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Formation of Dextran Deposits in Brazilian Sugar Cane Spirits

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Supporting Information

ABSTRACT: The formation of dextran deposits in sugared Brazilian cachaça was studied as a function of the time considering the effects of temperature, molecular weight (M_w), visible light, pH, and the presence of Ca, Mg, Cu, and Fe ions in the concentrations at which they are usually present in this beverage. At 25 °C and pH 4.4, the experimental half-lives ($t_{1/2}$) for precipitation are 73 and 124 days for dextrans with M_w 5.9 × 10^6 and 2.1 × 10^6 Da, respectively. For dextrans with M_w 5.0 × 10^5 and 4.0 × 10^4 Da, the experimental $t_{1/2}$ values are >180 days. For a dextran with M_w 2.1 × 10^6 Da a change in pH from 4.4 to 5.5 at 25 °C resulted in a $t_{1/2}$ decrease from 124 to 25 days. At pH 4.4 the visible light and the presence of metal ions in average concentrations usually found in cachaças do not exhibit noticeable influence on the rate of dextran precipitation.

KEYWORDS: dextrans, deposits, sugar, sugar cane spirit

INTRODUCTION

The Brazilian sugar cane spirit commonly known as cachaça is the third most important product of the Brazilian sugar cane industry, surpassed only by sugar itself and ethanol fuel. Brazilian cachaça has an official production of 1.3 billion liters per year, there being more than 40,000 producers and over 4000 brands, and the industry generates around 650,000 direct and indirect jobs.[1]

Despite current improvements of the quality of cachaças, important problems remain to be solved. The formation of insoluble deposits in cachaças is the most frequent visual defect in such product,[2] and diverse variables involved with dextrans precipitation have not been well described so far.[1,3] Dextrans are glucose homopolymers formed mainly by α-(1→6) glucosidic bonds with side chains linked with α-(1→3), α-(1→4), and, occasionally, α-(1→2) glucosidic bonds synthesized by bacteria such as *Leuconostoc* and *Streptococcus*.[4,5] Their occurrence in sugar cane is mainly caused by the contaminant bacterium *Leuconostoc mesenteroides*, which is almost omnipresent in sugar cane fields and sugar mills.[6,7]

The molecular masses of dextrans vary widely, from thousands to sometimes millions of daltons.[1] For this reason, even when the sugar cane juice is obtained and fermented under unfavorable conditions, dextrans are not transferred to cachaças by distillation. Thus, dextrans in cachaças originate from sucrose addition during standardization processes, which are usually carried out in industrial production plants of cachaças. Dextrans are innocuous to humans, such that they are even used in pharmaceutical formulations.[5,8] However, dextran precipitations in cachaça bottles are undesirable, because consumers commonly associate this with a lack of hygiene in the production process or with low-quality raw materials.[3,9]

Recently, Aquino and Franco[1] discussed the profiles of the distribution of dextran molecular masses in Brazilian sugars and in deposits formed in sugared cachaças. The present work deals with the formation of dextran precipitates in sugared cachaças as a function of the most representative dextran molecular masses found in Brazilian sugar and under different experimental conditions, such as temperature, visible light, pH, and the presence of Ca, Mg, Cu, and Fe ions. The data obtained from this work may then be applied in the development of procedures to solve or minimize this problem in sugared alcoholic beverages.

MATERIALS AND METHODS

Chemicals. Dextran reference standards of weight-average molecular masses of M_w 2.1 × 10^6 and 5.9 × 10^6 Da were purchased from American Polymer Standards (Mentor, OH), and reference standards of M_w 5.0 × 10^5 and 4.0 × 10^4 Da were purchased from Sigma-Aldrich (St. Louis, MO). Anhydrous ethanol (99.9% HPLC grade), sodium sulfate, and sodium hydroxide (ACS grade) were acquired from J. T. Baker (Xalostoc, Mexico). Reference standards of metal ions (copper, iron, calcium, magnesium) were purchased from Carlo Erba (Milan, Italy). The water used was first redistilled and then deionized using a Millipore Milli-Q system (Bedford, MA).

Sample Preparation, Analytic Conditions, and Apparatus. To reproduce the most representative conditions to evaluate the influence of the experimental parameters, dextran precipitation model systems were prepared using a real cachaça sample. This sample, designated "base cachaça", was distilled in columns, unsugared (consequently, without dextrans), and, in accordance with Brazilian chemical specifications for cachaça commercialization,[10] was kindly provided by a traditional cachaça producer and exporter.

