Effect of γ -irradiation on physico-chemical and antioxidant properties of galactan exopolysaccharide from *Weissella confusa* KR780676

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Abstract Exopolysaccharide (EPS) produced by *Weissella confusa* KR780676 from an Indian fermented food (*Idli*) has been characterized as galactan in earlier report. In this study, the galactan EPS was irradiated at different doses (0, 4, 8, and 16 kGy) using ^{60}Co as γ -source and effect of γ -irradiation on physico-chemical and antioxidant potential of galactan EPS was studied. Color and flow properties showed moderate change after the γ -irradiation treatment. Particle size analysis and scanning electron microscopy exhibited significant breakdown of the EPS particles into smaller average hydrodynamic diameter size from 495.54 to 198.44 nm with increasing irradiation doses. Overall, pH showed decrease, while the water activity showed an increasing trend with the increasing irradiation dose. No significant effect was observed in the functional groups of EPS as revealed in the FT-IR spectroscopic analysis. After the treatment, semi-crystalline nature of the EPS changed to amorphous as shown in XRD analysis due to the breakdown. Antioxidant activities of galactan EPS increased marginally after the treatment. However, hydroxyl radical scavenging activity increased significantly from 48 to 67%. Changes in physico-chemical and antioxidant properties of galactan EPS with γ -irradiation treatment altered the functional properties of EPS positively, making it more suitable for wider application in the food industry.

Keywords Galactan EPS · *Weissella confusa* KR780676 · γ -Irradiation · Physico-chemical · Antioxidant properties

Introduction

Bacterial EPS derived from wide sources are broadly studied and employed in the food industries as viscosifiers, emulsifiers, gelling agents, stabilizers and water-binding agents. In recent times, bacterial EPS especially from lactic acid bacteria (LAB) have attained more consideration due to its generally recognized as safe (GRAS) status and wide structural diversity (Patel and Prajapat 2013; Ryan et al. 2015). Also, EPS from LAB have been noted for several potential bioactivities viz. immunomodulatory, anti-inflammatory, immune stimulatory, antitumor, anti-diabetic, antioxidant and cholesterol-lowering activities (Li et al. 2015; Lynch et al. 2018).

Depolymerisation using chemical or enzymatic treatment shown to improve the functional and physico-chemical properties of the polymer (Bohn and BeMiller 1995; Ilyina et al. 2000). Even though these methods reduce the molecular weight, there are few drawbacks such as reduced yield, more processing time, high cost and acidic wastes after treatment (Byun et al. 2008). Gamma irradiation employed in sterilization of final biological materials also reported for depolymerisation by cleaving glycosidic bonds, thereby modifying their optical, electrical, mechanical and chemical properties. Ionizing radiation has also been known to enhance the bioactivities in carbohydrates like starch, chitosan, carrageenan, cellulose and sodium alginate without causing environmental pollution (Kume et al. 2002). The main advantages of radiation treatment are its potential to promote changes quantitatively and reproducibly, without any chemicals or special

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equipments/system that regulates environment and additives thereby making the technology more simple and environment friendly compared to conventional approaches (Charlesby 1981).

In our earlier studies, we have characterized the galactan EPS produced by *Weissella confusa* KR780676 from an *Idli batter* (traditional Indian fermented food) (Kavitate et al. 2016). Current study was undertaken to evaluate the effect of gamma irradiation on physico-chemical and antioxidant properties of galactan EPS in order to expand its utilization for diverse food applications.

Materials and methods

Microbial culture and EPS production

EPS producing probiotic strain *Weissella confusa* KR780676 formerly isolated from an Indian traditional fermented food was employed in the current study (Kavitate et al. 2016; Sharma et al. 2018). The EPS production was carried out according to the method explained in our previous report (Kavitate et al. 2016).

γ -Irradiation of galactan EPS

Purified galactan EPS (1 g) packed in a polyethylene bags were subjected to gamma irradiation ($^{60}\text{Co}\gamma$ - source irradiator) at an ambient temperature of $25 \pm 0.5^\circ\text{C}$. Galactan EPS was treated for various doses (4, 8, and 16 kGy) at a dose rate of 3 kGy/h and the dose was assured by a ceric-cerous dosimeter. This treatment was carried out in Central Instrumentation Facility (CIF), Pondicherry University, Puducherry, India.

Physical properties

Physical properties of both irradiated and native galactan EPS were studied viz. bulk density, tapped density, compressibility index and Hausner ratio (Oliveira et al. 2010).

