FORMATION OF COLOR AND SUCROSE DEGRADATION PRODUCTS ACROSS EVAPORATORS IN A SUGARBEET FACTORY.

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ABSTRACT
Sucrose chemical loss and degradation product formation including color compounds, across a beet factory's five-step evaporator were investigated. Kinetic samples of thin juice and evaporation syrups were obtained hourly, over a six hour sampling period. This sampling period occurred once in the 1995/96 and 1996/97 campaigns. Ion chromatography with integrated pulsed amperometric detection (IC-IP AD), an accurate carbohydrate analysis technique was used to measure sucrose, glucose and fructose levels, using a NaOH gradient method. IC-IP AD, with a strong NaOH/NaAcetate gradient method, was used to determine oligosaccharide degradation products. Conventional sugar factory analyses for monitoring sugar losses, i.e., pol, purity, pH and color, were compared to IC-IP AD analyses; purity was shown not to be viable for determining losses across the evaporator process. In the 1995/96 campaign, pH dropped -0.22 pH units across the evaporators and there was a concomitant increase in color of 418 ICUMSA_{420nm} units, with color increasing the most across the first two evaporator stages. In the 1996/97 campaign, pH dropped further, by ~1.08 pH units and color increased by 694 ICUMSA_{420nm} units. Color formation is due to a complex of reactions, including Maillard color reactions and alkaline thermal degradation reactions of sucrose and/or invert sugars. Maillard reactions are more dominant at the early stages of evaporation and alkaline degradation reactions at the latter stages. Excellent correlations existed between polarizations measured at 589 and 880nm for the 1995/96 (r^2=.997) and 1996/97 (r^2=1.000) campaigns.

INTRODUCTION
Sucrose and monosaccharide degradation causes chemical loss of sucrose, and also the formation of colored and non-colored degradation products. Such products can reduce unit process efficiencies affect end product quality. Understanding the extent of degradation and the processes causing it, is necessary to assess the methods of reducing or eliminating degradation. Color formation across evaporators in sugarbeet factories is a common problem and this study was, therefore, undertaken to assess color forming processes and measure sucrose losses across a sugarbeet factory's evaporation process. Conventional sugar factory analyses (e.g., changes in pol, purity and pH) were compared with the more accurate technique of ion chromatography with integrated pulsed amperometric detection (IC-IP AD) which can directly analyze for sucrose and invert levels. The formation of oligosaccharide degradation products across the process was also investigated, to
further assess sucrose loss, and hopefully reveal the identity of possible sucrose loss markers.

MATERIALS AND METHODS

This investigation of sucrose loss and color formation across the evaporation process was performed on samples obtained from a European sugarbeet factory during the 1995/96 and 1996/97 campaigns. The set of five evaporators are falling-film, single body types, except for the 2nd stage evaporator which is a Robert’s type with three bodies in series. Temperature and pressure both decreased, across the five evaporator effects. The approximate temperature (°C) and pressure (Bar Absolute) in the 1st evaporator was 130 °C, 2.70 BA; in the 2nd 123 °C, 2.03 BA; in the 3rd 117 °C, 1.56 BA; in the 4th 101 °C, 1.07 BA; and in the 5th 93 °C, 0.796 BA.

Sampling. Samples of thin juice (TJ) entering the evaporators, and syrups exiting the 1st, 2nd, 3rd, 4th and 5th evaporators, were obtained hourly over a six hour period. The retention times of the syrups in each evaporator were taken into account. Consequently, there was a 3 min delay between sampling TJ and the 1st syrup, 12 min between sampling the 1st and 2nd syrups, 2min between the 2nd and 3rd, 3min between the 3rd and 4th, and 4min between the 4th and 5th evaporators. The six hour sampling period occurred twice, once near the end of the 1995/96 campaign and once near the end of the 1996/97 campaign. All samples were immediately quenched and stored in dry ice, to prevent further decomposition, until they were air transported (in dry ice) to the laboratory and stored in a -43°C laboratory freezer.

