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Agricultural and Biosystems Engineering Department

Improving the Storage Life of Beet Juice for Ethanol Production

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EXECUTIVE SUMMARY

Studies were carried out to quantify and characterize the behavior of stored raw thick juice from sugar beets as a first objective of this research project. The results obtained through experimentation suggest that it is possible to store raw thick juice in a stable manner through pH and refractometric dissolved solids (RDS) content adjustments.

Raw thick juice was stored at acidic and alkaline pH. The steady RDS and pH values in raw thick juice stored for up to 12 weeks under acidic pH values indicate a high probability of successful long-term storage. High acid conditions (pH 2 or 3) actually increased the content of fermentable sugar through hydrolysis of complex carbohydrates. Under alkaline conditions, abrupt drops in pH values are a concern in the sugar industry when storing thick juice. However, a pH decline does not actually represent a significant loss of total fermentable sugar in this study. As part of the oncoming research, a storage test will be conducted for six months (Objective 2) and the viability of the storage conditions used will be evaluated through a fermentability test (Objective 3).

A small companion study was carried out to quantify total fermentable sugars in beets stored anaerobically at a temperature of 4°C for 10 weeks. Results showed a sugar loss of less than 10% under the storage conditions used. However, techniques such as modified and controlled atmosphere storage have been successful for the preservation of many perishable crops. A study combining a modified atmosphere with temperature adjustment is recommended to determine its viability for the storage of whole energy beets.

INTRODUCTION

The storage of thick juice at commercial scale was first implemented in 1960 in the United States as an alternative for storing sucrose. This sucrose was later processed and sold in its crystal form. This form of storage is still implemented in most of the sugar producing facilities around the US and some European countries. The equipment required for the storage of crystal sucrose is much more expensive than that required for thick juice storage.

The most influential factors in the quality of thick juice during storage are pH, water activity (related to refractometric dissolved solids content), and temperature. The combined effect of these factors has been previously studied at alkaline pH values ranging from 8.5 to 9.5. Nevertheless, what has been determined to be an optimal combination of factors (69°Bx, pH=9, 10°C<T<15°C) could be somewhat conservative when analyzed from the perspective of food preservation theory. On the other hand, storage of thick juice at acidic pH values is rejected by the beet sugar industry due to the inversion effect that acid has over sucrose. Nonetheless, invert sugars are not a problem when the stored juice is to be fermented for ethanol, and may actually benefit fermentation, since glucose is the most readily fermentable sugar.
STATEMENT OF OBJECTIVES

The overall objective of this research is to determine optimum conditions to maintain the quality of fermentable sugars during long-term storage of energy beet juice for ethanol production. The optimum conditions should be low cost, energy efficient, and compatible with the fermentation of the juice. The specific objectives of this research are:

I. Quantify and characterize the behavior of stored raw thick juice. *(Completed)*

II. Develop an empirical model useful to predict the loss of fermentable sugars in stored raw thick juice. *(To be completed by May 2012)*

III. Develop and apply a fermentability test to study the effects of chemical additives and storage conditions on the production of ethanol from raw thick juice. *(To be completed by May 2012)*

IV. Evaluate the quality of fermentable sugars in energy beets stored anaerobically. *(Completed; however, further, systematic study is recommended)*

MATERIALS AND METHODS

- Quantification and characterization of the behavior of stored raw thick juice

Raw beet juice with an RDS content of approximately 16°Bx was obtained from American Crystal Sugar Co. in Moorhead, MN and two systems for concentration were tested. A portion of the raw juice was concentrated without pretreatment using a rotovap. The remaining portion of beet juice was concentrated to a target RDS content of approximately 69°Bx using a rising-film evaporator (Fig. 1) operated with steam at 136 kPa (gauge), vacuum of 60 kPa (gage) inside evaporating tube, input juice temperature of 20°C, input juice flow rate of 16 L/hr, output juice temperature of 81°C. Prior to feeding into the rising-film evaporator, the raw beet juice was screened with cheese cloth to remove beet particles that could clog the feed valve. The rising-film evaporator was selected for future processing as it resulted in a much higher concentration rate.
Experiments were conducted to quantify and characterize the behavior of raw and raw thick juice stored anaerobically at a temperature of approximately 23°C under different combinations of pH and RDS content. A factorial design was used to study the effect of RDS content adjusted to 15, 40, 50, 60, 65, and 69°Bx in combination with pH values in the acidic (2 to 6) and alkaline (8 to 11) ranges.

