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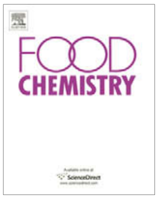

Molecular mass distribution of dextran in Brazilian sugar and insoluble deposits of cachaça

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ABSTRACT

The dextran molecular mass distribution profile in 77 sugar samples from Brazil and twelve insoluble deposits (alcoholic flocks) samples from sugared cachaças (Brazilian sugar cane spirit) is described in terms of number-average molecular mass M_n, weight-average molecular mass M_w, Z-average molecular mass M_z, and polydispersity. The analyses were performed by size-exclusion chromatography, using a refractive index detector. In most of the sugar samples, it was possible to identify two major groups of dextrans with M_w averages of 5×10^6 and 5×10^4 Da. Based on the evaluated parameters, the dextran distribution profile is about the same in samples analyzed over five seasons, and, therefore, it is likely that the Brazilian product pattern will not change very much over the years. In insoluble deposits from sugared cachaças, dextrans with M_w values in the order of the 10^5 Da were the most frequent ones, being present in 58% of the samples.

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1. Introduction

Brazilian sugar production in the 2006/2007 crop season was approximately 30.7 million tons, and the Brazilian southeast region accounted for 26.4 million tons. São Paulo state, which is located in the southeast, accounts for about 70% of the production of its region. The Brazilian northeast region accounts for the remaining 4.3 million tons (Rocha, 2007; Brasil – Ministério da Agricultura, Pecuária e Abastecimento, 2008). The ethanol production in the same period was about 22.2×10^9 m^3; and 91.1% of production was in the southeast.

Brazilian sugar cane spirit, which is commonly known as cachaça, is the third most abundant industrial product from sugar cane with an estimated production of 2×10^9 l per year. It is only overcome by vodka and soju productions (Aquino, Boso, Cardoso, & Franco, 2008). At this time, less than 20 million litres of cachaça are exported per year (Aquino et al., 2008; Fernandes et al., 2007).

Today, Brazil is the world's largest sugar cane producer and exporter, respectively accounting for 13% and 45% of the world's production and exportation (ISO – International Sugar Organization, 2007; Rocha, 2007). Brazil is also the largest sugar consumer, with an average *per capita* consumption of 59.4 ± 0.2 kilogrammes within the period 2004 to 2006 (ISO – International Sugar Organization, 2007). Food manufacturers, including those that produce carbonated drinks, chocolate, ice cream, and the like, account for approximately 35–45% of the sugar consumption, while domestic use accounts for the remaining 55–65% (Bolling & Suarez, 2001).

Considering Brazil's large territorial area, the suitable climate for sugar cane production, the accumulated knowledge of sugar cane cultivation, the development of new sugar cane varieties, and the fact that crops are continuously harvested over the year (from April to November in the southeast, and from September to March in the northeast), Brazil is enhancing its production and exportation potential in sugar and sugar cane derivates (Bolling & Suarez, 2001; Baldani, Reis, Baldani, & Döbereiner, 2002; Rocha 2007). Therefore, strict quality policies to control sugar cane and its products should be rigorously followed and improved, whenever possible.

The quality of the sugar cane crops supplying the factories is a critical point in production costs and product quality (Eggleston, Legendre, & Tew, 2004; Rauh, Cuddihy, Falgout, & Marquette, 2003). In sugar cane juices, compounds, such as mannitol and isomaltooligosaccharides (Eggleston & Harper, 2006; Eggleston et al., 2004), were suggested as chemical indicators of cane degradation, and, consequently, they have been used to predict and control processing problems in sugar production plants. However, in Brazil, dextran is the most common contaminant indicator used for quality control purposes in the sugar cane industry (Oliveira, Rinaldi, Tamanini, Voll, & Hauly, 2002).