The preparation of cachaça−dextran model solutions was carried out under the following conditions. The ethanolic concentration of base cachaça was measured through picnometry and then adjusted to 40.0% v/v by adding ultrapure water. Next, the cachaça was filtered through a mixed cellulose ester membrane (Millipore; 0.22 μm pore size × 47

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mm) to remove any insoluble material, followed by the addition of the dextran standards M_w 4.0×10^4, 5.0×10^5, 2.1×10^6, and 5.9×10^6 Da (distinct stock solutions were used for each dextran standard: for dextrans M_w 5.9 and 2.1×10^6 Da, 2.5 g/100 mL, and for dextrans M_w 5.0×10^5 and 4.0×10^4 Da, 1.00 and 0.10 g/100 mL). The selection of the reference standards was based on the M_w range of dextrans found in Brazilian sugars.[1] The initial concentration of the above dextran standards in all model solutions was 1.0×10^{-7} M.

After preparation, the apparent pH was measured in each model solution. To minimize junction potential errors before pH measurements, all samples were diluted 10-fold with ultrapure water (conductivity maximum at 25 °C, 0.05 μS/cm). For all solutions, the pH values were in the 4.4 ± 0.2 range. This value is comparable with the average pH value of 4.3 obtained from the analysis of 20 commercial cachaças from different producers. In the experiments performed with model solutions of pH 5.5 ± 0.2 and 3.4 ± 0.2, a model solution, initially with pH 4.4 ± 0.2 before the addition of the dextran standards, had its pH adjusted to the desired values by the addition of 0.10 M NaOH or 0.10 M CH_3COOH solutions. The pH measurements were carried out using a combined pH glass electrode model CW711 (Qualxtron, São Paulo, Brazil) coupled with a Materlab PHM 2540 pH/ion meter (Radiometer Analytical, Villeurbanne, France).

The influence of temperature, visible light, pH, presence of Ca, Mg, Cu, and Fe ions, and average molecular mass of dextrans on the precipitation of dextrans was evaluated using turbidimetry and analysis of the remaining masses of dextrans in solution as a function of time.

The model solutions were maintained at temperatures of 15 ± 1, 25 ± 1, and 35 ± 1 °C and distributed into 150 mL bottles filled with 125 mL. These bottles were sealed and placed in thermostatic baths (Tecnal model TE-184, Piracicaba, Brazil). For analytical purposes, only one flask of each dextran standard was removed from the thermal bath at the time of analysis.

The turbidimetric measurements were executed using a 10.0 mL aliquot of each model solution (collected without stirring). A Hack turbidimeter model 2100P (Loveland, CO) was used for these measurements. Prior to each analysis, the turbidimeter was calibrated with certified formazin turbidity standards.

When formation of the deposits was visually evident, the remaining mass of the dextran in solution was determined. Thus, the volume of each model solution (125.0 mL) was filtered through a mixed cellulose ester membrane (Millipore; 0.22 μm pore size × 47 mm) to retain the precipitated dextran, and the filtrate was investigated by size exclusion chromatography (SEC). The mass of precipitated dextran over time was estimated by subtracting the initial weight mass of dextrans dissolved in each starting model solution (1.0×10^{-7} M) from the dextran contents remaining in solution.

The SEC analysis (LQ = 25.0 mg/L) did not show the presence of dextrans in the filtrate portion of the filtered solutions. Accordingly, the photomicrographs obtained by scanning electron microscopy indicated that all of the precipitated dextran standards exhibited particle diameters of >0.22 μm membrane pore size (Figure I, Supporting Information). The SEC analyses were performed using 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. The setup for chromatographic runs and columns was previously described by Aquino and Franco.[1]

To quantify the dextrans in solution, the external standard method was used. Stock calibration solutions of dextrans were prepared by weighing, separately, 20.0 ± 0.1 mg in four volumetric flasks of 5 mL (4000 mg/L). The standards were transferred to volumetric flasks, dissolved, and diluted with the mobile phase (0.025 M Na_2SO_4). The calibration curve was constructed by sequential injections of 25.0, 50.0, 100.0, 250.0, 500.0, and 1000.0 mg/L of each standard obtained through appropriate dilutions of the stock solution. For all calibration curves, the correlation coefficient (r^2) was always >0.9965.

During the quantifications, when necessary, the filtered samples injected in the chromatographic system were concentrated by evaporation in a hot water bath. The maximum concentration factor was obtained when the volume of the sample was reduced from 125 to 5.0 mL, thus resulting in samples 25-fold more concentrated.

