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Insoluble Starch Contributes to Lower Sugar Exhaustion from Molasses During the Manufacture of Raw Sugar

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ABSTRACT

The greatest loss of sucrose in a raw sugar factory is that which resides in the final molasses. A 2-year (2020 and 2021 Louisiana processing seasons) study of weekly final molasses from three sugarcane factories has recently thrown up new information on the effect of starch on molasses exhaustion. Target purity difference (TPD) of final molasses is an important metric for factory performance to estimate final molasses exhaustion; generally, a lower TPD indicates greater recovery of sugar. During the 2021 processing season, LA factories experienced markedly higher target purity differences (TPDs) and thus lower exhaustion compared to the 2020 season (normal year). Differences in molasses exhaustion among the factories could not all be explained by conventional reducing sugar/ash (RS/ash) ratios, particularly in 2021. With the advent of new methods for the sugar industry to measure the amounts of soluble and insoluble starch as well as total starch, these three forms of starch were analyzed in weekly composite C molasses from the three LA factories in both seasons. For the first time, the starch form was shown to strongly impact molasses exhaustion. The greater the amount of insoluble starch in the molasses the lower the TPD value ($R^2=0.886$). This is most likely because the insoluble swollen starch increases the viscosity of the molasses which, in turn, impedes sucrose exhaustion. The factory that added high-temperature (HT) stable amylase to the clarification settling tank consistently had the lowest TPD values in both years. The risk of carry-over amylase activity in raw sugar, however, increases with the addition of HT amylase, but this problem could be solved at the refinery with the use of activated carbon.

INTRODUCTION

Since 2006 there has been growing concern in Louisiana and worldwide over increased amounts of starch in sugarcane entering factories mostly because of the (i) mechanical harvesting of green (unburnt) sugarcane, (ii) introduction of new varieties of cane with higher concentrations of starch, (iii) addition of chemical ripeners (depends on the sugarcane variety) and (iv) varying environmental conditions. Starch granules contain two glucopolysaccharides (glucans linked mostly by $\alpha 1 \rightarrow 4$ glycosidic linkages): linear amylose and branched amylopectin. With the recent development of a method capable of measuring total, soluble, and insoluble starch forms, it has been found that in the presence of water and heat and with time during sugarcane processing not all the insoluble starch granules swell, disintegrate, and cause amylose and amylopectin to become soluble (Cole et al., 2013). By utilizing digital microscopy and chemical methods it has been

unequivocally observed that the solubilization of insoluble starch is severely limited after the juice clarification because of increased Brix values, short retention times, and reduced temperatures in the later evaporation stages, all of which inhibit starch solubilization. Thus, some insoluble starch granules only become swollen and, along with soluble starch, can persist into factory raw sugars and even refinery white sugars, and this is illustrated in Fig. 1 (Cole et al., 2013).

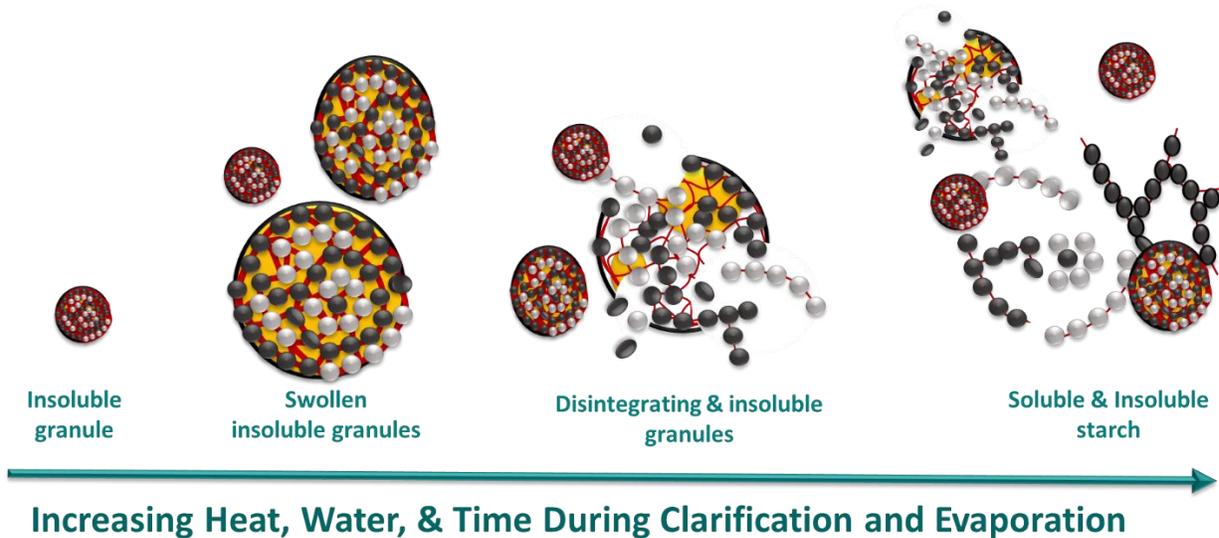


Fig. 1. Graphic illustration of how sugarcane starch behaves in the factory after being extracted as granules (1 to 5 μm) during extraction by milling.

