EVALUATION OF STORAGE TECHNIQUES TO PRESERVE FERMENTABLE SUGARS FROM SUGAR BEETS FOR ETHANOL PRODUCTION

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Juan Manuel Vargas-Ramirez

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Juan Manuel Vargas-Ramirez

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dennis P. Wiesenborn
Chair

Zhulu Lin

Scott W. Pryor

David M. Saxowsky

Approved:

October 03, 2012
Date

Sreekala Bajwa
Department Chair

ABSTRACT

New sugar beet varieties may qualify as an advanced biofuel feedstock in the U.S., but new alternatives to conventional pile storage are necessary to preserve fermentable sugars and allow yearlong beet ethanol production. Fermentable sugar preservation was assessed in sugar beets stored under aerobic and anaerobic atmospheres and in raw thick juice stored at acidic ($2 \leq pH \leq 5$) and alkaline ($8 \leq pH \leq 11$) conditions. Aerobic storage of sugar beets at 4°C for 14 wk resulted in higher fermentable sugar retention (99±4%) than at 25°C or anaerobic storage at 4°C and 25°C. Raw thick juice retained ≥99% of fermentable sugars at pH 3.5 and 9.5 and refractometric dissolved solids content of 64.5°Bx. The changes in fermentable sugars in raw thick juice stored for 24 wk at acidic and alkaline pH were modeled by response surface methodology. Although raw thick juice was stored successfully at acidic and alkaline pH, conditions for high-efficiency fermentation must be developed.

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LIST OF ABBREVIATIONS

α_w.....Water activity

µL……...Microliters

µm……..Micrometers

BTY……....Billion tons per year

BGY……....Billion gallons per year

d……..Days

EISA……....Energy Independence and Security Act (2007)

g……...Grams

GHG……....Greenhouse gas

h……..Hours

ha……Hectare

kg……Kilograms

kPa……...Kilopascals

L……...Liters

m……..Meters

mM……..Millimolar (concentration)

mL……....Milliliter

M……..Molar (concentration)

min……Minutes

MT……Million tons

MTY……Million tons per year

N……...Normal (concentration)

psi…….Pounds per square inch

RDS……..Refractometric dissolved solids

RPM……..Revolutions per minute

T……Tons

v v^{-1}……...By volume (concentration)

wk…….Weeks

w w^{-1}……..By weight (concentration)

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GENERAL INTRODUCTION

For over a century, petroleum has been the primary feedstock for liquid transportation fuels. The dependence of humankind on petroleum and the overexploitation of petroleum reserves are increasingly contributing to the scarcity of this non-renewable natural resource. Furthermore, the combustion of petroleum and other fossil fuels results in greenhouse gas (GHG) emissions of 8.5 BTY of carbon as carbon dioxide (CO_2), which contributes to global warming (Camill, 2010). Alternatives to petroleum-based liquid fuels, such as bio-based ethanol, have been proposed as promising options to displace petroleum use worldwide. Ethanol may be more environmentally friendly due to its potential to reduce net GHG emissions. Among GHG, CO_2 is of importance as it is the primary waste product of many industrial and biological processes. Ethanol has the potential to reduce net CO_2 emissions since it is readily produced from renewable feedstocks such as energy crops and agricultural residues. These renewable feedstocks take up atmospheric CO_2 to fulfill carbon requirements for growth and energy production through photosynthesis, thereby partially offsetting CO_2 released through combustion of the ethanol.

Countries such as Brazil, Germany, and the United States have focused on developing new technologies and feedstocks for ethanol production. Ethanol may be readily blended with gasoline for use in automotive engines. Despite its lower energy content compared to that of gasoline (Treu, 1996), ethanol has important properties such as low toxicity, low volatility, high heat of vaporization, and high octane number (U.S. Dept. of Energy, 1997; Wyman, 1996).

Countries such as Brazil and the United States have enacted laws that mandate a minimum percentage of ethanol be blended into their gasoline to reduce GHG emissions and the dependence on petroleum. The Brazilian government requires all gasoline sold within its

territory to contain at least 20% ethanol by volume (Sandalow, 2006). In the United States, the 2007 Energy Independence and Security Act (EISA) mandated the production and blending of 36 BGY of biofuels into transportation fuels by 2022. The EISA classifies biofuels into three categories based on their potential to reduce net GHG emissions. Conventional, advanced, and cellulosic biofuels have the potential to reduce net GHG emissions by 20%, 50%, and 60%, respectively. The EISA also specifies that advanced and cellulosic biofuels should account for almost 60% of the 36 BGY of biofuels to be produced by 2022. This has compelled researchers to identify potential feedstock alternatives to achieve the production goals stated under the EISA.

Sugar cane and corn are established ethanol feedstocks due to their high sugar and starch content, respectively. Brazil is well-known for producing ethanol from sugar cane, and for years has blended this fuel with gasoline. In the United States, sugar prices are high; therefore, the production of ethanol from cane and sugar beet is not economically viable (Wyman, 1996). For this reason, 97% of the annual ethanol production comes from corn starch (Hettinga et al., 2009). Nonetheless, corn grain is a basic food and feed crop, and its use as an ethanol feedstock has generated a controversy over the years due to its increasing price and limited impact of corn grain ethanol on GHG emissions. Corn grain ethanol results in an average GHG emission reduction of 24% in comparison to gasoline emissions (Wang et al., 2011). Therefore, the ethanol potential of renewable feedstocks that may result in more sustainable alternatives to corn grain ethanol is under evaluation at many research institutions throughout the United States.

Sugar beet varieties with high crop yields have become attractive for ethanol production in the United States as they may qualify as an advanced biofuel feedstock under the EISA. Sugar beets selected for ethanol production are also known as *energy beets*, and are not appropriate for table sugar production due to their high non-sucrose content. Research trials suggest that *energy*

beets have higher crop and ethanol yields than corn grain and typical sugar beets (Albus, 2010) (Table 1). Additionally, *energy beets* may require up to 46% less fresh water per unit volume of ethanol than corn grain (Gerbens-Leenes, 2009).

Table 1. Crop and ethanol yields compared between two of the most suitable feedstocks for ethanol production and energy beet as a new potential feedstock

	Energy Beet	Sugar Beet	Corn Grain
Crop yield (T ha^{-1})	91.4[1]	68.2[2]	9.6[3]
Crop yield (dry T ha^{-1})	22.9[a]	17.1[a]	7.5[b]
Ethanol yield (L ha^{-1})	9237[4]*	6750[5]	3300[5]

[1]Albus, 2010; [2]USDA, 2011b; [3]USDA, 2011c; [4]PSU, 2010; [5]Cuff and Goudie, 2009.
[a]Assumes 75% moisture content (Asadi, 2007); [b]Assumes 22% moisture content (Lauer, 2001).
*Estimate of 0.1 L of ethanol per kg of beets was used for calculation.

Storage techniques are essential to allow the yearlong operation of ethanol plants based on energy beets. One approach for storing energy beets for short periods may be the conventional storage technique followed by the sugar industry. In regions with harsh winter conditions, the sugar industry conventionally stores sugar beets in piles exposed to freezing temperatures. For example, winter conditions in the Red River Valley of the North Central United States allow storing sugar beets for up to 6 months with minimal sugar loss. Nevertheless, conventional pile storage has disadvantages including the formation of internal hot spots due to insufficient ventilation which creates a suitable environment for microbes to thrive and generate sugar losses. Additionally, freezing enhances the rupture of cell walls in the beet tissue making the cell contents susceptible to leaching during the thawing and washing of the beets prior to sugar extraction. Moreover, thawing the sugar beets requires large quantities of warm water which increases the overall process energy requirements. The conversion of energy beets to ethanol should be an energy efficient process to qualify this energy crop as an advanced biofuel feedstock. Hence, conventional storage may not be accepted by the ethanol industry; therefore,

alternative storage techniques should be evaluated to improve process energy efficiency and avoid risks associated with conventional storage.

The storage of pure thick juice is an alternative storage technique implemented by the sugar industry. Pure thick juice may be stored in a stable form by adjusting its refractometric dissolved solids (RDS) content in addition to controlling its pH and temperature to reduce the risk of microbial degradation (Willems et al., 2003). A similar approach which consists in storing concentrated raw beet juice, also known as *raw thick beet juice*, has been previously proposed as an alternative technique to store sucrose for table sugar production (Fiedler et al., 1993; Hein et al., 2002). Such a technique may be applicable to store fermentable sugars in sugar beets for ethanol (Hinkova et al., 2000). Unfortunately, there is limited literature regarding raw thick juice storage as an alternative technique for preserving sugars. Moreover, previous studies have solely dealt with increasing the pH of raw thick juice to between 8.5 and 9.5 (Fiedler et al., 1993; Hinkova et al., 2000; Hein et al., 2002). Storage at acidic pH values has been avoided by the sugar industry due to the hydrolyzing effect of acids on sucrose. Nevertheless, a low pH may improve raw thick juice storage by inhibiting microbial growth and thus minimizing sugar loss. Additionally, in contrast with table sugar industry requirements, hydrolysis of sucrose to glucose and fructose should not be a concern to the ethanol industry since these sugars are also readily fermented by yeast.

Objective of the Study

The overall objective of this study was to identify the best practices to store fermentable sugars from sugar beets to allow yearlong ethanol production in regions with short beet growing seasons such as the Red River Valley of the North Central United States. The storage techniques

considered for the satisfactory completion of this objective should require low energy inputs and chemical additives. The specific objectives of this research were:

I. To identify best combinations of pH and RDS for fermentable sugar preservation in raw thick juice stored for up to 24 weeks.

II. To evaluate the fermentability of stored raw thick juice to determine the effect of storage conditions on overall ethanol yield.

III. To evaluate the effect of storage temperature and initial oxygen content of storage atmosphere on the retention of fermentable sugars in whole sugar beets.

Thesis Organization

The thesis consists of two research papers preceded by a literature review. The literature review introduces the reader to processing steps typical of the sugar beet industry that may be applicable to the processing of sugar beets to ethanol. Concepts related to the preservation of perishable products, which are used throughout the thesis, are also defined in the literature review. Paper 1, entitled "Determination of Suitable Storage Conditions to Preserve Fermentable Sugars in Raw Thick Beet Juice for Ethanol Production: A Response Surface Methodology Approach", discusses the effect of pH and RDS content on the preservation of fermentable sugars in raw thick juice. The pH and RDS combinations that resulted in best fermentable sugar preservation are also presented in Paper 1. Finally, Paper 1 also describes the effect of storage conditions on the fermentability of stored raw thick juice.

Paper 2, entitled "Change in Fermentable Sugars in Sugar Beets Stored under Aerobic and Anaerobic Atmospheres", details the combined effect of aerobic and anaerobic storage atmospheres and temperature on the retention of fermentable sugars in sugar beets. Subsequent to the papers, recommendations are given for future research pertaining to the development of

techniques to store fermentable sugars from sugar beets. The appendices of the thesis present a preliminary raw thick juice storage study, as well as original and supplemental data from all experiments.

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LITERATURE REVIEW

What Are Sugar Beets?

Sugar beets (*Beta vulgaris*) are part of the *Chenopodiaceae* family along with red beets, spinach, and others. When grown in soft soil, sugar beet roots can extend to a depth of seven feet. Beets grow most successfully in northern latitudes, but are capable of adapting to various climates and a wide range of soils. In the United States, sugar beets are grown on sandy, silty clay, or silty clay loam soils, as well as on soils with high organic content and/or high clay content (Cattanach, 1991).

Sugar beets have been grown for sugar production since Andreas Margraff, a German chemist of the 1700s, extracted and crystallized the sucrose contained in sugar beet roots (U. S. Beet Sugar Association, 1959). In 1811, Napoleon Bonaparte encouraged the cultivation of sugar beets and construction of processing facilities to produce beet sugar to feed his armies (The Beet Sugar Story, 1959). By the late 1800s, sugar beets had become the main source of sugar in Europe.

Energy beets are varieties of sugar beets developed for use as a feedstock to produce ethanol and other biofuels and industrial chemicals. Energy beets are characterized by much higher sugar yields per unit area than typical sugar beets; however, their high non-sucrose content per unit mass makes energy beets not suitable for sugar production. Business groups in North Dakota have envisioned the development of the first ethanol facilities in the United States to produce ethanol from energy beets by 2014 (AgWeek, 2012).

General Characteristics of Sugar Beets

Sugar beets are a biennial crop, namely, their complete life cycle lasts two years. During the first year of growth, sugar beets accumulate sucrose, whereas during the second year, they begin reproductive growth when exposed to temperatures >6°C. Reproductive growth of sugar beets lasts approximately 12 weeks and seed production takes place during this period. However, if sugar beets are exposed to temperatures <6°C they remain vegetative (McGinnis, 1982).

A sugar beet can be divided into two major sections: the shoot and the root. The shoot is constituted of petioles and leaf blades; the latter are indispensable for the synthesis of sucrose via photosynthesis. The root is made of insoluble cell wall material and soluble compounds, and is responsible for storing sucrose during growth. Sucrose accounts for approximately 70% of the dry substance in the beet root and it is stored within the beet cells (Asadi, 2007). The cell wall is formed in approximately equal portions of cellulose, hemicellulose, and pectin which support cell contents (McGinnis, 1982).

Where Are Sugar Beets Grown?

Most European countries grow sugar beets for the production of table sugar and the foliage is utilized for protein isolation. In 2010, France became the world's leading producer of sugar beets, followed by the United States (Table 2) (FAO, 2010). Sugar beets are successfully grown in the United States within five regions encompassing 11 states (Fig. 1). Sugar beets grow well in the temperate summers of North Dakota and Minnesota; some varieties also adapt to the hot climate of Arizona (McGinnis, 1982).

Production in the North Central Region of the United States

The North Central Region of the United States is the greatest contributor to the total national production of sugar beets. Total production of sugar beets in the United States decreased from 29.05 MT in 2010 to 26.12 MT in 2011 (USDA/ERS, 2012a). North Dakota and Minnesota, together, are the major contributors to sugar beet production in the United States with approximately 50% of the total production. In 2011, the production of both states accounted for 47% of the total sugar beet production in the nation (Table 3). In the same year, Idaho and Michigan, together, contributed 34% of the total U.S. production (Table 3).

Table 2. Primary sugar-beet producing countries in the world in 2010 (FAOSTAT, 2012)

Country	Production (MTY)
France	31.91
United States	26.12*
Germany	23.86
Russia	22.26
Turkey	17.94
Ukraine	13.75

*U.S. production in 2012 (USDA/ERS, 2012a)

Figure 1. Sugar beet production in the United States (USDA/ERS, 2007)

Table 3. States with highest contribution to U.S. sugar beet production (USDA/ERS, 2012b)

State	Production (MT)
Minnesota	8.08
North Dakota	4.18
Idaho	5.51
Michigan	3.33
U.S. Total	26.11

Table Sugar Production from Sugar Beets

Storage, Cleaning and Slicing Of Beets

After harvest, sugar beets are washed to remove dirt and trash that has been carried along with them before they are sent to storage sites surrounding processing factories. In regions with harsh winter conditions, sugar beets may be stored for up to 6 months before being processed. Sugar beets are stacked in piles with dimensions of up to 5 meters tall by 50 meters wide by 300 meters long before being supplied to the processing facilities as required. The beets are conveyed to the facilities through water flumes in which rocks and other trash are removed by density difference. Rocks impose a great risk of damage to the beet slicing equipment and may seriously delay processing.