Composite Samples. 15g of each hourly sample, for each period of sampling, were added together to form a physical composite for comparison purposes.

Pol Clarification of 26g/100ml samples was undertaken by mixing with Filtercel™ and filtering through Whatman 91 paper. Clarified samples were measured, in duplicate, at 589 and 880nm in a 20cm cell, on a Rudolph Autopol 880 Polarimeter calibrated in ISS (Z scale) at 20°C.

Brix was measured using a Leica Abbe Mark II Refractometer with a crosshair reticule.

pH was measured at room temperature (~23 °C), using an Ingold™ combination pH electrode calibrated at room temperature using two different pH buffers. The electrode was connected to a Metromh 716 DMS pH meter.

Color and turbidity were measured as the absorbance at 420nm and calculated according to the official ICUMSA method GS2/3-9 (1994). Samples were diluted in triethanolamine/hydrochloric acid buffer (pH 7) and filtered through Filtercel™.

Nitrogen Analyses. Total Kjeldahl nitrogen content in TJ samples was determined by methods 351.3 and 351.4 EPA-600/4-79-020.

Sucrose, glucose and fructose. Diluted samples were filtered through a 0.45μm filter. Sucrose, glucose and fructose concentrations were determined by ion chromatography (IC) using a Dionex (Sunnyvale, CA, USA) BioLC instrument. Carbohydrates were separated on Dionex CarboPac PA-1
guard (25 x 4 mm) and analytical (250 x 4 mm) anion exchange columns, at a flow rate of 1.0 mL/min at ambient temperature (~25 °C). Column eluant conditions were: 16 mM NaOH isocratic (inject; 0.0-2.0 min), a gradient of 16-160 mM NaOH (2.0-35.0 min), followed by isocratic 200 mM NaOH (35.1-40.0 min), and return to 16 mM NaOH (40.0-49.0 min) to re-equilibrate the column with the initial mobile phase prior to the next sample injection. Carbohydrates (25 μl injections) were detected using integrated pulsed amperometric detection (IPAD). The detector was equipped with Au working and Ag/AgCl reference electrodes, operating with the following working electrode pulse potentials and durations: E1=+0.05 V (t1=0.00 s), E2=0.05 V (t2=0.42 s), E3=+0.75 V (t3=0.43 s), E4=+0.75 V (t4=0.60 s), E5=0.60 V (t5=0.61 s), E6=-0.60 V (t6=0.96 s). The duration of the IPAD integration interval was set at 0.2-0.4 s. Using a Spectra-Physics SP8880 autoinjector and Dionex Peaknet chromatography software, runs were accumulated of multiple samples and standards. The standards were myo-inisitol, glucosamine-HCl (internal standard), glucose, fructose, sucrose, raffinose and stachyose. Seven different levels of the standards were run first, and standard curves were generated (sucrose ranged from 1 to 25 ppm) to test linearity in multiple runs and generate area response factors. Weight diluted samples were run in duplicate. Glucose and fructose were quantified in different runs than sucrose, due to the very different concentrations of these carbohydrates in the samples. There was a much larger dilution for sucrose quantification. Response factors were generated for each of the carbohydrates, using internal standard calibrations and check standards.

Glucose%E Sucrose Ratios were calculated following the method of Purchase et al. Glucose and sucrose were on a brix basis, and Glc%E Sucr increase = \[ \frac{[G/S]_{\text{out}} - [G/S]_{\text{in}}}{\text{MW}_{\text{sucrose}}} \times 100 \times \text{MW}_{\text{glucose}} \]

Oligosaccharide degradation products in diluted samples were separated by IC-IPAD on a Dionex™ CarboPac PA-1 column, using a NaOH/NaOAc gradient. See Eggleston for complete method.

Statistical Analyses: For each sample type, a separate analysis of variance was performed on sample characteristics using PROC GLM in PC-SAS (SAS Institute, Inc., Cary, NC). The design was a two-factor ANOVA without replication with sample date and sample time both being considered random effects. Factor means were separated using Duncan’s New Multiple Range Test. For each sample type, correlation coefficients were calculated on data combined over sample dates.