It is of great interest to refiners of raw sugars that both (i) the total amount of starch in raw sugar and (ii) the ratio of soluble to insoluble starch can severely affect the refinery process, particularly carbonatation clarification (Cole et al., 2016b,c, 2019). Phosphatation clarification processes in refineries are less sensitive to starch. Fig. 2 illustrates the effects of various ratios of soluble and insoluble starch on the formation of fine and large particles of calcium carbonate particles during carbonatation clarification of raw liquors (simulated experiments) (Cole et al., 2019). High starch concentrations have caused some carbonatation refineries to add amylase in the refinery but this has multiple disadvantages to the refiner, including:

- Inefficient breakdown of starch due to the high Brix values of refinery liquors since water is a reaction requirement for the catalyzed hydrolysis of starch with amylase,
- Increased risk of possible carry-over amylase in the refined sugar (this risk is much greater if high-temperature stable amylase is added) (Eggleston et al., 2016, 2017), and
- Increased protein concentrations are caused by adding amylase protein which can contribute to floc formation between phenolic colorants and protein. This is more severe in alcoholic beverages since the alcohol denatures the protein and, unfortunately, makes it more “floc reactive” (Eggleston and Triplett, 2017).

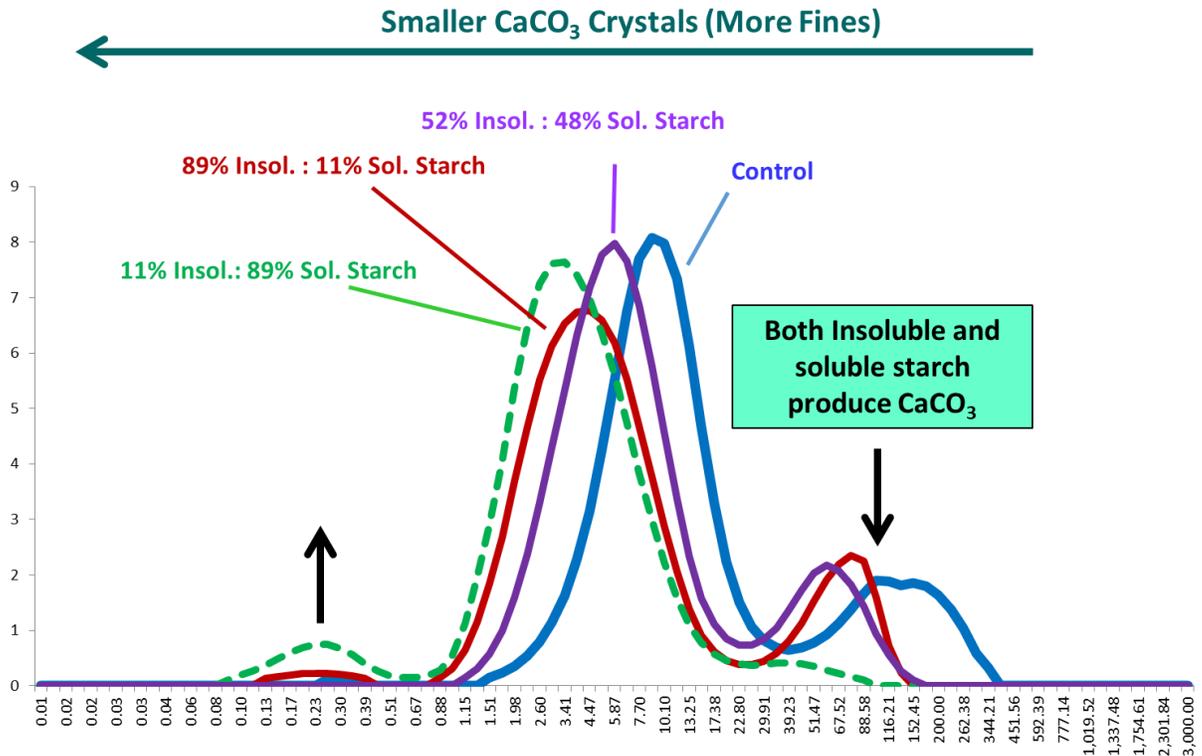


Fig. 2. The effect of various ratios of insoluble and soluble starch on the production of calcium carbonate particles during carbonation clarification simulated experiments with refinery raw liquors. Particle size distributions (μm) were measured with a Laser Diffraction Particle Size Analyzer LA-950 (Horiba, Houston, TX, USA) and adapted from Cole et al. (2019).

The effect of starch on the exhaustion of molasses at the factory has been unknown which the current authors believe is because the traditional methods of starch measurement are neither able to solubilize all the starch present nor detect insoluble starch (Cole et al., 2017, 2018). Unfortunately, the greatest loss of sucrose in raw sugar manufacture occurs in final molasses and, although many improvements have occurred in LA sugarcane factories over the past 50 years, the improvement of molasses exhaustion has been relatively limited (Birkett, 2022). To aid processors in Louisiana, the Audubon Sugar Institute (ASI) conducts a yearly molasses exhaustion service for all eleven sugarcane factories. Composite molasses samples provided weekly from each factory are analyzed and results are used to calculate a “target purity” (TP) and a true purity for the molasses. The TP is an arbitrary target concentration of sucrose where no further sugar can be expected to crystallize in the industrial setting. The model that is used to calculate the TP in Louisiana originates from South Africa (Rein, 2007). The true purity is determined directly by high-performance liquid chromatography (HPLC) and is free of interferences, e.g., reducing sugars *RS*, that can offset the accuracy of polarimetric determinations, particularly in molasses where purities are very low. The formula for TP is given below, where *RS* is the total reducing sugar (glucose + fructose) determined by HPLC and *Ash* is the approximate sulfated ash via conductivity (Saska et al., 1999).