Sugar beets are washed carefully within the factory to remove soil and clay residues. Several washing system designs are commercially available, but all operate under the same principle: increasing contact between beets while water enhances the removal of residues. The clean beets are subsequently crinkle-cut into thin pieces known as *cossettes*. Cossettes have a maximum thickness and length of 0.6 cm and 6 cm, respectively, which increases the diffusion of sugar out of the beet cells (Asadi, 2007).

Sugar Extraction

Sugars are contained within the sugar beet cells, surrounded by a protein substance known as *protoplasm* which is denatured at temperatures above 50°C (McGinnis, 1982). The sugars in the beet cells of the cossettes are conventionally extracted by diffusion in counter-current diffusers operated with water at 70°C to increase extraction efficiency (Asadi, 2007). The diffusion rate of sugars out of the beet cells and into the extraction water is directly proportional to the concentration gradient between the two mediums. Other factors that impact the efficiency of diffusion are particle size, as well as temperature and viscosity of the extraction medium.

The liquid that results from the diffusion process is commonly referred to as *raw beet juice*. Raw beet juice contains approximately 15% (w w^{-1}) dry substance from which 90% is sucrose, glucose, and fructose. The de-sugared cossettes are removed from the diffuser and screw pressed to expel about 80% of the remaining water. The press water typically contains a significant portion of sucrose; hence, it is recycled to the diffuser to maximize sucrose recovery from the beets. The pressed pulp has a high nutrient content and thus is dried, densified into pellets, and sold as livestock feed.

Raw Beet Juice Purification

Raw beet juice from the diffuser is screened to remove beet pulp particles and heated to approximately 85°C. Raw beet juice contains approximately 2% insoluble solids including sand and fine pulp particles that interfere with sucrose recovery (Asadi, 2007). These insoluble compounds are removed by purifying the raw beet juice in a process that uses calcium oxide (*lime*) mixed with water (*milk of lime*) (Fig. 2).

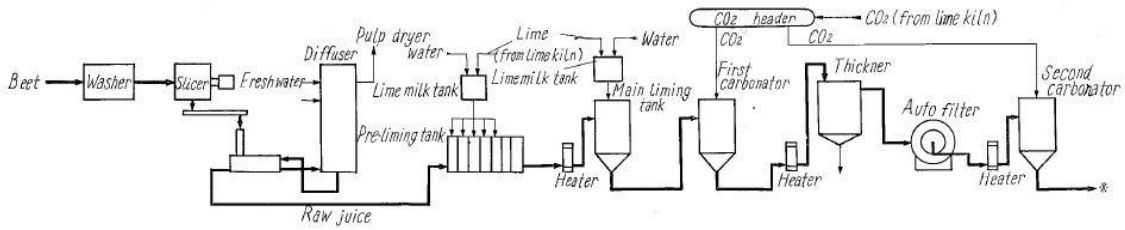

Figure 2. Flow diagram of raw beet juice purification (Yoshida, 1963)

Purification begins in a pre-liming tank in which a portion of limed juice taken from the main liming tank is added to the raw beet juice. The mixture is then sent to the main liming tank where more lime is added at 2% beet weight (Yoshida, 1963) to precipitate the non-sugars. The limed juice is subsequently sent to the first and second carbonation tanks where carbon dioxide is injected at the bottom of the tanks and into the limed juice to precipitate the lime in the form of calcium carbonate. The precipitating calcium carbonate sweeps colloidal material on its way, and adsorbs other impurities. The precipitated solids are removed in a clarifier and by filtration. Purification removes 20 to 30% of non-sucrose soluble solids from the juice, most of them being invert sugars, colloids, and coloring substances (Asadi, 2007; Kearney et al., 1995). The purified juice is referred to as *thin juice.*

In recent years, new purification technologies have been explored to increase sugar recovery, reduce chemical use, and simplify the purification of raw beet juice (Kearney et al., 1995; Hinkova et al., 2002; Hakimzadeh et al., 2006; Seres et al., 2008).

Concentration of Thin Juice

Thin juice with a typical RDS content of 16°Brix is concentrated by water removal in a multiple-effect evaporator. The thin juice is preheated before being sent to the multiple-effect evaporator which operates with steam as the heating medium. Steam produced in an industrial boiler is used in the first effect and the energy content of the vapor produced in that effect is

sequentially reused in the following effects (McGinnis, 1982) (Fig. 3). Hence, multiple-effect evaporators conserve energy used to remove water. The concentrated thin juice is known as *thick juice* and has a pH of 9.0 and an average RDS content of 68°Brix (Justé, 2008).

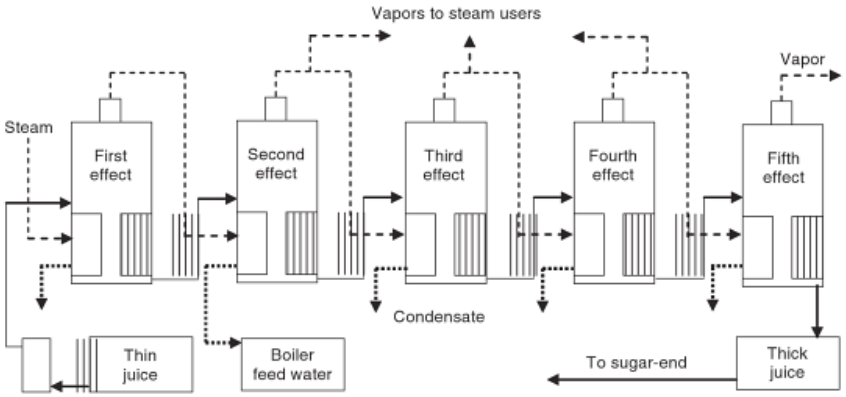

Figure 3. Five-effect evaporator typical of sugar processing plants (Asadi, 2007)

Crystallization of Sucrose

Crystallization is an effective method used to separate the sucrose from the impurities in thick juice. Sucrose is a disaccharide formed by a molecule of glucose bound to a molecule of fructose through a glycosidic bond (Fig. 4).

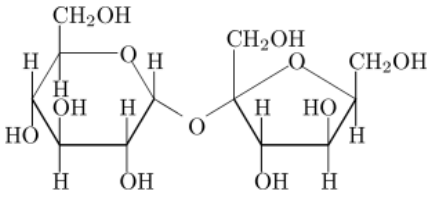

Figure 4. Sucrose molecule

Sucrose crystallization takes place under conditions of supersaturation (80°Brix at 75°C) by mass transfer when sucrose molecules move from the thick juice to the surface of fine sugar crystals, known as *seeds*, which are added to the juice. The product of the crystallization step is known as *massecuite* and is separated by centrifugation into syrup and sugar crystals of which

sucrose represents 99.9% of the dry weight (Asadi, 2007). The syrup portion is known as *molasses* and may be further de-sugared by chromatography (Munir, 1976). Molasses may also be used as livestock feed and as ethanol feedstock since it contains approximately 50% (w w^{-1}) sucrose (McGinnis, 1982).

Raw Beet Juice as an Ethanol Feedstock

The process of converting sucrose, glucose, and fructose to ethanol is much simpler than that to convert corn starch into the same product (Jacobs, 2006). Sucrose is readily broken down into readily fermentable glucose and fructose molecules by the hydrolytic enzymes of yeast. In contrast, the conversion of corn starch to ethanol requires additional energy-intensive steps, namely cooking, liquefaction, and saccharification, to break down starch into glucose. The cooking and liquefaction of corn mash is carried out at 88°C and the further saccharification of starch occurs optimally at 60°C (McAloon, 2000). Conventional ethanol production from corn starch requires double the amount of energy needed to produce ethanol from sucrose (Jacobs, 2006).

The theoretical maximum ethanol yield from sucrose is 0.68 L kg^{-1} sucrose. However, in realistic scenarios in which *S. cerevisiae* is used, the ethanol yield achieved is typically 90-93% of the theoretical maximum yield (Bai et al., 2008). Grahovac et al. (2011) reported an ethanol yield of 0.64 L kg $sucrose^{-1}$ (94% of theoretical maximum yield) when fermenting raw beet juice. The productivity of fermenting microorganisms may be inhibited by factors such as sugar and/or inorganic salt concentrations, temperature, and pH. The growth of yeast, which is coupled with the production of ethanol as a byproduct, is also completely interrupted by ethanol above certain concentrations. Growth of some strains of *S. cerevisiae* ceases at a concentration of 14% (v v^{-1}) ethanol (Ghareib, 1988).

Grahovac et al. (2011) reported that the optimum fermentation time for raw, purified, and thick juices was 36 h when fermented by *S. cerevisiae* at 30°C, pH 5, and agitation rate of 200 RPM. The fermentable sugar contents of the fermentation media were adjusted to 130 g kg media^{-1} and ethanol yields of approximately 0.64, 0.62, and 0.62 L kg sugar^{-1} were obtained for raw, purified, and thick juices, respectively.

Factors that Influence the Growth Rate of Spoiling Microorganisms

Water activity, pH, temperature, and storage atmosphere are factors that influence the rate of microbial growth in biological materials. These factors can be manipulated to inhibit the growth of microbes such as bacteria, yeast and mold, with only a slight alteration in the quality of a product.

Water Activity

Water is an essential constituent of all living organisms. W.J. Scott (1953) identified the relationship between the activity of water as a medium in food and the deterioration of food due to the action of microorganisms. Water activity (α_w) is a measure of the water available for microbial growth and is expressed as the ratio of the vapor pressure of water in a biological material to that of pure water at the same temperature. In terms of quality preservation, the α_w of biological materials is more significant than their water content (Rahman, 2007), and it can be modified without altering the water content of a material. The α_w of a material is typically adjusted by adding hygroscopic compounds such as sugar or salt to reduce the portion of water available for microorganism proliferation. Alternatively, the α_w of biological materials may be adjusted by removing a portion of water from the matrix of the materials by evaporation or drying.

For most biological materials, the minimum α_w value at which microorganisms may grow is in the range of 0.6-0.7 (Russel, 2003). The α_w ranges in which various spoiling microorganisms may grow are shown in Figure 5. The lower limit of α_w at which a given microorganism may grow can be shifted upwards or downwards with the adjustment of factors such as pH and temperature.

Water activity is a factor that is not usually studied and reported in the literature regarding thick juice storage. However, α_w is inversely related to the RDS content, a property that is extensively measured during beet juice analyses. Increasing the RDS content of a material by evaporation (or other means) results in a decrease of the α_w of that material.

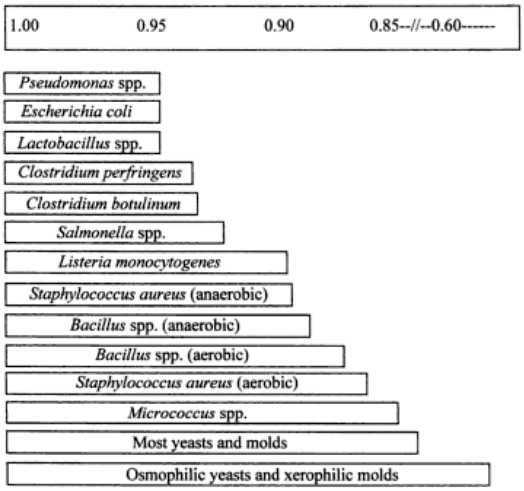

Figure 5. Water activity ranges for various spoiling microorganisms (Russel, 2003)

Instruments designed to measure the α_w of biological materials are commercially available; however, measuring the α_w of a material is a time-consuming task. A. A. Gabriel (2008) developed a predictive model to estimate the α_w of simulated food solutions at 25°C as a function of pH and RDS content (°Brix) values:

$$a_w^{25^\circ C} = [0.95 + 0.03x_1 + (1.02 \times 10^{-2})x_2 + (5.21 \times 10^{-4})x_1x_2 - (3.95 \times 10^{-3})x_1^2 - (1.07 \times 10^{-4})x_2^2]^{1/2} \quad \text{[Eq. 1]}$$

where x_1 and x_2 represent actual pH and RDS (°Brix), respectively. This model was validated using real food samples with pH values ranging from 2 to 7 and RDS contents from 0 to 80°Brix.

Hydrogen Ion Concentration

The hydrogen ion concentration, referred to as pH, has an important influence on food preservation. A high concentration of hydrogen ions (high acidity; pH<3.5) halts the proliferation of most bacteria in biological materials; however, many yeasts and molds remain active at pH>2 (Fig. 6).

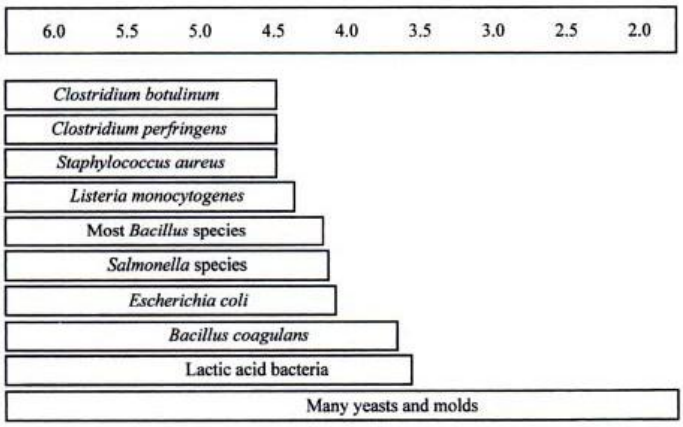

Figure 6. Low pH ranges for various types of microbial growth (Russel, 2003)

Organic acids, such as acetic and lactic acid, have a greater inhibitory effect on microbes compared to mineral acids such as hydrochloric and phosphoric acids (Rahman, 2007; Troller, 1985). Nevertheless, organic acids may decrease the yeast ethanol productivity (Phowchinda et al., 1995; Limtong et al., 2000; Huang et al., 2012) making them less attractive than mineral acids for the preservation of fermentation feedstocks. The preservative potential of mineral acids is based on their denaturing effect on enzymes involved in the metabolism of biological materials (Rahman, 2007).

A concurrent manipulation of the pH and α_w of biological materials may result in an additive or synergistic preservation effect (Fig. 7) (Troller, 1987; Dodds, 1989). Additionally, not only high concentration of hydrogen ions have an inhibiting effect on microbial growth (starting at pH<5), but high concentrations of hydroxide ions (pH>10) are also known to inhibit microbial proliferation (Tewari, 2007).