RESULTS AND DISCUSSION

Conventional Factory Analyses - Pol and Purity Values as Indicators of Sugar Degradation

Statistical analyses of the conventional factory data was undertaken and sampling time (0 to 5h) was shown not to be significant for all the different sample types or points (TJ and evaporator syrups). It was interesting to note that there was a highly significant (P<.01) variation between campaign years in the early stages of the evaporation process for all variables, especially in the TJ samples. This reflects variable genetic and environmental factors of the beets processed. As expected, on progression from one sampling point to the next, less year to year variability was evident, due to the evaporation process creating homogeneous high Brix syrups. The largest changes occurred during the periods between the 1st and 2nd, and 2nd and 3rd evaporators, where temperature is the highest. By
the 3rd sampling point, year to year variation became insignificant for all variables except pH.

Excellent correlations existed between pols measured at 589 and 880nm, with 96/97 data slightly higher (see Table 1). This confirms the observations of the factory personnel that the beet was of higher quality in the 96/97 campaign, and obviously had fewer interfering optical impurities. As expected, purity values measured at 589 and 880nm were also very good, but less than the respective pol correlations (Table 1). This reflects the error in measuring Brix, even though Brix values were averages of at least triplicate samples. Moreover, the significant moderate correlation between purity at 589nm and Brix was negative (Table 1), which further suggests that Brix is more difficult to measure at the higher Brix concentrations, and that most error occurred at the later stages of evaporation where Brix values are higher.

**Table 1. Some Significant Correlations Across All Sample Types**

<table>
<thead>
<tr>
<th>Correlation</th>
<th>Campaign Dates</th>
<th>$r^2$</th>
<th>$P$ level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pol$<em>{589}$ v Pol$</em>{880}$</td>
<td>1995/96</td>
<td>0.997</td>
<td>.0001</td>
</tr>
<tr>
<td>Pol$<em>{589}$ v Pol$</em>{880}$</td>
<td>1996/97</td>
<td>1.000</td>
<td>.0001</td>
</tr>
<tr>
<td>Purity$<em>{589}$ v Purity$</em>{880}$</td>
<td>1995/96</td>
<td>0.965</td>
<td>.0001</td>
</tr>
<tr>
<td>Purity$<em>{589}$ v Purity$</em>{880}$</td>
<td>1996/97</td>
<td>0.953</td>
<td>.0001</td>
</tr>
<tr>
<td>Purity$_{589}$ v Brix$^a$</td>
<td>1995/96</td>
<td>-0.539</td>
<td>.0012</td>
</tr>
<tr>
<td>Purity$_{589}$ v Brix$^a$</td>
<td>1996/97</td>
<td>-0.540</td>
<td>.0014</td>
</tr>
</tbody>
</table>

$^a$Purity$_{880nm}$ v Brix correlations were very similar

Fig. 1 shows that purity values varied randomly across the evaporators. The lower purity values of the 95/96 campaign reflect the poorer beet quality. Purity values are dependent on two analytical techniques, neither known to be highly accurate, and the variation observed is caused not only by measurement errors in both techniques, but chemical changes. Sucrose degradation will decrease the pol value and the purity, but subsequent loss of fructose leads to an increase in pol and therefore, an underestimation of sucrose degradation. From this data, it can be concluded that purity values are not viable to determine sucrose degradation/losses at levels that occur in the evaporation unit process. This confirms similar observations in model studies undertaken under simulated industrial conditions (including constant pH)$^{14}$. 

