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New insights on the hard-to-boil massecuite phenomenon in raw sugar manufacture

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ABSTRACT

Louisiana hard-to-boil (HTB) massecuites (mixtures of sucrose crystals in molasses) with markedly low heat transfer properties are a sporadic problem in sugarcane factories, which cause raw sugar and molasses production to decrease and increase, respectively. This usually occurs after severely deteriorated sugarcane has been processed, but the specific cause is unknown and only limited correction has been achievable. At the end of the 2006 sugarcane processing season, HTB and normal massecuites and molasses were collected from four Louisiana factories. Compared to normal samples, the HTB samples had 9.1–33.2% lower heat conductivity and 10.0–49.2% higher heat resistivity. The more HTB a sample is, the greater the increase in heat resistivity compared to the corresponding decrease in heat conductivity. Excess lime addition to neutralise acids during juice clarification is *not* the direct cause of hard boiling. Oscillatory deformation rheology applied at 20 °C to normal molasses samples gave typical mechanical spectra of concentrated solutions. In contrast, a highly viscous, intermolecular (gel) network was present in the HTB molasses, which would explain the difficulty of removing entrapped water on boiling. Polysaccharides in the samples were characterised. GFC, TLC, and methylation analyses suggested the presence of an arabinogalactan and endo-dextranase-resistant dextran structures. The HTB phenomenon may have different causes and mannitol is a contributing factor.

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1. Introduction

In sugarcane processing, juice extracted across tandem mills or a diffuser is subsequently purified in the clarification unit process. The clarified juice (13–18 Brix or % dissolved solids) is then concentrated through a series of multiple-effect evaporators to yield final evaporator syrup (FES) of ~65 Brix. FES is further concentrated under vacuum to ~90–95 Brix (at lower temperatures than in evaporation to minimise the chemical degradation of sucrose) and crystallized. The vacuum pans are seeded with finely ground sucrose to allow larger sucrose crystals to form. The mixtures of sucrose crystals and mother liquor (molasses) discharged from a vacuum pan are massecuites, which are subsequently separated in centrifuges; the mother liquor is further re-concentrated and re-crystallized to give two more crops ("B and C crops") of crystals. The final liquor is the by-product molasses (also known as blackstrap or final molasses).

Boiling of massecuites is the process of removing water from impure syrup containing seed grains, which results in dissolved sucrose molecules being deposited onto seed sugar crystals for crystal growth. There have been sporadic episodes in Louisiana (LA) sugarcane factories of "hard-to-boil" (HTB) massecuites and molasses where mild to severe slow boiling difficulties were encountered. HTB massecuites have also been reported in South Africa (Koster, Vermeulen, Getaz, & Lionnet, 1978). This can be so severe that it can cause a factory stand-still, which happened at New Iberia, LA factory in 2002 for approximately 3 weeks. The HTB phenomenon usually occurs after severely deteriorated sugarcane has been delivered to a factory. This can occur after prolonged, heavy rains or after a severe sugarcane freeze. There have been few studies on this phenomenon (Duffaut & Godshall, 2004; Koster et al., 1978; Saska, 2003) and the cause is still unknown. As a consequence, few solutions to minimise the problem have been developed or implemented and processers frequently do not know how to correct it in the factory boiling house.

Saska (2003) measured the heat transfer coefficients (HTC) of syrups, molasses, and massecuites boiled in pilot vacuum pans, and found that some LA HTB samples had less than one tenth of the heat transfer capability of normal samples. Heat transfer rates were reduced and these were unrelated to crystallization kinetics of sucrose and the presence of the common polysaccharides dextran and starch. Furthermore, Saska (2003) reported that

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increasing crystal content on boiling to >88 Brix had a negligible effect on the reduced heat transfer properties and may even have had a positive effect. Rotational viscosity measurements and starch and dextran polysaccharides were reported not to correlate with the drastic heat transfer reductions (Saska, 2003). Also, surfactants, lubricants, and soda ash had no effect on improving heat transfer in the HTB samples. In comparison, lowering the pH by adding hydrochloric acid directly to the vacuum pan had a progressive effect on improving the boiling rate (Saska, 2003). Duffaut and Godshall (2004) reported that occurrences of the LA HTB phenomenon may have different causes. Like Saska (2003), they reported that "starch and dextran polysaccharides did not seem to be the cause" of the HTB phenomenon, although elevated dextran levels sometimes were observed in HTB molasses. In South Africa, Koster et al. (1978) observed abnormally high dextran and ethanol levels, indicators of severe sugarcane bacterial and yeast deterioration, respectively, in HTB massecuites with relatively high viscosities. Duffaut and Godshall (2004) reported that LA HTB samples exhibited higher pH, calcium, lactic acid, and ash levels than did normal samples. Hydrogen peroxide and hydrochloric acid were the most effective agents for improving boiling characteristics. Unfortunately, Duffaut and Godshall (2004) did not directly measure the heat transfer properties of samples which they studied.

At the end of the 2006 LA processing season, an early freeze caused HTB massecuites to occur at a few factories. Samples of HTB and normal massecuites and molasses were collected from four LA factories. The purpose of this research was to try to identify the cause(s) of the hard-to-boil massecuites and molasses and to underpin efforts to alleviate the problem at the factory. As Saska (2003) had reported that reduced heat transfer rates in HTB samples were unrelated to crystallization kinetics of sucrose, this was not studied. Using more sophisticated techniques than were used previously, including oscillatory deformation rheology, original insights are reported, including the presence of intermolecular networks in HTB products.

2. Materials and methods

2.1. General sampling

Abnormal HTB molasses and massecuites were collected from three LA raw sugar factories (Alma, St. Mary, and Lafourche) after deteriorated sugarcane was processed near the end of the 2006 processing season. As controls, normal molasses samples were collected from a fourth LA factory (Cora Texas or CT) that was operating under normal conditions. All four factories operated tandem mills. Samples were transported to the Southern Regional Research Center laboratory in New Orleans, LA for analyses.

2.2. Brix (per cent dissolved solids)

The mean Brix of triplicate samples was measured using an Index Instruments TCR 15-30 temperature controlled refractometer accurate to ±0.01 Brix.

2.3. Thermal properties

The thermal conductivity k ($W\,m^{-1}\,C^{-1}$) and resistivity R ($mC\,W^{-1}$) were measured using a KD2 Thermal Properties Analyser (Decagon, US) with an accuracy of 5%. The single needle probe was held by a clamp to minimise vibrations and the needle inserted into the middle of a beaker (100 ml) of massecuite or molasses. Results are expressed as averages of six measurements.