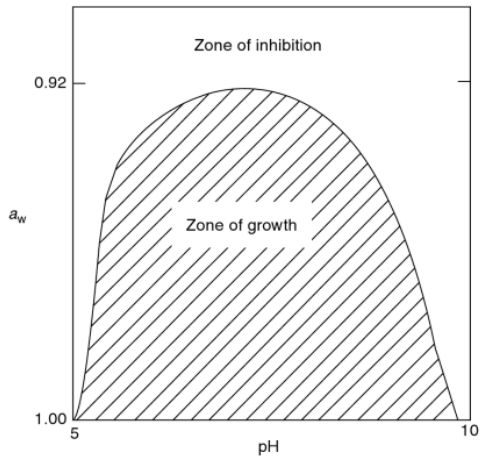

Figure 7. General interacting effect of pH and α_w on bacterial growth (Rahman, 2007)

Storage Temperature

Storage temperature is another factor that may be modified to halt or reduce the risk of microbial proliferation and minimize the effects of enzymes present in biological materials. Different types of microorganisms grow optimally at specified temperature ranges and, hence, can be classified into four general categories: psychrotrophs (0-5°C), psychrophiles (12-15°C), mesophiles (30-40°C), and thermophiles (55-65°C) (Tewari, 2007). Most microorganisms and enzymes become more active as temperature increases within one of the above ranges. Nonetheless, many spoilage microorganisms are capable of growing at slow rates when exposed to temperatures <0°C and free water is present (Russel, 2003).

Temperature, α_w, and pH may be modified concurrently to achieve a combined effect of greater magnitude than the sum of the individual effects.

Storage Atmosphere

The storage atmosphere of a biological material may also influence the proliferation of microorganisms. Agricultural crops are commonly stored under modified atmospheres to preserve their quality by halting the proliferation of aerobic microbes and reducing the respiration rate of the crops (Brody, 1989; Blakistone, 1999; Russel, 2003). Inert gases such as carbon dioxide and nitrogen are typically used to modify storage atmospheres. Modified atmospheres are generated by partially or completely flushing the air out of storage containers using inert gases. However, not all crops store best under the same atmospheric composition. For example, a complete removal of air from a storage unit may be detrimental to certain crops due to their ability to undergo anaerobic respiration. Through anaerobic respiration, crops metabolize valuable substrates within their matrix to produce energy required to repair wound tissue and increase the defense against pathogens. Pathogens, such as fermenting bacteria and yeast, proliferate in the absence of oxygen by metabolizing crop substrates.

A modified atmosphere may be controlled with modern systems that monitor the atmospheric composition of a storage unit and re-adjust it if necessary (Thompson, 1998). This technique is used in the storage of large quantities of agricultural crops in countries with climate conditions that restrict long harvesting seasons.

Storage of Sugar Beets and Thick Juice

In regions with extreme winter conditions, such as the Red River Valley of the North Central United States, sugar beet seeds are typically planted in May and the crop is harvested in

October. To extend the processing campaigns of sugar beet factories, a continuous supply of beets is required beyond the period in which they are harvested (van der Poel et al., 1998). For example, American Crystal Sugar Co. (Moorhead, MN) requires approximately 5,900 T beets d^{-1} over a processing campaign of 275 days (American Crystal Sugar, 2012). To maximize the supply of beets to the factory, sugar beet storage is indispensable. Sugar beets are conventionally stored in piles on storage fields adjacent to the factory. Piled sugar beets are frozen with cold ambient air from extreme winter conditions which is pumped through tunnels underneath the piles. Alternatively, the sugar may be extracted from the beets and stored in the form of thick juice, a technique that has been recently studied and incorporated into sugar beet factories.

Storage of Whole Beets

Sugar beets remain metabolically active after harvest; hence, effective storage techniques are required to maximize the preservation of sugar in the beet tissue. Sugar beets are conventionally stored in piles of approximately 300 m long and 5-12 m high on open fields during winter in the northern regions of the United States. In these regions, the winter temperatures are typically below -7°C, the temperature at which sugar beets freeze due to their high dry substance content (Asadi, 2007). Freezing reduces the water activity in the beet tissue and consequently decreases the ability of microorganisms to proliferate. The activity of beet enzymes is also halted below the beet freezing point. Nevertheless, as temperature increases due to seasonal changes and beets begin to thaw, microorganisms and enzymes become more active metabolizing and hydrolyzing sugar, respectively.

Under normal frozen storage conditions, sucrose loss in beets is in the range of 100-200 g T $beets^{-1}d^{-1}$, but it can reach up to 2 kg T $beets^{-1}d^{-1}$ under unfavorable storage conditions (Asadi, 2007). An example of an unfavorable condition is the lack of ventilation due to the compacting

of beets in the piles, thus generating hot spots within the piles. The storage piles are commonly monitored through aerial photographs taken with infrared cameras to detect hot spots (Asadi, 2007). When a hot spot is detected, the further loss of sucrose is prevented by sectioning the pile and processing beets that have begun to undergo spoilage.

Storage of Thick Juice

Thick juice storage was introduced in 1960 by Holly Sugar Company in the United States. Since then, many sugar factories around the world have expanded their processing capacity and campaign by storing thick juice (van der Poel et al., 1998). Thick juice is stored in cylindrical steel containers with a diameter of 40-55 m and height of 15 m, and a capacity of 18,000 to 30,000 m^3.

Under best conditions, thick juice may be stored for over a year with little or no microbial degradation of sucrose (Sargent, 1997). Microbial activity in thick juice is decreased by the reduced water activity associated with the high RDS content of the juice. The minimum safe RDS content of the juice varies depending on its purity and storage temperature. An RDS content of 69°Brix is sufficient for thick juice storage at a temperature between 10°C and 15°C (van der Poel et al., 1998).

In the sugar industry, the pH of stored thick juice is constantly monitored and maintained at $pH \geq 9$ by adding a solution of NaOH to prevent microbial growth (Asadi, 2007). The storage of thick juice under acidic pH values is avoided by the sugar industry due to the hydrolytic effect that low pH has on sucrose. However, raw thick juice storage under acidic pH may be an effective technique for ethanol feedstock preservation.

Storage of Raw Thick Juice

Thick juice is produced by evaporating a portion of water from the thin juice that exits the clarifiers in the purification process (see *Raw beet juice purification*). In contrast to thick juice, *raw thick juice* results from the concentration of raw beet juice. Literature on raw thick juice storage is scarce; hence, this storage option deserves more attention as it may reduce energy expenditure in beet ethanol plants. As with thick juice storage, RDS content (inversely related to α_w), pH, and temperature are three determinant factors for successful long-term storage of raw thick juice. These factors require careful control to reduce the risk of sugar loss due microbial contamination and avoid the crystallization of sucrose in the storage unit.

Scientific literature on raw thick juice storage is limited. Fiedler (1993) reported that raw thick juice was storable for 300 days at a temperature between 15°C and 20°C when its surface was sprayed with formalin. Nevertheless, formalin is a carcinogenic substance that may be introduced into the environment through the wastes generated in an ethanol plant if they are not disposed of properly. Additionally, the levels of formalin required to prevent microbial growth during storage may restrict yeast fermentation.

Indicators of Thick Juice Deterioration

The pH value is a reliable and sensitive indicator of microbial activity within stored thick juice (van der Poel et al., 1998). Most microorganisms produce organic acids (such as lactic and acetic) as byproducts of the aerobic metabolism of carbohydrates (Ray, 2004), thereby reducing pH. The production levels of these acids depend on the rate of deterioration of the thick juice. Therefore, sugar beet factories rely on pH measurement as a quick technique to monitor and evaluate the quality of stored thick juice.

Sucrose, glucose, and fructose in thick juice are often quantified as a means of monitoring sucrose hydrolysis. The table sugar industry considers the hydrolysis of sucrose a decrease of thick juice quality since the final product of this industry is crystal sucrose. In the case of stored raw thick juice for ethanol production, the hydrolysis of sucrose is not a concern since glucose and fructose are also readily fermented by yeast. Nevertheless, a decrease in total fermentable sugars (defined in this study as sucrose, glucose, and fructose) may not be accepted by the ethanol industry.

The indicators used to monitor stored thick juice and evaluate its quality, may be used to monitor stored raw thick juice. Monitoring pH and sugar contents is inexpensive compared to the sugar losses that may be caused by microbial spoilage. If microbial contamination is detected, corrective measures may be taken to halt microbial proliferation and prevent major sugar losses that would ultimately translate into economic losses.

The condensation of vapor within storage tanks forms a zone of low RDS content on the surface of the thick juice, increasing the risk of microbial growth. Antimicrobial agents such as sodium hydroxide (Hein et al., 2002) and formaldehyde (Justé, 2008; van der Poel et al. 1998) have been satisfactory for sugar preservation in thick juice. Hein et al. (2002) and Justé et al. (2008b) studied the effect of hop extracts (hop β-Acids) on thick juice preservation and their results suggest that thick juice may be stored satisfactorily for a period of at least 272 days. Thick juice has also been successfully stored in floating roof tanks for periods over three years (van der Poel et al., 1998).

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USDA, NASS, Crop Production; FSA, Sweetener Market Data. Sugar and Sweeteners: Recommended Data. U.S. Sugar Supply and Use. Table 14 (Updated on 3/31/2011).

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PAPER 1. DETERMINATION OF SUITABLE STORAGE CONDITIONS TO PRESERVE FERMENTABLE SUGARS IN RAW THICK BEET JUICE FOR ETHANOL PRODUCTION: A RESPONSE SURFACE METHODOLOGY APPROACH

Paper 1 is a revised version of a paper presented at the 2012 ASABE Annual International Meeting in Dallas, TX, July 29 – August 1, 2012. Title: Improving the Storage Life of Raw Beet Juice for Ethanol Production. Paper No.: 121337525. Authors: Juan M. Vargas-Ramirez, Darrin M. Haagenson, Scott W. Pryor, and Dennis P. Wiesenborn. Juan M. Vargas-Ramirez designed and conducted the experiments for this study, and is the first author of Paper 1. The co-authors assisted in the editing of Paper 1 which will be submitted to the journal *Biomass and Bioenergy* for publication after thesis defense.

Abstract

New long-term storage techniques are necessary to preserve fermentable sugars from new sugar beet varieties; those varieties may help qualify the ethanol from such feedstock as an advanced biofuel in the United States. The focus of this study was to evaluate the combined effect of pH and refractometric dissolved solids (RDS) content on fermentable sugar preservation in raw thick beet juice stored for 24 wk at 23±1°C. Response surface methodology was applied to model the change in fermentable sugars in raw thick juice stored under controlled acidic ($2 \leq pH \leq 5$) and alkaline ($8 \leq pH \leq 11$) conditions in combination with $60°Brix \leq RDS \leq 69°Brix$. Combinations of $pH \leq 3.5$ and $pH \geq 9.5$ with $RDS \geq 64.5°Brix$ were effective in preserving up to 99% of fermentable sugars in stored raw thick juice. Following storage, yeast fermentation of acidic treatments achieved efficiencies of <82% relative to raw juice, whereas alkaline treatments hindered yeast fermentation to a greater extent resulting in efficiencies of <54%.

Introduction

A life cycle analysis conducted by the Environmental Protection Agency shows that corn-starch-based ethanol results in a net reduction of greenhouse gas (GHG) emissions of approximately 20%, relative to 2005 gasoline (EPA, 2010). The need for greater reductions in GHG emissions has prompted the search for cleaner alternative fuels. The Energy Independence and Security Act of 2007 mandated the production and blending of 36 BGY of biofuels into liquid transportation fuels by the year 2022. From the 36 BGY, no less than 5 BGY must be advanced biofuels that achieve at least a 50% net reduction in GHG emissions relative to gasoline and are produced from feedstocks other than corn starch.

High-yielding sugar beets may qualify as an advanced biofuel feedstock, and thus have become attractive for the production of ethanol in the United States. The storage and preservation of fermentable sugars from sugar beets would be required for yearlong operation of beet ethanol plants. In the Red River Valley of North Dakota and Minnesota, sugar beets are typically planted in May and the crop is harvested in October. Conventional storage of sugar beets for table sugar production consists of piling the crop on open storage fields and freezing the beets by forced ventilation, taking advantage of the extreme winter conditions. However, there are risks and disadvantages associated with conventional storage. For example, storing sugar beets in open piles can lead to the formation of hot spots and microbial degradation of sugars due to inadequate ventilation. In addition, freezing enhances the rupture of cell walls making the cell contents susceptible to leaching during the thawing and washing of the beets before sucrose extraction. Moreover, the thawing of sugar beets before processing requires large quantities of energy which contributes to a less favorable GHG life cycle assessment. The processing of sugar beets to ethanol should be highly energy efficient to qualify sugar beets as an advanced biofuel feedstock. Thus, conventional pile storage may not be a suitable technique for beet ethanol plants.

The storage of concentrated purified beet juice (*pure* thick juice) is one alternative technique to conventional pile storage of beets for ethanol production. However, the purification of raw beet juice is yet another energy-intensive processing step, and may not be essential if the juice is to be used for ethanol production. Therefore, the storage of *raw* thick beet juice may be a better practice to allow conversion to ethanol throughout the year. Front-end beet processing facilities for the extraction of sugars and concentration of raw beet juice may be located

strategically near sugar beet fields. In addition to the preservation benefits to storage, the concentration of raw beet juice may reduce transportation costs.

Pure thick juice for table sugar production is already stored in a stable form by controlling the refractometric dissolved solids (RDS) content, pH, and temperature, which influence microbial growth (Willems et al., 2003). Unfortunately, there is clearly a lack of literature regarding the storage of *raw* thick juice as an alternative technique for preserving sugars. Moreover, previous studies have solely focused on increasing and controlling the pH of *raw* thick juice at 8.5<pH<9.5 (Fiedler et al., 1993; Hein et al., 2002). Acidic pH values are not acceptable to the table sugar industry due to their hydrolyzing effect on sucrose, its product of interest. Nevertheless, acidic pH values may improve the retention of fermentable sugars in stored *raw* thick juice by halting microbial degradation. The glucose and fructose that result from partial sucrose hydrolysis should be acceptable to the ethanol industry since those sugars, along with sucrose, are readily fermented by yeast.

The overall objective of this study was to identify the best practices to store *raw* thick juice with minimal fermentable sugar loss. The storage techniques considered for the satisfactory completion of this objective should require low energy inputs and chemical additives.

Materials and Methods

Raw Thick Juice Storage

Raw Beet Juice Collection and Storage

Raw beet juice was collected from the diffuser at American Crystal Sugar Co. (Moorhead, MN, USA). The juice was analyzed within the same day of collection for RDS content and pH at 23°C, and total fermentable sugars (sucrose, fructose, and glucose) at 15°C.

Subsequently, the juice was stored for 1 to 3 d in sealed, 19-L plastic pails at 4°C before it was concentrated through evaporation.