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Fig. 1. Average Purity Values Across The Evaporators

![Graph showing average purity values across evaporators.](image)

**Color Formation Across the Evaporators**

It is known that purified juice darkens across evaporators and juice levels in the evaporator bodies have a critical effect. Significant color formation occurred across the evaporators, in both campaigns, as shown in Fig. 2, and there was an overall color increase of 418 and 694 ICUMSA units across the evaporators in the 95/96 and 1996/97 campaigns, respectively. The higher initial color of the TJ samples in 95/96, is most likely because of the poorer beet quality which was more acidic and, therefore, more protonated hydrolysis (inversion) of sucrose occurred with subsequent color formation. Color formation is evidence of sucrose degradation and can be due to the formation of Maillard color compounds (melanoidins), from reactions between carbonyl and amino compounds. In the evaporation system, the carbonyl compounds could be partly glucose and fructose and partly even a number of alkaline degradation products from these invert sugars; the amino compounds are amino acids from the sugar beet. Color can also be formed from the thermal degradation of sucrose and invert sugars, particularly through alkaline degradation under these evaporation conditions, and color is formed from the condensation reactions of color precursors.

There were significant (P<.0001) correlations between color and Brix for each campaign with 96/97 (r²=.878) higher than for 95/96 (r²=.641), and this is further illustrated in Fig 3. This relationship between color and Brix suggests color formed is due to the prevalent concentration of color precursors which react to produce color, and are most likely Maillard color precursors. (Note: The concentration of color compounds due to the increase in Brix across evaporation can be discarded as ICUMSA color measurements take Brix into account). It is interesting to note that in the 95/96 campaign, color increased the most across the first two evaporator stages which reflects the higher temperatures there and also reaction product concentrations, as there was a concomitant increase in Brix (see Fig 3).
Fig. 2. Average Color Formation Across The Evaporators

![Graph showing average color formation across the evaporators]

**Evaporator stages**

- ▲ - av color 9697
- ■ - av color 9596

Fig. 3. Relationship Between Color and Brix

![Graphs showing relationship between color and Brix for 9596 and 9697]

**Brix**

- 0
- 20
- 40
- 60
- 80

**ICUMSA color (420 nm)**

- 0
- 500
- 1000
- 1500
- 2000
- 2500
Although there was approximately half the amount of nitrogen containing compounds in the 95/96 campaign compared to 96/97 (personal communication, factory personnel), there was only slightly less total nitrogen in the 95/96 TJ samples in this study, than in the 96/97 samples, and nitrogen varied more in the latter samples (see Table 2). No significant relationships were found between TJ nitrogen levels and TJ and 5th evaporator syrup color, in both campaigns, but there was a moderate correlation ($r^2=0.535$) between TJ nitrogen levels and color increase in each evaporation stage, for the 95/96 campaign. This further suggests that color is not caused by one sole chemical process, such as Maillard color formation, but involves more processes.

### Table 2. Total Nitrogen content in Thin Juice Samples

<table>
<thead>
<tr>
<th>Thin Juice Sample</th>
<th>Total Nitrogen (mg/Kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1995/96 campaign</td>
</tr>
<tr>
<td>Reaction time (h)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>945</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>813</td>
</tr>
<tr>
<td>3</td>
<td>749</td>
</tr>
<tr>
<td>4</td>
<td>797</td>
</tr>
<tr>
<td>5</td>
<td>738</td>
</tr>
</tbody>
</table>

ND= not determined

**pH Drop**

A drop in pH is also evidence of sucrose and monosaccharide degradation because it is mostly caused by the formation of organic acids (i.e., from degradation). A pH drop will also occur if the saponification of amides has been insufficient in juice purification and this will continue in the evaporators. Fig 4 shows that pH dropped across the evaporators in both campaigns. The initial pH of TJ samples was higher in the 95/96 campaign, because the lower quality beet was more acidic, thereby causing more lime to be added at the clarification stage to compensate. There was also an anomalous, but consistent increase in pH at the 5th evaporator in the 96/97 campaign which can be attributed to the “salting out” of organic acids. (Note: dilution of samples to the associated Brix of the TJ samples had no significant affect on the pH measurement, which indicates good buffering properties).
Fig. 4. pH Drop Across The Evaporators

![Graph showing pH drop across the evaporators]