2.4. Conductivity ash

Inorganic, soluble ash content was determined by electrical conductivity according to ICUMSA method GS1/3/4/7/8-13.

2.5. Sulfated ash

Total soluble and insoluble ash contents after successive incinerations at 550 and 650 °C with sulphuric acid were determined according to ICUMSA method GS1/3/4/7/8-13.

2.6. Monoclonal antibody dextran

The Rapid Dextran Test (SucroTest™; Midland, US) was used (Rauh, Cuddihy, & Falgout, 2001). A conversion factor was calculated for each batch of antibody used.

2.7. Mannitol

Mannitol was measured using the enzymatic method of Eggleston (2009). Massecuite and molasses samples (20 g) were first diluted in 80 ml of de-ionised water.

2.8. Total polysaccharides

Total soluble polysaccharides were calculated, using the SPRI method (Roberts, 1981). Soluble polysaccharides were precipitated by 80% ethanol and the carbohydrate content of the precipitate then determined spectrophotometrically after reacting with phenol and sulphuric acid (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.9. Mechanical spectra

Mechanical spectra were recorded on an AR1000 Advanced [Oscillatory deformation] rheometer (TA Instruments, Houston, TX), using cone and plate geometry of angle 2° and diameter 4.0 cm. Sample temperature was 20 °C, controlled to within ±0.01 °C by a Peltier cell. Readings were taken 1 min after the sample had attained thermal equilibrium. A frequency sweep of 0.1–1000 rad/S was applied to each sample.

2.10. Preparation of polysaccharides

For isolation of polysaccharides, the massecuite and molasses samples were dissolved in de-ionised water (20 g/100 ml), clarified by centrifugation, and polysaccharides precipitated by addition of 4–5 volumes of 80% ethanol. The procedure was repeated twice on re-dissolved polysaccharide to remove residual sugars.

2.11. Anionic polysaccharides

A 1% aqueous solution of cetyl pyridinium chloride, a quaternary cationic surfactant, was added to a solution of 10 g sample in 50 ml of distilled or de-ionised water. Haze formation was noted visually, and any precipitate formed was collected by centrifugation.

2.12. Gel filtration chromatography (GFC)

High molecular weight (HMW) and low molecular weight (LMW) polysaccharide fractions were separated by gel filtration chromatography. Aqueous solutions of the precipitated polysaccharides were filtered and separated over 2.5 × 140 cm columns of Bio-Gel A-5m (exclusion limit = 5 million Da) and, in some cases, Sephacryl S-1000 (exclusion limit = 50 million Da for dextrans)

and/or Sephacryl S-200 (exclusion limit = 80,000 Da for dextrans), using water as the eluant. Fractions were collected from the eluate, analysed for total carbohydrate content (Dubois et al., 1956) and appropriate fractions combined and freeze-dried.

2.13. Thin-layer chromatography (TLC)

Polysaccharides were analysed for monosaccharide composition by hydrolysing samples in 2 M trifluoroacetic acid for approximately 2 h at 100 °C. Hydrolysates were analysed by thin-layer chromatography on Whatman K5 silica gel plates, which were irrigated for four ascents in 90% acetonitrile. Carbohydrate spots were detected, using the technique described by Bounias (1980).

2.14. Polysaccharide linkage analysis

This was performed via methylation (Ciucanu & Kerek, 1984) and GC–MS of the peracetylated aldononitrile derivatives (Seymour, Plattner, & Slodki, 1975). Two different isomaltodextranases were prepared and applied according to published procedures, namely, *Arthrobacter globiformis* NRRL B-4425 enzyme (Sawai, Toriyama, & Yano, 1974) and *Actinomadura* sp. NRRL strain B-11411 enzyme (Sawai et al., 1981). Several hydrolytic enzymes were tested for their abilities to hydrolyse isolated massecuite polysaccharides. Reaction mixtures consisted of 10 μl of polysaccharide solution (50 μg μl) mixed with 5 μl of enzyme solution in 20 mM pH 5.4 sodium acetate buffer with 0.01% sodium azide, and were incubated at room temperature. Reactions were monitored by TLC for up to 5 days. Enzymes tested included: α-amylase from *Bacillus licheniformis*, 10,000 U/ml (Sigma); endo-dextranase L, 30,000 U/ml (Amano); purified *Klebsiella pneumoniae* pullulanase, 18 U/ml (Sigma); starch saccharifying/transferase enzyme (Megadex, activity not assayed); crude *Aspergillus niger* hemicellulase (Sigma, 0.1 g/ml); hemicellulase (Amano, source organism unknown, 0.1 g/ml); cytolase M102 (Gist-Brocades, not assayed); and gamanase (galactomannanase, Novo, not assayed). Enzymes were shown by TLC to be active on their respective "natural" substrates.

2.15. Statistics

Pearson correlation coefficients were calculated to investigate relationships among the different chemical and thermal parameters using Microsoft Excel 2003™. Mean separations of values were obtained using Duncan's new multiple range test at the 5% probability level using PC-SAS (SAS Institute, Cary, NC).

3. Results and discussion

3.1. Heat transfer properties

A portable, thermal analyser was used to measure the thermal conductivity (k) or resistivity (R) of the samples. Thermal conductivity is the ratio of heat flux density to temperature gradient in a material, and it measures the ability of a substance to conduct heat. Thermal resistivity is a measure of the ability of a substance to prevent heat flowing through it. With this technique, a 30 s heat pulse was applied to a needle inserted into the sample. The temperature dissipation response versus time, which is a result of the thermal conduction rather than thermal convection properties of the material, was monitored at the needle and computed. In viscous materials, such as massecuites and molasses, heat transfer by conduction is much greater than that by convection.

The thermal conductivity and resistivity of the HTB and control samples are listed in Table 1 with the Brix values. In LA sugarcane processing, final evaporator syrups typically range from 63 to 68 Brix. Syrups and massecuites in vacuum pans typically range from 70 to 95 Brix (A–C pan boiling) (Saska, 2003), and final A–C massecuites from 89 to 96 Brix. The heat transfer coefficient (HTC) of such samples usually steadily decreases with an increase Brix in the respective pan. Saska (2003) reported that, for LA HTB molasses, the decrease in HTC was much more precipitous (in 79–81 Brix range) than that for normal molasses, and this was attributed to a "precipitation on, or fouling of, the heat transfer surface" of the pan (i.e., a boundary layer) where the local temperature and concentration can be expected to be higher than those of the bulk sample. Another explanation may have been the precipitous concentration of low heat transfer substance or substances in the bulk sample.

