distance from the beam source to the sample was 50 cm. Tamarind seed powder was irradiated to 3 mm of thickness due to its low penetration.

#### 2.3. Extraction of TSP

Tamarind seed polysaccharide was prepared in three batches on a laboratory scale. To 20 g of tamarind kernel powder, 200 ml of cold distilled water was added and slurry was prepared. The slurry was poured into 800 ml of boiling distilled water. The solution was boiled for 20 min under stirring condition in a water bath. The resulting thin clear solution was kept overnight so that most of the proteins and fibers settled out. The solution was then centrifuged at 5000 rpm for 20 min. The supernatant was separated and poured into twice the volume of absolute ethanol by continuous stirring. The product was pressed between felts. The precipitate was washed with absolute ethanol, diethyl ether and petroleum ether and then dried at 50–60 °C under vacuum. The dried material was ground and sieved to obtain granules of different particle size range. The particle size range 150–75  $\mu m$  was used for preparation of tablets.

#### 2.4. Scanning electron microscope (SEM)

TSP samples were coated with gold by using a carbon coater (108-CA, Jeol, Tokyo, Japan). Microscopic observation of the cross-section was performed by using a 30 kV, 10  $\mu \text{Å}$  scanning electron microscope (JSM-6335F, Jeol, Tokyo, Japan).

#### 2.5. Measuring the molar mass by gel permeation chromatography

Gel permeation chromatography (GPC) was conducted to monitor the changes in the molar mass of the TSP by the irradiation. GPC was performed by a Waters system (Milford, MA) equipped with a separation module (Waters 2690), a refractive index detector (RI, Waters 2410), and a PLaquagel-60 column, -40 column, -30 column ( $300 \times 7.5 \ \text{mm}^2$ , 8  $\mu \text{m}$ , Polymer laboratories, UK). The mobile phase was 0.1 M sodium nitrate at a flow rate of 1 mL/min, and the column was operated at 40 °C. The injection volume was 200  $\mu \text{L}$  and the calibration was carried out using a pullulan standard (Showa Denko, Tokyo, Japan). The weight average molar mass was calculated using Empower software (System Software, Empower option GPC, Waters Co.).

#### 2.6. Superoxide radical scavenging ability

To  $0.3\,\mathrm{ml}$  of sample solution, added  $2.16\,\mathrm{ml}$  of  $50\,\mathrm{mM}$  of phosphate buffer pH 8.24, then added  $90\,\mathrm{\mu l}$  of  $3\,\mathrm{mM}$  pyrogallol. Zero and  $10\,\mathrm{min}$  readings were taken and the difference in the 0.D at  $325\,\mathrm{nm}$  was calculated for the superoxide radical scavenging activity, capacity was estimated from the difference in the absorbance for the samples or sample blank and expressed as a percentage of the superoxide radical scavenging activity.

#### 2.7. Total antioxidant capacity

The assay is based on the reduction of Mo (VI)–Mo (V) and subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto et al., 1999). The tubes containing sample solution and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95  $^{\circ}$ C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank.

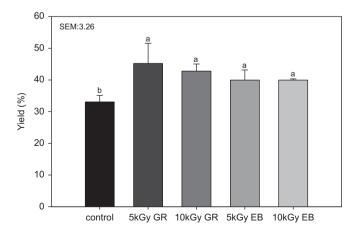
#### 2.8. Statistical analysis

The data were analyzed by SAS software (V. 8.02, SAS Institute, Cary, NC, USA). The general linear model procedure was processed and the Duncan's multiple range test was used to compare the mean values at P < 0.05.

#### 3. Results and discussion

#### 3.1. Extraction yield of TSP

The extraction yields of tamarind seed polysaccharide with 0, 5 and 10 kGy irradiation dose by electron beam and gamma ray showed in Fig. 1. Both irradiation treatments resulted in significant increases in the extraction yields for all samples (p<0.05) compared to control. But, there was no significant difference between the extraction yields from GM-treated TSP and EB treated. Also, there was no difference between 5 and 10 kGy of absorbed doses. The increase in the dry weights of TSP extracts following irradiation might be due to degradation of high molecular weight TSP, and changing these TSPs from non-soluble to soluble ones and/or easy to releasing from other attached components such as proteins or lipid. The increase in extraction yields with radiation treatment has also been reported



**Fig. 1.** Extraction yield of gamma ray (GR)- and electron beam (EB)-irradiated tamarind seed polysaccharide (TSP).

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