



Physico-chemical characterization of galactan exopolysaccharide produced by *Weissella confusa* KR780676



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ABSTRACT

Physico-chemical properties of a galactan exopolysaccharide (EPS) produced by *Weissella confusa* KR780676 isolated from an idli batter were studied. In our previous paper, we had reported the isolation and characterization of a linear galactan (containing α -(1 $\rightarrow$ 6)-linked galactose units) isolated from an acidic fermented food. In this manuscript, we are reporting the physico-chemical properties viz., Colour evaluation (L^* 81.79, a^* 3.15, b^* 15.04), flow properties (compressibility index 38.46%), syneresis, light transmittance (50.7% for 5 days), pasting properties (temperature 64.6 °C), texture (hardness 9.876 N) and particle size (549.81 d nm) for the galactan were carried out. This galactan also possessed high oil absorption capacity (5.09 mL/g) with good emulsifying activity (69.4%) and emulsion stability up to 15 days. Flocculating activity of 65.3–81.7% was observed with 0.2 mg/mL EPS in a wide range of pH (2.0–12.0). These functional properties will make the galactan EPS a good candidate for use as thickening and gelling agent and emulsifier to form long-term emulsions in food, pharmaceutical and cosmetic products, as well as for bio-treatment of wastewater and hydrocarbon-polluted environments.

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1. Introduction

Exopolysaccharides (EPS) are biological high-molecular weight extracellular biopolymers that are produced during the metabolic process of microorganisms, such as bacteria, fungi and blue-green algae [1]. Recently, EPS from lactic acid bacteria (LAB) have attracted more and more attentions as they are generally regarded as safe. Also the ever increasing demand for natural products is raising interest in use of EPS-producing LAB as natural bio-thickeners, especially due to its functional properties and potential bioactivities such as immunomodulatory, immunostimulatory, antitumor, anti-inflammatory and antioxidant activities [2]. EPS from LAB can also be utilized as prebiotics by human intestine bacteria to promote the proliferation of other probiotics [3,4]. EPS-producing LAB are industrially important microorganisms in the development of functional food products and used as starter cultures or coadjutants to develop fermented foods such as yoghurt, cheese and cereal-based products [3,5–8] due to their viscosity and mouth-feel enhancement properties.

EPS producing LAB have been isolated from various Indian traditional fermented foods [9,10]. Idli is one such popular cereal-legume based fermented food known for its sensory appeal, easy digestibility and good nutritive value [11]. Some of the known EPS producing LAB isolated from idli batter are *Leuconostoc mesenteroides*, *Weissella confusa*, *W. cibaria* and *Pediococcus parvulus* [9,12]. Recently, we reported the galactan EPS production by *Weissella confusa* KR780676 isolated from an acidic fermented food, which is the first study reporting a 100% linear galactan (containing α -(1 $\rightarrow$ 6)-linked galactose units) from lactic acid bacteria [13].

Galactans are defined as polymers of galactose, may be homopolymers of galactose (galactans) or heteropolymers containing variable amounts of galactose, besides other sugar residues such as glucose, mannose, rhamnose, arabinose and/or fructose. Many of these polymers contain, besides neutral sugars, acidic sugars (glucuronic acid, galacturonic acid) or amino-sugars (N-acetylglucosamine, N-acetyl-galactosamine). The presence of non-sugar components, such as acyl groups (acetate esters, pyruvate ketals, succinyl half esters) or inorganic residues (ex. sulphate, phosphate) is also quite common [14]. Sulfated galactans and galactan-containing polysaccharides are widely distributed in terrestrial plants and macroalgae; possessing therapeutic applications such as anticoagulant, immunomodulatory, antithrombotic, antiviral and antitumor effects [15]. Galactan structures (D-galactopyranose) are also reported from *Methylobacterium* sp. [16] and *Lactobacil-*

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lus plantarum 70810 as linear and branched galactosyl residues possessing antitumor activities [17]. Galactose rich heteropolysaccharide are reported by *Lactococcus lactis* subspecies *cremoris* H414 [18] and *Lactococcus lactis* subsp. *cremoris* B891 [19]. The structural and physico-chemical properties of EPS molecules determine their technological applications and biological activities. Hence, this investigation was aimed at studying the physico-chemical properties of galactan from *Weissella confusa* KR780676.

2. Materials and methods

2.1. Microorganisms and materials

The EPS produced by *Weissella confusa* KR780676 isolated from idli batter (an Indian acidic fermented food) which has been characterized as galactan was used for the present study [13]. All reagents used were of analytical grade.

2.2. Extraction of galactan EPS

The galactan-EPS was isolated and purified as stated in our previous study [13]. Briefly, freshly prepared inoculum (10%) of *W. confusa* was grown in 2% sucrose supplemented MRS broth at 30 °C for 48 h under static condition. The suspension was subsequently centrifuged (12000 × g for 15 min) to separate the biomass and further treated with tri-chloro acetic acid to removing the protein moieties. The galactan-EPS was precipitated using ice cold ethanol (thrice the volume), centrifuged (19200 × g for 15 min) and the resultant crude EPS was dissolved in Milli-Q water. Finally, the crude galactan was subjected to dialysis (12–14 kDa) and freeze dried for further physico-chemical analysis.