Density, water content, temperature and composition of a material affect thermal conductance and resistivity (Campbell & Norman, 1998). Water has a much higher thermal conductivity (0.57) than have air (0.025) and organic matter (0.25), which is why the thermal properties of foods are often manipulated by changing the water or air contents (Campbell & Norman, 1998). In this study, for all samples, there was no significant relationship between Brix and thermal conductivity or resistivity, but this was mainly because of the deviant nature of the St. Mary B molasses. Thus the water content of the samples was not responsible for HTB behaviour, but may have been a contributing factor in some samples, particularly the Alma C massecuite which had a 95.6 Brix. HTB Alma C massecuite was noted by the factory staff to have abnormal "clumping" tendencies, and the pan had to be cleaned more frequently than usual with acid. The Brix values of Alma A and B massecuites were lower than usual (Table 1), because they were hard-to-boil and caused a reduction in the factory processing rate. Consequently, the massecuites were collected early, i.e., with low Brix values and high water contents, to maintain an acceptable rate.

Ninela and Rajoo (2006) recently reported that high Brix (normal) massecuites may result in long pan boiling and crystal collection times in batch pans, poor mixing, mobility, and cooling in the C crystallizers, and may necessitate high re-heating temperatures for massecuites to improve flow and curing. Furthermore, high Brix massecuites may slow the massecuite circulation rate and increase the occurrence of Maillard colour-forming reactions which, in turn, increase C massecuite viscosity and adversely affect final molasses exhaustion (recovery of sucrose from molasses). South Africa now recommends a C massecuite Brix of 96.5–97.5 (Ninela & Rajoo, 2006). If C massecuite Brix is extremely high and causes a marked increase in viscosity, there could be retardation in migration of the sucrose molecules from the mother liquor onto the growing crystals. This could have contributed to the slower boiling of the 95.6 Brix Alma C massecuite (Table 1).

The reciprocal relationship between thermal conductivity and resistivity was polynomial ($R^2 = -0.998$; $y = 26.933x^2 - 27.119x + 9.050$). Thus, as samples became less able to conduct heat, the resistance to heat transfer became even higher than the reciprocal, or it just became more difficult to measure heat resistivity with the Thermal Analyser. This was markedly accentuated for HTB St. Mary A and B molasses, and Lafourche and Alma C massecuite, that had significantly ($P < 0.05$) lower thermal conductivity values than the rest of the samples (Table 1). Compared to the control CT A molasses, the St. Mary A molasses had 9.1% lower heat conductivity and a 10% higher heat resistivity. Compared to the control CT B molasses, the St. Mary B molasses had a 33.2% lower heat conductivity and 49.2% higher heat resistivity. This was in good agreement with the factory staff observation of ~30% slower boiling in the vacuum pans. The Alma A and B massecuites had only slightly lower thermal conductivities than had the normal molasses (Table 1). However, when their thermal conductivities were calculated on a Brix basis (not shown) they had pronouncedly lower values than had

Table 1
Average thermal conductivity and resistivity of the hard-to-boil and control samples.

Sample	Processing characteristic	Brix ± std dev[a]%	Average thermal conductivity ± std dev[a] (W m^{-1} C^{-1})	Average thermal resistivity ± std dev[a] (mC W^{-1})
Cora texas A molasses	Normal	63.81 ± 0.13 b	0.385 ± 0.008b	2.598 ± 0.042d
Cora texas B molasses	Normal	59.87 ± 0.01a	0.397 ± 0.014a	2.520 ± 0.103d
St. Mary A molasses	Hard-to-boil	77.94 ± 0.01d	0.350 ± 0.000 cd	2.854 ± 0.005c
St. Mary B molasses	Hard-to-boil	78.17 ± 0.01e	0.265 ± 0.007f	3.760 ± 0.000a
Alma A massecuite	Hard-to-boil [b]	76.35 ± 0.01c	0.360 ± 0.000c	2.816 ± 0.005c
Alma B massecuite	Hard-to-boil [b]	80.43 ± 0.01f	0.380 ± 0.000b	2.651 ± 0.010d
Alma C massecuite	Hard-to-boil	95.62 ± 0.02 h	0.340 ± 0.000d	2.920 ± 0.008c
Lafourche massecuite[c]	Hard-to-boil	95.48 ± 0.00 g	0.308 ± 0.020f	3.253 ± 0.214b

[a] The same lower case letters represent no statistical differences ($P < 0.05$) among the samples for each separate parameter measured.
[b] Unusually low Brix values – see explanation in the text.
[c] Had the consistency of toffee.

the controls. This was the case for all the other HTB samples, as well.

Overall, these results suggest that the more hard-to-boil a sample, the greater is the increase in heat resistivity compared to the corresponding decrease in heat conductivity. This strongly indicates that a substance (or substances) that suppresses heat transfer is responsible for the increased thermal resistance. These results also demonstrate the use of the portable Decagon KD2 Thermal Properties Analyser™ to confirm HTB samples as long as controls are included. It is easier to measure the thermal properties of molasses, as crystals in massecuites could interfere with the measurement.

3.2. Inorganic ash content

Soluble ash content reflects salts, minerals and ionised acids delivered to the factory from (i) soil associated with harvested sugarcane, (ii) sugarcane tissues, especially leaf tissues and (iii) lime added during the juice clarification process. The inorganic, soluble ash content of the samples ranged from 4.3% to 10.4% and the relationship between thermal conductivity and % soluble ash is illustrated in Fig. 1. The expected trend of thermal conductivity increasing as ash increases [because inorganic substances have relatively high heat transfer capacities compared to organic substances (Campbell & Norman, 1998)] occurred for the normal samples (Fig. 1). In strong contrast, all the confirmed HTB samples deviated from this expected trend (Fig. 1), i.e., the higher the soluble ash content the lower was thermal conductivity. Similar results (not shown) were observed for the relationship between thermal conductivity and sulfated ash (a measure of soluble and insoluble inorganic ash after incineration), although Lafourche HTB massecuite contained more insoluble ash than did St. Mary B molasses.