$$TP = 33.9 - 13.4 \cdot \text{Log}_{10} \frac{RS}{Ash}$$

The TP is subtracted from the true purity to give a target purity difference or TPD. The TPD is used by the factories to determine how well they are recovering sugar from their massecuites (which is reflected by residual sugar in the molasses). “True purity” is the sum of the non-crystallizable sugar and that which was crystallized but lost across the centrifuges. A lower TPD indicates greater efficiency as it relates to the recovery of sugar. *RS* and *ash* contents are part of the TP formula because they influence the amount of sucrose extracted from massecuites into molasses (Chen, 1993). In the presence of *RS*, the solubility of sucrose is decreased whereas in the presence of mineral salts, the solubility of sucrose is increased. Thus, a relative proportion or ratio of these two components influences the purity of the final exhausted molasses (Chen, 1993). Additionally, viscosity can have a strong effect on molasses exhaustion, since it influences and limits the handling capabilities of crystallizers and centrifugals (Chen, 1993). Viscosities are known to vary widely due to the nature and amounts of non-sugars, and the influence of these components on viscosity increases rapidly with an increase in their content.

Sugarcane processing seasons in Louisiana usually occur from late September to early January, with the length of processing being affected by crop yields and the weather. In 2021, the LA processing season was unusual because (i) the cane was short in height (ii) a freeze occurred in early 2021, (iii) there was excess rain in the growing season, and (iv) Hurricane Ida occurred on the 29 August 2021 which affected a lot of the field cane in the southeastern region of the State. Additionally, the chemical ripener glyphosate (Polado®), was sprayed late in some areas due to the rain and the hurricane in the growing season. Starch concentrations are typically highest at the beginning of a season due to the immaturity of the cane and decrease in December with the onset of colder weather, although varietal effects are strong. Even though the beginning of the LA processing season was late in 2021, the starch concentrations were noticeably higher than normal. Furthermore, at the same time, the molasses exhaustion was sub-normal when compared to previous LA processing seasons. The objective of this current study was, therefore, to evaluate the role of insoluble and soluble starch on molasses exhaustion as well as the roles of dextran and fructan polysaccharides.

EXPERIMENTAL

Molasses exhaustion analyses. During the 2021 processing season, as part of the annual Audubon molasses exhaustion survey, weekly final (C) molasses samples, i.e., 7-day composites were received and analyzed in duplicate from eleven LA factories (denoted by uppercase letters A to K). Analyses included (i) refractometer Brix (ii) sucrose, glucose, and fructose by HPLC, (iii) apparent purity by polarimetry, and (iv) conductivity ash, as described below.

Refractometric Brix of the molasses was measured following the ICUMSA GS4-13 (2007) method using a Bellingham and Stanley RFM340+ sugar refractometer (Tunbridge Wells, UK) temperature controlled at 20 °C.

Sucrose, glucose, and fructose by high-performance liquid chromatography (HPLC). True purity of the molasses was measured using HPLC with refractive index detection, following the ICUMSA method GS7/4/8-23 (2002). Sugars were separated on an Agilent (Santa Clara, CA, USA) 1200 HPLC instrument, using a Bio-Rad (Hercules, CA, USA) Aminex HPX-87C column (300 x 7.8 mm) and guard column at 85 °C. The eluent conditions were de-ionized water at an isocratic flow rate of 0.6 mL/min. An Agilent refrigerated auto-sampler at 5 °C was used to prevent the

degradation of sugars in samples while waiting for injection into the column. An Agilent Series 1200 refractive index detector at 45 °C was used to detect the sugars. Agilent Openlab chromatography software (CDS ChemStation Edition for LC/MS Systems; Rev. C.01.04) was used to accumulate multiple samples and standards. The samples were analyzed in duplicate.

Apparent purity by polarimetry. Pol was obtained using a Rudolph AutoPol 880 Polarimeter (Hackettstown, NJ, USA) at 589 nm. Each molasses sample was first diluted 1:1 with de-ionized water then 26 g/200 mL with de-ionized before being clarified with Octapol™ (Baddley Chemicals, Baton Rouge, LA, USA) clarification agent. Each prepared sample was poured through a 200 mm flow tube and a measurement in °Z was taken.

Conductivity ash. The conductivity of the molasses was measured with ICUMSA GS1/3/4/7/8-13 (1994) method using an Oakton CON 700 (Vernon Hills, IL, USA) conductivity meter. Each sample was measured at 20 °C ± 0.2 °C.

Analyses of final molasses from three Louisiana sugarcane factories across the 2020 and 2021 grinding seasons. Weekly samples of final molasses across the 2021 season were further analyzed from three Louisiana factories denoted E, H, and I. The three factories were selected because they represented a range of molasses exhaustion performances. Results were compared to equivalent samples across the more normal 2020 season, since ASI stores samples from the molasses survey from the previous processing season

Total, insoluble, and soluble starch. Molasses samples were analyzed for total, soluble, and insoluble starch using the microwave-assisted sonication/iodometric USDA Research method (Cole et al., 2016a). Molasses were first adjusted to ~15 Brix before analyses and starch was assayed in duplicate. Occasionally, insoluble results were negative which indicated that the sample had been “over-solubilized” with the accompanied breakdown of some soluble starch; as a result, these samples were considered outliers. Results are quoted as average ppm/Brix values.