Raw Thick Juice Preparation

A single-effect rising film evaporator (Wiesenborn et al., 1995) was used to concentrate the raw beet juice. The juice was first screened through three layers of cheese cloth (Grade 50 – Lymtech; Chicopee, MA, USA) to remove suspended beet particles that would potentially clog the feed valve of the evaporator. The evaporator was operated with steam at 107°C, vacuum of 60 kPa (gauge) inside the evaporating tube, input juice flow rate of 0.8 kg min^{-1} at 20°C, and output juice at 81°C. The juice was passed through the evaporator two times to increase its RDS content from 16.5 to 62°Brix. The RDS content of the resulting raw thick juice was adjusted to 70°Brix using a vacuum shelf dryer (Buflovak; Buffalo, NY, USA) operated at 65°C under a vacuum of 67 kPa (gauge) for 12 h. The final raw thick juice was stored in sealed 19-L pails at 4°C for less than 2 wk, until used for storage experiments. Total fermentable sugars in the final raw thick juice were quantified to determine changes due to processing.

Experimental Design

A two-factor, five-level central composite design (Myers et al., 2002; Gabriel, 2008) was used to evaluate the combined effect of pH and RDS content on the change of total fermentable sugars in raw thick juice stored for 24 wk at 23±1°C. The design consisted of 12 treatments, including 4 factorial points, 4 axial points, and 1 center point replicated 4 times; each treatment was carried out in duplicate. Two separate experiments were conducted to study the effect of acidic and alkaline pH values. The coded and uncoded levels of pH and RDS content are

presented in Table 4. Each raw thick juice sample, as described by the pH and RDS combinations, represented a treatment within the study.

Table 4. Central composite designs conducted to evaluate the combined effects of pH and RDS content (°Brix) on the change of fermentable sugars in stored raw thick juice

			Acidic Treatments		Alkaline Treatments	
	Coded Variables		Uncoded Variables		Uncoded Variables	
Treatments	pH	°Brix	pH	°Brix	pH	°Brix
1	-1	-1	2.4	61.3	8.4	61.3
2	1	-1	4.6	61.3	10.6	61.3
3	-1	1	2.4	67.7	8.4	67.7
4	1	1	4.6	67.7	10.6	67.7
5	-1.414	0	2.0	64.5	8.0	64.5
6	1.414	0	5.0	64.5	11.0	64.5
7	0	-1.414	3.5	60.0	9.5	60.0
8	0	1.414	3.5	69.0	9.5	69.0
9	0	0	3.5	64.5	9.5	64.5
10	0	0	3.5	64.5	9.5	64.5
11	0	0	3.5	64.5	9.5	64.5
12	0	0	3.5	64.5	9.5	64.5

Experimental Setup

Individual treatments (600 mL) were stored in 950-mL amber jars (storage units) sealed with screw caps before storage. This allowed 350 mL of headspace to simulate a storage scenario in a processing plant using conventional, fixed-roof storage tanks. Each filled unit was weighed before storage and after each sampling time to track the mass and headspace.

Analytical Methods

The pH of the stored treatments was measured on a weekly basis using a Thermo Scientific Orion 2-Star benchtop pH meter (Thermo Fisher Scientific Inc.; Beverly, MA, USA) equipped with automatic temperature compensation. Prior to analyses, the pH meter was calibrated with fresh standard buffer solutions of pH 1.7, 4, and 7 for acidic treatments, and pH

7, 10, and 12 for alkaline treatments. The pH meter was checked after every 10 measurements, using one calibration standard to ensure accurate readings. Throughout the study, the pH of the treatments was adjusted to within ±0.2 of the original value, if necessary, using 8 M HCL or 8 N NaOH solutions. The amounts of HCl and NaOH added were recorded and used to determine dilution factors to correct the fermentable sugar content of each treatment at the end of storage.

Aliquots (10 mL) were collected on a biweekly basis to determine the RDS content and water activity (α_w) of each treatment. RDS were quantified by pipetting 1 mL of sample into the chamber of a Pocket Digital Refractometer Mod. 300053 (SPER Scientific; Scottsdale, AZ, USA). The instrument was zeroed with distilled water before use. The α_w was measured using a ROTRONIC AG Version 4 water-activity meter (Rotronic AG; Bassersdorf, Switzerland). Prior to initiating the study, the device was calibrated according to manufacturer's recommendations using 35%, 80%, and 95% relative humidity standards. The water-activity meter was checked bimonthly using the calibration standards to ensure accurate readings.

The total fermentable sugars (defined here as sucrose, glucose, and fructose) in the treatments were quantified by HPLC (Waters Corporation; Milford, MA, USA) at the initiation and completion of the storage period. The HPLC system was equipped with an Aminex HPX-87P (300x7.8 mm) carbohydrate column (Bio-Rad Laboratories; Hercules, CA, USA), an isocratic pump, autosampler, and refractive index detector (Model 2414 – Waters Corporation). The injection volume into the column was 20 μL and the samples were eluted with 18.2-mΩ nano-pure water at a flow rate of 0.6 mL min^{-1} and elution time of 25 min. The column and detector temperatures were 85°C and 50°C, respectively. The total fermentable sugars were reported as the average of the duplicated storage treatments.

Fermentability of Raw Thick Beet Juice Samples after Storage

The fermentability of all acidic and alkaline treatments was evaluated after storage. The purpose of the fermentability test was to assess the effect of storage conditions on fermentation efficiency and ethanol yield of the treatments. Aliquots of the original raw beet juice (stored frozen) were fermented in parallel with the stored samples and used as a baseline for comparison of ethanol yields and fermentation efficiencies.

Inoculum Preparation

Saccharomyces cerevisiae (Fermentis Ethanol Red yeast) obtained in dry granule form from POET (Sioux Falls, SD, USA) was used for the fermentability test. The inoculum seed was prepared by inoculating 0.15 g of *S. cerevisiae* dry granules in a sterile broth of distilled water containing yeast extract (2 g L^{-1}) and glucose (15 g L^{-1}), at pH 5.0. After inoculation, the culture flask was incubated in a rotary shaker (MaxQ7000 – Thermo Scientific; Dubuque, IA, USA) at 30°C and 150 RPM for 24 h.

Fermentation of Samples

Treatments were fermented in triplicate in 250-mL Erlenmeyer flasks with 100 mL of culture media. The total fermentable sugars of the treatments were adjusted to 130 g kg $juice^{-1}$ by adding distilled water. The pH of the media was adjusted to 5.0 with either 8 M HCl or 8 N NaOH solutions. The flasks with the fermentation media were sterilized in an autoclave at 121°C and 220 kPa for 20 min. All the fermentation flasks were inoculated with 3% (v v^{-1}) of inoculum seed.

Six-chamber plastic airlocks (Brew PS, Inc.; Moorpark, CA, USA) were used to maintain anaerobic conditions within the fermentation flasks. The flasks were incubated in a water-bath

rotary shaker at 30°C and agitated at 150 RPM for 96 h. Sample aliquots of 1.5 mL were collected in micro-centrifuge tubes at 0 and 96 h for fermentable sugar and ethanol quantification. The aliquots were centrifuged (Galaxy 16 Micro-centrifuge – VWR International; Bristol, CT, USA) at 13,000 RPM for 5 min and filtered through 0.2-µm nylon filters (Pall Corporation; West Chester, PA, USA) into HPLC vials.

Analytical Methods

Fermentable sugars were quantified following the HPLC method described in sub-section 2.1.5. Ethanol, lactic acid, and acetic acid were quantified by HPLC (Waters Corporation; Milford, MA, USA) using an Aminex HPX-87H (300x7.8 mm) ethanol column (Bio-Rad Laboratories; Hercules, CA, USA), an isocratic pump, autosampler, and refractive index detector (Model 2414 – Waters Corporation). The injection volume into the column was 12 µL and the samples were eluted with 5-mM sulfuric acid at a flow rate of 0.6 mL min^{-1} and elution time of 30 min. The column and detector temperatures were 60°C and 50°C, respectively.

The ethanol yield of the treatments was expressed as g ethanol per g glucose equivalents in the original raw thick beet juice (before storage). The residual sugars in the fermentation media were reported as percentage of initial sugars.

Statistical Analysis

Response surface methodology (Myers et al., 2002) was applied to model the change in total fermentable sugars (Y_i) in raw thick juice stored for 24 wk, and ethanol yields (Y_i^{EtOH}) of treatments fermented after storage. A regression analysis was conducted using Minitab® Statistical Software 16 (Minitab Inc.; State College, PA, USA) to fit a second-order polynomial model:

$$Y_i \left(or\ Y_i^{EtOH}\right) = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2 + \varepsilon \qquad \text{[Eq. 2]}$$

where x_1 and x_2 are the uncoded independent variables for pH and RDS content (°Brix), respectively; β_0 is a constant, and β_1 (β_2), β_{11} (β_{22}), and β_{12} are linear, quadratic, and interaction coefficients, respectively. Statistically insignificant (p-value>0.05) terms were identified and removed to test different forms of second-order polynomials before selecting the most significant model.

Results and Discussion

The storage of raw thick juice at acidic and alkaline pH values was evaluated in an effort to determine the best conditions for fermentable sugar preservation. The raw thick juice used throughout the study was produced by concentrating raw beet juice in a rising-film evaporator. Juice purification, a step required in the beet sugar industry to meet quality standards for human food use, was bypassed to reduce process energy requirements and waste products. Reduction of process energy requirements is desired to attain acceptable GHG emissions in the production of sugar beet ethanol. This benefit would help qualify sugar beet ethanol as an advanced biofuel.

Observations during the Preparation of Raw Thick Beet Juice

Two phenomena were observed while preparing the raw thick juice for this study. First, foaming was visible in the separator of the rising-film evaporator. High foam content reduces the efficiency of heat transfer operations and increases the retention time of juice in evaporating units (Asadi, 2007). Foaming may be overcome by adding an anti-foaming agent to the raw juice prior to concentration.

Also, some sucrose inversion was detected during the production of raw thick juice. Invert sugars increased from 3.9±0.6 to 9.6±0.3% of the total fermentable sugar content in the juice.

Some inversion of sucrose is inevitable during concentration of purified juice in sugar factories (Chou, 2000). Sucrose inversion is not a concern to the ethanol industry; however, if desired, it may be reduced by decreasing the residence time of the juice in the evaporating units.

Acidic Storage

Effect of Storage Parameters

Raw thick juice treatments stored under acidic conditions showed a 1 to 30% decrease in fermentable sugar content depending on pH and RDS content combinations (Table 5). The greatest decrease was detected in treatments characterized by a high pH and low RDS content (treatments 2 and 6; Table 5), conditions least prohibitive to microbial activity throughout storage. Microbial activity was corroborated visually by the presence of microbial colonies on the juice surface. In contrast, all other treatments showed little or no evidence of microbial activity with a <6% decrease in fermentable sugars.

Table 5. Volume of 8 M HCl and 8 N NaOH required for pH control of acidic and alkaline treatments, respectively, during 24-wk storage, and decrease in fermentable sugars after storage

	Volume required (% of initial treatment volume)		Decrease in fermentable sugars (% of initial sugar content)	
Treatments[a]	Acidic	Alkaline	Acidic	Alkaline
1	0.07	1.48	2.0	40.9
2	0.03	1.82	29.3	1.5
3	0.03	0.70	2.1	-3.3
4	0.12	2.42	4.5	-0.3
5	0.03	0.77	3.5	21.7
6	0.07	2.67	30.1	2.1
7	0.05	2.52	2.1	2.9
8	0.07	1.03	5.8	-2.5
9	0.10	1.27	1.4	-0.5
10	0.08	1.38	1.3	0.0
11	0.07	1.28	1.0	-0.6
12	0.08	1.23	2.3	-1.1

[a]Refer to Table 4 for treatment details.

Despite clear evidence of microbial activity in treatments 2 and 6, the pH of those treatments remained stable. Lactic acid, acetic acid, and ethanol are common products of the microbial degradation of sugars (Sauer et al., 2008). Treatments 2 and 6 were examined for lactic acid, acetic acid, and ethanol, but these were not detected.

The pH of all acidic treatments was stable throughout storage minimizing the need for addition of HCl for pH adjustment (Table 5). Consequently, the RDS contents of the acidic treatments did not decrease significantly due to dilution throughout storage. However, the α_w of some treatments showed a slight, linear decrease over time (p-value<0.005). This change in α_w may be attributed to the increase in invert sugars due to sucrose inversion at acidic conditions (Gabriel, 2008). A decrease in α_w may improve storage as a result of the more pronounced inhibitory effects of lower α_w on microorganisms (Rahman, 2007).

Model Fitting

The change in fermentable sugars during acidic storage (Y_{Acidic}) was modeled in the format of Equation 2. Most model coefficients were statistically significant with a p-value<0.03 (Table 6) except for the main effect (x_2) and quadratic (x_2^2) terms associated with RDS. The latter term was removed from the model; however, the main effect term was kept in the model due to the significance of the interaction term (x_1x_2). The resulting model (p-value<0.003) was:

$$Y_{Acidic} = -315.46 + 82.43x_1 + 5.72x_2 + 6.42{x_1}^2 - 1.85x_1x_2 + \varepsilon \qquad \text{[Eq. 3]}$$

where x_1 and x_2 represent actual pH and RDS content (°Brix), respectively. The high significance of regression (R^2=0.896 and R^2_{Adj}=0.836) indicates model accuracy in representing data within the design space.

Table 6. ANOVA summary for the response surface model of acidic storage

Source	DF	F-value	P-value
Regression	4	15.02	0.002
pH (x_1)	1	30.45	0.001
RDS (x_2)	1	2.56	0.154
pH*pH (x_1x_1)	1	18.71	0.003
pH*RDS (x_1x_2)	1	8.39	0.023

The combined effect of acidic pH and RDS content on the preservation of fermentable sugars may be easily seen in Figure 8. Conditions of pH>4 and RDS<67°Brix appeared not to sufficiently protect against microbial degradation, resulting in >5% losses of fermentable sugars over 24 wk. The contour plot (Fig. 8) shows combinations of acidic pH and RDS content that resulted in best fermentable sugar retention in stored raw thick juice. Predictions on the change in fermentable sugar content are only reliable within the design space delimited by the experimental points.