There have been reports (Duffaut & Godshall, 2004; Eggleston, unpublished results) that abnormal LA HTB massecuites and molasses have greater levels of calcium and, therefore, inorganic ash contents, than have normal samples. Higher calcium levels occur because of greater-than-normal lime addition during factory clarification of juice extracted from deteriorated sugarcane. This greater acidity of deteriorated sugarcane compared to fresh sugarcane necessitates more lime to neutralise acids and form calcium

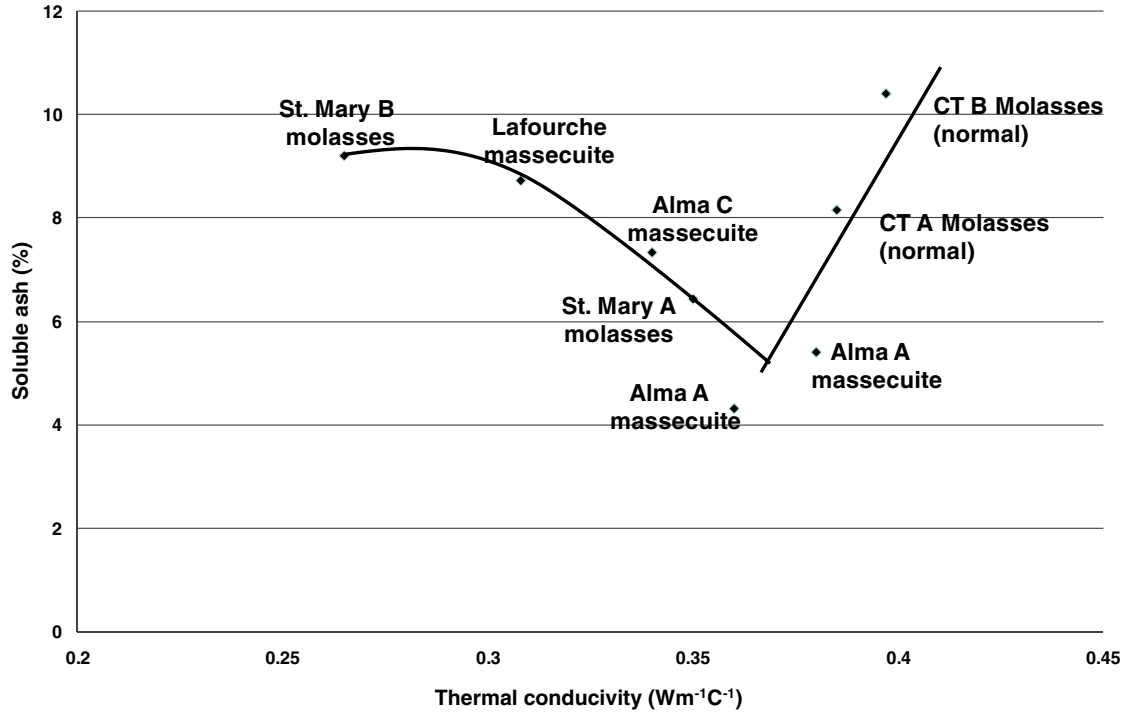

Fig. 1. Relationship between thermal conductivity and soluble ash content of HTB and normal (CT A and B molasses) samples.

phosphate flocs. Thus, increased lime addition is an indirect indicator of the processing of deteriorated sugarcane. However, as shown in Fig. 1, the additional lime is *not* the direct cause of slower boiling (lower thermal conductivity). Instead, a sugarcane deterioration product *must* be responsible for suppressing thermal conductivity (increasing thermal resistance) in HTB samples. Furthermore, Fig. 1 indicates that, the more severely deteriorated the sugarcane (indirectly demonstrated by the higher lime and subsequently higher inorganic ash content), the greater were the abnormal, unexpected heat transfer properties.

3.3. Total polysaccharides

Saska (2003) and Duffaut and Godshall (2004) both reported that starch and dextran polysaccharides did not correlate with drastic heat transfer reductions in LA HTB samples. Duffaut and Godshall (2004) did, however, observe that some LA HTB samples still had elevated levels of total polysaccharides and dextran. Koster et al. (1978) also observed abnormally high dextran levels in slow boiling, highly viscous HTB massecuites in South Africa. However, there are polysaccharides, other than starch and dextran, present in sugarcane, such as indigenous cane polysaccharide (arabinogalactan) (Blake & Clarke, 1984), and the sum of dextran and starch polysaccharides is always considerably lower than total polysaccharides.

Total soluble polysaccharides (TSP) in the samples are listed in Table 2 and were significantly ($P < 0.05$) different among all the samples. As expected, the less pure B molasses contained greater amounts of TSP than did A molasses, and TSP increased from A to C massecuites. Nevertheless, the HTB molasses still had considerably greater TSP than had the normal molasses (Table 2). TSP content for all samples was also markedly higher than antibody dextran (a measure of HMW dextran; Eggleston, Monge, Montes, & Stewart, 2007a) (Table 2). There are more polysaccharides in the leaves than in the stalk and growing point region of sugarcane (Eggleston, Grisham, Tew, Triche, & Antoine, 2009a). As more soluble impurities (Brix) are extracted from deteriorated green leaves than deteriorated brown leaves (Eggleston, Klich, Antoine, Viator, & Gober, 2009b), the deterioration of green leaves may contribute to the hard-to-boil phenomenon. However, brown leaves that have fallen on the ground of the sugarcane field and deteriorated, can contain very high levels of *Leuconostoc* bacteria and dextran polysaccharide (Eggleston et al., 2009b).

Moderate, but significant ($P < 0.05$), polynomial relationships existed between thermal conductivity and TSP values ($R^2 = -0.717$; $y = 290{,}455x^2 - 289{,}440x + 81{,}933$) and between thermal resistivity and TSP ($R^2 = 0.687$; $y = 43.78x^2 + 9970.5x - 12{,}646$), although they were only slightly better than linear relationships. Therefore, the greater content of soluble polysaccharides in the HTB samples contributed to their higher thermal resistivity.