Dextran and fructan. Dextran was measured by an antibody dextran method using a kit purchased from Guangzhou Sugarcane Industry Research Institute, Guangdong, China, and a Midland MCA SucroTest™ Turbidimeter was used. Dextran was assayed in duplicate and concentrations were quoted as average ppm on a Brix basis. Fructan (levan) in molasses was measured using a specific enzymatic kit (K-FRUC) from Megazyme® (Bray, Wicklow, Ireland), which is based on AOAC Method 999.03 and is specific for all types of fructans. Sucrose and starch were first removed with enzymes and the resulting reducing sugars plus existing reducing sugars and fructooligosaccharides in the molasses were reduced to non-interfering sugar alcohols and reduced fructooligosaccharides. Fructans were then specifically hydrolyzed with exo- and endo-inulinase and endo-levanase. An external standard of levan flour was used to correct the final concentrations of fructan. All polysaccharides were assayed in duplicate and concentrations were quoted as average ppm on a Brix basis.

Statistics

Pearson correlation coefficients were calculated to investigate relationships among the various parameters using Excel Microsoft Office Professional Plus 2016 (Microsoft, Redmond, WA, USA). The same software was used to calculate means, standard deviations, and coefficient of

variations (% CV). Yearly results were also compared with one-way ANOVA, with means ranked using Tukey’s HSD at the 5% confidence level. Analyses were conducted using R (R Core Team, 2022) and RStudio (Posit Team, 2023).

RESULTS AND DISCUSSION

Analyses of Final Molasses from Three Select Louisiana Factories Across the 2020 and 2021 Processing Seasons

The variation in TPD, total starch, dextran, and fructan values across the 2020 and 2021 seasons at LA Factories E, H, and I are illustrated in Fig. 3. For each factory, the mean TPD values were higher in 2021 than in 2020. The TPD values, generally, were highest at the start of grinding, but in 2021 factories E and H exhibited an increase in TPD values at the end of the season.

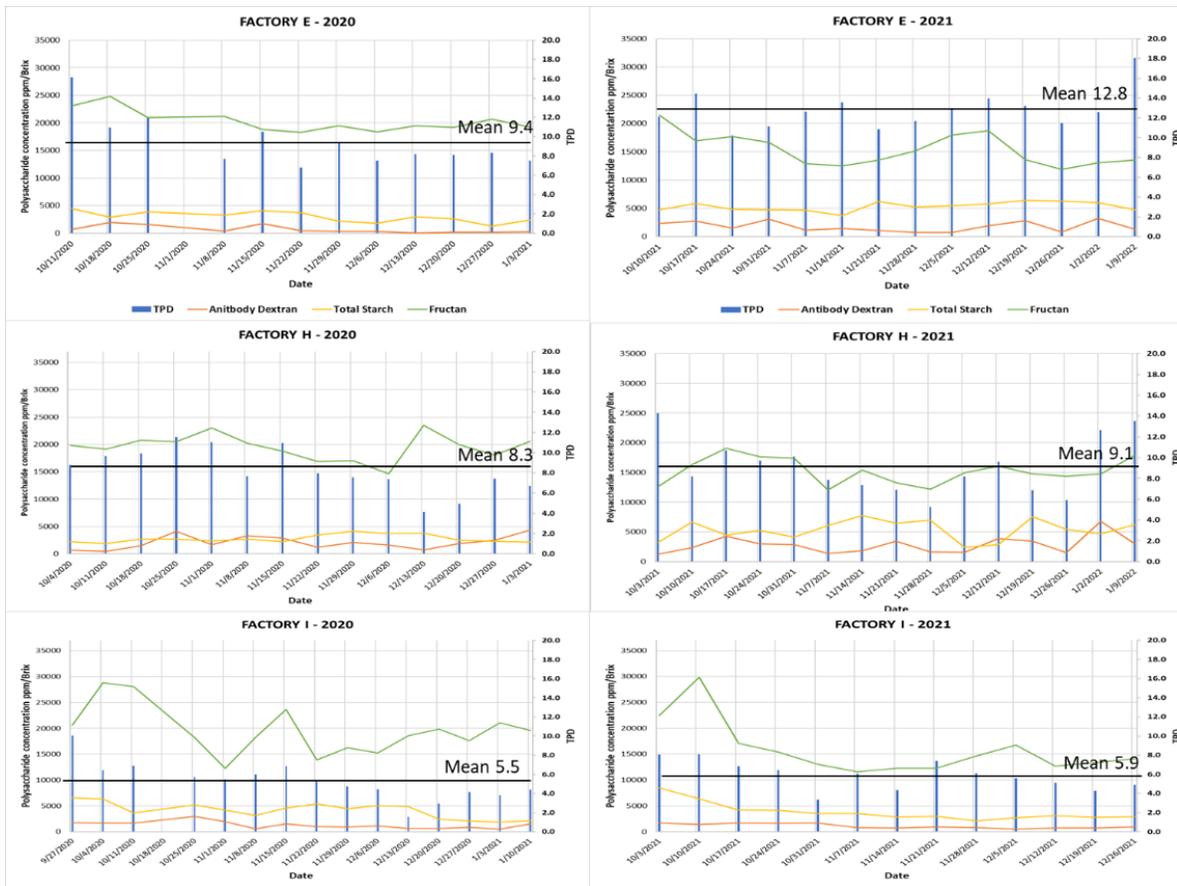


Fig. 3. Variations in TPD, dextran, total starch, and fructan concentrations in three LA sugarcane factories across the 2020 and 2021 grinding seasons. Season mean TPD values are directly shown on each graph.