Bulk density and tapped density

To measure the volume, galactan EPS (1 g) was subjected in a 5 mL glass graduated cylinder. The bulk density is the ratio between total weight (M) and initial volume of powder (V_0) whereas the tapped density (tap) is the ratio of total weight (M) and final volume of powder (V_f). Densities are calculated as per Eqs. (1) and (2) then expressed in g/mL.

$$\text{Bulk density} = \frac{M}{V_0} \quad (1)$$

$$\text{Tapped density} = \frac{M}{V_f} \quad (2)$$

Compressibility index and Hausner ratio

Bulk and tapped densities were used to calculate the compressibility index and Hausner ratio of galactan EPS as mentioned below in Eqs. (3) and (4).

$$\text{Compressibility index} = \frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}} \quad (3)$$

$$\text{Hausner ratio} = \frac{\text{tapped density}}{\text{bulk density}} \quad (4)$$

Colour analysis

The colour of galactan EPS was measured using Hunter Lab Colour Flex instrument A60-1012-312 (Hunter Associates Laboratory, Reston, VA). using Hunter colour values of L^* , a^* , b^* indicate lightness, green to red and blue-yellow color respectively (Tuyen et al. 2010). Chroma (indicating colour intensity) and hue angle (H°) were calculated using the following Eqs. (5) and (6).

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} \quad (5)$$

$$H^\circ = \arctan(b^*/a^*) \quad (6)$$

pH

For pH determination, EPS sample (0.2 g) was dissolved in 10 mL water at 25°C and measured using a digital pH meter (Hanna, USA).

Water activity (a_w)

Water activity of galactan EPS were estimated using a water activity meter (AquaLab PawKit, Decagon Devices, USA).

Water solubility index

WSI of the galactan EPS was measured according to Anderson et al. (1970) as mentioned in Eq. (7) and expressed in percentages. EPS (60 mg) was mixed with 6 mL distilled water, stirred continuously and kept in a water bath at 60°C for 30 min to get a homogeneous suspension. Then the suspension was centrifuged at

3000 rpm for 10 min and the supernatant was transferred into a petri dish and dried for 4 h at 105 °C to get the dry solid weight.

$$\text{WSI (\%)} = \frac{\text{Weight of dry solids in supernatant}}{\text{Weight of dry sample}} \times 100 \quad (7)$$

Fourier-transformed infrared spectroscopy (FT-IR) analysis

The FT-IR analysis of the galactan EPS has been carried out for assessing the impact of irradiation on structural and functional groups of the EPS. Dry potassium bromide (KBr) was mixed with the EPS powder (1 mg) to make a pellet. The spectrum peaks were observed at the wavelength ranges from 400 to 4000 cm^{-1} using FT-IR (Thermo Nicolet: 6700).

Particle size analysis

Galactan EPS samples (0.5% w/v) were dissolved in distilled water homogeneously and average particle size was measured by using a particle size analyser (Zetasizer, UK) at 25 °C.

X-ray diffraction (XRD) analysis

XRD analysis of both irradiated and native galactan EPS was carried out as described by Kavitha et al. (2016) with diverse ranges scan of two-theta angles (10–70 °C). XRD was accomplished on X-ray powder diffractometer (Shimadzu XRD 7000) at speed of 2°/min with a diffraction angle 2θ of 4° and 50° at 40 kV and 30 mA. Crystallinity index (CI_{xrd}) was measured from the area under crystalline peaks stabilized with corresponding to the total scattering area.

Scanning electron microscopy (SEM)

SEM (Hitachi, Model: S-3400N) was used to study the impact on morphology of galactan EPS after irradiation treatment. EPS was fixed on the aluminium stub and gold sputtered to examined through SEM at an accelerating voltage of 10 kV (Oliveira et al. 2010).

Antioxidant activity

Hydroxyl radical scavenging activity

Scavenging ability of galactan EPS sample was measured as per the method described by Kunchandy and Rao (1990)

with slight modifications. The reaction mixture consists of 200 μL of 2-deoxy-D-ribose (10 mM), 1.2 ml phosphate buffer (10 mM, pH 7.4), 1 mL of sample, 200 μL EDTA (10 mM), 200 μM FeCl_3 (10 mM) and 100 μL of H_2O_2 (10 mM) and incubated at 37 °C for 4 h. After incubation, 1.0 ml of trichloroacetic acid (2.8%) and 1.0 ml of thiobarbituric acid (1%) are added and incubated for 10 min at 100 °C. After cooling, the absorbance was measured at 532 nm, against a blank.