2.3. Colour evaluation

The colour evaluation of galactan EPS (5 g) was assessed using Hunter Lab Colour Flex instrument A60-1012-312 (Hunter Associates Laboratory, Reston, VA). The values of lightness (L^*), redness (a^*), and yellowness (b^*) were measured and the colour related parameters, such as chroma and hue angle ' θ ', were calculated.

2.4. Flow properties

Flow properties of galactan were measured using parameters such as bulk density, tapped density, compressibility index and Hausner ratio [20,21].

2.4.1. Bulk density and tapped density

Two grams of powdered galactan was placed in a 10 mL glass graduated cylinder and their volume was measured. The bulk density (ρ_{bul}) was calculated as the ratio of net weight (M) and initial volume of powder (V_0). The tapped density (ρ_{tap}) was calculated as the ratio of net weight (M) and final volume of powder (V_f). Both the densities were expressed in grams per milliliters (g/mL).

$$\rho_{bul} = M/V_0$$

$$\rho_{tap} = M/V_f$$

2.4.2. Compressibility index and hausner ratio

Compressibility index and Hausner ratio for galactan was calculated by using following equations:

$$\text{Compressibility index} = (\rho_{tap} - \rho_{bul})/\rho_{tap}$$

$$\text{Hausner ratio} = \rho_{tap}/\rho_{bul}$$

2.5. Syneresis and light transmittance

The ability of galactan-EPS to minimize the liquid separation (syneresis) of cooked starch (2%) paste during refrigeration was

investigated as described by Ismail and Nampoothiri [22]. Thick paste of 2% starch was prepared by cooking for 10–15 min in a boiling water bath with gentle mixing. Different concentrations (0.1, 0.25 and 0.5%) of the galactan-EPS were blend with 2% starch paste, stirred using a magnetic stirrer until complete dissolution and allowed to reach room temperature. Sodium benzoate (1 g/L) was added as an antimicrobial agent. The samples (10 mL) were poured into screw-cap plastic tubes and kept vertically at 4 °C for 10 days. The extent of syneresis was measured at one-day interval along with positive (2% starch) and negative (2% galactan-EPS) controls. The degree of syneresis was calculated by $\Delta h/h_0$, where h_0 stands for initial height of the sample dispersion and Δh stands for height of the liquid phase above the sediment.

The light transmittance of galactan polymer was determined using the method described by Wani et al. [23]. Galactan polymer solutions (1 & 2%, w/v) was prepared in water and heated at 90 °C in a water bath for 30 min with constant stirring. The suspension was cooled for 1 h at 30 °C and stored for 5 days at 4 °C in a refrigerator. The transmittance was determined at every 24 h by measuring the absorbance at 640 nm against water blank in a UV–vis Spectrophotometer (UV-1800, Shimadzu, North America).

2.6. Oil absorption capacity (OAC)

OAC was measured as described by Abbey and Ibeh [24]. Ten mL of oil (Soy bean oil) was added to 1 g of galactan polymer in a centrifuge tube of known weight. The mixture was allowed to stand for 30 min and centrifuged at 3500g, 15 min. After discarding the supernatant, the tube along with residue was weighed and the gain in weight was recorded as OAC.

$\text{OAC (mL/g)} = \text{Weight of wet sample residue} - \text{Weight of dry sample}$

2.7. Emulsifying properties of galactan

2.7.1. Determination of emulsifying activity (EA)

Three mL of sunflower oil was added to 2 mL of EPS solution (1%, w/v) in a screw cap glass tube (100 mm × 13 mm), vortexed for 3 min at 40 Hz and stored at 25 °C. After 1, 24, 168 and 360 h, the emulsion index (EA_1 , EA_{24} , EA_{168} and EA_{360}) was determined as given below:

$$\text{Emulsifying activity (EA)} = (h_e/h_t) \times 100$$

where, h_e is the height of the emulsion layer and h_t is the overall height of the mixture.

2.7.2. Microscopic evaluation of emulsions

A volume of 100 μ L emulsion formed after 24 h storage at 25 °C was examined through a 10 × objective lens of light microscope using a cavity microscope slide.

2.8. Pasting properties

The viscoamylographic property of galactan powder was performed as described by Reddy et al. [25] with Rapid Visco Analyser (RVA starch master 2, Newport Scientific, Warriewood, NSW, Australia). The following parameters such as paste temperature, peak viscosity; breakdown viscosity, final viscosity and setback viscosity were determined.

2.9. Texture studies

Back extrusion (BE) test was performed for investigating the textural properties of the galactan polymer with texture profile analyzer (Texture analyzer (TA) TA-HD plus, Stable Micro Systems,

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