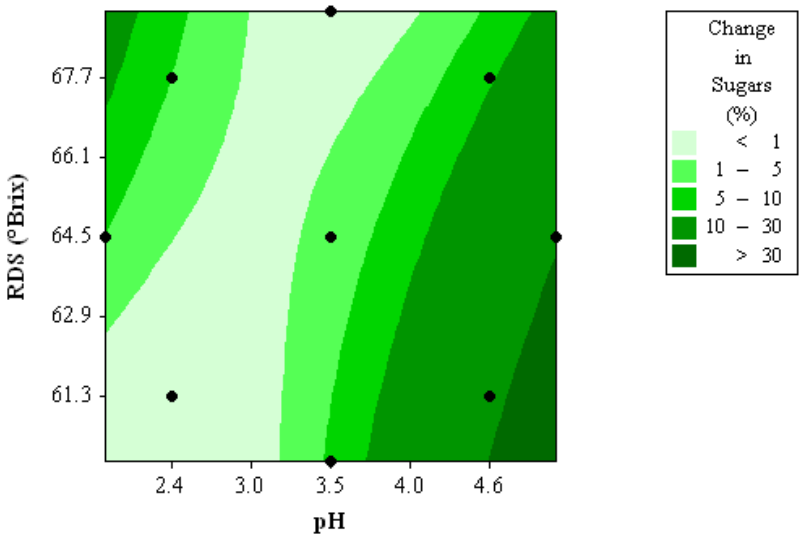

Figure 8. Contour plot showing the combined effects of pH and RDS on the decrease in fermentable sugars in raw thick beet juice stored under acidic conditions for 24 wk at 23±1°C
•: Experimental points

Storage at strongly acidic pH values (pH<2.4) and high RDS contents (>64.5°Brix) showed a slightly negative impact on fermentable sugars (Fig. 8). Dehydration of glucose by mineral acids (e.g. hydrochloric acid) results in the production of levulinic and formic acids, with 5-hydroxymethylfurfural (HMF) as an intermediate product (Fitzpatrick, 1997; Zeng et al., 2010). Therefore, treatments stored at strongly acidic pH (treatments 1, 3, and 5; Table 4) were examined for levulinic acid and HMF following the HPLC method described by Xie et al. (2011). However, neither of these degradation compounds was detected in the samples.

Alkaline Storage

Effect of Storage Parameters

Most alkaline treatments, with the exception of treatments 1 and 5, showed a <3% fermentable sugar loss during storage (Table 5). A pH>9.5 ensured the retention of fermentable sugars in raw thick juice by inhibiting microbial activity. Treatments 1 and 5, characterized by a pH<9 and RDS<65°Brix, showed the greatest fermentable sugar losses during storage accounting for 40.9 and 21.7% of the initial content, respectively.

In contrast to the stable pH of acidic treatments, pH of alkaline treatments was susceptible to decrease; thus, alkaline treatments required a much more frequent pH adjustment during storage. The volumes of 8 N NaOH required for pH control in the alkaline treatments are reported in Table 5. The RDS contents of the treatments showed a slight, linear decrease (*p*-value<0.005) caused by the dilution effect of NaOH addition. In contrast to acidic treatments, the α_w of the alkaline treatments remained fairly constant throughout storage.

Microbial degradation of sugars is known to be a major cause of pH drop in thick juice stored under alkaline conditions (Fiedler, 1993; Hein et al., 2002). If pH is not controlled during

storage, it may reach a value suitable for rapid microbial growth, which would result in substantial fermentable sugar loss. Treatments 4 and 6 had slight, repeated pH drops even though storage conditions (pH>10, RDS>64.5°Brix) were expected to protect the juice from microbial degradation. This phenomenon may have been caused by a chemical reaction inherent to highly alkaline storage conditions; a similar observation in sterile thick juice was reported by Justé (2008).

Treatment 7 (pH 9.5, 60.0°Brix) required a significant, repeated addition of NaOH for pH adjustment during storage (Table 5). The steady pH drop of treatment 7 was attributed to its low RDS content which did not adequately inhibit microbial growth. Nevertheless, a weekly pH adjustment was an effective strategy that held the loss of fermentable sugars in treatment 7 to <3% over 24 wk.

The buffering capacity of beet juice is directly proportional to its ash and amino acid contents (Van der Poel et al., 1998). Raw thick beet juice, which has not undergone purification, contains high amounts of ash and amino acids that increase the buffering capacity of the juice. The buffering capacity of the raw thick beet juice became more pronounced at highly alkaline pH values. For example, alkaline treatments 2, 4, and 6 required more NaOH addition for pH adjustment than treatments 1 and 5 (Table 5). However, the former treatments showed less evidence of microbial activity as supported by less pronounced pH fluctuations and higher sugar retention.

Model Fitting

The change in fermentable sugar content during alkaline storage ($Y_{Alkaline}$) was modeled, and as in the acidic storage model (Eq. 3), the quadratic term associated with RDS content (x_2^2) was not significant and thus omitted. Additionally, the main effect term (x_1) associated with pH

was not significant; however, this term was kept in the model since the interaction term (x_1x_2) was significant with a *p*-value<0.02 (Table 7). The resulting model was:

$$Y_{Alkaline} = 2687.34 - 326.45x_1 - 32.01x_2 + 6.10{x_1}^2 + 3.15x_1x_2 + \varepsilon \qquad \text{[Eq. 4]}$$

where x_1 and x_2 represent actual pH and RDS content (°Brix), respectively. The model showed a high level of statistical significance with a *p*-value<0.004 and was adequate for data representation (R^2=0.876 and R^2_{Adj}=0.805).

Table 7. ANOVA summary for the response surface model of alkaline storage

Source	DF	F	P
Regression	4	12.33	0.003
pH (x_1)	1	15.46	0.006
RDS (x_2)	1	10.82	0.013
pH*pH (x_1x_1)	1	9.46	0.018
pH*RDS (x_1x_2)	1	13.58	0.008

Unfavorable fermentable sugar retention was detected in raw thick juice at combinations of moderate pH and low RDS contents and strongly alkaline pH and high RDS contents (Fig. 9). Similarly, raw thick juice stored at moderate acidic pH and low RDS contents and strongly acidic pH and high RDS contents retained the least amount of sugars during storage (Fig. 8). However, these behaviors are more accurate and reliable within the design space delimited by the experimental points. The best alkaline storage conditions are represented by the light-colored circumscribed area in Figure 9. Comparing Figure 9 with the acidic storage contour plot in Figure 8, it is evident that raw thick juice may be stored with minimal sugar loss over a wider range of alkaline pH and RDS combinations. However, other factors, such as pH stability during storage and effects of storage conditions on juice fermentability should be considered when selecting best combinations of pH and RDS content for storage.

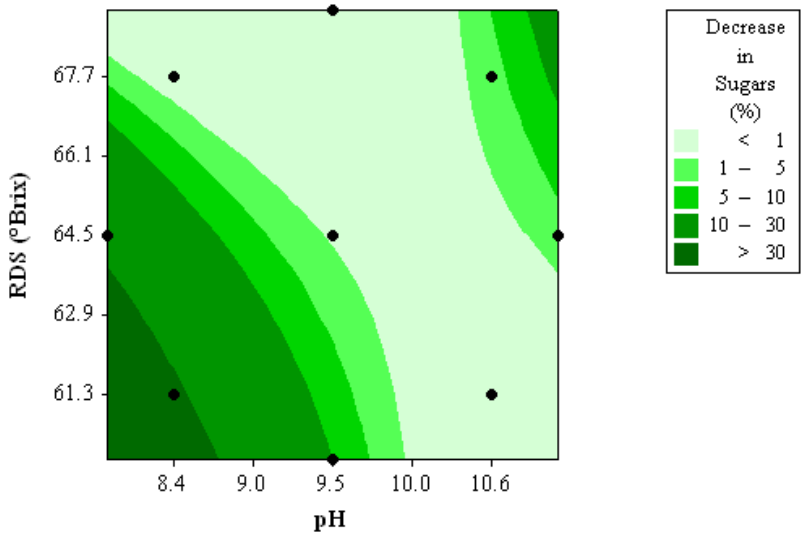

Figure 9. Contour plot showing the combined effects of pH and RDS on the decrease in fermentable sugars in raw thick beet juice stored at alkaline pH for 24 wk at 23±1°C
•: Experimental points

Fermentability of Raw Thick Juice after Storage

Acidic Treatments

After fermentation under experimental conditions, raw beet juice achieved a fermentation efficiency of 86% relative to maximum theoretical yield (0.51 g ethanol g glucose^{-1}). All raw thick juice treatments stored under acidic conditions, with the exception of those that retained the lowest amount of sugars throughout storage (treatments 2 and 6; Table 5), showed a fermentation efficiency of 80±3% relative to raw juice (Table 8). The residual sugars detected at the end of fermentation (Table 8) indicated that the treatments had not completely fermented. Nevertheless, a fermentation process of more than 96 h is impractical from an industrial point of view.

Table 8. Ethanol yield, efficiency, and residual sugars from the fermentation of acidic and alkaline treatments after storage

	Acidic Treatments			Alkaline Treatments		
Sample	Ethanol Yield *(g/g glucose eq.[a])*	Efficiency[b] (%)	Residual Sugars (% of Initial Sugars)	Ethanol Yield *(g/g glucose eq.[a])*	Efficiency[b] (%)	Residual Sugars (% of Initial Sugars)
1	0.36	81.8±2.5	16.1±0.6	0.13	28.9±0.4	76.4±0.4
2	0.27	61.4±0.1	27.8±0.3	0.08	17.8±2.5	80.5±4.1
3	0.35	79.5±2.1	16.3±0.3	0.24	53.3±0.4	57.7±0.2
4	0.35	79.5±0.5	16.4±0.3	0.09	20.0±1.4	76.4±3.5
5	0.36	81.8±0.3	16.7±0.03	0.19	42.2±1.2	62.9±1.0
6	0.24	54.5±0.1	26.2±0.1	0.04	8.9±0.4	90.7±0.3
7	0.35	79.5±1.2	18.3±0.5	0.07	15.6±0.6	83.7±0.1
8	0.36	81.8±0.8	11.9±0.3	0.16	35.6±2.1	69.9±0.4
9	0.36	81.8±2.5	18.0±0.5	0.17	37.8±0.3	67.5±0.2
10	0.36	81.8±1.4	19.5±0.4	0.15	33.3±2.0	69.3±0.2
11	0.36	81.8±3.6	18.0±1.1	0.16	35.6±2.1	63.1±0.3
12	0.35	79.5±1.5	19.3±0.2	0.17	37.8±1.4	66.9±0.7
Raw Juice	0.44	100±2.6	6.7±0.2	0.45	100±1.4	7.8±0.6

[a]Glucose equivalents are those in the treatments before undergoing storage.
[b]Efficiency of stored treatments is relative to raw beet juice efficiency.
NOTE: Appendix A presents detailed data showing initial fermentable sugar content in fermentation media and ethanol concentrations obtained from fermentation.

Grahovac et al. (2011) reported that raw beet juice diluted to a fermentable sugar content of 130 g/kg juice achieved 96% maximum theoretical yield after 38 h of fermentation at 30°C and agitation rate of 200 RPM. In contrast to the present study, their inoculum was prepared by rehydrating yeast in a small quantity of fermentation media for 2 h at the same experimental conditions. Hence, the inoculum preparation method followed in this fermentability test may have impacted yeast fermentation rate, resulting in low ethanol yields. However, the objective of this test was solely to compare the fermentability of the treatments using a set of standard fermentation conditions. The fermentation efficiency of stored raw thick juice can likely be substantially improved.

The ethanol yield of the acidic treatments (Y_{Acidic}^{EtOH}) was modeled to evaluate the effect of storage conditions on the fermentability of stored raw thick juice. Neglecting the statistically insignificant terms, the ethanol yield from the fermentation of the acidic treatments was represented by the following model (p-value<0.001; Table 9):

$$Y_{Acidic}^{EtOH} = 1.459 - 0.289x_1 - 0.020x_2 - 0.024{x_1}^2 + 0.007x_1x_2 + \varepsilon \qquad \text{[Eq. 5]}$$

where x_1 and x_2 represent actual pH and RDS content (°Brix), respectively. The high coefficient of determination (R^2=0.926 and R^2_{Adj}=0.884) indicated that the model was accurate for data representation within the design space.

Table 9. ANOVA summary for the response surface model of ethanol yield of acidic treatments

Source	DF	F-value	P-value
Regression	4	21.93	<0.001
pH (x_1)	1	4.62	0.069
RDS (x_2)	1	7.64	0.028
pH*pH (x_1x_1)	1	27.0	0.001
pH*RDS (x_1x_2)	1	10.84	0.013

The contour plot generated by the model is shown in Figure 10. The response pattern coincides with that of the change in fermentable sugars during storage (Figure 8). Nevertheless, the ethanol yields of the treatments were much lower than the maximum theoretical value expected. The presence of 12-28% residual sugars at the end of the fermentability test suggests that the yeast fermentation rate was negatively impacted by raw thick juice storage conditions.

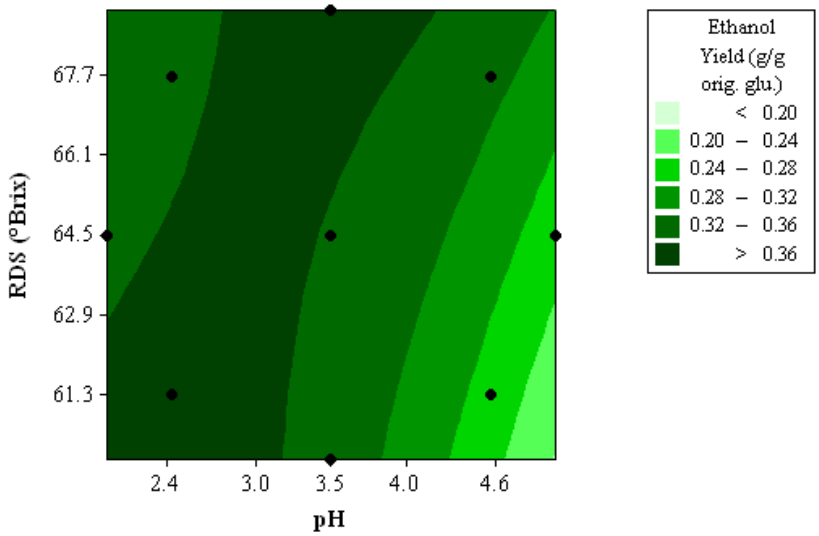

Figure 10. Contour plot showing the effect of storage conditions (pH and RDS content) on the ethanol yield of raw thick juice stored under acidic conditions
•: Experimental points

Alkaline Treatments

In contrast to acidic treatments, alkaline treatments with highest fermentable sugar retention throughout storage (>99%; Table 5) achieved lower ethanol yields and showed higher residual sugar contents after fermentation. As a result, these treatments achieved an average fermentation efficiency of only 37±10% relative to raw juice. Additionally, those treatments with highest sugar retention during storage (Table 5) achieved the highest fermentation efficiencies among all alkaline treatments (Table 8). Treatments stored at pH 9.5 achieved a fermentation

efficiency of 37±0.8% which coincides with the average efficiency of treatments 3 and 4 (37%; Table 5).