Thiobarbituric acid (TBA) method

TBA estimation for galactan EPS was carried as described by Ottolenghi (1959) with minor modifications. The final sample concentration of 1% w/v was used in this method. For assay, 2 mL of 0.67% of thiobarbituric acid and 2.0 mL of 20% trichloroacetic acid was added to 1 mL of sample solution and kept in a boiling water bath for 10 min. After incubation, the reaction mixture was allowed to cool and then it is centrifuged for 20 min at 3000 rpm. The absorbance was measured at 552 nm for all the samples of respective doses.

Statistical analysis

All the experiments were carried out in triplicates and the results were represented as mean $\pm$ SD. The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($p < 0.05$) using SPSS 24.0.

Results and discussion

Physical properties

Bulk density, tapped density, compressibility index and Hausner ratio of the galactan EPS were determined as a part of flow properties (Table 1). The bulk and tapped densities are used to study not only for packing and arrangement of particles but also the compaction profile of any given material. The bulk and tapped densities of galactan EPS increased gradually with increasing irradiation doses, inverse trend has been reported in Horse Chestnut starch (Wani et al. 2014) which may be due to the difference in the structure, morphology and degrading pattern of the samples. Whereas, compressibility index and Hausner ratio decreased at 16 kGy (20.06 and 1.25) compared to 0 kGy (36.78 and 1.581), respectively which revealed the improvement in flow ability of EPS after γ -treatment.

Table 1 Flow properties of γ -irradiated galactan EPS

Dose (kGy)	Bulk density (g/mL)	Tapped density (g/mL)	Compressibility index (%)	Hausner ratio
0	0.223 $\pm$ 0.03 ^a	0.343 $\pm$ 0.02 ^a	36.78 $\pm$ 0.04 ^a	1.581 $\pm$ 0.01 ^a
4	0.225 $\pm$ 0.02 ^a	0.353 $\pm$ 0.04 ^a	34.98 $\pm$ 0.10 ^a	1.538 $\pm$ 0.03 ^b
8	0.224 $\pm$ 0.04 ^a	0.345 $\pm$ 0.03 ^a	35.01 $\pm$ 0.08 ^a	1.538 $\pm$ 0.01 ^b
16	0.380 $\pm$ 0.03 ^b	0.476 $\pm$ 0.04 ^b	20.06 $\pm$ 0.06 ^b	1.25 $\pm$ 0.02 ^c

Results are mean of three determinations $\pm$ SD. Mean values bearing different letters a, b, c within the same column are significantly different ($p < 0.05$) on application of Duncan's multiple range test

Colour characteristics

The colour parameters of the galactan EPS have a slight impact after irradiation treatment (Table 2). The maximum value for lightness (L^*) is 100 indicating white and L^* of galactan EPS (0, 4, 8 and 16 kGy) is 81.16, 80.57, 79.93 and 78.33 respectively, which is near to lightness with a slightly decreasing manner with respect to doses. The positive a^* and b^* values of control and irradiated galactan indicates the reddish and yellowish colour. Also, increased significantly ($p < 0.05$) in C^* from 17.17 to 29.11 of the galactan EPS (0–16 kGy) revealed increased color intensity. A high value for lightness and low value for chroma are desired for the product to meet consumer preference. Hue angle for all the irradiated samples has been decreased significantly ($p < 0.05$) to 74.64 when compared to control (78.58), but still in the same range of yellowness and redness. Similar irradiation effect on lightness has been reported for freeze dried *Amorphophallus paeoniifolius* flour by Suriya et al. (2017).

pH

The pH of γ -irradiated galactan EPS (Fig. 1) showed a significantly ($p < 0.05$) decreasing trend (6.76, 6.52, 6.48 and 6.36) with the increase in irradiation doses (0, 4, 8 and 16 kGy). Similar trend has been observed by Bashir and Haripriya (2016) with chickpea starch after γ -irradiation. The changes in pH have been attributed to free radical formation and cleavage of large starch molecules during irradiation (Sabularse et al. 1991). Formation of acetic,

Fig. 1 pH (a) and water activity (b) of native and γ -irradiated galactan

formic, glucuronic and pyruvic acids during starch irradiation could also result in decrease of pH (Ghali et al. 1979).