To confirm the efficiency of the filtration process in retaining the dextrans precipitated in the model solutions, dextrans of each molecular mass were totally precipitated from the samples of these models by raising the ethanol concentration to 80%. These solutions were then centrifuged at 7826*g* for 30 min at 4 °C (Hitachi Himac centrifuge model CR20b2, rotor RPR20-2). Next, 2.0 mL aliquots from the supernatant of all models were collected for analysis by SEC. The remaining liquid phases were removed by rotoevaporation (Büchi rotavapor model R-205; Büchi Laboratory Equipments, Flawil, Switzerland) at 45 °C, and the solids obtained were dried (in a Schlenk line) for subsequent scanning electronic microscopy (SEM) analysis.

The SEM and energy dispersive X-ray (EDX) analyses were carried out using a LEO Leica-Zeiss microscope model 440 (LEO Electron Microscopy, Cambridge, U.K.) using an Oxford detector model 7060 (Oxford Instruments, Concord, MA), operating with primary electron beams of 20 keV using samples previously coated with 10 nm gold.

To evaluate the influence of the metal ions (Ca, Mg, Cu, Fe) on the dextran precipitation, a model solution was prepared with the same base cachaça as used in the previous experiments with the following characteristics: 40.3% v/v ethanol, pH 4.4, 2.0×10^{-7} M dextran of M_w 2.1×10^6 Da, and concentrations of 2.6×10^{-4}, 3.6×10^{-4}, 7.9×10^{-5}, and 4.4×10^{-5} M for Ca, Mg, Cu, and Fe, respectively, obtained by the addition of the standards of these metal ions (special grade for ICP analysis, 1000 mg/L). These concentrations correspond to the average concentrations found for Ca and Mg, 10-fold the average content of Fe determined in Brazilian cachaças,[11] and the maximum concentrations permitted for Cu according Brazilian legislation.[10]

The analyses of the metal ions in the base cachaça and in the model solutions were performed as follows: a sample (50.0 mL) was placed into an open 250.0 mL beaker and then evaporated with 5.0 mL of HNO_3 (5%) under controlled heating until approximately 5.0 mL sample volume. After cooling to room temperature, the sample was quantitatively transferred to a 25.0 mL volumetric flask, diluted to a volume of 5% nitric acid solution, and then analyzed. The analyses of the metal ions were done by ICP-AES (Optima 3000 dual view, Perkin-Elmer). The instrumental conditions and analytical lines for each element are given in Table I in the Supporting Information. The calibration curves were constructed using the external standard method, and all analyses were carried out in triplicate.

To probe the influence of visible light on dextran precipitation, six bottles with aliquots of the same model solution prepared for the assays on the influence of cachaça acidity reduction in the dextran insolubilization (125 mL/bottle; pH 5.5) were kept at 22 ± 1 °C for 120 days. Three bottles were directly exposed to light, and the other three bottles were protected from light, covered by aluminum foil. The light sources were two fluorescent white lamps, Philips "Day light" TLDRS 32W-S840-ECO, with a spectral distribution between 320 and 740 nm and peak emissions at 400, 440, 500, 550, 600, 630, and 650 nm. This light source proved suitable, because such lamps present the same characteristics as the lamps that are frequently used to illuminate cachaça bottles in supermarket shelves and other cachaça retailers.

For the analyses of the filtration residue from the industrial production of a sugared cachaça, an aliquot (50 mL) was centrifuged at 10,000 rpm for 30 min, the solid portion was dried to fully remove ethanol; the residue was resuspended in ultrapure water and held under magnetic stirring at 30 °C for 24 h and then filtered through a quantitative paper (Whatman no. 41) and an ester cellulose membrane

Table 1. Turbidity Variation (NTU[a]) of Model Solutions Containing Dextran Standards as a Function of Time at Temperatures of 15, 25, and 35 °C