The ethanol yield of the fermentation of alkaline treatments ($Y_{Alkaline}^{EtOH}$) was modeled in the format of Equation 2. All model coefficients were statistically significant (p-value<0.02; Table 10) and the experimental data was represented by the following model:

$$Y_{Acidic}^{EtOH} = -13.6 + 0.71x_1 + 0.32x_2 - 0.02{x_1}^2 - 0.002x_2^2 - 0.007x_1x_2 + \varepsilon \qquad \text{[Eq. 6]}$$

where x_1 and x_2 represent actual pH and RDS (°Brix), respectively. The model showed a high level of statistical significance (p-value<0.001) and was accurate for data representation within the design space (R^2=0.971 and R^2_{Adj}=0.948).

Table 10. ANOVA summary for the response surface model of ethanol yield of alkaline treatments

Source	DF	F-value	P-value
Regression	5	40.69	<0.001
pH (x_1)	1	22.66	0.003
RDS (x_2)	1	22.84	0.003
pH*pH (x_1x_1)	1	14.7	0.009
RDS*RDS (x_2x_2)	1	14.7	0.009
pH*RDS (x_1x_2)	1	12.38	0.013

The contour plot (Figure 11) suggests that, apart from the effect of pH and RDS content on the preservation of fermentable sugars, pH conditioning of the fermentation media has a detrimental effect on yeast fermentation rate. The ethanol yields of the alkaline treatments decreased by a progressively greater extent as storage pH increased. The use of HCl (or NaOH) for raw thick juice pH adjustment during storage, followed by pH neutralization before fermentation, resulted in the synthesis of NaCl. The amount of NaCl formed during pH neutralization likely resulted in an increasing negative impact on yeast fermentation rate as salt

concentrations increased. Inorganic salts, such as NaCl, are known to decrease yeast fermentation rate (Wei et al. 1982).

The low ethanol yields in this study would be a concern to the beet ethanol industry. Nonetheless, fermentation conditions and inoculum preparation can be readily improved to maximize ethanol yields. Also, the use of other acids and alkalis for storage pH adjustment and pH conditioning prior to fermentation would result in salts other than NaCl that may improve yeast fermentation rate.

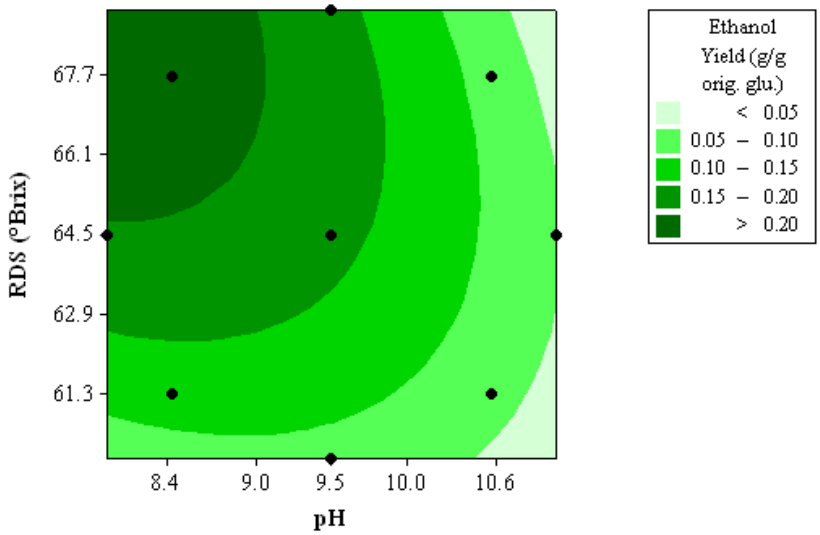

Figure 11. Contour plot showing the effect of storage conditions (pH and RDS content) on the ethanol yield of raw thick juice stored under alkaline conditions
•: Experimental points

Conclusion

Combinations of pH≤3.5 and pH≥9.5 with RDS≥64.5°Brix were effective in preserving up to 99% of fermentable sugars in raw thick juice stored for 24 wk at 23±1°C. Raw thick juice stored under alkaline conditions required much more frequent pH adjustment than juice stored under acidic conditions, which would result in higher storage cost. Acidic treatments fermented

under experimental conditions achieved fermentation efficiencies of <82% relative to raw juice. In contrast to acidic treatments, alkaline treatments achieved fermentation efficiencies of <54%. Fermentation conditions and inoculum preparation can be readily adjusted to maximize fermentation efficiencies of stored raw thick juice.

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PAPER 2. CHANGE IN FERMENTABLE SUGARS IN SUGAR BEETS STORED UNDER AEROBIC AND ANAEROBIC ATMOSPHERES

Paper 2 is a revised version of a paper presented at the 2012 ASABE/CSBE North Central Intersectional Conference in Fargo, ND, March 30 – 31, 2012. Title: Change in Fermentable Sugars in Sugar Beets Stored Anaerobically. Paper No.: RRV12100. Authors: Juan M. Vargas-Ramirez, Darrin M. Haagenson, Shafiqur Rahman, and Dennis P. Wiesenborn. Juan M. Vargas-Ramirez designed and conducted the experiments for this study, and is the first author of Paper 2. The co-authors assisted in the editing of Paper 2.

Abstract

High-yielding sugar beets may be an attractive feedstock for ethanol production in the Red River Valley of the North Central United States. Long-term storage methods are necessary to preserve fermentable sugars in beets and allow successful yearlong operation of beet ethanol plants. Anaerobic storage of sugar beets was evaluated as an alternative to conventional pile storage used in beet sugar factories. Experimental results indicated >85% fermentable sugar retention in sugar beets stored anaerobically for 14 wk at 4°C. After 14 wk of storage, 99±4% of the initial fermentable sugar content was preserved in beets stored aerobically at 4°C. Aerobic and anaerobic storage of sugar beets at 25°C preserved 82±9% and 48±11% of the fermentable sugar content, respectively. After 7 wk of anaerobic storage at 25°C, CO_2 was detected at 61±2% (v/v) within the storage units, and reached a maximum concentration of 97±3% after 10 wk before dropping to a plateau of 79±1% by week 12. Temperature reduction improved fermentable sugar retention in beets; however, the absence of oxygen in the storage atmosphere resulted in increased fermentable sugar degradation. Fermentable sugars and ethanol in beet exudates recovered after storage may significantly boost overall ethanol yield of stored sugar beets by 3±0.5%.

Introduction

The 2007 Energy Independence and Security Act (EISA) mandated the production and blending of 36 BGY of biofuels into transportation fuel by 2022. The primary objective is to reduce net greenhouse gas (GHG) emissions that result from fossil fuel combustion. Biofuels are classified under the EISA based on their potential to reduce net GHG emissions. Conventional, advanced, and cellulosic biofuels are required to reduce net GHG emissions by 20, 50, and 60%,

respectively. Sugar beets are of interest for ethanol production in the United States as they may qualify as an advanced biofuel feedstock under the EISA.

Sugar beets have the ability to adapt to numerous climate conditions (McGinnis, 1982). North Dakota and Minnesota are the major producers of sugar beets in the United States. In 2011, both states accounted for 47% of the total sugar beet production in the nation (USDA/ERS, 2012a). The ethanol potential of experimental sugar beet varieties with high harvest yields is currently under study in North Dakota. Sugar beet trials have been carried out in 11 locations across the state: Carrington, Hannaford/Dazey, Oakes, Turtle Lake, Williston, Langdon, Minot, Colgate, Litchville, Jamestown, and Harvey. The best trial with 16 experimental beet varieties yielded an average of 93 T beets ha^{-1} and 16 T sugar ha^{-1} at Carrington (NDSU Carrington REC, 2010). In contrast, conventional sugar beets yielded an average of 65 T beets ha^{-1} and 10 T sugar ha^{-1} in North Dakota during 2010 (USDA/ERS, 2012a & b).

Sugar beet is a biennial crop, but it reaches its maximum sugar content during the first year of growth. In the Red River Valley, sugar beets are typically planted in May and harvested in October. Conventional storage consists of piling sugar beets on open storage grounds adjacent to the factories and freezing them by forced ventilation using cold, ambient air during harsh winter conditions. However, there are risks and disadvantages associated with conventional storage. Storing sugar beets in exposed piles can lead to the formation of hot spots within the piles due to insufficient ventilation and hence, the microbial degradation of beet sugars. In addition to this, freezing enhances the rupture of cell walls making cell contents susceptible to leaching during sugar beet thawing and washing before sugar extraction. Also, beet thawing before processing requires large quantities of warm water which increases overall process energy requirements. Ethanol production from sugar beets should be highly energy efficient to qualify

beets as an advanced biofuel feedstock. Hence, alternative technologies to conventional storage should be explored to address the above issues.

Preservation technologies have been developed throughout the years to increase the shelf-life of perishable produce. Modifying the composition of storage atmospheres has been successful for minimizing quality loss of stored produce. Inert gases such as CO_2 and N_2 are commonly used to modify atmospheres in storage facilities. The preservation effects of modified atmospheres can be improved in concert with reduced temperatures (<25°C) to decrease plant respiration and metabolism. These principles have increased the shelf-life of fresh produce such as apples and pears from several days to as long as 9 months (Brody, 1989).

Sugar beets typically contain 15 to 20% (w w^{-1}; w.b.) sucrose, 0.2 to 0.5% raffinose, and 0.05 to 0.1% glucose and fructose (Asadi, 2007). Cole & Bugbee (1976) assessed the hydrolysis of sucrose into glucose and fructose in sugar beets stored aerobically and under a non-ventilated atmosphere at 5°C and 26°C. Yet, their study was based on the importance of high sucrose retention in sugar beets as a requirement for acceptance in sugar processing facilities. In contrast with sugar industry requirements, the hydrolysis of sucrose into glucose and fructose should not constitute a problem to the ethanol industry since these sugars are readily fermented by yeast.

The objective of this study was to determine the effect of storage temperature and initial oxygen content of the storage atmosphere on retention of fermentable sugars (sucrose, glucose, and fructose). The findings may aid in the design of subsequent, larger experiments to develop improved storage technologies for fermentable sugar preservation in sugar beets.

Materials and Methods

Sugar Beets - Collection and Storage

Sugar beets from a single variety, Beta 1301R, (Betaseed, Inc.; Shakopee, MN, USA) were harvested in a 2011 field trial in Fargo, North Dakota and were provided by the USDA Agricultural Research Service (Fargo, ND, USA). Soil was removed by washing the beets in a pilot scale tumbler. The washed beets were stored in perforated polyethylene bags for 4 wk at 5°C until the storage treatments were initiated.

Experimental Setup

At the initiation of the storage study, eight beets were selected randomly and immediately assayed in pairs for total fermentable sugars. The fermentable sugar content was averaged among the four pairs of beets and used as a baseline throughout the experiment. The experimental treatments were prepared using beets of uniform size and shape which were weighed individually before being randomly assigned to treatment bags. The experimental units consisted of either a 3-sided seal vacuum pouch (anaerobic treatment) with a thickness of 75 μm (Ultravac Solutions; Kansas City, MO, USA) or a perforated Ziploc® freezer bag (S.C. Johnson & Son, Inc.; Racine, WI, USA) (aerobic treatment) containing 1 beet each. The beets for the anaerobic portion of the experiment were vacuum packaged using an Ultravac® 2100 manual double chamber vacuum packaging machine (Ultravac Solutions; Kansas City, MO, USA) operated with a vacuum in the range of 20 to 30 psi to extract 97 to 99% of the air in the pouches. The packaged beets were stored at 4°C or 25°C and each storage treatment was run in triplicate. The stored beets were analyzed for total fermentable sugars at 2, 4, 7, 10, 12, and 14 wk of storage.

Analytical Methods

Beet tissues were collected at the specified time intervals (2, 4, 7, 10, 12, and 14 wk of storage) by drilling each beet with a power drill equipped with a 1.6-cm spade bit. Drilling was initiated below the lowest leaf scar and proceeded in a transversal direction below the root crown extending toward the root tip. Drilling provided a representative tissue sample for sugar analyses, and approximately 50 g of beet tissue were collected from each beet. Tissue samples were thoroughly mixed to ensure sample homogeneity. Samples were immediately placed in individual Ziploc® freezer bags (S.C. Johnson & Son, Inc.; Racine, WI, USA) and frozen overnight to enhance the rupture of cells and facilitate the extraction of sugars.

The cold digestion method for cossettes (Asadi, 2007) was followed to extract the sugars from the beet tissue samples. Methods to quantify total fermentable sugars (sucrose, glucose, and fructose) were based on modifications of the Glucose UV Liquid Reagent kit (Cliniqa Corp.; San Marcos, CA, USA). Sugars were quantified following end-point spectrophotometric (340 nm) enzyme assays modified for use with a microplate reader (SpectraMAX Plus –Molecular Devices Corp.; Sunnyvale, CA, USA). Sucrose was hydrolyzed to glucose plus fructose by digesting with invertase (Sigma I4504) and fructose was assayed after isomerization with phophoglucose isomerase (Sigma P5381), according to manufacturer's recommendations. The total fermentable sugars were reported on a dry basis and as the average of the triplicate storage treatments.

Individual samples of the gas formed and entrapped within the anaerobic treatment units were collected from each replicate in sampling syringes and quantified by gas chromatography (GC). The gas samples were characterized using a GC unit (Model No. 8610C – SRI Instruments; Torrance, CA, USA) equipped with a flame ionization detector (FID) and an electron capture detector (ECD). Prior to injecting a sample into the sampling loop, the

temperatures of the FID and ECD detectors were adjusted to 300°C and 350°C, respectively. The ECD was operated using N_2 as the carrier gas at 140 kPa (20 psi). Hydrogen and air were supplied at 140 kPa (20 psi) to the FID/Methanizer using a built-in air compressor. In this GC system, the ECD detected N_2O while the FID/Methanizer detected CH_4 and CO_2. Gas chromatographs were recorded and analyzed with the PeakSimple Chromatography Data System Software (Version 3.72 – SRI Instruments; Torrance, CA, USA). Three-point calibration curves were generated using CH_4 (20, 100, and 1000 ppmv), CO_2 (100, 1000, and 2500 ppmv), and N_2O (0, 1, and 10 ppmv) gases. Calibration gases were analyzed before and after sample analysis to ensure proper functioning of the GC unit.

Ethanol in exudates was quantified by HPLC (Waters Corp.; Milford, MA, USA) using an Aminex HPX-87H (300x7.8 mm) ethanol column (Bio-Rad Laboratories, Hercules, CA, USA), an isocratic pump, autosampler, and refractive index detector (Model 2414 – Waters Corp.). The injection volume into the column was 12 μL and the samples were eluted with 5-mM sulfuric acid at a flow rate of 0.6 mL min^{-1} and elution time of 30 min. The column and detector temperatures were 60°C and 50°C, respectively.

Statistical Analysis

SigmaPlot Version 8.0 (SPSS Inc.; Chicago, IL, USA) was used to determine best fit regression functions to model the change in total fermentable sugars for each treatment condition.

Results and Discussion

The change in total fermentable sugars was assessed in sugar beets stored in the presence and absence of oxygen at 4°C and 25°C. As storage time increased, gas and exudate were

detected in the anaerobic storage units while mold growth was observed on the skin tissue of several beets stored aerobically at 25°C. Storing sugar beets in an aerobic environment at 25°C resulted in better retention of total fermentable sugars after 4 wk in comparison to anaerobic storage at the same temperature (Figure 12).