Fig. 5. Relationship Between pH Drop Across Evaporators and Thin Juice pH

**pH Drop from Thin Juice to 4th and 5th Evaporators**

For 95/96:
- $y = -0.8119x + 7.1664$
- $R^2 = 0.5518$

For 96/97:
- $y = -1.1252x + 9.9783$
- $R^2 = 0.9209$

**pH Drop from Thin Juice to 4th Evaporator**

For 96/97:
- $y = -1.1757x + 9.3216$
- $R^2 = 0.8778$

**pH of Thin Juice**
When the alkaline pH value of the incoming evaporator thin juice (TJ) was plotted against the total pH drop across the evaporation process, there was a significant highly negative correlation, for both campaigns, which is illustrated in Fig 5. This strongly indicates that alkaline thermal degradation of sucrose and/or monosaccharides (with the subsequent formation of organic acids) occurred across the process. The pH of TJ is, therefore, critical. This would also indicate that color formation results not solely from Maillard color formation but from alkaline degradation as well.

**Sucrose Decomposition and Invert Formation/Decomposition**

As the statistical analysis of the conventional factory data indicated that no significant variation existed between the sampling times, reaction time composites of the TJ and five evaporator stage samples, for both campaigns, were analysed for sucrose and invert levels by IC-IPAD, with a NaOH gradient. It can be seen in the example chromatogram in Fig. 6 that mannose was present in the TJ and evaporator syrup samples. This verifies that alkaline degradation occurred across the evaporators. Under alkaline conditions, glucose, fructose and mannose are in initial equilibrium via an enediol anion intermediate, which subsequently undergoes further non-reversible degradation reactions and subsequent color formation. The mechanism of this reversible isomerization is known as the Lobry de Bruyn-Alberda van Ekenstein rearrangement.

**Fig. 6. Alkaline Degradation Products in 96/97 Campaign**

The levels of glucose, fructose and mannose in each evaporator stage are illustrated in Fig 7. The very low levels of invert in the TJ samples reflect the efficiency of invert removal in juice purification. This is undertaken to minimise subsequent Maillard reactions with amino compounds. The initial increase in glucose at the first evaporator stage is most likely because of protonated...
hydrolysis of sucrose. Although the pHs of the initial evaporator stages (see Fig 4) were ~9.0 and 8.7 pH units, for the 95/96 and 96/97 campaigns, respectively, these pH values were measured at ambient (room) temperature on a pH meter calibrated at room temperature, whereas the actual process streams were at ~130°C. At such initial high temperatures the dissociation of sucrose would be high, thus actual process pHs would be lower and more protons would be available to catalyze sucrose hydrolysis. Moreover, protonated catalyzed degradation of sucrose is known to occur at low alkaline pHs (up to pH 8.3). This also explains the increase in fructose levels. The apparent plateaus in glucose levels, particularly across evaporator stages 2 to 4, reflect the decreasing temperatures with concomitant reductions in sucrose dissociation and water activity. Such conditions are less favorable for the formation of invert from sucrose protonated hydrolysis, and alkaline degradation processes would dominate. Therefore, alkaline degradation particularly of invert sugars, initiated by the Alberda van Ekenstein rearrangement, dominate over the latter stages of evaporation, and this is further evident in both campaigns where mannose increased at the latter stages. The subsequent decrease in glucose levels at the final evaporator (stage 5) is likely due to even further reduced temperature and water activity, and the complex degradation and color forming reactions occurring. Fructose levels were always lower than those of glucose, and did not exhibit the plateau levels. These differences are because fructose is more reactive than glucose under alkaline conditions, and reacts faster than glucose in the Maillard color reaction at alkaline pH. As alkaline degradation of invert sugars dominates at the latter stages of the evaporation system, more fructose and glucose would have been available at the initial stages for Maillard reactions. Therefore, Maillard reactions likely dominated over the initial stages of evaporation. As fructose is formed and then further reacted at a higher rate than glucose, the pol values would have been artificially high and, therefore, sucrose degradation was underestimated by pol and purity values.
Glucose and fructose levels were generally both lower in the 95/96 than 96/97 samples. This was likely due to the slightly higher TJ pH values which would have slightly increased alkaline degradation and more color forming reactions. The initial higher increase in glucose formation in 95/96 is possibly due to higher temperatures or localized heat spots, and explains the relatively higher increase in color across the early stages of evaporation in the 95/96 samples, due mainly to the predominant Maillard color formation.

