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Characterisation of a new exopolysaccharide obtained from of fermented kefir grains in soymilk

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ABSTRACT

Microbial exopolysaccharides (EPSs) have been widely studied in recent decades due to similarity to gums used in the food industry. Exopolysaccharides can be used in food processing as a thickener and/or stabiliser. This study aimed to investigate the physicochemical properties, thermal behaviour and structural composition of the lyophilised EPS obtained from the fermentation of kefir grains in soymilk. The EPS in concentration 18 mg/mL exhibited water activity of 0.204 and pH = 6.20 at 25 °C, reducing sugars content of 22.10% (v/v) and protein content of 2% (v/v). The thermogravimetric curve obtained was similar to those reported in the literature for other EPSs. The degradation temperature was 351.84 °C and showed that the EPS in this study had a high thermal stability. Characteristic polysaccharide bands were observed in the infrared spectrum. The analysis by liquid chromatography coupled to electrospray ionisation mass spectrometry (LC–ESI-MS) showed that the EPS is only composed of glucose.

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1. Introduction

Exopolysaccharides (EPSs), also called extracellular polysaccharides, are defined as carbohydrate polymers that can be produced by widely different micro-organisms. These biopolymers are associated with the cell surfaces in the form of capsules or are secreted into the extracellular medium in the form of mucus. Microalgae and bacteria produce large amounts of EPSs, while yeasts and fungi produce small amounts. Some micro-organisms produce EPSs in amounts satisfactory for use on an industrial scale, including curdlan, xanthan and dextran gums (Silva et al., 2006; Sutherland, 1990).

Exopolysaccharides are water soluble gums that have long linear or branched chains consisting of monosaccharides, mainly glucose, galactose, mannose and glucuronic acid or derivatives of these sugars such as rhamnose, in different proportions (Welman and Maddox, 2003). These biopolymers are used as emulsifiers, stabilisers, gelling agents, binders, lubricants, coagulants and thickeners, and they are also used in the production of suspensions and films. These applications rely on the chemical structure of the biopolymers. The food industry has widely used these biopolymers, especially to alter the texture, viscosity and elasticity of food (Duboc and Mollet, 2001; Rottava et al., 2009).

The EPS obtained from the fermentation of kefir grains in soymilk has been minimally studied. In a previous study, it was observed that this EPS from the fermentation of soymilk is composed of the monomers glucose and galactose in the ratio of 1.00:0.43. This composition was compared to the monosaccharide composition of the polysaccharide obtained from kefir in cow's milk, which has a ratio of 1.00:1.14 (glucose:galactose) (Liu, Chen, and Lin, 2002). Using the above information as a starting point, this study aimed to verify some of the physicochemical properties of the EPS extracted from fermented kefir grains in soymilk and structurally characterise this EPS for possible further applications in the food and cosmetic industries.

2. Methods

2.1. Materials

The glucose, anthrone reagent and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (Saint Louis, MO, USA).

2.2. Obtaining the exopolysaccharide (EPS)

The EPS was obtained from the commercial kefir product called Kefir BioLogicus® Ind. e Com. de Produtos Naturais S.A., which is located in the city of Recife, State of Pernambuco, Brazil. The extraction was performed according to the procedure used by Yokoi,

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Watanabe, Fujii, Toba, and Adachi (1990), with some modifications. The obtained solution was precipitated with cold ethanol and centrifuged in a refrigerated CIENTEC CT-6000R 115 mm centrifuge (10,000 × g, 20 min, 20 °C) to remove most other compounds present in the solution and obtain only the EPS. The obtained EPS was then subjected to lyophilisation. The lyophilised EPS was macerated and stored under refrigeration at 5 °C for further analysis.

2.3. Physicochemical analysis

The concentrations of the reducing sugars were determined by the method of Antrona (Yemm and Folkes,1954), using glucose as a standard. The protein content was verified by the method of Bradford (Bradford, 1976), using bovine serum albumin as a standard. The water activity (A_w) of the lyophilised EPS was determined using a Water Activity AquaLab 4TEV meter. The pH of the solution containing the EPS at a concentration of 10% (w/v) was read directly by a Tecnal pH meter, using buffers with pH values of 4 and 7 as references.

2.4. Infrared (IR) spectroscopic analysis

The IR spectra were obtained by a 640-IR spectrometer (Varian), using the total internal reflection or attenuated total reflectance (ATR) technique. The spectra were recorded over the wavenumber interval of 600–4000 cm^{-1}, using 32 scans to achieve the best signal noise ratio.

2.5. Thermogravimetric analysis (TGA)

To study the thermal properties of the obtained EPS, we used a SHIMADZU TGA system under atmospheric pressure. A sample of 5.5 mg of EPS was placed in a Pt crucible. The system was programmed with a heating rate of 10 °C/min under a N_2 atmosphere, a flow rate of 50 mL/min and a sweep range from 25 to 600 °C.