Table 1. Molasses exhaustion (conventional) data from three LA sugarcane factories across the 2020 and 2021 seasons.

| Factory | Season Statistical Parameter | % on Total Solids | | | | | | |
|-------------|------------------------------|---------------------|----------------------|---------|----------|--------|-----------|---------|
| | | TPD | Sucrose [†] | Glucose | Fructose | RS* | Cond. Ash | RS/Ash |
| 2020 | | | | | | | | |
| E | Mean ± | 9.43aB [§] | 44.2aB ± | 4.5aB ± | 7.1aA ± | 11.6aB | 15.7bA | 0.748aA |
| | SD | ± 2.6 | 2.2 | 0.6 | 0.6 | ± 1.1 | ± 1.5 | |
| E | CV % | 28.0 | 5.1 | 13.9 | 7.8 | 9.2 | 9.6 | 17.8 |
| E | Max | 16.1 | 47.8 | 5.9 | 8.1 | 14.0 | 17.5 | 14.0 |
| E | Min | 6.8 | 38.4 | 3.7 | 6.5 | 10.6 | 12.8 | 10.6 |
| H | Mean ± | 8.3aA ± | 44.0aA ± | 3.9aA ± | 7.7aA ± | 11.6aA | 16.0abA | 0.738aA |
| | SD | 2.2 | 1.6 | 1.1 | 0.6 | ± 1.7 | ± 1.2 | |
| H | CV % | 26.8 | 3.7 | 28.2 | 8.2 | 14.6 | 7.7 | 21.1 |
| H | Max | 11.6 | 46.6 | 6.5 | 9.3 | 15.8 | 17.4 | 1.112 |
| H | Min | 4.1 | 41.4 | 2.4 | 6.5 | 8.9 | 13.8 | 0.512 |
| I | Mean ± | 5.5bA ± | 41.3bA ± | 4.4aA ± | 7.7aA ± | 12.1aA | 17.3aA | 0.730aA |
| | SD | 2.3 | 1.5 | 1.6 | 1.8 | ± 3.4 | ± 2.0 | |
| I | CV % | 41.4 | 3.6 | 36.7 | 23.7 | 28.2 | 11.4 | 41.0 |
| I | Max | 10.5 | 43.5 | 7.5 | 12.0 | 19.5 | 19.8 | 1.313 |
| I | Min | 1.5 | 38.3 | 2.6 | 5.5 | 8.1 | 13.1 | 0.429 |
| 2021 | | | | | | | | |
| E | Mean ± | 12.8aA | 48.0aA ± | 5.5aA ± | 7.4aA ± | 12.9aA | 16.0aA | 0.797aA |
| | SD | ± 2.0 | 2.4 | 0.6 | 1.5 | ± 1.5 | ± 1.7 | |
| E | CV % | 15.3 | 4.9 | 10.6 | 20.1 | 11.8 | 6.6 | 17.7 |
| E | Max | 18.1 | 54.3 | 6.5 | 10.6 | 15.7 | 18.4 | 1.098 |
| E | Min | 10.2 | 45.0 | 4.0 | 5.2 | 10.7 | 14.3 | 0.582 |
| H | Mean ± | 9.1bA ± | 45.0bA ± | 4.2bA ± | 7.3aA ± | 11.5aA | 15.7aA | 0.735aA |
| | SD | 2.7 | 2.3 | 0.6 | 1.5 | ± 1.9 | ± 1.5 | |
| H | CV % | 29.8 | 5.1 | 14.4 | 21.0 | 16.7 | 10.8 | 24.8 |
| H | Max | 14.3 | 50.3 | 5.0 | 10.8 | 15.6 | 18.9 | 1.130 |
| H | Min | 5.3 | 42.2 | 3.0 | 5.4 | 9.0 | 12.7 | 0.487 |
| I | Mean ± | 5.9cA ± | 42.0cA ± | 4.4bA ± | 7.5aA ± | 11.9aA | 17.1aA | 0.707aA |
| | SD | 1.5 | 1.3 | 0.4 | 1.6 | ± 1.9 | ± 1.5 | |
| I | CV % | 25.1 | 3.1 | 9.4 | 21.8 | 16.3 | 8.7 | 24.7 |
| I | Max | 8.1 | 44.0 | 5.1 | 11.5 | 16.1 | 19.0 | 1.019 |
| I | Min | 3.4 | 39.0 | 3.9 | 5.8 | 6.2 | 14.7 | 0.521 |

[†]True purity sucrose by HPLC

*RS = reducing sugars (glucose + fructose)

[§] Lowercase letters indicate statistical differences among factories for the same year

[§] Upper case letters indicate statistical differences between years for each factory

The mean molasses exhaustion data for the three factories from the 2020 and 2021 seasons are listed in Table 1. In both years, the mean TPD value followed the same order: Factory E > Factory H > Factory I, with values worse in 2021 than in 2020. (Note: In 2020 only, the mean

TPD value for Factory E was not significantly different from the value for Factory H). As expected, a similar order occurred for sucrose (true purity) values (Table 1). The glucose and fructose concentrations, however, did not follow this order and were not as significantly different among the factories and between the two years. This is most likely because these values are governed by Maillard color reactions which occur in later evaporators, vacuum pans, and crystallizers. Glucose is preferentially used over fructose in Maillard reactions which explains the higher fructose concentrations in Table 1.