	dextran													
	M_w 5.90 × 10^6 Da			M_w 2.10 × 10^6 Da					M_w 5.00 × 10^5 Da			M_w 4.00 × 10^4 Da		
days	15 °C	25 °C	35 °C	15 °C	25 °C	35 °C	25 °C[b]	25 °C[c]	15 °C	25 °C	35 °C	15 °C	25 °C	35 °C
0	0.32	0.32	0.32	0.26	0.26	0.26	0.32	0.32	0.23	0.23	0.23	0.23	0.23	0.23
1	0.31	0.36	0.30	0.26	0.25	0.28	0.51	0.26	0.25	0.21	0.20	0.23	0.24	0.25
6	3.62	3.27	2.63	1.21	1.08	1.01	5.36	1.10	0.81	0.42	0.32	0.45	0.36	0.32
12	3.81	3.49	2.99	1.35	1.31	1.16	12.4	1.35	1.02	0.47	0.47	0.59	0.41	0.47
30	5.01	4.24	3.67	1.71	1.77	1.36	3.91	1.59	1.03	0.61	0.50	0.62	0.47	0.50
60	7.82	4.74	4.06	2.18	2.11	1.72	3.29	1.98	1.16	0.63	0.63	0.66	0.54	0.63
90	13.6	5.39	4.32	7.79	2.59	2.06	1.68	2.62	1.83	0.82	0.60	0.75	0.57	0.60
120	6.56	7.48	4.58	4.86	3.09	2.35	0.62	2.90	3.42	0.87	0.67	0.84	0.63	0.67
160	4.39	4.97	5.07	3.89	2.37	2.32	0.43	3.17	2.55	0.99	0.71	0.85	0.74	0.71
210	0.47	4.49	5.38	1.87	2.34	2.54	0.38	2.53	3.03	1.18	0.76	1.38	0.71	0.76
240	0.41	1.14	4.92	0.56	1.71	2.13	0.41	2.21	2.36	1.36	1.09	1.91	0.80	1.09

[a] Nephelometric turbidity unit. [b] pH 5.5. [c] pH 3.5.

(Millipore; 0.45 μm pore size × 47 mm). This filtrate was concentrated using a vacuum rotoevaporator and analyzed by SEC following the procedure described by Aquino and Franco.[1]

NMR experiments were performed using a Varian Inova spectrometer (9.4 T), at ^{13}C and ^{1}H frequencies of 100.5 and 400.0 MHz, respectively, using a 5 mm Jackobsen probe with magic angle spinning (MAS) and a rotation frequency of 10 kHz. The ^{13}C spectra were obtained via cross-polarization magic angle spinning (CPMAS) with a contact time of 1 ms and a repetition time of 5 s.

RESULTS AND DISCUSSION

The effects of temperature and average molecular mass of the dextrans on the formation of deposits in cachaças were monitored in the model solutions containing dextrans with M_w 4.0 × 10^4, 5.0 × 10^5, 2.1 × 10^6, and 5.9 × 10^6 Da at 15, 25, and 35 °C (individual flasks for each dextran standard) through turbidity measurements and by means of the dextran quantification remaining in solution.

Table 1 shows the turbidity variations for the different model solutions as a function of time. As expected, the turbidity variations showed a behavior that is dependent on the molecular mass of the dextrans, in agreement with an increase in insolubility of the dextrans in ethanol as their molecular masses increase.[12]

According to the turbidimetric data, lower temperatures account for cloud development (Table 1) and precipitation of dextrans (Table 2). The influence was more pronounced in systems that had higher molecular mass dextrans. In solutions containing dextrans with M_w 5.9 × 10^6 and 2.1 × 10^6 Da, the turbidities increased at 15 and 25 °C to a maximum at 90 and 120 days, respectively (13.6 and 7.48 NTU for M_w 5.9 × 10^6 Da and 7.79 and 3.09 NTU for M_w 2.1 × 10^6 Da) and then exhibited a continuous decrease over time. For the models containing dextrans with M_w 5.0 × 10^5 and 4.0 × 10^4 Da, cloud development was substantially less efficient and slower than for the other models with M_w 10^6 Da, achieving a maximum of 3.42 NTU at 15 °C in 120 days for a dextran with M_w 5.0 × 10^5 Da. This value is less than half the turbidity observed for a dextran with M_w 2.1 × 10^6 Da at this temperature during 90 days. The turbidity reduction observed over time is coherent, because in all systems the insoluble dextrans do not remain disperse in the liquid but gathered at the bottom of the bottles (Figure II, Supporting Information).

Even at a temperature as low as 15 °C, for the solutions containing dextrans with a mass of 10^6 Da (less soluble than the other dextrans), the visible precipitation of the dextrans could be observed only after 30 days of monitoring (Table 2). At temperatures of 25 and 35 °C, the time elapsed for the first visible precipitations of 2.1 × 10^6 Da dextrans was 60 days. At this time, at 25 °C, the precipitated amounts of the dextrans represent 37.1% and only 6.0% for dextrans with M_w 5.9 × 10^6 and 2.1 × 10^6 Da, respectively. Table 2 gives the decrease of dextrans in solution as a function of time.