2.6. Liquid chromatography–electrospray ionisation mass spectrometry (LC–ESI-MS)

The monosaccharide composition of the EPS was analysed by LC–ESI-MS using an Esquire 3000 Plus chromatograph (Bruker Daltonics) with a capillarity of 4000 V, a gas flow of 5 L/min, a temperature of 320 °C and a pressure of 12 psi. The EPS was hydrolysed with 2 M trifluoroacetic acid (TFA) at 120 °C for 2 h and derivatised with *p*-aminobenzoic acid ethyl ester (ABEE) (Gomis, Tamayo, and Alonso, 2001; Kwon and Kim, 1993) before being subjected to LC–ESI-MS.

3. Results and discussion

3.1. Physicochemical analysis

The pH of the EPS solution was slightly acidic but near neutral (pH = 6.20 ± 0.05). The pH of kefir reported in the literature is between 4.2 and 4.6. This lower pH value can be influenced by the other constituents present, such as CO_2, acids, lactose, ethanol and the amounts and types of protein and the fat content (Dadkhah, Pourahmad, Assadi, and Moghimi, 2011; Irigoyen, Arana, Casteilla, Torre, and Ibanez, 2005; Kesenkaş et al., 2011). The difference between the pH values of the EPS solution and the kefir is most likely related to the extraction process. The extraction process removes compounds that decrease the pH, such as CO_2 and lactic acid, which are present in the fermented beverage.

The EPS showed an A_w value of 0.204 ± 0.001 at 25 °C, which places it in zone A according to Fennema, Damodaran, and Parkin (2010). In this zone, food supplies have good shelf life stabilities and are therefore resistant to deterioration over time.

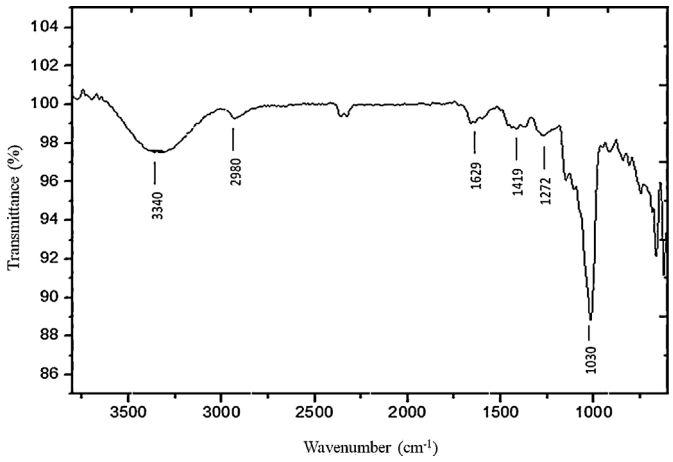

Fig. 1. Infrared absorption spectrum of EPS.

The protein analysis using the method described by Bradford (1976) showed that the EPS exhibited a low protein content of approximately 2% (v/v) of the total sample. Values near those found in this investigation (2.3% protein) were found by Liu et al. (2002) in the EPS obtained by the fermentation of kefir grains in soymilk after precipitation with ethanol and by Piermaria, La Canal, and Abraham (2008). Using the same method of analysis, Piermaria et al. (2008) also identified a low protein content for a solution of EPS obtained from the fermentation of kefir grains in bovine milk. The concentration of the reducing sugars or total soluble sugars (ATS) of the studied EPS was 221.03 mg/L, or 22.10% (v/v) of the sample. This result can be attributed to the small amount of terminal reducing sugars present in the EPS solution. This low reactivity can also be explained by the partial hydrolysis of the sugars using the proposed method of analysis, which is insufficient to break all of the glycosidic linkages present in the EPS.

3.2. IR spectroscopic analysis

In the IR spectrum shown in Fig. 1, a band was found at 3440 cm^{-1} that corresponded to the hydroxyl groups (Bramhachari et al., 2007; Howe, Ishida, & Clark, 2002; Wang, Ahmed, Feng, Li, & Song, 2008; Wang et al., 2010). The band at 2980 cm^{-1} can be attributed to methyl and methylene groups (Bramhachari et al., 2007; Wang et al., 2008, 2010). The bands in the range between 1660 and 1220 cm^{-1} approximately, are asymmetric and symmetric characteristics stretching of links C—O (Haxaire, Maréchal, Milas, and Rinaudo, 2003; Wang et al., 2008, 2010). The region of 1000–1200 cm^{-1} has showed an intense band which is characteristic of stretches C—O—C and C—O of alcohol groups in carbohydrates. The band at 1030 cm^{-1} is characteristic of polysaccharide compounds (Haxaire et al., 2003; Wang et al., 2008, 2010). These bands indicate that the substance is a polysaccharide, according to the findings of Cho, Amy, Pellegrino, and Yoon (1998) and Nataraj, Schomacker, Kraume, Mishra, and Drews (2008).

3.3. Thermogravimetric analysis (TGA)

The literature reports that certain common substances, both volatile and non-volatile, are formed by the thermal degradation of different types of carbohydrates. According to Fagerson (1969), during the initial temperature increase, the primary events that appear in the thermograms are gelatinisation and swelling. Increasing the temperature further causes the following three events to occur: dehydration, pyrolysis and the reorganisation of linkages.

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