Graphs in Fig. 4 show that for all three factories in 2020, there were significant relationships (R^2 values were >0.390) between TPD and RS/ash ratios, which were strongest in Factory E. Thus RS/ash ratios mostly governed TPD values in 2020. In strong contrast, in 2021 the relationships between TPD and RS/ash ratios were considerably weaker than in 2020, and in Factory E non-existent. Thus, other factors than RS/ash ratios were governing TPD values in 2021.

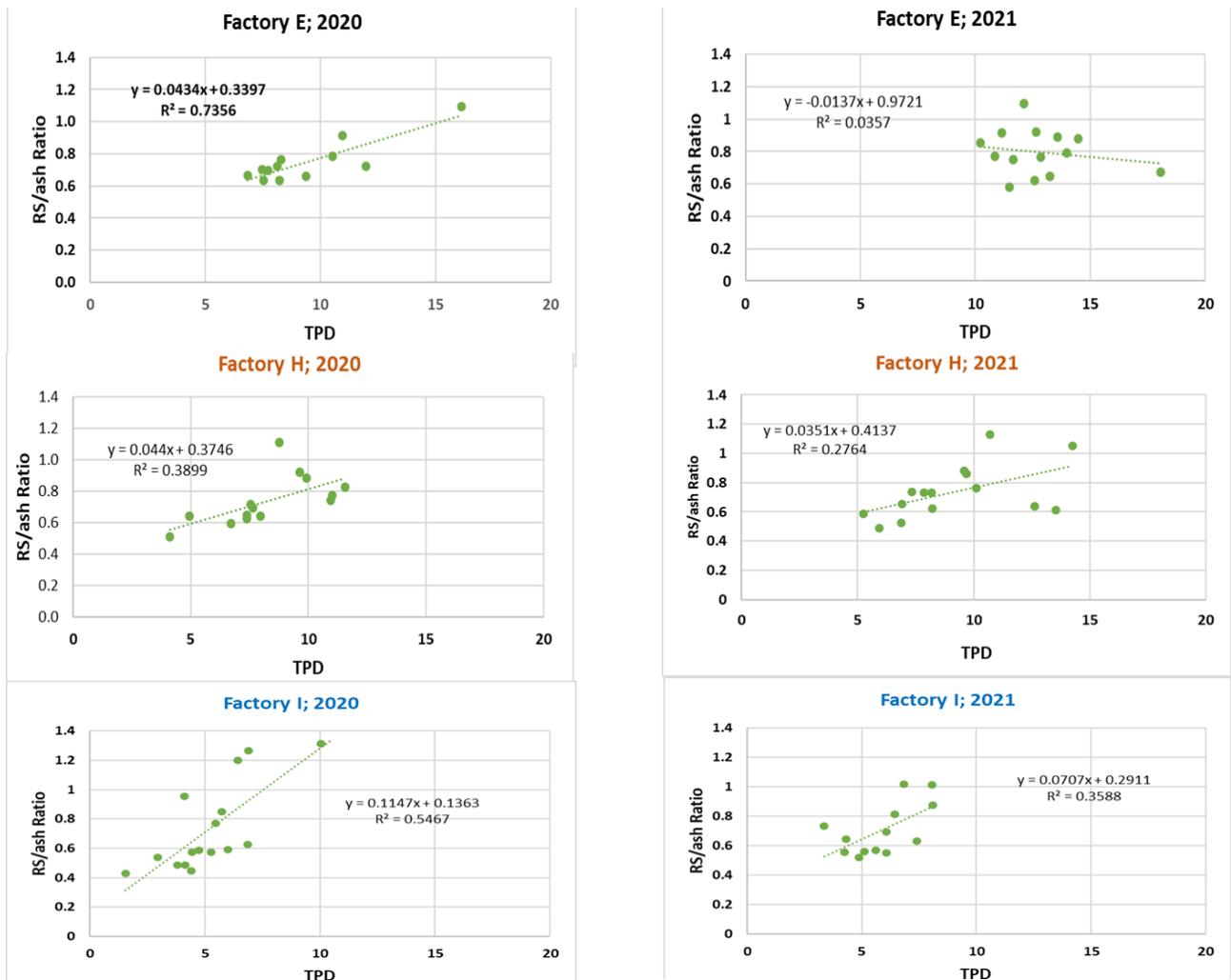


Fig. 4. Relationship between conventional TPD values and RS/ash ratios for three LA factories across both the 2020 and 2021 grinding seasons.

Total, Insoluble, and Soluble Starch Contents of Final Molasses

Total starch concentrations in the final molasses from the three LA factories, during the 2020 and 2021 seasons, are also shown in Fig 3, and the statistical analyses of the data, including % soluble and % insoluble starch values are listed in Table 2. The mean concentrations for total starch in molasses for the three factories in 2020 ranged from 2752 to 4130 ppm/Brix and, except for Factory I, were consistently higher ($P < 0.05$) in 2021 than in 2020 with a range of 3824 to 5343 ppm/Brix. Moreover, for Factories E and H, the mean concentrations of total starch nearly doubled from 2020 to 2021 (Table 2). The median concentrations for total starch from factories E and H in 2021 were all higher than in 2020. The maximum and minimum starch concentrations for all three factories were higher in 2021 than in 2020 which reflects the extreme values at the start of the season (Fig. 3; Table 2).