Therefore, the monitoring of turbidity during the first 2 months after cachaça bottling (at temperatures around 15 °C) may be a good indication of product stability during its first year of commercialization.

Figure 1 illustrates the precipitation curves for dextrans with different average molecular masses at the same temperature. The plots point out the relevance of this parameter for dextran precipitation in cachaças. As shown in Figure 2, the precipitation of dextrans with the same molecular mass decreases as the temperature increases.

The experimental half-lives ($t_{1/2}$) were determined from the plots of Figures 1−3. For dextran with M_w 5.9 × 10^6 Da (pH 4.4) the calculated half-life values are 56, 73, and 119 days at 15, 25, and 35 °C; for dextran with M_w 2.1 × 10^6 Da (pH 4.4) $t_{1/2}$ values are 102, 124, and 162 days, respectively. For dextran of M_w 5.0 × 10^5 Da (pH 4.4) the half-life at 15 °C is 187 days, whereas for 25 and 35 °C $t_{1/2}$ values are estimated as >250 days. The same behavior was observed for dextran with M_w 4.0 × 10^4 Da at 15, 25, and 35 °C.

In addition to the influence of the molecular mass of the dextrans that are critical to the half-life of the precipitation of the dextrans, the ethanol concentration in cachaça (on average 40% v/v) is crucial.[13] Indeed, this concentration is half of the recommended concentration for precipitation of all dextrans, including the low molecular mass dextrans as applied in Robert's analytical methodology adopted by the Association of Official Analytical Chemists (AOAC) and up to <50% approved by the

Table 2. Concentration (M) of Dextrans Remaining Soluble in the Model Solutions

		dextran in solution				
dextran M_w (Da)	days	15 °C, pH 4.4	25 °C, pH 3.5	25 °C, pH 4.4	25 °C, pH 5.5	35 °C, pH 4.4
5.9×10^6	12	*a*	*b*	*a*	*b*	*a*
5.9×10^6	30	7.4×10^{-8}	*b*	9.5×10^{-8}	*b*	9.7×10^{-8}
5.9×10^6	60	5.1×10^{-8}	*b*	6.3×10^{-8}	*b*	8.9×10^{-8}
5.9×10^6	90	1.7×10^{-8}	*b*	3.5×10^{-8}	*b*	7.3×10^{-8}
5.9×10^6	120	5.8×10^{-9}	*b*	1.2×10^{-8}	*b*	4.8×10^{-8}
5.9×10^6	160	3.9×10^{-9}	*b*	6.5×10^{-9}	*b*	2.7×10^{-8}
5.9×10^6	210	4.4×10^{-9}	*b*	6.3×10^{-9}	*b*	1.7×10^{-8}
5.9×10^6	240	3.8×10^{-9}	*b*	5.8×10^{-9}	*b*	9.7×10^{-9}
2.1×10^6	12	*a*	*a*	*a*	8.9×10^{-8}	*a*
2.1×10^6	30	9.8×10^{-8}	*a*	*a*	3.3×10^{-8}	*a*
2.1×10^6	60	8.4×10^{-8}	*a*	9.4×10^{-8}	1.4×10^{-8}	9.6×10^{-8}
2.1×10^6	90	6.5×10^{-8}	9.2×10^{-8}	8.2×10^{-8}	1.0×10^{-8}	8.8×10^{-8}
2.1×10^6	120	3.1×10^{-8}	5.9×10^{-8}	4.7×10^{-8}	4.3×10^{-9}	7.5×10^{-8}
2.1×10^6	160	1.8×10^{-8}	3.7×10^{-8}	3.2×10^{-8}	3.7×10^{-9}	5.1×10^{-8}
2.1×10^6	210	6.3×10^{-9}	2.4×10^{-8}	1.6×10^{-8}	2.2×10^{-9}	2.9×10^{-8}
2.1×10^6	240	6.7×10^{-9}	1.8×10^{-8}	1.2×10^{-8}	2.1×10^{-9}	2.1×10^{-8}
5.0×10^5	12	*a*	*b*	*a*	*b*	*a*
5.0×10^5	30	*a*	*b*	*a*	*b*	*a*
5.0×10^5	60	*a*	*b*	*a*	*b*	*a*
5.0×10^5	90	8.8×10^{-8}	*b*	*a*	*b*	*a*
5.0×10^5	120	7.2×10^{-8}	*b*	*a*	*b*	*a*
5.0×10^5	160	6.4×10^{-8}	*b*	*a*	*b*	*a*
5.0×10^5	210	4.1×10^{-8}	*b*	8.6×10^{-8}	*b*	9.4×10^{-8}
5.0×10^5	240	2.4×10^{-8}	*b*	7.9×10^{-8}	*b*	9.1×10^{-8}
4.0×10^4	12	*a*	*b*	*a*	*b*	*a*
4.0×10^4	30	*a*	*b*	*a*	*b*	*a*
4.0×10^4	60	*a*	*b*	*a*	*b*	*a*
4.0×10^4	90	*a*	*b*	*a*	*b*	*a*
4.0×10^4	120	9.3×10^{-8}	*b*	*a*	*b*	*a*
4.0×10^4	160	8.7×10^{-8}	*b*	*a*	*b*	*a*
4.0×10^4	210	8.2×10^{-8}	*b*	8.9×10^{-8}	*b*	*a*
4.0×10^4	240	7.4×10^{-8}	*b*	8.3×10^{-8}	*b*	*a*

