

The chemical composition of ‘canjica’, a polymeric material found in Brazilian cane sugar industry

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Summary

A polymeric water-insoluble material, the so called ‘canjica’, was collected in nine Brazilian sugar and alcohol plants. It was found that all the samples were very similar to each other regarding the moisture and variable in ash contents, whereas CHNS analysis showed that they were carbohydrates. Trace amounts of nitrogen and sulphur compounds were also found to be present, and their source were attributed to proteinaceous material from sugarcane juice contaminants. Paper chromatography and gas chromatography-mass spectrometry (GC-MS) analysis showed that the polymers are formed by repeated glucose moieties bond by 1,6-linkages. Infrared spectroscopy revealed a dextran polysaccharide structure, while X-ray fluorescence determined that various other elements (Ca, Al, Fe, K, Cl, Mn, P and traces of Si and Ti) were also associated with the polymer, in amounts that varied considerably from sample to sample. X-ray diffraction analysis confirmed the polysaccharide dextran structure while identifying a certain amount of different (polysaccharide) material present in the ‘canjica’.

Introduction

Over 4 million hectares of farmland are used for sugarcane cultivation in Brazil, and nearly 70% of the crop is for fuel alcohol production (Oliveira *et al.* 1995). This amounts to an annual production of about 13 million m³ of alcohol and 7 million tonnes of sugar. Recently, considerable efforts have been made to improve the technology of sugar processing. Yet, much has to be done to reduce sugar loss by the activities of enzymes and microbes in the processing.

As a perishable item, sugar stalk undergoes various forms of deterioration due to chemical, biochemical and microbiological activity (Bruijn 1966). Alcohol, organic acids and aminoacids are increased during this process, but the amount of soluble oligosaccharides is shown to be the best parameter to measure deterioration (Bruijn 1970). Dextran is known to be the major polysaccharide detected in stale cane (De Vos 1996), but other polysaccharides have also been detected such as pullulan, sarcaran and levan (Hide *et al.* 1976; Blake & Littlemore 1984a, b; Blake & Clarke 1984b). Water-insoluble polymers are formed at different intensity and may cause serious problems to the industrial operation of sugarcane-processing factories. These polymers are

known among sugar technologists in Brazil as ‘canjica’, a name derived that of a popular food made out of corn which displays similar shape and consistency. ‘Canjica’ may have the same structure as the so called ‘tapioca-like gum’ described by Bevan & Bound (1971). The incidence of ‘canjica’ is frequently associated with dextran formation during sugarcane processing (Lima *et al.* 1982). Some are regarded as a result of interaction between dextran of *Leuconostoc mesenteroides* and bacteria and yeast cells (Bevan & Bound 1971). However, the exact nature of ‘canjica’ is unknown. In this study we have used different analytical techniques to characterize the chemical composition of nine samples of ‘canjica’ collected in the Piracicaba area, State of São Paulo, Brazil.

Materials and methods

Samples

Samples of ‘canjica’ were collected from the cane juice heat exchanger unit of sugar factories. Nine samples were collected in the Piracicaba area during 1993/94 season. The ‘canjica’ samples were washed with water to

remove excess dirt and dried with hot air (50 °C), for conservation.

Chemical analysis

The contents of water and ash in 'canjica' were determined according to the method of AOAC (1995). Carbon, sulphur, nitrogen and hydrogen contents were measured by using a Perkin-Elmer PE-2400 elemental analyser on dried samples (105 °C/24 h). The following elements, Al, Si, P, Cl, K, Ca, Ti, Mn and Fe, were analysed on dried samples (105 °C/24 h) by using a X-ray fluorescence spectrometer Spectrace 5000.

Paper chromatography was used to identify the sugar monomers according to the method of Chaplin & Kennedy (1986). Glucose, mannose, galactose and fructose were used as the standards.

Gas chromatography-mass spectrography (GC-MS)

(a) *Methylation*: Five milligram of the dried polysaccharide (105 °C/24 h) was placed in a Corex tube and 3 ml of previously dried dimethyl sulphoxide was added. The mixture was agitated for 20 min in an N₂ atmosphere at room temperature. Then, 3 ml of methylsulphonyl carbocation (prepared according to Chaykovsky & Corey 1963) was added with a glass syringe. Agitation was maintained for an additional 6 h and 1 ml ice-cooled methyl iodate was then added. The mixture was agitated for another 16 h at room temperature and normal atmosphere. The extraction was carried out with 1 ml of deionized water and 4 ml of chloroform. The chloroform layer was washed five times with 3 ml of deionized water and them completely dried under N₂ flow.