Table 2. Statistical data of total, soluble, and insoluble starch concentrations in final molasses from three LA sugarcane factories across the 2020 and 2021 seasons, as well as the percentage of each starch form.

| Factory | Season Statistical Parameter | Concentration (ppm/Brix) | | | Soluble Starch % | Insoluble Starch % | Soluble: Insoluble Starch Ratio |
|-------------|------------------------------|----------------------------------|-------------------|-------------------|------------------|--------------------|---------------------------------|
| | | Total Starch | Soluble Starch | Insoluble Starch | | | |
| 2020 | | | | | | | |
| E | Mean \pm SD | 2952bB [§] \pm 961 | 2273aA \pm 1035 | 740aB \pm 674 | 74 \pm 19 | 26 \pm 19 | 2.85 |
| E | CV % | 33 | 46 | 91 | 26 | 73 | |
| E | Median | 2932 | 2065 | 578 | 73 | 27 | 2.70 |
| E | Maximum | 4418 | 4268 | 2036 | 98 | 53 | |
| E | Minimum | 1281 | 921 | 53 | 47 | 2 | |
| H | Mean \pm SD | 2762bB \pm 702 | 2086aB \pm 841 | 863aB \pm 413 | 68 \pm 14 | 32 \pm 14 | 2.13 |
| H | CV % | 25 | 40 | 48 | 21 | 44 | |
| H | Median | 2581 | 1953 | 680 | 64 | 36 | 1.78 |
| H | Maximum | 4099 | 4099 | 1625 | 90 | 49 | |
| H | Minimum | 1894 | 1214 | 349 | 51 | 10 | |
| I | Mean \pm SD | 4130aA \pm 1524 | 3514aA \pm 1417 | 616aA \pm 570 | 85 \pm 15 | 15 \pm 15 | 5.67 |
| I | CV % | 37 | 40 | 93 | 17 | 96 | |
| I | Median | 4467 | 3386 | 368 | 89 | 11 | 8.09 |
| I | Maximum | 6538 | 5635 | 2041 | 99 | 55 | |
| I | Minimum | 1861 | 1088 | 32 | 45 | 2 | |
| 2021 | | | | | | | |
| E | Mean \pm SD | 5343aA \pm 796 | 2534bA \pm 963 | 2808aA \pm 1173 | 48 \pm 19 | 52 \pm 19 | 0.92 |
| E | CV % | 15 | 38 | 42 | 40 | 37 | |
| E | Median | 5342 | 2342 | 3141 | 39 | 61 | 0.64 |
| E | Maximum | 6442 | 5105 | 4392 | 85 | 74 | |

| | | | | | | | |
|----------|------------------|---------------|---------------|---------------|---------|---------|-------|
| E | Minimum | 3697 | 1213 | 792 | 26 | 15 | |
| H | Mean ± SD | 5326aA ± 1693 | 4056aA ± 1923 | 1905bA ± 1145 | 64 ± 22 | 36 ± 22 | 1.78 |
| H | CV % | 32 | 47 | 60 | 34 | 61 | |
| H | Median | 5445 | 3009 | 1793 | 54 | 46 | 1.17 |
| H | Maximum | 7746 | 7045 | 3672 | 95 | 60 | |
| H | Minimum | 2407 | 1519 | 361 | 40 | 5 | |
| I | Mean ± SD | 3824bA ± 1750 | 3526aA ± 1614 | 353cB ± 222 | 91 ± 6 | 9 ± 6 | 10.11 |
| I | CV % | 46 | 46 | 63 | 6 | 62 | |
| I | Median | 3109 | 2992 | 302 | 93 | 7 | 13.29 |
| I | Maximum | 8466 | 7887 | 748 | 96 | 25 | |
| I | Minimum | 2109 | 2011 | 98 | 75 | 4 | |

§ Lowercase letters indicate statistical differences among factories for the same year

§ Upper case letters indicate statistical differences between years for each factory

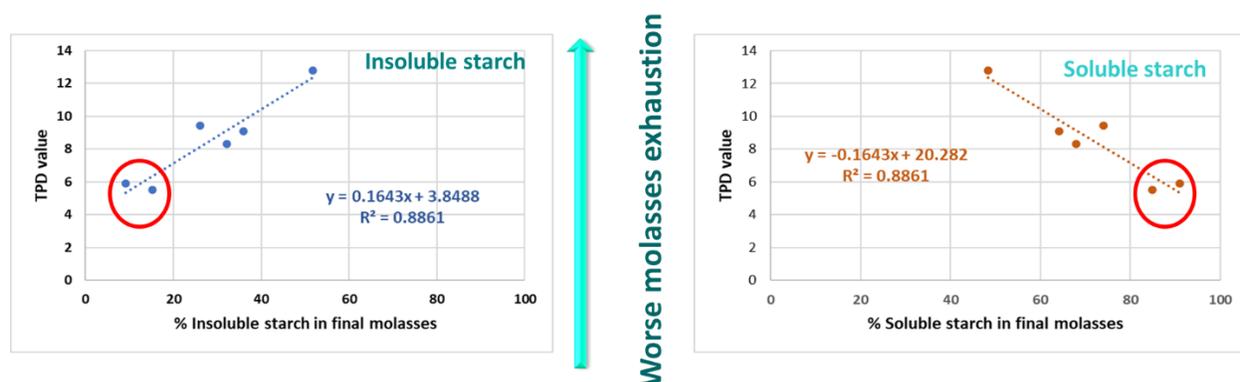


Fig. 5. Effect of percent insoluble (right) or soluble (left) starch on the season mean TPD values for three LA factories for both 2020 and 2021.