Dextran $(C_6H_{10}O_5)_n$ is synthesized from sucrose by dextransucrase enzymes which are excreted by microorganisms such as *Streptococcus*, *Lactobacillus* and *Leuconostoc mesenteroides*, the last of these being the predominant species in sugar cane fields. The glucose monomers are predominantly linked by α(1,6) bonds in

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their major chains with a variable percentage of α(1,3) and occasional α(1,2) or α(1,4) branched linkages (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005). The presence of dextran is associated with operational problems in sugar mills (Jiménez, 2005; Khalikova, Susi, & Korpela, 2005; Rauh et al., 2003; Ravno & Purchase, 2006), and with spoilage in other food industries, such as candy and chocolate manufacture (Edye, 2004; Haynes, Zhou, & Hopkins, 2004; Ravno & Purchase, 2006). Furthermore, since sugar cane is used as a sweetener in alcoholic and soft drinks, the presence of dextran could lead to the formation of haze and precipitations (Edye, 2004; Rodrigues-Filho et al., 2007).

Despite the relevance of the problems caused by the presence of dextrans in sugar in the food and beverage industries, the dextran molecular mass distribution profile in Brazilian processed sugar has not yet been reported. Aiming to contribute to the development of sugar cane technology, we present here the dextran molecular mass distribution profile in Brazilian sugar and isolated insoluble deposits (alcoholic flocks) from sugared cachaças in terms of number-average molecular mass M_n, weight-average molecular mass M_w, Z-average molecular mass M_z, and polydispersity (M_w/M_n) (Chmelík, Chmelíková, & Novotny, 1997; Karmarkar, Garber, Klusa, & Koberda, 2006; Kostanski, Keller, & Hamielec, 2004).

2. Materials and methods

2.1. Samples

A general picture of the dextran molecular mass distribution profile was obtained for the 2006/2007 season by analyzing 25 sugar samples from São Paulo (SP) state, which is the largest producer in the southeast (responsible for 70% of the production in its region), and twelve sugar samples from the northeast. The production sites in São Paulo state and their respective number of samples were: Catanduva (6), Jaboticabal (4), Sertãozinho (2), Quatá (2), Rincão (2), Lençóis Paulista (2), Ribeirão Preto (2), São Carlos (1), Araras (1), Cerquilho (1), Itapira (1), Mococa (1). The samples were provided by Centro de Tecnologia Canavieira – CTC (Piracicaba, SP). The providers of sugar samples from the northeast, which are sugar mills, and their production sites are: Alteza I (Ipojuca, PE), Alteza II (Rio Formoso, PE), Caeté (São Miguel dos Campos, AL), Coruripe cristal (Coruripe, AL), Coruripe demerara (Coruripe, AL), Estrela (Arês, RN), Titara (São Luiz do Quitunde, AL), Sublime (Primavera, PE), Coceal (Vitória da Conquista, BA), Singular (Alvorada, BA), and Padin (Itabuna, BA). Regarding the samples from the northeast, all the sugar mills provided one sugar sample, except for Caeté, which provided two sugar samples.

The possible variations in the dextran molecular weight distribution profile in sugar samples during four consecutives cane crops (seasons 1997/1998, 1998/1999, 1999/2000, 2000/2001), were evaluated in an additional forty samples, which were collected from ten representative sugar mills in the southeast region. These sugar samples from the producers listed were supplied and certified by the Instituto de Tecnologia de Alimentos – ITAL (Campinas, SP). The producers were: Alvorada (Araporã, MG), Cresciumal (Leme, SP), Da Pedra (Descalvado, SP), Ipiranga (Descalvado, SP), Santa Rosa (Boituva, SP), São Luiz (Ourinhos, SP), Quatá (Quatá, SP), Santa Adélia (Jaboticabal, SP), Rafard (Rafard, SP), and Jacarezinho (Jacarezinho, PR). Thus, for every season, one sample from each plant was collected.