(b) *Hydrolysis*: The methylated polysaccharide was dissolved in 2 ml of 90% formic acid solution and heated to 100 °C for 4 h. The solvent was then evaporated with N₂ flow and the residue treated with an aqueous 2 M solution of trifluoroacetic acid for 6 h at 100 °C. Again, the solvent was evaporated under N₂ flow.

(c) *Reduction of the partially methylated sugar*: To the residual solid, 1.5 ml of a solution containing 10 mg of sodium borohydride dissolved in 10 ml 0.5 M NH₄OH was added and the mixture which was kept at 40 °C for 1 h. Then, glacial acetic acid was added dropwise until no gas evolution was observed. The solvent was evaporated with N₂ flow and the residue washed five times with methanol followed by evaporation under N₂ flow.

(d) *Acetylation*: To the residue, 1 ml of acetic anhydride-pyridine (1:1 by volume) solution was added and kept at room temperature for 18 h. Then, 2 ml of toluene was added and evaporated under N₂ flow. To the residue, 5 ml of dichloromethane was added and the solution was washed three times with 1 ml of water. The solvent was totally evaporated under N₂ flow and the solids were kept in the freezer until analysed.

(e) *GC-MS analysis*: The dried sample was dissolved in dichloromethane and analysed with Hewlett-Packard

GC-MS equipment, model 5988 A, using the following operating conditions: electron ionization energy of 70 eV; column temperature of 130 °C for 5 min and then raised by a rate of 7 °C min⁻¹ up to 300 °C; injector and detector temperatures of 250 and 280 °C, respectively; gas flow of helium; and volume of sample of 1 µl.

Infrared spectroscopy

Spectra were taken from a standard dextran sample (Sigma Aldrich) and 'canjica' - KBr capsules prepared as following: 100 mg of dried KBr was added to 2-3 mg of samples, ground in mortar and pestle to homogeneity and pressed to form a capsule. The capsule was immediately placed on a Perkin-Elmer infrared spectrometer, model FT-IR 1605, and the spectra registered from 4000 to 500 cm⁻¹.

X-ray diffraction

Dried 'canjica' sample and dextran standard (Sigma Aldrich) were analysed directly by Rigaku X-ray diffractometer with horizontal goniometer ray flow of 30 kv/20 mA; CuK_α collimator and Kβ filter.

Results and discussion

General features

The collected 'canjica' samples were composed of water-insoluble isolated and linked granules with size varying from 1 to 15 mm in diameter. The colour varied from white to grey, with some reddish tint in some samples probably due to incorporation of soil pigments. The surface of the grains was usually smooth but rather irregular in shape. In the natural form, the 'canjica' grains were soft to the touch and contained about 80% of water. Upon drying with hot air (ca. 50 °C), they became hard and the water content dropped to about 10%. Figure 1 shows three types of commonly found 'canjica'.

Analytical results

Table 1 shows the chemical composition of dried 'canjica'. Water, carbon and hydrogen contents among different samples varied just slightly, from 8 to 10%, 41.0-43.5% and 6.0-7.0%, respectively. Paper chromatography (data not shown) on acid hydrolysed 'canjica' detected only glucose. Larger variations were shown in contents of nitrogen, sulphur and ash, which varied from 0.51 to 1.40%, 0.04-0.13% and 0.7-3.0%, respectively.

The close correlation between the amount of nitrogen and sulphur ($r = 0.99$) indicates that most of these elements were present in organic forms. If the nitrogen contents are converted to protein figures, they amount