Not only were the total, soluble, and insoluble starch concentrations in molasses higher for Factories E and H in 2021 compared to 2020, but less starch was solubilized in the factory as evidenced by the higher percentage values of insoluble starch and lower values for soluble starch, as well as their ratios (Table 2). Since most of the starch extracted into juice during milling is in the insoluble form, these differences must be due to variations in factory performances and operations, including the addition of amylase to control starch. The strong effect of the percent insoluble or soluble starch on TPD is shown in Fig. 3, which clearly shows that the starch form strongly impacted molasses exhaustion. The greater the amount of insoluble starch in the molasses the lower the TPD value. This is most likely because the insoluble swollen starch increases the viscosity of the molasses which, in turn, impedes sucrose exhaustion. Results in this paper reinforce the observations of Eggleston et al. (2020) where total, soluble, and insoluble starch concentrations in final molasses from LA factories in the 2018 grinding season were approximately one-third (37%) of those in 2017 (normal year) which caused the TPD values to be much lower.

Results in Table 2 and Fig. 5 (highlighted by a red circle) also showed that, in both seasons, Factory I performed considerably better than the other two factories by not only being able to better solubilize the starch in the factory but also by better controlling the starch concentrations (all

forms) in the final molasses (Table 2). This is most likely because Factory I added a high temperature (HT) stable amylase to clarified juice, rather than the typical intermediate temperature (IT) stable amylases in Factory E and H.

Eggleston et al. (2016) previously reported that HT amylases, compared to IT stable amylases, broke down considerably more total starch (both insoluble and soluble starch) in both factories clarified juices and model clarified juices at 96 °C, even when added at a very low dose of 1 ppm. Furthermore, irrespective of what type of amylase was applied, i.e., IT or HT amylase, most starch hydrolysis was reported to occur “over the first 10 min, with no or little hydrolysis thereafter.” This was attributed to considerable denaturation/deactivation of the amylase at 96 °C (Eggleston et al., 2016). The downside, however, of using HT amylase even at a very low dose of 1 ppm or less is the reported risk of a small amount of carry-over (residual) amylase activity in the raw sugar and even subsequent refined sugar (Eggleston et al., 2016). This risk can be mitigated at the refinery with the use of activated carbon to remove the amylase protein (Lima et al., 2016). Additionally, there are reports from Brazil that a new enzyme from Novozymes named Premira™ can reduce starch and improve molasses exhaustion without causing carry-over amylase in raw sugars, but this needs to be studied in Louisiana (Anon, 2019).

Dextran Content

Dextran (glucopolysaccharide consisting mainly of α 1→6 glycosidic linkages) is formed from the deterioration of sugarcane mostly, but not solely, from *Leuconostoc* lactic acid bacteria. It is known to increase viscosity but does not form highly viscous gels. Variations in the dextran content of the molasses in this study are also illustrated in Fig. 3. Eggleston et al. (2020) reported that dextran in molasses that was measured using the same monoclonal antibody method as in this study was strongly and positively correlated ($R=0.907$) with TPD in 2018 and moderately correlated ($R=0.722$) in 2017. In contrast, in this study, the dextran values were not significantly correlated with TPD values for each factory in either the 2020 or 2021 seasons. It must be noted that no freezes, which are often responsible for severe dextran formation in cane, occurred during the 2020 or 2021 harvest/processing seasons and the dextran concentrations were not especially elevated.

Concentration of Fructans

Fructans (also known as levans and inulins) are fructoligo- and fructopolysaccharides connected mainly by β 2→6 and β 1→6 glycosidic linkages, that are formed from sucrose by some microorganisms and plants. Moreover, some lactic acid bacteria in Louisiana cane juice can form both dextran and fructan (Aita and Moon, 2023). Eggleston et al. (2020) reported considerable amounts of fructan (>9000 ppm/Brix) in Louisiana final molasses formed during the whole season and these results were confirmed in the current study (Fig. 3). Average season values for fructan in the molasses varied little among the three factories in 2020 (19454 – 20303 ppm/Brix) and 2021 (15257 to 15800 ppm/Brix) but were lower in 2021 compared to 2020. Nevertheless, these values were still higher than for average fructan concentration in 2017 (12260 ppm/Brix) and 2018 (10970 ppm/Brix) molasses (Eggleston et al., 2020).

In 2020, there was only a significant but moderate correlation ($R^2=0.421$) between fructans and TPD for Factory E. In 2021, only Factory I exhibited a correlation ($R^2=0.635$) between

fructans and TPD. These results indicate that fructan, like dextran, is still likely involved in reducing molasses exhaustion in Louisiana, but fructan was not a dominant factor in these years.

CONCLUSIONS

- All factory products contain starch in both soluble and insoluble forms
- A 2-year (2020 and 2021) study of final molasses from three LA factories showed that the amount of solubilization of starch in the factory affects sugar exhaustion from molasses. Insoluble starch directly reduces the exhaustion of sucrose.
- High-temperature stable amylase can better control insoluble starch than intermediate stable-temperature amylase but there is a risk of carry-over (residual) amylase in the raw sugar
- Possible solutions to removing carry-over amylase are adding Premira™ enzyme at the factory but more studies are required, and/or use of activated carbon at the refinery

ACKNOWLEDGEMENTS

Mention of commercial products is solely for providing specific information and does not imply recommendation or endorsement by the Audubon Sugar Institute.

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