Water activity (a_w)

Generally, foods with $a_w < 0.6$ are considered to be microbiologically stable and if spoilage occurs, it is mainly due to chemical reactions rather than by microorganisms. High water activity specifies more free water available for biochemical reactions and hence shorter the shelf life. Irradiation (0, 4, 8 and 16 kGy) moderately increased the water activity (0.42, 0.46, 0.48 and 0.57) (Fig. 1) of galactan EPS, nevertheless still values are in microbiologically stable range.

Table 2 Colour characteristics of γ -irradiated galactan EPS

Dose (kGy)	L^*	a^*	b^*	Chroma (C^*)	Hue ($^\circ$)
0	81.16 $\pm$ 0.03 ^a	3.43 $\pm$ 0.04 ^a	16.83 $\pm$ 0.02 ^a	17.17 ^a	78.58 ^a
4	80.57 $\pm$ 0.10 ^a	3.61 $\pm$ 0.02 ^c	18.19 $\pm$ 0.06 ^b	17.61 ^a	74.52 ^b
8	79.93 $\pm$ 0.04 ^a	3.57 $\pm$ 0.06 ^b	18.30 $\pm$ 0.03 ^b	17.76 ^a	74.35 ^b
16	78.33 $\pm$ 0.05 ^a	3.64 $\pm$ 0.03 ^c	17.63 $\pm$ 0.04 ^b	29.11 ^b	74.64 ^b

Results are mean of three determinations $\pm$ SD. Values with same letters (a, b, c within columns) are not significantly different at $p < 0.05$

Water solubility index (WSI)

During radiation treatment, depolymerisation and breakdown of the polysaccharide could induce the production of shorter fragments (low molecular weight) leading to increase in solubility (Graham et al. 2002). The WSI of irradiated galactan EPS (0, 1, 4, 8 and 16 kGy) remained at 100% and does not have any negative impact after γ -irradiation treatment. This high degree of water solubility may be due to interaction of EPS with water through hydrogen bonding, which remains same even after at higher dose of γ -treatment due to the intensive hydrogen bonding of EPS with water. These results exhibit a potential choice for galactan EPS as biothickener and stabilizing agent.

FT-IR analysis

During irradiation, carbohydrate deformation might occur due to cleavage of glycosidic bonds. To confirm this effect, galactan EPS has been analysed using FT-IR before and after irradiation (Fig. 2). The broad absorption peak at 3425 cm^{-1} was recognized with the extend vibration of hydroxyl group (O–H) existing in all polymers. The strong peak at 2919 cm^{-1} indicates the C–H stretching vibration in the sugar ring (Li et al. 2014) and the strong absorption peak at 1621 cm^{-1} exhibit the C=O and carboxyl group stretching vibration (Ye et al. 2014). The presence of solid

absorbance in the area $1200\text{--}950\text{ cm}^{-1}$ showed the polysaccharide character of the EPS. A strong stretch at $1000\text{--}1200\text{ cm}^{-1}$ (C–O–C, C–O) corresponds to the presence of carbohydrates (Kavitake et al. 2016). The solid stretching absorption peak at 1153 and 1012 cm^{-1} affirmed the polysaccharide and pyranose ring nature of the EPS (Chen et al. 2015). In addition, the band at 867 cm^{-1} showed the presence of glycosidic linkages of the EPS (Li et al. 2015). Based on the above results, the native and irradiated galactan EPS seems to depict a similar FT-IR, revealing that no new functional groups were added by irradiation process. The increased (4 kGy and 8 kGy) and decreased (16 kGy) intensities by irradiation exhibited alteration in the ordered structure of irradiated galactan EPS. This result is in agreement with previous report of Byun et al. (2008) about irradiated and non-irradiated β -glucan.