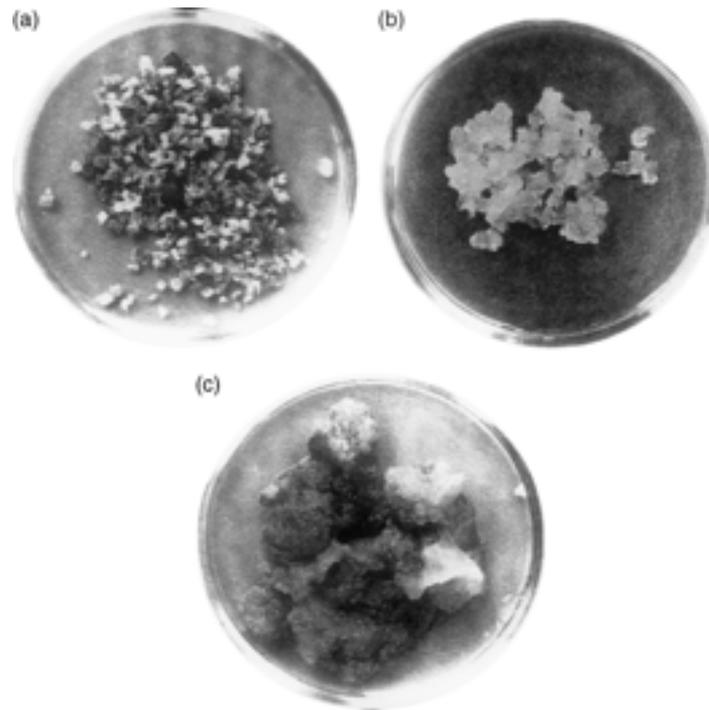


Figure 1. Freshly collected 'canjica' of different origin. (a), (b) isolated grains and (c) clumped grains.

Table 1. Chemical composition of 'canjica' from different sugar industries.

Sample	Water (%)	Ash (%)	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulphur (%)
1	8.90	0.72	43.0	7.10	0.87	0.08
2	8.80	2.60	41.4	6.80	0.79	0.08
3	9.70	0.62	41.6	6.80	0.72	0.07
4	8.80	0.71	41.6	6.80	0.43	0.04
5	9.70	3.00	41.0	7.00	0.44	0.04
6	10.1	0.60	43.4	7.00	0.48	0.05
7	8.30	0.64	43.6	7.20	0.65	0.06
8	8.00	1.00	41.3	6.00	1.39	0.13
9	8.70	0.65	42.0	6.80	0.51	0.05

to 3.2–8.7%, and if one assumes that 40–45% of cell solids are protein, the amount of cell components becomes 7.6–20% of the total dry matter of 'canjica'. This indicates that microbial cells are an important part of the 'canjica' structure.

The low correlation between nitrogen (or sulphur) and ash contents ($r = 0.07$) indicate that they are from different origins. Ash was normally entrapped in raw materials with soil and its removal varied from samples collected from industry to industry. This finding is in agreement with the results of Fernandez *et al.* (1993) and Sai *et al.* (1995).

Other elements analysis

Table 2 shows the content of other elements in the different samples of 'canjica', which vary considerably among samples. These variations could be attributed to different cane varieties or types of soil where the sugarcane was grown, as indicated by Sai *et al.* (1995).

This variation could also result from the type of harvesting system (Kumar *et al.* 1989), processing procedures, mainly regarding the washing water composition (Fernandez *et al.* 1993) and physiological stage of maturation at harvesting time (Sharma & Gupta 1969).

Gas chromatography-mass spectrometry

'Canjica' samples after methylation, hydrolysis, and acetylation and the 1,4- and 1,6-glycosyl standards were analysed under identical conditions by GC-MS. The 1,4- and 1,6-glycosyl standards display retention times of 18.093 and 18.394 min, respectively. The hydrolysed and derivatized 'canjica' samples display the most intense peak at a retention time of 18.241 min. The MS spectra showed that the 1,4- and 1,6-standards formed 1,4,5 tri-*O*-acetyl-2,3,6-tri-*O*-methylglucitol and 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol, respectively (McLafferty & Stauffer 1989). The mass spec-

Table 2. Metal ion, phosphate and chloride contents of 'canjica'.

'Canjica'	Al	Si	P	Cl (%)	K	Ca	Ti	Mn	Fe
1	0.44	0.44	0.17	0.02	0.01	0.09	0.01	0.00	0.09
2	0.85	0.26	0.14	0.03	0.04	0.09	0.02	0.01	1.19
3	1.05	0.06	0.13	0.02	0.01	0.10	0.01	0.00	0.05
4	0.87	0.07	0.11	0.02	0.08	0.11	0.01	0.02	0.05
5	0.46	1.72	0.09	0.02	0.02	0.09	0.08	0.01	0.41
6	0.89	0.11	0.10	0.04	0.06	0.16	0.00	0.01	0.06
7	0.97	0.16	0.12	0.05	0.04	0.17	0.01	0.00	0.16
8	0.81	0.08	0.26	0.03	0.06	0.18	0.00	0.01	0.06
9	0.82	0.03	0.18	0.17	0.34	0.69	0.00	0.03	0.16