[a] Precipitation not observed. [b] Experiment not realized.

International Commission for Uniform Methods of Sugar Analysis (ICUMSA) for analysis of high molecular mass dextrans.[14,15]

As a result of the presented data, it is likely that at 25 °C (the usual temperature in cachaça storerooms) the nondevelopment of noticeable clouds or dextran deposits in sugared cachaça during the first 4 months after sugar addition will by no means ensure the absence of this problem during the cachaça's shelf life or during its average time for marketing of about 6−8 months. This observation concurs with the frequent formation of deposits in sugared cachaças during display on shelves at sale points.[1]

The hypothesis that changes in the solution pH might lead to changes in the rate of dextran precipitation was evaluated by monitoring the model solutions with dextrans M_w 2.1×10^6 Da at pH 3.5 and 5.5, which is the pH range usually found in cachaças. Dextrans with M_w 2.1×10^6 Da were used in these experiments due to their experimentally convenient precipitation rate.

Acid concentrations $>10^{-3}$ M in cachaças are usually the result of bacterial contamination of the sugar cane juice during the fermentation, leading to increase of the concentration of acids such as acetic and lactic.[16−18] In these cases it is common for some producers to add sugar, aiming to mask the sensory defects caused by presence of these acids. This procedure results in contamination of the cachaças with dextrans. Figure 3 shows for comparison purposes the precipitation profiles obtained for solutions with pH 3.5, 4.4, and 5.5 as a function of time at 25 °C.

It is evident from Tables 1 and 2 and Figure 3 that an increase in pH speeds precipitate formation. At pH 5.5, the half-life for dextran precipitation was 25 days, whereas, at pH 4.4, it was 124 days; therefore, the precipitation rate was increased 5-fold. At this point it is interesting to comment on the observed effects of changes in temperature and pH on the precipitation of dextrans. The increase of 1.1 pH units (from 4.4 to 5.5) has a more

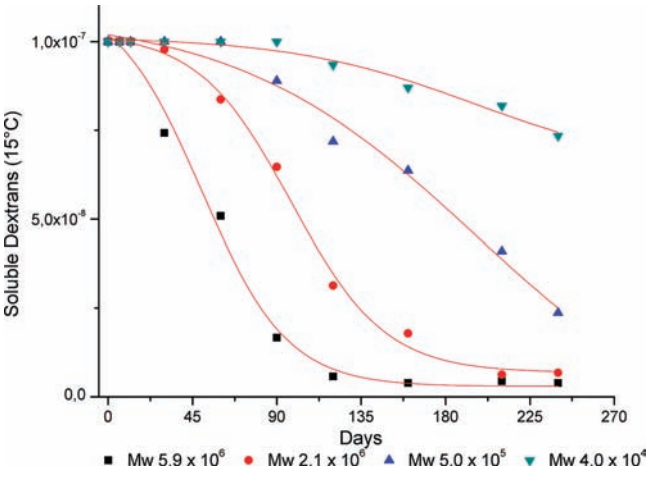

Figure 1. Precipitation curves for dextrans of M_w 4.0 × 10^4, 5.0 × 10^5, 2.1 × 10^6, and 5.9 × 10^6 Da at the same concentrations (1.0 × 10^{-7} M) in cachaça model solutions at 15 °C.