3.4. Mechanical spectra

Saska (2003) reported that viscosity measurements did *not* correlate with the drastic heat transfer reductions in Louisiana HTB samples. In contrast, Koster et al. (1978) did report higher viscosity in HTB massecuites in South Africa. However, viscosity measurements in these two studies (Koster et al., 1978; Saska, 2003) were obtained from rotational viscometers of the Brookfield type and, therefore, only indicated the resistance of a sample to a rotational torque. In contrast, oscillatory deformation rheology (ODR), whereby an oscillatory force of different frequencies is applied to a sample, provides more information about viscosity and intermolecular network associations within the sample (Rees, Morris, Thom, & Madden, 1982, chap. 5).

In ODR, the sample under test is subjected to a sinusoidal deformation and the resistance to deformation is measured (Eggleston, 1989; Rees et al., 1982 chap. 5). For true solids, the greatest deformation occurs at maximum applied stress, i.e., at either extreme of the oscillatory cycle (in phase; G′). In contrast, for a perfect liquid the greatest resistance to flow (stress) occurs when the rate of deformation is greatest, i.e., in the middle of the oscillatory cycle (out of phase; G″). However, many materials, including polysaccharide solutions and gels, show intermediate properties, between solid-like and liquid-like (viscoelastic) behaviour (Rees et al., 1982 chap. 5).

Permanent networks, e.g., true gels, show properties approaching those of elastic solids; hence G′ predominates over G″ at all frequencies applied. The greater G′ over G″, the stronger is the gel network. Dynamic viscosity (η^*) decreases steeply with increasing frequency. At high frequencies of oscillation (where interchain entanglements do not have sufficient time to come apart within the period of one oscillation), concentrated polymer solutions behave similarly to true gels, i.e., G′ > G″. In contrast, for dilute polymers such as dextran structures typically found on sugarcane deterioration (not a true gel), G″ predominates over G′. However, with increasing frequency, intramolecular motions become more important and G' approaches G".

The quality, not the quantity, of polysaccharides often reflects or governs their physical properties. The mechanical ODR spectra for select normal and HTB samples at 20 °C are shown in Figs. 2a and b, respectively. Generally, normal molasses (Fig. 2a) showed mechanical spectra of concentrated solutions (Rees et al., 1982 chap. 5). (Crystals in massecuite samples often interfered with the production of mechanical spectra, but this may be remedied by using parallel plate geometry). Sometimes, G′ approached G″ at higher frequencies, due to entanglement of molecules. None of the normal samples exhibited intermolecular (gel) networks where G′ > G″ across all frequencies. However, the HTB molasses showed the presence of intermolecular networks, particularly the St. Mary A and B molasses (Fig. 2b), which were *not because of the higher Brix values of the HTB samples*. This could explain why factory

Table 2
Total polysaccharide and antibody (HMW) dextran contents of normal and hard-to-boil molasses and massecuites.

Sample	Processing characteristic	Average total polysaccharides ± std dev[a] (ppm/Brix)	Average antibody dextran ppm/Brix
Cora texas A molasses	Normal	11,556 ± 94a	287
Cora texas B molasses	Normal	14,213 ± 133b	92
St. Mary A molasses	Hard-to-boil	15,472 ± 121c	1564
St. Mary B molasses	Hard-to-boil	25,339 ± 49d	3332
Alma A massecuite	Hard-to-boil[b]	10,821 ± 121e	1117
Alma B massecuite	Hard-to-boil[b]	15,886 ± 85f	1626
Alma C massecuite	Hard-to-boil	21,474 ± 50 g	2282
Lafourche massecuite	Hard-to-boil	20,141 ± 196 h	1472

[a] Different lower case letters represent statistical differences ($P < 0.05$) among the samples for total polysaccharides.
[b] Unusually low Brix values – see explanation in the text.

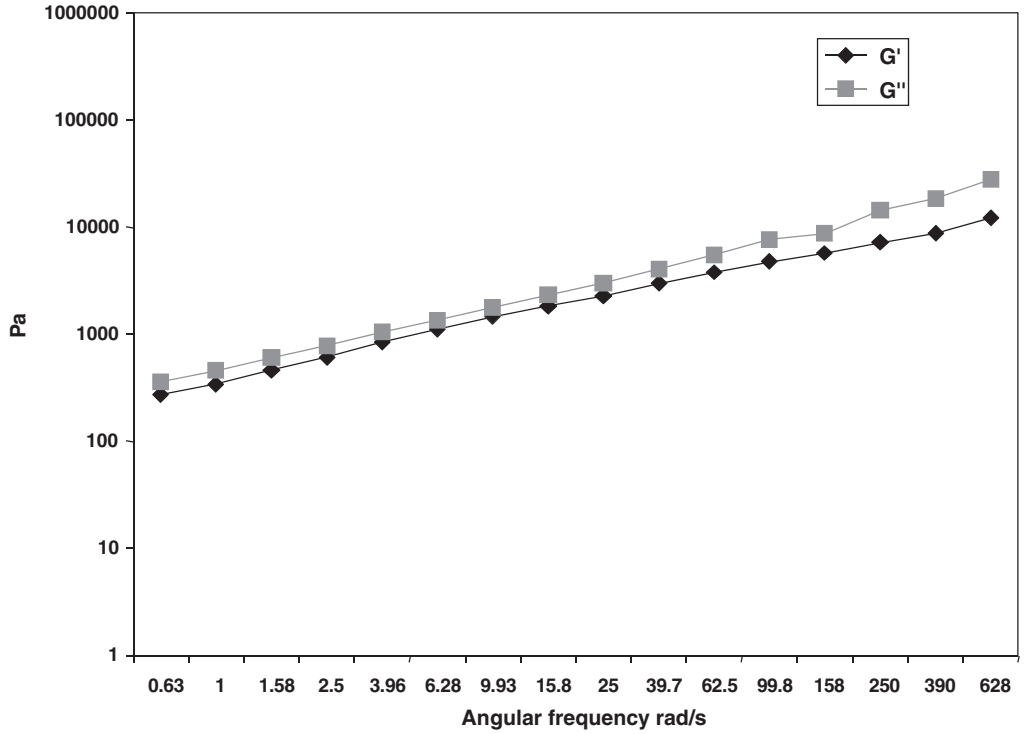

Fig. 2a. Mechanical ODR spectra of normal Cora Texas A molasses (63.8 Brix) at 20 °C.