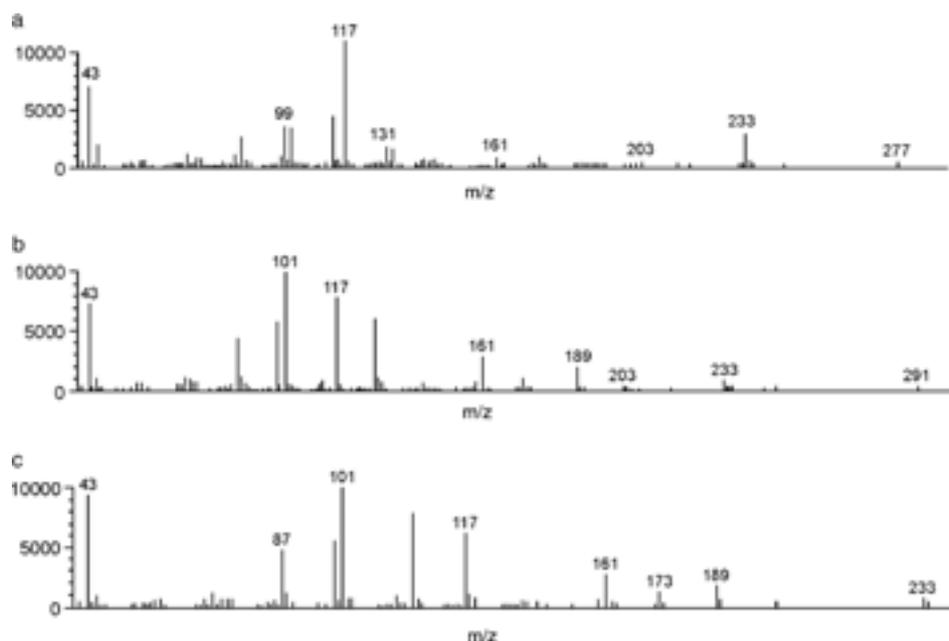


Figure 2. Mass spectrogram obtained from (a) 1,4 standard; (b) 1,6 standard and (c) 'canjica'.

Table 3. GC-MS data of alditol acetates from 'canjica' and the standards with 1,4- and 1,6-glycosidic bonds.

Compounds	Retention time (min)	Major ions (m/z) ^a
1,5,6-tri- <i>O</i> -acetyl-2,3,4-tri- <i>O</i> -methylglucitol	18.394	43,101,117,161,189,203,233,291
1,4,5-tri- <i>O</i> -acetyl-2,3,6-tri- <i>O</i> -methylglucitol	18.093	43,99,117,131,161,203,233,277
'canjica'	18.241	43,87,101,117,161,173,189,233

^a m/z = mass/charge ratio.

trum of the major compound detected in the 'canjica' sample (Figure 2c) shows similar patterns to that of 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol (Figure 2b), indicating that the predominant polysaccharide bond in 'canjica' is of the 1,6-glycosidic type. When the 1,6-glucosyl standard was spiked into the 'canjica' sample, the chromatographic peak of the standard was intensified, a co-injection confirmation of composition. The comparison with the mass spectrum presented by McLafferty & Stauffer (1989) shows that this chromatographic peak at 18.394 min is due to detection of 1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylglucitol. This finding confirms that the predominant polysaccharide glycosidic bond of 'canjica' is of the 1,6-type. Table 3

shows the primary fragments and the retention times of alditol acetate, derived from samples of 'canjica' and treated standards.

X-ray diffraction analysis

The technique of X-ray diffraction is useful in detecting the crystalline shape of samples. If X-ray diffraction was applied to purified dextran and 'canjica', a highly organized structure was observed for dextran (Figure 3), while 'canjica' showed two extra bands, one at 9.580 and other at 21.200 (2θ). These two bands must therefore belong to other compounds associated with dextran,