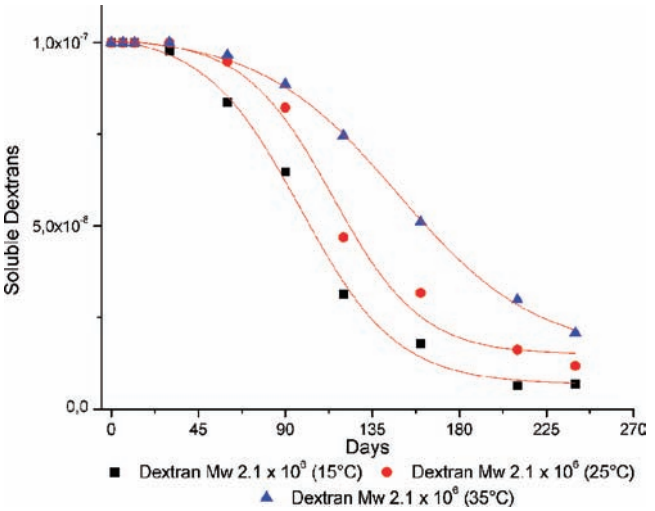

Figure 2. Precipitation curves for dextrans of M_w 2.1 × 10^6 Da at 15, 25, and 35 °C, at an initial concentration of 1.0 × 10^{-7} M.

pronounced influence on cachaças than a temperature decrease of 10 °C (from 25 to 15 °C).

Aiming to identify possible chemical alterations in the dextran precipitates caused by pH changes, a ^{13}C solid state NMR analysis was performed comparing a dextran standard and precipitates obtained at pH values of 4.4 and 5.5 (Supporting Information, Figure III), no noticeable chemical changes were detected in the ^{13}C NMR spectra. The shoulders observed in the NMR spectra for dextran precipitates at pH 4.4 and 5.5 (mainly for C1 and C6 signals 97.8 and 66.1 ppm, respectively) would be attributed to local differences in chain conformations, probably associated with the increase in dextran precipitation rates.[19,20]

With regard to the behavior of dextrans when the pH was reduced from 4.4 to 3.5, the half-life decreased only by 10 days. This small reduction was not a surprise because the dextrans are known to resist depolymerization in moderately acidic conditions,[21−23] which will reduce its molecular weight and consequently increase its solubility.[12] Conditions for acidic hydrolysis at pH values of 1−2 require temperatures of 70−100 °C during a time period of up to 2 h.[21−23]

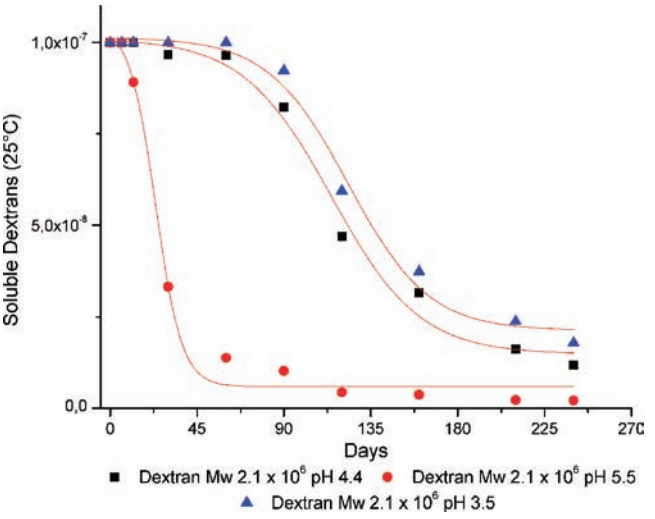

Figure 3. Precipitation curves for dextrans of M_w 2.1 × 10^6 Da at 25 °C as a function of pH (3.5, 4.4, and 5.5).

Several metallic ions such as aluminum, calcium, magnesium, zinc, cobalt, and mainly copper and iron are known to associate with dextrans and therefore might induce changes in the dextran precipitation rates.[24−27] Experiments with calcium, magnesium, copper, and iron were carried out on the basis of their more representative abundances when compared with the other metals found in Brazilian cachaças[11,28] and the recognized capacity of dextrans to form complexes with these metal ions.[24,27,29,30] It is known in the literature that interactions occur already in slightly acidic or neutral conditions and strongly increase when the solutions are alkalinized.[26,29−32] The results of these experiments are summarized in Table II of the Supporting Information.