Meaningful attempts to accurately measure sucrose losses across evaporators in sugar cane factories have been undertaken by Purchase et al., and Edye and Clarke, using GC and IC techniques, respectively. Because low levels of invert can be measured more accurately than the relatively larger quantities of sucrose, both studies measured sucrose loss by increases in glucose to sucrose ratios (Glc%Suc). However, inversion of sucrose is mostly responsible for sucrose loss in the sugar cane system which operates at lower pHs, and as described in this paper, alkaline degradation is more dominant in the sugar beet factory where glucose and fructose levels are markedly lower. Therefore, glu%Suc ratios across the whole evaporator system were low as shown in Table 3, and are not very meaningful. Furthermore, measured changes in glucose % sucrose cannot give an entirely accurate evaluation of sucrose degradation, because the exact amount of glucose that is destroyed during evaporation is unknown and can only give an indication of minimum degradation.

Table 3. Glucose:Sucrose Ratios and % Sucrose Losses Across Total Evaporation Process

<table>
<thead>
<tr>
<th>Campaign</th>
<th>Total Glc%Suc Increase (on brix basis)</th>
<th>Approx. Total % Sucrose Decrease (on brix basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>95/96</td>
<td>0.143</td>
<td>2.639b</td>
</tr>
<tr>
<td>96/97</td>
<td>0.151</td>
<td>0.911b</td>
</tr>
</tbody>
</table>

* Sucrose and glucose levels determined by Ion Chromatography (IC)

b Probably an overestimate due to experimental error

Percentage sucrose losses across the total evaporator system, as measured by IC-IPAD are also shown in Table 3. Sucrose levels, at the high concentrations which occur in the evaporator are very difficult to measure and the author is currently optimizing the IC-IPAD method for accurate quantification of sucrose in industrial samples. The results shown here are approximations. Nevertheless, sucrose loss was obviously higher in the 95/96 campaign and reflects the poorer beet quality. The most accurate determination of sucrose loss across the evaporation process would be to analyse for a stable degradation product, i.e., a marker compound, and this is currently under investigation.

**Oligosaccharide Degradation Products: Possible Sucrose Loss Markers**

Oligosaccharides are formed in the breakdown of sucrose and monosaccharides, under alkaline conditions, and have strong potential as possible stable markers. An IC-IPAD method, using a strong sodium acetate (NaOAc) gradient, was developed to separate oligosaccharides (up to 12
Fig. 8. “Fingerprint” Oligosaccharides Present in Composite Thin Juice and 5th Evaporator Syrup Samples (96/97 Campaign)

IC-IPAD: NaOH/NaOAc Gradient

CONCLUSIONS

Color formed across the evaporators in the 95/96 and 96/97 campaigns, with a concomitant decrease in pH. Results obtained indicate that color is due to both the formation of Maillard color (melanoidins) from the reaction of nitrogen containing amino acids with invert sugar, and the alkaline degradation of sucrose and/or invert sugar. Maillard reactions dominate at the earlier stages of evaporation and alkaline degradation reactions at the latter stages. Analyses of syrups by gel permeation chromatography will further delineate the extent of formation of color types. Purity and
glucose:sucrose ratios (as measured by IC), were shown not to be viable to determine sucrose losses in these samples.

Low levels of oligosaccharides, or other degradation products can be measured more precisely and accurately than the relatively larger quantities of sucrose. Therefore, identification of a stable marker compound which correlates directly with sucrose loss, would be more suitable to evaluate the sucrose losses which occur across evaporators. This work is part of an ongoing USDA project being undertaken in collaboration with the Sugar Processing Research Institute (SPRI), and one of the major aims of this project is to identify such a stable marker compound(s). GC-MS and photo-diode array spectroscopy techniques with HPLC are currently being used to separate and identify makers.

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LITERATURE CITED