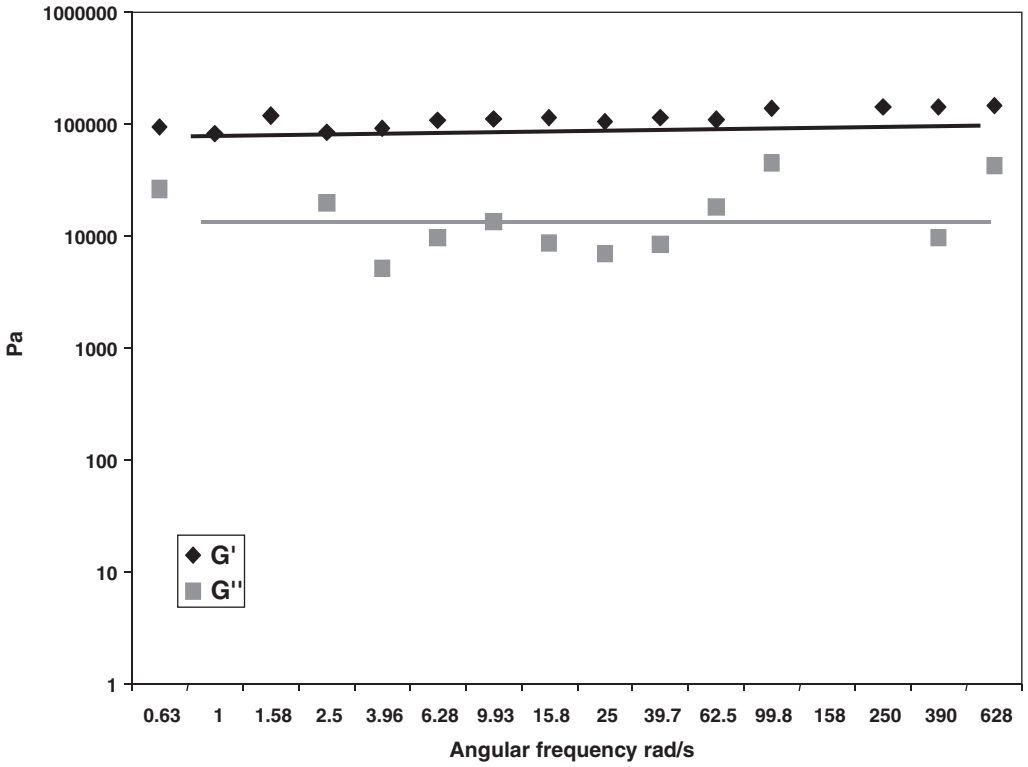

Fig. 2b. Mechanical ODR spectra of HTB St. Mary A molasses (76.5 Brix) at 20 °C.

processers often complain that they cannot evaporate off enough water in HTB massecuites – because the water is trapped in an intermolecular, cross-linked network. Furthermore, the gel networks dramatically increased viscosity. For example, viscosity at 2.5 rad/s was 221 Pa s for normal Cora Texas A molasses (Fig. 2a) compared to 53,010 Pa s for St. Mary A molasses (Fig. 2b). Sucrose is also known to strengthen polysaccharide gel networks (Eggleston, 1989). The presence of a polymer (most likely polysaccharide; Table 2) gel network in at least some HTB samples would make sense. Molasses associated with massecuites can also display

mechanical spectra of very concentrated solutions which can also markedly increase viscosity and reduce heat transfer properties of molasses (Eggleston, unpublished results). Recently, Muir, Eggleston, and Barker (2009) ruled out excess leaves and tops from fresh sugarcane delivered with stalks to the factory contributing to gel networks in downstream factory products, such as final evaporator syrups and molasses.

3.5. High molecular weight (HMW) dextran

Duffaut and Godshall (2004) observed that some HTB samples had elevated dextran polysaccharide (combined HMW, MMW and LMW dextran), but the dextran content did not correlate with the drastic reduction in heat transfer properties. In this study, HTB samples had higher antibody dextran (HMW dextran) levels than had normal samples (Table 2) and a moderate, polynomial relationship existed between antibody dextran and thermal conductivity ($R^2 = -0.723$) and thermal resistivity ($R^2 = 0.707$). This suggests that HMW dextran may have contributed to the HTB phenomenon, which is further evidenced by a moderate, polynomial relationship between HMW dextran and total polysaccharides ($R^2 = 0.771$) which was weaker in the samples that contained less dextran.

3.6. Polysaccharide structural analyses

Crude ethanol precipitates of polysaccharides from the normal and HTB massecuites and molasses were first analysed for anionic polysaccharide contents by attempting to precipitate them with quaternary detergent. A slight amount of haze formed in some samples but no recoverable amount of precipitate was formed; therefore, only trace amounts of anionic polysaccharides were present. The crude ethanol precipitates were then subjected to further purification and analysis. Total acid hydrolysis of the crude polysaccharide precipitates yielded mainly glucose and galactose, with lesser amounts of arabinose.

The crude polysaccharides were subjected to size-exclusion chromatography. Upon gel filtration (Bio-Gel A-5m) chromatography, most samples exhibited two carbohydrate peaks: one