The treated juice samples were stored for periods of up to 12 wk in 15-mL Corning graduated plastic tubes with minimal headspace. A set of samples was stored frozen as a control for each treatment. Analyses of pH, RDS content, and fermentable sugars (sucrose, glucose, and fructose) were performed during weeks 1, 2, 4, 8, and 12. Sucrose, glucose, and fructose concentrations were determined by HPLC equipped with an Aminex HPX-87P (300x7.8 mm) carbohydrate column (Bio-Rad Laboratories, Hercules, CA, USA) with a mobile phase of 18.2 mΩ nano-pure water at flow rate of 0.6 mL/min. The column and detector temperatures were 85°C and 50°C, respectively (National Renewable Energy Laboratory).

- **Evaluation of the quality of fermentable sugars in energy beets stored anaerobically**

A small companion study was performed to determine if anaerobic storage at low temperature (4°C) of beets is a viable technique for quality preservation. Sugar beet halves were weighed, vacuum-packed and stored for a period of 10 wk. Analyses of the beet halves were performed every 2 wk. Samples were weighed to determine moisture loss.

* Analyses Methods

The pH measurement was carried out using a Thermo Scientific Orion 2-Star benchtop pH meter (Thermo Fisher Scientific Inc., Beverly, MA, USA, 2008) equipped with automatic temperature compensation (ATC). The pH meter was calibrated with buffer solutions of pH 4.01, 7, and 10.01, prior to analyses to ensure accurate readings. In a similar fashion, the soluble solids content (°Bx) and water activity (\(a_w\)) will be measured with the use of a Pocket Digital Refractometer Mod. 300053 (SPER Scientific, Scottsdale, AZ) and a ROTRONIC AG Version 4 water-activity meter (Bassersdorf, Switzerland), respectively.
An estimate of the sucrose content in beets was carried out following the Sugar in Cossettes Procedure from the Laboratory Procedures Manual of American Crystal Sugar (Test No. 3001.10) and using a polarimeter Autopol 880 – Automatic Saccharimeter Purity Option (Rudolph Research Analytical, Hackettstown, NJ, USA).

Sucrose, glucose and fructose concentrations were determined by HPLC equipped with an Aminex HPX-87P (300x7.8 mm) carbohydrate column (Bio-Rad Laboratories, Hercules, CA, USA) with a mobile phase of 18.2 mΩ nano-pure water at flow rate of 0.6 mL/min. The column and detector temperatures were 85°C and 50°C, respectively (National Renewable Energy Laboratory).

RESULTS AND DISCUSSION

- Acidic pH storage of raw thick juice

A set of experiments regarding the storage of raw thick juice under acidic pH is useful to understand the behavior of the fermentable sugars in the juice as the storage time increases. Table 1 presents the variation of fermentable sugars in the raw thick juice. The fermentable sugars in raw thick juice at 65°Bx seem essentially unchanged. On the other hand, the decrease of fermentable sugars might be of concern in raw thick juice at 60°Bx and pH 5 and 4.

Table 1. Variation of fermentable sugars in raw thick juice stored under acidic pH and RDS of 60 and 65°Bx

<table>
<thead>
<tr>
<th>Initial pH</th>
<th>Initial °Bx</th>
<th>60</th>
<th>65.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>(1.57%)</td>
<td>(0.50%)</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>(2.56%)</td>
<td>(0.56%)</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>1.51%</td>
<td>6.27%</td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>5.69%</td>
<td>4.24%</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: The values in parenthesis indicate the amount of sugar lost by microbiological activity, whereas the remaining values account for the sugar gained due to a possible hydrolysis of complex carbohydrates.

It has been observed that a pH of approximately 3.0 or less causes the inversion of sucrose to glucose and fructose. The increase in sugar content detected at the end of the storage period suggests that some of the complex carbohydrates within the raw thick juice were hydrolyzed to monosaccharides. Invert sugars are not a problem to fermentation and actually enhance it since glucose is the most readily fermentable sugar for ethanol producing microorganisms.