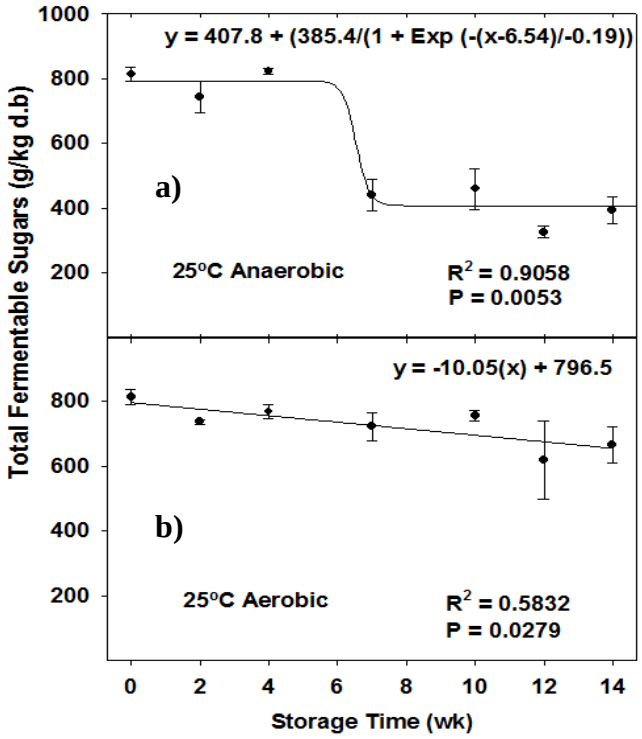

Figure 12. Change in total fermentable sugars in sugar beets a) stored anaerobically and b) aerobically at 25°C

Sugar beets stored anaerobically at 25°C showed an abrupt >45% decrease in total fermentable sugars between 4 and 7 wk of storage. This abrupt decrease coincided with the appearance of exudate within the storage units. Bacteria present in the beet tissue are responsible for metabolizing most of the sugars lost during storage (Cole & Bugbee, 1976). In addition to bacterial degradation, sucrose is metabolized in the beets to fulfill substrate required for plant respiration and to heal wounds which originate during harvest (Klotz, 2004). After 7 wk, the concentration of sugars dropped to a plateau of 48±11% of the initial fermentable sugar content.

The change in fermentable sugars in beets stored anaerobically at 25°C was best fitted by a sigmoid function. Many natural processes that have a slow initial progression followed by an abrupt acceleration before reaching a plateau are best represented by a sigmoid function (Majumder *et al*., 2010).

Cole and Bugbee (1976) detected a rapid increase in sucrose-hydrolyzing bacteria in freshly harvested beets stored under a non-ventilated atmosphere at 26°C for 7 d. In their study, bacterial counts decreased after the pH of the beet tissue declined to 4-5. Similarly, in the present study, a pH decline may have halted the activity of bacteria residing in the tissue of beets stored anaerobically. Furthermore, hydrolytic enzymes, in conjunction with the low pH of the tissue, could have contributed to the hydrolysis of sucrose explaining the increase in glucose and fructose beyond 7 wk of storage (Figure 13).

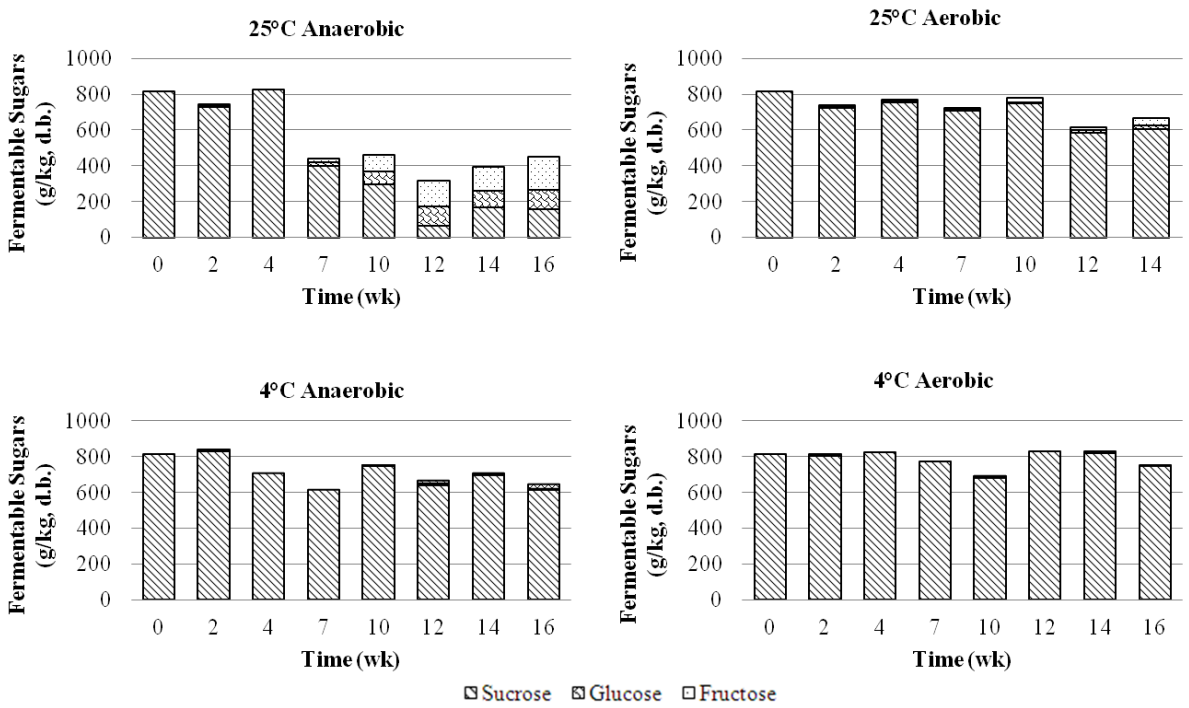

Figure 13. Fermentable sugars in sugar beets stored under aerobic and anaerobic atmospheres at 4°C and 25°C

Sugar beets stored aerobically at 25°C showed a gradual decrease of 1.3% wk^{-1} in fermentable sugars and retained 82±9% after 14 wk of storage (Figure 12b). After 2 wk of storage, mold was visually detected on the surface of several beets, and spread slowly for the remaining storage period (Figure 14). Mold secretes hydrolytic enzymes that aid invasiveness by hydrolyzing complex carbohydrates in healthy tissue into readily digestible sugars.

Figure 14. Mold growth on sugar beets stored aerobically at 25°C for 14 wk

Sugar beets stored aerobically and anaerobically at 4°C retained 99±4% and 87±0.1% of the initial fermentable sugars, respectively (Figure 15). In contrast, sugar beets stored at 25°C under aerobic and anaerobic atmospheres retained 82±9% and 48±11% of the initial fermentable sugars, respectively (Figures 12 and 15). Low temperatures are known to reduce the growth rate of microorganisms and suppress enzyme activity. Sugar beets stored anaerobically at 4°C showed a decrease of 1.2% wk^{-1} in fermentable sugars in comparison with a decrease of 3.7% wk^{-1} in beets stored under a similar atmosphere at 25°C. An average decrease of 0.3% wk^{-1} in fermentable sugars was estimated for sugar beets stored aerobically for up to 14 wk at 4°C, and was not statistically significant.

Gas was observed within all the vacuum packages after 2 wk of storage, and its volume increased visibly with storage time (Figure 16a). After 7 wk, the CO_2 concentration averaged 61±2% within the vacuum packages of sugar beets stored at 25°C. The CO_2 concentration reached a maximum of 97±3% on week 10 before dropping to 79±1% by week 12 to remain

stable thereafter. The high level of CO_2 should result in carbonic acid (H_2CO_3) in the exudate entrapped within the vacuum packages, which would decrease pH and consequently improve fermentable sugar retention. Methane was also detected at a concentration of 1±0.2% within the vacuum packages at 10 and 12 wk of storage.

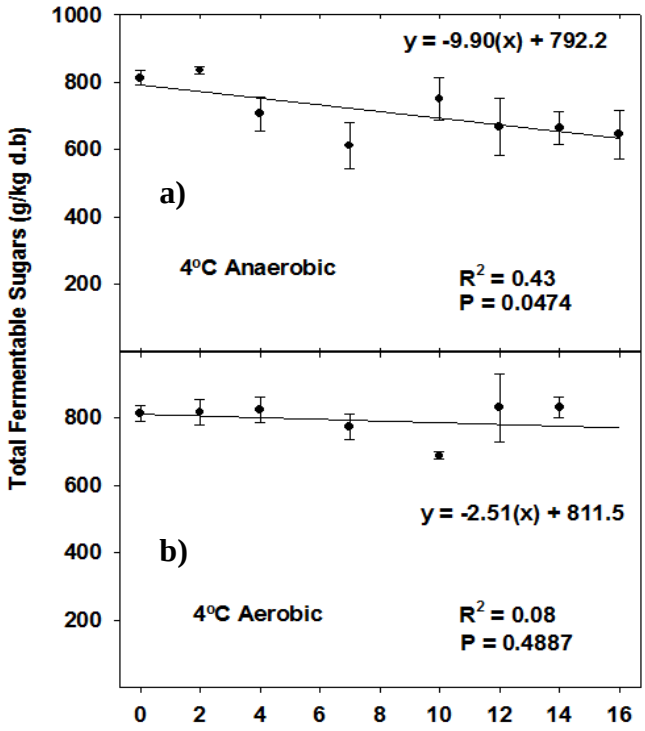

Figure 15. Change in fermentable sugars in sugar beets stored a) anaerobically and b) aerobically at 4°C

Figure 16. a) Gas accumulated within vacuum package after 4 wk of storage and b) exudate released by beets stored under an initial anaerobic atmosphere

Sugar beets stored under an initial anaerobic atmosphere and at 25°C released exudate after 7 wk of storage (Fig. 16b). After 10 wk of storage, exudate was present in all vacuum packages containing beets stored at 4°C and 25°C. Exudation may have been enhanced by pressure differential between the interior and exterior of the packages, expulsing cell content from the beet cells. However, water formed as a by-product of beet respiration or the microbial fermentation of sugars, may have contributed to the exudate volume.

The exudates were collected from all storage units, weighed, and analyzed for fermentable sugars and ethanol to determine their contribution to overall ethanol yield. Appendix B presents detailed data on fermentable sugar and ethanol contents in exudates. After 7 wk of storage, the exudates of the anaerobic treatments stored at 25°C accounted for 3% of the initial beet weight, and reached 5% by the end of storage. Exudates were recovered from treatments stored at 4°C for 12 and 14 wk, and accounted for 4% and 6% of the initial beet weights, respectively. Fermentable sugars in exudates collected at 14 wk of storage accounted for 2±0.4% and 1±0.1% of the initial fermentable sugars in beets stored at 4°C and 25°C, respectively. Ethanol contents were converted to glucose equivalents, and accounted for 1±0.5% and 0.3±0.1% of the initial fermentable sugars in beets stored at 4°C and 25°C, respectively. Hence, the recovery of exudates after anaerobic storage of sugar beets at 4°C may boost fermentable sugar retention from 87±0.1 to 90±0.5%.

Conclusion

The impact of the presence and absence of oxygen on the preservation of fermentable sugars in sugar beets was evaluated for 14 wk at 4°C and 25°C. Beet storage atmospheres contained ambient (21%) initial O_2 in one set of samples; vacuum packaging was employed to achieve anaerobic conditions in another set. At 14 wk of storage at 4°C, 87±0.1% and 99±4% of

initial fermentable sugars in beets were preserved under anaerobic and aerobic atmospheres, respectively. Conversely, beets stored under similar atmospheres for 14 wk at 25°C retained only 48±11% and 82±9% of their initial fermentable sugar content, respectively. Exudates recovered after anaerobic storage of sugar beets at 4°C may boost overall ethanol yield by 3±0.5%. The results suggested that the loss of fermentable sugars was a result of sugar beet respiration in combination with microbial fermentation that yielded low, yet significant ethanol that may contribute to overall ethanol capacity.

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USDA/ERS. 2012b. Sugar and Sweeteners: Recommended Data. U.S. Sugar Supply and Use. Table 17 – U.S. sugarbeet are, yield, and production (Updated on 01/12/2012). Available at: http://www.ers.usda.gov/Briefing/Sugar/Data.htm

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GENERAL CONCLUSIONS

Conventional pile storage of sugar beets possesses disadvantages such as risk of beet sugar degradation during storage and limited application period (4-5 mo. during winter). Alternatives to conventional pile storage are necessary to allow yearlong beet ethanol production. Therefore, this study focused on the development and evaluation of two storage techniques to preserve fermentable sugars from sugar beets for ethanol production: *raw thick juice* storage and whole beet storage.

Raw thick juice may be stored at alkaline and acidic conditions with minimal fermentable sugar loss (<1%) for at least 24 wk. Storage conditions of pH≤3.5 and pH≥9.5 in combination with an RDS≥64.5°Bx resulted in effective fermentable sugar preservation (>99%). However, raw thick juice stored at alkaline conditions required more frequent pH adjustment than at acidic pH, which would result in higher storage cost. Raw thick juice storage was shown to be a promising technique in terms of fermentable sugar preservation. Yet, fermentation conditions and inoculum preparation have to be improved to maximize ethanol yields from stored raw thick juice. Additionally, storage should be extended to longer periods and alternative acids and alkalis should be evaluated for pH adjustment to maximize overall ethanol yields after storage.

Aerobic and anaerobic sugar beet storage for 14 wk at 4°C resulted in 99±4% and 87±0.1% fermentable sugar retention in beets, respectively. Conversely, beets stored under similar conditions, but at 25°C, retained 82±9% and 48±11% of their initial fermentable sugars, respectively. Accounting for sugars and ethanol in sugar beet exudates generated during anaerobic storage at 4°C boosted the overall fermentable sugar retention from 87±0.1 to 90±0.5%. Application of surface treatments prior to storage may improve sugar retention during increased storage periods necessary to comply with ethanol industry requirements.

FUTURE WORK

Stored raw thick juice showed an apparent loss in fermentation efficiency, relative to raw juice. Further research should be conducted to determine if processing of raw juice to raw thick juice results in the production of compounds which inhibit yeast. Fermentation of freshly prepared raw thick juice in parallel with raw juice may provide new insight on this phenomenon. Optimum fermentation parameters such as media pH, temperature, agitation rate, and inoculum loading, should also be established.