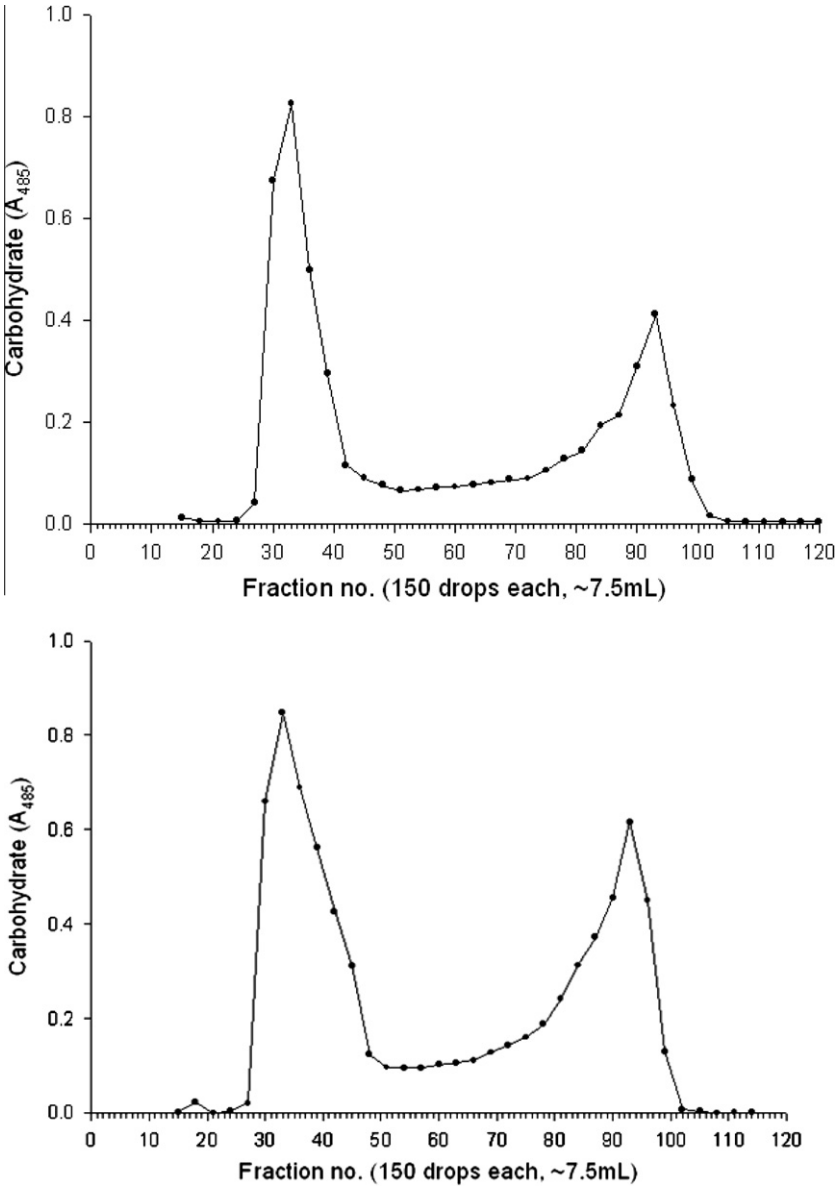

Fig. 3. Bio-Gel A-5 m gel permeation chromatograms of crude polysaccharide isolates from Alma A massecuite (top) and HTB St. Mary B molasses (bottom). These are typical of most samples.

corresponding to a large (HMW) polysaccharide and one to a smaller (LMW) polysaccharide (Fig. 3). Chromatography of the crude polysaccharides on Sephacryl S-200 yielded a large single peak, eluting near the void volume, indicating that all fractions were larger than 80 kDa, whereas chromatography on Sephacryl S-1000 yielded a very broad peak eluting entirely in the non-void volume, indicating an average MW for all fractions of less than 500 MDa. This result, along with the evidence that the crude mixture was precipitated by ethanol, leads to the conclusion that the low-MW fraction was mainly polysaccharide, rather than a mixture of oligosaccharides. We hypothesize that this low-MW fraction contains fragments of the larger polysaccharide that were produced from the enzymatic or other biological breakdown of the larger fraction. The LMW fraction, when subjected to acid hydrolysis, followed by TLC, gave glucose as the main product, with only trace amounts of galactose. Furthermore, methylation analysis of the LMW fractions showed mainly 6-linked glucose, indicative of a dextran-like polysaccharide, although significant proportions of 4-linked glucose were also detected, particularly in the normal samples, indicative of smaller starch (α-1,4-glucan), and possibly small cellulose (β-1,4-glucan) or pullulan (α-1,4, α-1,6-glucan), fragments. Therefore, it can be further speculated that the glucan molecules in the high-MW fraction are more likely to be broken down than are the other portion(s), and that they have at least some dextran-like structural features.

The HMW fractions (Fig. 3) were also subjected to monosaccharide analysis by acid hydrolysis and TLC. All HMW fractions contained glucose, galactose, and small amounts of arabinose and other pentoses, suggesting that the HMW fractions were composed of a mixture of polysaccharides. Densitometry of the TLC plates showed that the ratios of glucose to arabinose + galactose were much lower in the normal than in HTB samples (Fig. 4). This strongly indicates that all samples contained arabinogalactan, that is most likely an indigenous sugarcane polysaccharide (Blake & Clarke, 1984), but the HTB massecuites and molasses also contained large amounts of unknown glucan(s). Methylation analysis of the mixtures indicated that the glucans were mainly 1,6-linked, with smaller and variable amounts of 1,3 and possibly other secondary linkages also present. This is indicative of dextran (1,6-α-D-glucan), although resistance to endo-dextranase (1,6-α-D-glucan 6-glycanhydrolase; EC 3.2.1.11), i.e., dextranase added to sugar factories to break down dextran (Eggleston et al., 2007a) action, suggests that some type of secondary linkages may be interrupting the usual α-(1, 6)-linked chain structures.

Further evidence of unusual dextran-like structures was provided through the application of an exo-dextranase (isomaltodextranase; 1,6-α-D-glucan isomaltohydrolase; EC 3.2.1.94) to the mixtures. Isomaltodextranase is known to remove isomaltose from the non-reducing ends of dextran and, at least partially, hydrolysed HTB samples, to isomaltose. Thus, the most likely source of polysaccharide in the HTB samples is an endo-dextranase-resistant form of dextran and *not* indigenous sugarcane polysaccharide. Unfortunately, isomaltodextranase is only available for analytical uses and is not a commercial, industrial enzyme. It is, therefore, not available for factories to break down HTB massecuites. Other enzymes, namely, amylases, starch glucotransferases (4-α-D-glucotransferase; EC 2.4.1.25), pullulanase (EC 3.2.1.41), endo-dextranase and mutanase (α-1,3-glucanase; EC 3.2.1.59), were used to try to break down the HTB massecuite polysaccharides into oligosaccharides. However, the polysaccharides were resistant to degradation by these enzymes.

Although dextran is defined as a predominantly α-1,6-linked D-glucan, there are always secondary linkages present to some extent. Jeanes et al. (1954) described dextrans from 96 strains of *Leuconostoc* and related species, and provided some early evidence demonstrating the remarkable structural variability among the dextrans. The type, extent and distribution of these secondary linkages play a major role in determining the susceptibility of dextrans to hydrolysis by endo-dextranase (Sidebotham, 1974). Furthermore, the structural features of dextrans drastically affect their immunological reactions (Jeanes, 1986), and may influence the antibody dextran reaction (Rauh, Cuddihy, & Falgout, 2001) in the dextran method used in this study. It should come as no surprise that some of the more dextranase-resistant types are likely to be present in *Leuconostoc*-contaminated sugar syrups. We suspect that this may be the case with some HTB massecuites.