The insoluble deposits were collected from commercial sugared cachaças available in our Laboratory collection, produced in the following cities: Tanabi (SP), Campina Grande (PB), Patus (PB), Candido Mota (SP), Fortaleza (CE), Colônia de Leopodina (AL), São José dos Pinhais (PR), Jandaia do Sul (PR), São Paulo (SP), Tabatinga (SP), Vitória do Santo Antão (PE), and Sorocaba (SP).

2.2. Chemicals

Dextran calibration reference standards M_w 2,100,000, 4,200,000, 5,900,000 and 7,400,000, were purchased from American Polymer Standards (Mentor, OH, USA), and those of M_w 11,600, 23,800, 48,300, 148,000, 410,000 and 1,100,000 were purchased from Waters (Milford, MA, USA). Ethanol (anhydrous) and sodium sulfate, both ACS grade, were acquired from J. T. Baker (Phillipsburg, NJ, USA). The water was previously bidistilled and then deionized using a Millipore Milli-Q system (Bedford, MA, USA).

2.3. Apparatus, analytic conditions and sample preparation

Size-exclusion chromatography (SEC) analyses were performed on a Shimadzu liquid chromatography system (Tokyo, Japan), consisting of an SLC-10AVP system controller supporting an LC-10AD pump, a refraction index detector RID-10A and a Rheodyne injection valve with a 100 μl loop. Data acquisition and processing was performed using Class-VP 6.12 and GPC 1.02 for Class-Vp softwares.

The best chromatographic conditions were achieved under aqueous solutions with 3.55 g of Na_2SO_4 per litre (0.5 ml/min, at room temperature) using three columns assembled in line: two Waters Ultrahydrogel linear (7.8 mm i.d. × 300 mm) packed with a blend of different pore size particles (ranging from 250 to 2000 Å), and one Tosoh Bioscience TSK-gel 3000PWxl column (7.8 mm i.d. × 300 mm, pore size of 200 Å).

Stock calibration solutions of dextrans and the dextrose were prepared by separately weighing 20.0 ± 1 mg of the desired standard in 10 volumetric flasks of 5 ml (4000 ppm). The standards in each flask were dissolved and diluted with the mobile phase (0.025 M Na_2SO_4). The calibration curve used to characterize the dextran molecular mass distribution ($\text{Log}\,M = 0.00021109X^3 - 0.03370649X^2 + 1.62437664X + 16.85697652$; dispersion: 0.08151) was built up by injecting 250 ppm of each standard obtained through the dilution of the stock solutions.

For dextran analysis in sugar samples, preparation was carried out as follows: A sample of 40.0 g of the sugar sample was dissolved in water, and then the volume was adjusted to 100 ml in a volumetric flask. 50 ml of this solution were filtered, using filter paper (80 g/m^2) to remove insoluble particles. 160 ml of anhydrous ethanol were added to a 40 ml aliquot of the filtered solution. After standing 24 h, this solution was centrifuged at 10,000RPM and 4 °C for 1 h (Hitachi Himac – CR20b2). The supernatant solution was discarded and the precipitate was dissolved in 1.5 ml of 0.025 M Na_2SO_4. This solution was cooled to 5 °C and filtered through mixed cellulose ester membrane (Millipore – 0.45 μm pore size × 25 mm Ø). Previously to the injection in the chromatography system, an aliquot of 0.3 ml was tested with iodine solution for the presence of starch (Aquino & Franco, 2008). The tests were all negative.

The insoluble deposits from sugared cachaças were collected directly from the bottles. For the SEC analysis, the deposits were separated from the cachaça through centrifugation at 10,000RPM at 4 °C for 1 h. The precipitate was then dissolved in 0.50 or 1.00 ml of 0.025 M Na_2SO_4 according to the it is amount, and filtered through the same type of membrane, as described above.

3. Results and discussion

A typical SEC dextran chromatogram obtained for a sugar sample, is illustrated in Fig. 1. In this chromatogram, two distinct peaks can be observed at the retention times of 47 and 61 min for dextrans. As will be discussed in this paper, this pattern has often been found in sugar samples used by Brazilian cachaça producers.

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