The concentration values for unprecipitated dextrans in both systems (with and without metal ion additions) during the study period of 60, 90, and 120 days using concentrations of 8.9 × 10^{-8}, 8.6 × 10^{-8}, and 5.2 × 10^{-8} M and 9.4 × 10^{-8}, 8.2, and 4.7 × 10^{-8} M did not differ significantly (the CV% were 3.9, 3.4, and 7.1%, respectively). These data and the small variation in the concentrations of monitored metal ions in solution throughout all of the experiments (secured by low CV%) suggest that the metal ions do not exert noticeable influence on dextran precipitation in cachaças at pH 4.4.

Illumination of cachaças is reported to delay the precipitation of dextrans, because visible light may promote bond cleavages that should yield smaller molecules, hence increasing its solubility in ethanol.[7,33,34] After a 120 day period storage at pH 4.4 and 25 °C, no significant differences were observed in the turbidity and in the masses of soluble dextrans with and without day light illumination. The turbidities were 2.97 and 3.11 NTU (CV% 3.3%), respectively, and the precipitated dextran masses were 59.5 ± 4.45 and 59.4 ± 4.10 mg, respectively.

Industrial filtration procedures are typically used in the production of Brazilian cachaças in big distilleries. To evaluate the efficiency of this procedure, a residue from filtration through a quartz sand filter of a 100,000 L of sugared cachaça was collected. This residue, collected from the upper section of the filter, consists of a material that upon decantation separates in two phases, which were analyzed on separate (see Materials and Methods). The solid phase showed the presence of dextrans with a high average molecular mass (M_w 1.8 × 10^6 Da), as can be

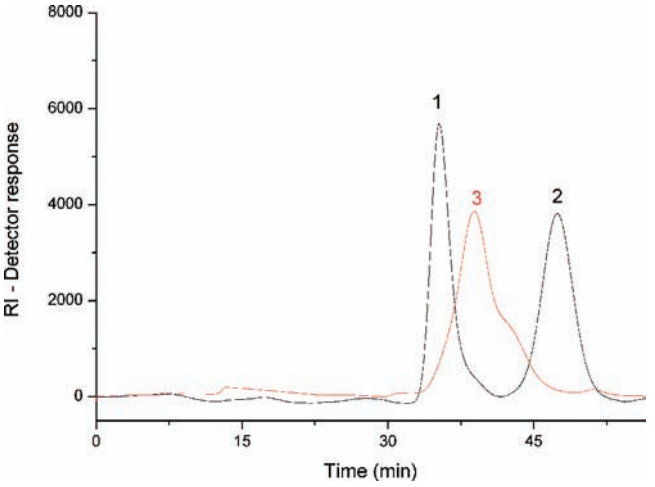

Figure 4. Size exclusion chromatograms of aqueous solutions containing dextran standards (black line: peak 1, dextran M_w 5.9 × 10^6 Da; peak 2, dextran M_w 4.8 × 10^4 Da) and solutions resulting from redissolution of a residue obtained from an industrial filtration using a quartz sand filter (red line: peak 3, dextran M_w 1.83 × 10^6 Da).

observed in the chromatogram in Figure 4. Absolute ethanol was added to the liquid phase until the concentration of 80% v/v was reached. The formation of a precipitate was quite evident after 24 h, in which the presence of dextrans having M_w on the order of 10^4 Da was experimentally verified.

Despite the content of dextrans being reduced in the cachaça upon filtration, this procedure did not ensure that the remaining dextrans would not lead to precipitation. Nowadays, aiming to circumvent this inconvenience, a second filtration is performed using diatomite filters (150 mesh size) by some producers.

The present study points out that a decrease in temperature and an increase in pH facilitate the precipitation of dextrans in cachaças. The effect of a pH change of 1.1 unit (from 4.4 to 5.5) was more pronounced than a reduction of the temperature by 10 °C (common storage temperatures vary from 25 to 15 °C). Visible light and the presence of metal ions of Ca, Mg, Cu, and Fe in average concentrations usually found in cachaças have no significant effects on the dextran precipitation rate at pH 4.4. At temperatures around 25 °C, the nondevelopment of perceptible clouds or dextran deposits in sugared cachaças during the first 4 months after sugar addition does not ensure the absence of this problem during the cachaça's shelf life or during its average time for marketing (about 6−8 months), because the experimental half-lives for precipitate formation for dextrans with M_w 10^5 and 10^4 Da are >180 days. A procedure to enhance the efficiency of the filtration process could be the inclusion of a previous filtration of the sugar syrup with a higher ethanol concentration and a pH value around 6.5.

■ ASSOCIATED CONTENT

Supporting Information. Additional figures and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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