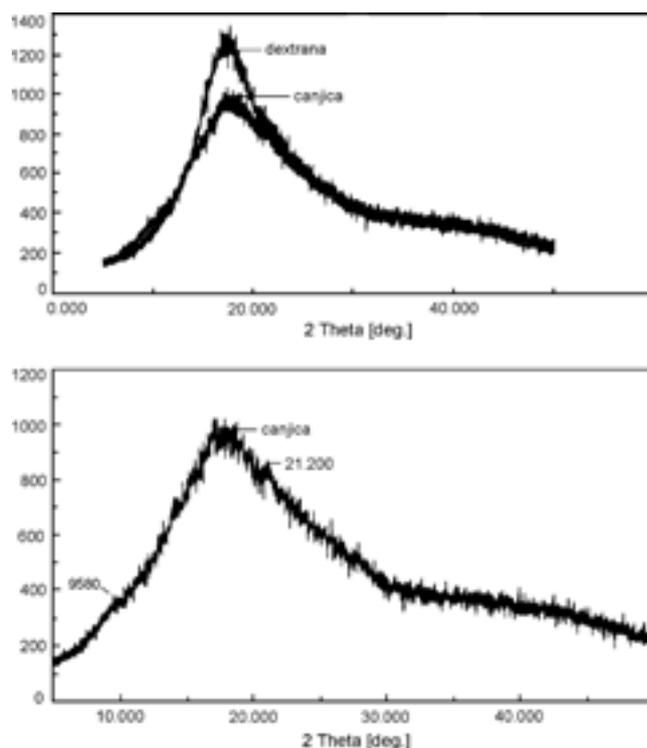


Figure 3. X-ray diffraction pattern of (a) dextran and 'canjica' and (b) 'canjica'.

which shows that 'canjica' is not solely made of dextran. The impurities could therefore be responsible for the differences observed in solubility.

Infrared spectroscopy

Infrared spectroscopy (i.r.) was applied to detect the presence of functional groups in the 'canjica' structure. A typical i.r. spectrum of 'canjica' and dextran is shown in Figure 4. Three important bands are observed at (1) 2926.44 and 2924.97 cm^{-1} which are characteristic of C—H bonds; (2) 1011.98 and 1011.43 cm^{-1} which are characteristic of the C=O bonds; and (3) 3433.03 and 3440.35 cm^{-1} which are characteristic of the O—H bonds. The similarity of this i.r. spectrum to that reported by Nakanishi & Solomon (1977), provides good evidence that dextran is indeed the major component of 'canjica'.

Conclusion

'Canjica' is a water-insoluble gum that occurs in the cane sugar industry at various intensities depending upon several factors, such as quality of raw material, sanitary condition of the industry, weather conditions and particularly the harvesting season. Whenever it appears at high intensity, the industrial loss is considerable, due to lower quality of the sugar, and frequent stoppage of the production line due to plugging and breakdown of equipment.

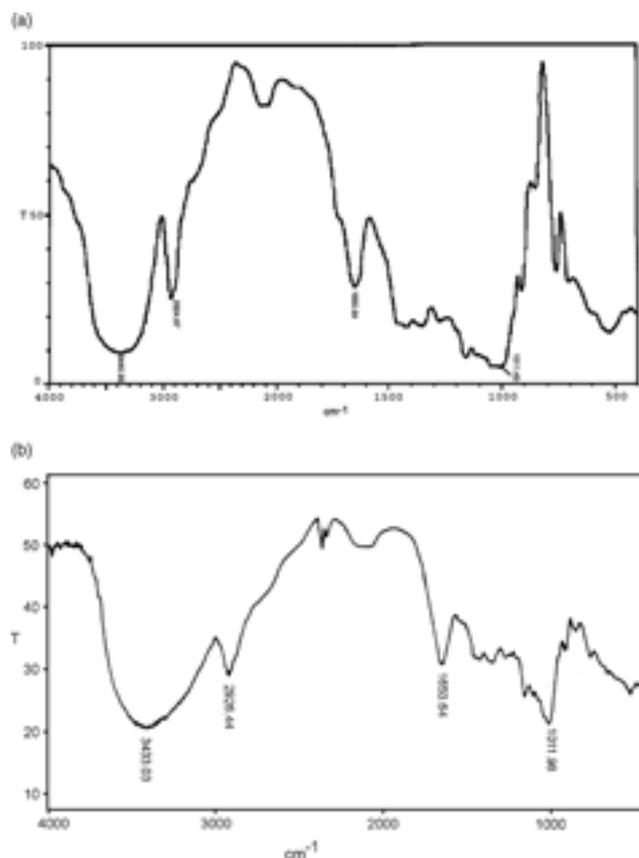


Figure 4. Infrared spectrogram of a standard dextran (a) and of a 'canjica' (b).

The chemical analysis performed for nine samples shows that 'canjica' is composed of dextran and a considerable part of non-dextran compounds. The GC-MS data has shown that dextran in 'canjica' is linear and displays predominantly 1,6-glycosidic linkages. This type of dextran is less water-soluble than the highly branched type (Seymour *et al.* 1979). X-ray diffraction analysis has shown that non-dextran compounds are also found in 'canjica'. The chemical analysis has also shown that a considerable portion of 'canjica' could be of microbial cell matter. The type of microbial association involved in 'canjica' formation still remains to be elucidated.

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