3.7. Mannitol

Mannitol, a sugar alcohol, is now known to be a major degradation product and sensitive indicator of *Leuconostoc* sugarcane deterioration (Eggleston, 2009; Eggleston, Basso, Amorim, De Lima Paulillo, & Basso, 2007b; Eggleston, Legendre, & Tew, 2004).

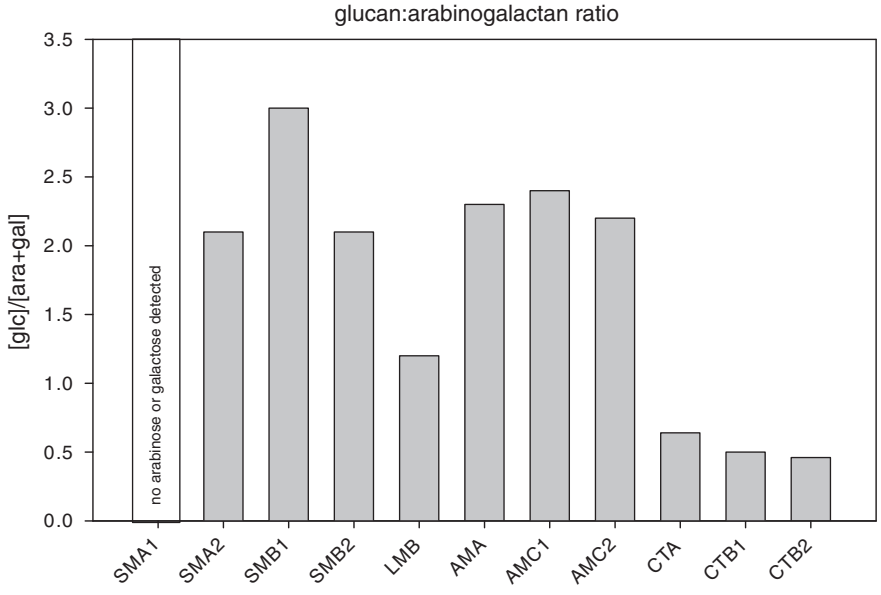

Fig. 4. Glucan:arabinogalactan ratios in normal and HTB HMW fractions. Ratios were determined by densitometry of TLC plates. CT samples are normal and the rest are HTB. Samples designated 1 and 2 are duplicates.

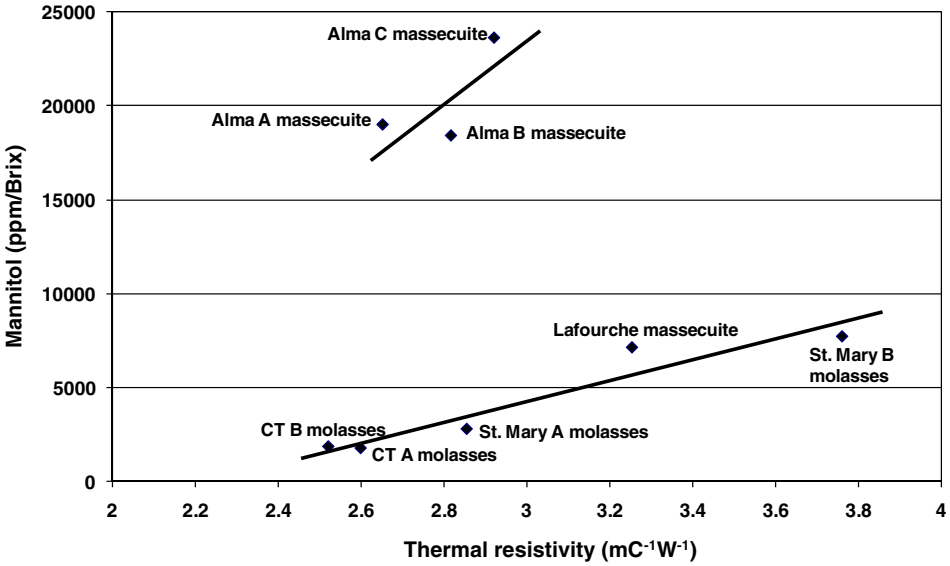

Fig. 5. Relationship between thermal resistivity and mannitol for normal (CT A and B molasses) and HTB samples. Linear trend lines are shown on the Figure for (top) Alma massecuites ($R^2 = 0.529$; $y = 15282x - 22{,}386$) and (bottom) the rest of the samples ($R^2 = 0.904$; $y = 5422.4x - 11{,}997$). Polynomial fits for (top) Alma massecuites ($R^2 = 1$; $y = 199193x^2 - 1E{+}06x + 2E{+}06$) and (bottom) the rest of the samples ($R^2 = 0.929$; $y = 2628.1x^2 + 21888x - 37{,}184$) are not shown.

Mannitol is also produced by *Lactobacillus* bacteria and a few fungi, although *Leuconostoc* is the greatest producer (Eggleston et al., 2007b). Mannitol does not degrade under processing conditions (Eggleston et al., 2004). The relationship between mannitol content and thermal resistivity is illustrated in Fig. 5.

Large amounts of mannitol occurred in the HTB samples compared to the normal samples (Fig. 5). This confirms that HTB massecuites and molasses were processed from deteriorated sugarcane. Overall, as mannitol increased, there was a concomitant resistance to heat transfer. This suggests that mannitol was, at the very least, a contributor to reduced heat transfer in HTB samples. We hypothesize that mannitol may have formed adducts (carbohydrate-salt complexes bound together by ion-dipole forces of attraction) (Rendleman, 1966) with calcium or another alkaline earth ions which caused an increase in viscosity and, hence, lower heat transfer properties (Rendleman, 1966). However, two distinct patterns existed (Fig. 5) – one for the three Alma HTB massecuites and one for the rest of the samples. This further suggests that that the LA HTB phenomenon may have different causes at different factories and, although mannitol is a contributing factor, it is not the major factor. Duffaut and Godshall (2004) similarly reported that occurrences of the HTB phenomenon may have different causes. Furthermore, when mannitol was plotted against total soluble polysaccharides (TSP), as mannitol increased, then TSP increased. However, two similar patterns (not shown) also existed, which further suggests that both mannitol and TSP are both involved in reducing the thermal transfer properties of HTB massecuites.

4. Conclusions

Additional research is now required to find an economical processing aid that is capable of (i) improving heat transfer, (ii) breaking down the intermolecular network, and (iii) increasing sucrose recovery in HTB samples at the factory.

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