The pH and RDS content (°Brix) are simple and useful indicators of change in sugar content in beet juice. For this reason, they are commonly used in the sugar industry. The Brix index can be used to monitor the behavior of total fermentable sugars in stored raw thick juice. The pH values within the raw thick juice stored for a period of 12 wk under acidic pH values were very steady (Fig 2a). RDS content remained about constant at pH 4 and 5, but actually increased at pH 2 and 3, again indicating hydrolysis of complex carbohydrates (Fig. 2b). Therefore, storage of raw thick juice shows promise. It remains to
be shown that juice stored in this way will result in excellent ethanol yield upon fermentation; this will be determined in the coming year.

- Alkaline pH storage of raw thick juice

The storage of thick juice under alkaline pH is already an accepted practice within the sugar industry. However, an energy-intensive process is used to purify the raw beet juice before it is concentrated. Much less has been reported on the storage of raw (not purified) thick juice. The following should distinguish between thick and raw thick juice.

The storage of raw thick juice under alkaline pH has been previously studied to determine optimal storage conditions (Fiedler et al., 1993). Raw thick juice has stored better at a pH of 9 rather than a pH of 6, and at a temperature of 5°C rather than one of 15-20°C or 30°C. However, in many researches regarding storage under alkaline pH, a drop of one unit in the initial pH value of the juice bulk has been considered an indicator of juice spoilage. Although a pH drop indicates the presence of microbiological activity, the corresponding loss of total fermentable sugars may be small (Fig. 3), which is of interest for the ethanol industry. The fermentable sugars content of this particular sample decreased by 2.2% during a storage period of 8 weeks. Similarly, a series of samples that showed a distinct drop in pH had stable RDS contents which suggested that fermentable sugars were retained (Fig. 4).

Figure 1. Behavior of pH and RDS content in raw thick juice with initial pH=9 and 60°Bx

Figure 2. a) pH behavior and b) variation of RDS content (°Brix) of raw thick juice at an initial RDS of 60°Bx

Figure 4, which illustrates the behavior of pH and RDS content in three different samples stored at pH 10, helps visualize the stability of the RDS content in the juice even when an abrupt pH decline occurs.

The fermentable sugars content decreased by 0.6, 4.9, and 9.1% during a storage period of 12 weeks in the samples with an RDS content of 60, 50, and 40°Bx, respectively.

In order to achieve the satisfactory completion of Objective 2, the pH of the stored samples will be periodically monitored and re-adjusted to its original value to forestall the effects of microbiological activity.

- **Evaluation of the quality of fermentable sugars in energy beets stored anaerobically**

A small companion study that involved the anaerobic storage of beets at a temperature of 4°C for a period of 10 weeks, gave the following results:

<table>
<thead>
<tr>
<th>Storage Time (Weeks)</th>
<th>Fermentable Sugars Lost (as a % of initial sugars)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>(4.2%)</td>
</tr>
<tr>
<td>4</td>
<td>(8.9%)</td>
</tr>
<tr>
<td>6</td>
<td>(6.4%)</td>
</tr>
<tr>
<td>8</td>
<td>(8.7%)</td>
</tr>
<tr>
<td>10</td>
<td>(9.2%)</td>
</tr>
</tbody>
</table>

NOTE: The values in parenthesis indicate the amount of sugar lost by microbiological activity.

A sugar loss of less than 10% can be observed at all the storage times considered for this experiment. It is important to mention that the beets used in this study were vacuum packed individually (Fig. 5) and analyzed individually at the different storage times. Also, from the limited number of beets that were available, some appeared to be in very good condition while others looked unhealthy. This helps explain the inconsistency of the percentage reported at week 6 of storage.
The effect of naturally modified atmospheres on the quality preservation of sugar beets has been studied previously (Cole, 1976). From results obtained during storage at 26°C, it is evident that the rate of sucrose loss in beets stored under this technique is higher than the rate of sucrose loss in beets stored aerobically. The sucrose content was determined to be stable when following both storage methods at a temperature of 5°C. A study regarding the effect of an artificially modified atmosphere in combination with temperature deserves more attention to determine the viability of such technique for quality preservation of whole beets.

**ONGOING RESEARCH**

The development of an empirical model useful to predict the loss of fermentable sugars in stored raw thick juice (Objective 2) will be initiated this month and is to be completed in February 2012. In addition to this, a fermentability test using *Saccharomyces cerevisiae* is under development to study the effects of chemical additives and storage conditions on the production of ethanol from raw thick juice (Objective 3). This fermentability test will also serve as an indicator of spoilage during the analyses of the samples for Objective 2.

**ACKNOWLEDGEMENTS**

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