Particle size analysis

Dynamic laser light scattering principle was used to calculate the average particle size of the galactan EPS (Zheng et al. 2015). The result of both γ -irradiated and un irradiated galactan EPS is plotted in Fig. 3 and it is clear that the average hydrodynamic diameter size of galactan polymer constantly decreases towards an increased dose of irradiation. Reduction in particle diameter is ascribed to

Fig. 2 FT-IR spectra of galactan EPS irradiated at different doses

Fig. 3 Particle size analysis of galactan EPS as measured by dynamic light scattering (DLS) method (**a** 0 kGy, **b** 4 kGy, **c** 8 kGy and **d** 16 kGy)

formation of smaller granules due to high energy radiations and generation of free radicals during irradiation (Yu and Wang 2007; Grant and D'apponia 1991). The average hydrodynamic diameter size of native galactan EPS is 495.54 nm, which is greatly reduced to 303.33, 235.30 and 198.44 nm at 4, 8 and 16 kGy respectively. It is clear that the non treated sample possess a maximum particle size, while the γ -irradiation treatment significantly reduced the particle size. Change in size of the galactan EPS with respect to γ -irradiation treatment could alter its functional properties that can lead to a wide range of applications in food industries.

X-ray diffraction (XRD) analysis

X-ray diffractograms of control (0 kGy) galactan EPS showed higher intensity of the peaks at 43.64 and 51.02 °C, with 0.23 crystallinity index (Kavitake et al. 2016). While,

irradiated galactan EPS (4, 8 and 16 kGy) did not show presence of any intensity peak, revealing the change in nature of EPS from semi-crystalline to amorphous (Fig. 4). Likewise, a decrease in the relative crystallinity of bean starches and potato after gamma-irradiation at 50 kGy was reported by Chung and Liu (2010).

Scanning electron microscopy (SEM)

The SEM analysis of irradiated galactan EPS samples (Fig. 5) clearly confirms the breaking down of galactan EPS particles as shown above in the particle size analysis. This kind of breakage resulted in an increased number of small sized EPS granules, in a dose dependent manner which in agreement with Singh et al. (2011) where an increase in the proportion of small size starch granules were observed after irradiation. Result showed a significant difference in the average granule size between irradiated

Fig. 4 X-ray diffraction patterns of native and irradiated galactan EPS

Fig. 5 SEM of gamma treated galactan EPS (**a** 0 kGy, **b** 4 kGy, **c** 8 kGy, **d** 16 kGy). Arrow indicates small molecules broken down due to irradiation process

EPS granules (4, 8 and 16 kGy) compared to control (0 kGy).

Antioxidant activities

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity depends upon the deoxyribose degradation by hydroxyl radicals produced during Fenton reaction. Under acidic conditions, boiling of degraded products with thiobarbituric acid results in a pink chromogen that can be measured at 532 nm (Koppenol et al. 2010; Abad et al. 2013). Irradiation doses of 4 kGy does not have any significant impact on the scavenging activity, which is supported with previous studies by Hussain et al. (2018) and Abad et al. (2013). While, higher doses of 8 and 16 kGy showed the maximum scavenging activity up to 51.60 and 67.21%, respectively at 10 mg/ml of galactan EPS compared to control (47.71%) (Supplement 1-a). Increased activity of polysaccharides could be either through hydrogen or electron donation or formation of different functional groups in irradiated galactan EPS due to structural degradation (Hussain et al. 2018).

Thiobarbituric acid (TBA) method

Thiobarbituric acid number assay is most commonly used for determination of lipid peroxidation (Anwar et al. 2015). Briefly, lipid oxidation proceeds via a free radical chain mechanism results in many end products, with aldehydes being prominent among them (Ghani et al. 2017). These aldehydes react with thiobarbituric acid to form TBARS (Thiobarbituric acid reactive substance) whose absorption is measured to determine the antioxidant activity. The TBA value of native and irradiated galactan shows not much difference in its antioxidant value (Supplement 1-b). Gamma irradiation at 4 and 8 kGy did decrease the absorption of galactan EPS marginally from 0.058 (0 kGy) to 0.055 and 0.053, respectively. This slight decrease in absorption correlates well with the hydroxyl radical scavenging activity. The antioxidant activity increased slightly up to 0.6 at 16 kGy, which could be due to the formation of TBA reacting substances and/or possibility that irradiation could activate the autoxidation chain reactions.

Conclusion

In this study, γ -irradiation was used as an effective method for breakdown of the galactan EPS into low particle size without causing any notable changes in the functional-group status. Water activity, pH and antioxidant potential of the EPS slightly increased with high irradiation doses.

Moderate changes in colour, flow properties and semi-crystalline nature of EPS was observed with irradiated galactan. Additional studies need to be carried out to evaluate the effect of irradiation treatment on the other biological properties of galactan EPS such as anti-microbial, immunomodulatory, immunostimulatory, anticoagulant and anti-inflammatory activity which will make this EPS a promising candidate for food and pharma industries.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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