The effect of NaCl formed during pH re-adjustment prior to fermentation should be evaluated. The use of inorganic (strong) acids and alkalis, other than HCl and NaOH, for pH adjustment, may help determine the effect of other synthesized salts on *S. cerevisiae* ethanol productivity. Unpublished results suggest that disodium phosphate (Na_2HPO_4) may represent a nutrient to *S. cerevisiae* as it increased the productivity of this yeast strain by 36%. Replacing HCl with phosphoric acid (H_3PO_4) to adjust raw thick juice pH may result in a storage benefit similar to that of HCl in terms of fermentable sugar preservation. Moreover, H_3PO_4 may be neutralized with NaOH prior to juice fermentation to produce Na_2HPO_4 which may enhance the productivity of *S. cerevisiae*.

The scaled-up storage of raw thick juice at acidic pH would be worthwhile to ensure the effectiveness of this storage technique in an ethanol facility. Extending the storage of raw thick juice to one year may confirm that fermentable sugars can be preserved in a risk-free manner. Additionally, more extensive research on the production of raw thick juice will help determine process energy requirements which may be used for overall lifecycle assessment and economic analysis of beet ethanol.

Storage of sugar beets under an environment with initial O_2 concentrations between 0% and 21% should be explored in combination with temperatures above freezing to assess the potential of fermentable sugar and ethanol yield retention. Displacing O_2 from the storage atmosphere using inert gases such as CO_2 and N_2 is worthwhile since these gases may reduce the respiration rate of sugar beets. A larger, whole beet storage experiment consisting of 20-L storage units and treatments conducted in triplicate is recommended. Larger replicates (e.g. 8 to 10 sugar beets per replicate) may help reduce variability within treatments and ensure more solid results.

The surface application of antimicrobial agents and senescence inhibitors on sugar beets may be effective for fermentable sugar preservation. Antimicrobial agents such as jasmonic acid, acetic acid, and acidic calcium sulfate have shown a preservative effect on fresh produce. The application of N^6-benzyladenine as a retardant of senescence in freshly-harvested produce and rose petals has also been effective for quality preservation. The use of these chemicals in combination with modified storage environments at reduced temperatures may result in a superior preservation effect and improve the retention of fermentable sugars in sugar beets. Finally, the effect of surface applied chemicals on sugar recovery during extraction and on yeast ethanol productivity should also be evaluated to ensure high ethanol yield per unit weight of beets entering storage.

APPENDIX A. TOTAL FERMENTABLE SUGARS IN RAW THICK JUICE TREATMENTS BEFORE FERMENTATION AND ETHANOL CONCENTRATIONS AFTER FERMENTATION

Table A1. Fermentable sugar contents in fermentation media prepared with acidic treatments

Treatment	(g kg media^{-1})	Average	SD	CV
1	129.31	129.67	0.34	0.26%
	129.97			
	129.73			
2	131.67	132.02	0.33	0.25%
	132.07			
	132.33			
3	130.05	129.60	0.42	0.33%
	129.53			
	129.21			
4	132.18	132.06	0.50	0.38%
	132.48			
	131.51			
5	129.79	130.27	0.55	0.42%
	130.16			
	130.87			
6	132.21	132.73	0.45	0.34%
	132.92			
	133.05			
7	113.04	116.09	7.37	6.35%
	110.74			
	124.50			
8	141.51	142.79	4.71	3.30%
	148.01			
	138.86			
9	128.52	128.74	0.19	0.15%
	128.90			
	128.79			
10	130.11	129.84	0.25	0.19%
	129.79			
	129.61			
11	135.12	135.53	0.35	0.26%

Table A1. Fermentable sugar contents in fermentation media prepared with acidic treatments (Continued)

Treatment	(g kg media^{-1})	Average	SD	CV
11	135.67			
	135.78			
12	129.67	129.70	0.04	0.03%
	129.74			
	129.68			
Raw beet juice	143.06	139.00	4.91	3.53%
	140.40			
	133.54			

NOTE: Total fermentable sugar contents are given in grams of glucose equivalents per kg of fermentation media.

Table A2. Fermentable sugar contents in fermentation media prepared with alkaline treatments

Treatment	(g kg media^{-1})	Average	SD	CV
1	119.79	117.24	2.21	1.89%
	116.14			
	115.80			
2	129.36	129.12	0.28	0.22%
	129.18			
	128.81			
3	115.62	115.81	0.17	0.15%
	115.96			
	115.85			
4	117.89	117.97	0.45	0.38%
	117.57			
	118.45			
5	131.20	130.66	0.47	0.36%
	130.44			
	130.33			
6	116.40	116.16	0.29	0.25%
	115.84			
	116.25			
7	115.38	115.53	0.29	0.26%
	115.87			
	115.34			
8	116.19	116.32	0.14	0.12%
	116.30			
	116.47			
9	115.38	115.50	0.12	0.10%
	115.62			
	115.48			
10	115.59	115.45	0.13	0.11%
	115.44			
	115.33			
11	115.60	115.48	0.34	0.29%
	115.75			
	115.10			
12	115.28	115.44	0.27	0.23%
	115.29			
	115.75			
Raw beet juice	123.85	124.98	1.07	0.86%
	125.99			
	125.08			

NOTE: Total fermentable sugar contents are given in grams of glucose equivalents per kg of fermentation media.

Table A3. Ethanol concentrations obtained from fermentation of acidic treatments

Trtmnt.	(g kg media^{-1})	Average	SD	(g kg juice^{-1} @ T.0)	Average	SD	CV
1	47.46	47.69	0.75	200.10	201.09	3.16	1.57%
	48.53			204.63			
	47.09			198.55			
2	49.84	50.43	1.53	211.88	214.41	6.52	3.04%
	49.28			209.52			
	52.17			221.82			
3	48.71	48.13	0.80	224.06	221.38	3.69	1.66%
	48.46			222.90			
	47.21			217.18			
4	48.85	48.27	0.94	217.20	214.65	4.18	1.95%
	48.78			216.92			
	47.19			209.83			
5	51.07	49.47	2.15	231.96	224.71	9.78	4.35%
	50.32			228.57			
	47.02			213.59			
6	51.19	51.39	0.19	223.63	224.50	0.85	0.38%
	51.58			225.32			
	51.40			224.55			
7	73.40	73.48	0.12	160.88	161.04	0.27	0.17%
	73.62			161.35			
	73.41			160.90			
8	50.69	49.99	1.33	235.83	232.59	6.18	2.66%
	48.46			225.47			
	50.83			236.48			
9	49.57	49.50	0.32	236.37	236.03	1.51	0.64%
	49.16			234.38			
	49.78			237.34			
10	52.53	53.05	0.52	246.24	248.68	2.46	0.99%
	53.04			248.64			
	53.58			251.16			
11	80.81	80.72	0.16	152.44	152.26	0.31	0.20%
	80.53			151.90			
	80.81			152.44			
12	47.73	49.47	1.51	218.74	226.71	6.90	3.05%
	50.36			230.79			
	50.32			230.60			
Raw J.	56.86	57.37	1.47	72.54	73.19	1.87	2.56%
	59.02			75.30			
	56.22			71.73			

Table A4. Ethanol concentrations obtained from fermentation of alkaline treatments

Trtmnt.	(g kg media^{-1})	Average	SD	(g kg juice^{-1} @ T.0)	Average	SD	CV
1	11.15	10.79	0.43	44.61	43.2	1.73	4.00%
	10.31			41.28			
	10.92			43.71			
2	48.14	47.41	0.63	78.35	77.17	1.03	1.33%
	47.09			76.64			
	47.01			76.51			
3	20.84	20.04	1.15	102.48	98.58	5.66	5.75%
	20.57			101.18			
	18.72			92.08			
4	22.05	21.64	0.84	109.29	107.29	4.19	3.90%
	22.21			110.11			
	20.67			102.48			
5	20.96	21.43	1.24	103.54	105.86	6.12	5.78%
	22.84			112.80			
	20.5			101.24			
6	39.15	40.42	1.1	120.44	124.34	3.39	2.72%
	41.14			126.57			
	40.96			126.00			
7	11.79	10.38	1.53	52.81	46.46	6.85	14.8%
	8.75			39.19			
	10.58			47.37			
8	29.21	29.45	0.23	157.47	158.78	1.24	0.78%
	29.49			158.96			
	29.66			159.92			
9	13.13	12.19	0.84	64.65	60.02	4.13	6.89%
	11.52			56.72			
	11.92			58.68			
10	18.49	19.9	1.21	99.74	107.31	6.55	6.11%
	20.62			111.21			
	20.57			110.96			
11	6.42	6.29	0.29	28.50	27.96	1.27	4.55%
	5.97			26.51			
	6.50			28.88			
12	22.38	22.2	0.16	111.39	110.47	0.8	0.73%
	22.10			110.00			
	22.11			110.00			
Raw J.	59.75	58.84	0.8	76.26	75.09	1.02	1.36%
	58.48			74.64			
	58.28			74.38			

APPENDIX B. FERMENTABLE SUGARS IN SUGAR BEETS STORED UNDER AEROBIC AND ANAEROBIC ATMOSPHERES AT 4°C AND 25°C

Table B1. Total fermentable sugars in sugar beets stored aerobically at 25°C for 14 wk

Week	Sample	Sucrose ($g\ kg^{-1}$)	Glucose ($g\ kg^{-1}$)	Fructose ($g\ kg^{-1}$)	Total ($g\ kg^{-1}$)	Sugar Average	Standard Deviation	CV
0	1A	747.4	10.0	9.8	767.2	773.0	8.2	1.06%
	1B*	887.7	9.1	7.3	904.2			
	1C	766.5	6.4	5.9	778.8			
2	2A*	979.7	2.5	7.2	989.3	829.8	22.0	2.65%
	2B	840.9	1.6	2.8	845.3			
	2C	779.8	13.1	21.3	814.2			
4	3A	870.7	0.6	3.0	874.2	837.9	43.0	5.13%
	3B	827.1	8.0	13.9	849.0			
	3C	751.7	14.1	24.6	790.4			
7	4A*	360.7	52.8	94.3	507.9	909.0	15.5	1.70%
	4B	913.5	2.9	3.5	919.9			
	4C	888.9	3.4	5.8	898.1			
10	5A	621.8	25.7	41.9	689.4	773.8	119.4	15.43%
	5B	835.5	8.0	14.7	858.2			
	5C*	1019.8	0.9	2.4	1023.1			
12	6A	737.9	26.5	45.9	810.3	875.3	56.6	6.47%
	6B	879.0	14.7	20.1	913.7			
	6C	761.0	54.4	86.5	901.8			

* indicates an outlier.

NOTE: Fermentable sugar contents are given in grams per kilogram of sugar beet (dry basis).

Table B2. Fermentable sugars in sugar beets stored anaerobically at 25°C for 14 wk

Week	Sample	Sucrose ($g\ kg^{-1}$)	Glucose ($g\ kg^{-1}$)	Fructose ($g\ kg^{-1}$)	Total ($g\ kg^{-1}$)	Sugar Average	Standard Deviation	CV
0	11A	683.4	6.4	5.1	694.9	748.7	49.1	6.56%
	11B	776.8	8.2	6.0	791.1			
	11C	744.8	7.9	7.5	760.2			
2	12A	834.9	0.0	0.0	834.9	829.0	8.3	1.00%
	12B	823.1	0.0	0.0	823.1			
	12C*	690.4	0.0	0.0	690.4			
4	13A*	220.9	33.2	54.2	308.3	453.8	48.2	10.62%
	13B	375.3	20.8	23.6	419.7			
	13C	441.0	21.5	25.4	487.8			
7	14A	307.8	90.4	123.0	521.1	476.3	63.4	13.31%
	14B*	268.5	34.1	48.3	350.9			
	14C	303.8	55.7	71.9	431.5			
10	15A	24.7	140.4	159.4	324.5	338.0	19.1	5.64%
	15B	106.3	97.3	147.9	351.4			
	15C*	287.4	92.9	160.1	540.4			
12	16A	237.5	88.6	115.5	441.6	423.8	42.4	10.01%
	16B	159.0	124.3	171.1	454.4			
	16C	131.4	100.8	143.2	375.4			

* indicates an outlier.

NOTE: Fermentable sugar contents are given in grams per kilogram of sugar beet (dry basis).

Table B3. Fermentable sugars in sugar beets stored aerobically at 4°C for 14 wk

Week	Sample	Sucrose (g kg^{-1})	Glucose (g kg^{-1})	Fructose (g kg^{-1})	Total (g kg^{-1})	Sugar Average	Standard Deviation	CV
0	101A	781.3	7.7	7.6	796.6	820.0	37.9	4.62%
	101B	783.5	8.7	7.4	799.6			
	101C	852.4	6.9	4.4	863.7			
2	102A	841.0	0.0	0.0	841.0	835.5	39.1	4.68%
	102B	793.9	0.0	0.0	793.9			
	102C	871.5	0.0	0.0	871.5			
4	103A	852.1	0.0	0.0	852.1	808.5	37.9	4.69%
	103B	789.5	0.0	0.0	789.5			
	103C	783.8	0.0	0.0	783.8			
7	104A	704.0	0.0	0.0	704.0	711.5	10.5	1.47%
	104B	701.5	6.6	10.8	718.9			
	104C*	881.1	0.0	0.0	881.1			
10	105A	953.0	0.0	0.0	953.0	880.7	102.2	11.60%
	105B	808.5	0.0	0.0	808.5			
	105C*	1010.7	0.0	0.0	1010.7			
12	106A	856.0	7.6	5.8	869.4	903.5	29.8	3.30%
	106B	909.0	8.2	7.3	924.5			
	106C	903.2	7.3	6.1	916.6			

* indicates an outlier.

NOTE: Fermentable sugar contents are given in grams per kilogram of sugar beet (dry basis).

Table B4. Fermentable sugars in sugar beets stored anaerobically at 4°C for 14 wk

Week	Sample	Sucrose (g kg^{-1})	Glucose (g kg^{-1})	Fructose (g kg^{-1})	Total (g kg^{-1})	Sugar Average	Standard Deviation	CV
0	111A	818.3	5.2	3.8	827.3	835.2	11.3	1.35%
	111B*	677.3	3.7	2.2	683.3			
	111C	839.5	3.0	0.7	843.2			
2	112A	742.6	0.0	0.0	742.6	706.1	51.6	7.31%
	112B	669.6	0.0	0.0	669.6			
	112C*	926.6	0.0	0.0	926.6			
4	113A	562.5	0.0	0.0	562.5	611.5	69.4	11.34%
	113B	660.6	0.0	0.0	660.6			
	113C	missing	missing	missing	missing			
7	114A	699.4	1.6	4.4	705.4	750.6	63.5	8.45%
	114B	823.2	0.0	0.0	823.2			
	114C	723.3	0.0	0.0	723.3			
10	115A	628.1	1.0	5.8	634.9	695.6	85.8	12.33%
	115B	707.9	12.0	36.3	756.2			
	115C*	310.5	20.0	115.6	446.1			
12	116A	29.3	20.5	145.9	195.6	196.0	0.6	0.28%
	116B	694.8	7.0	5.4	707.2			
	116C	19.3	30.6	146.5	196.4			

* indicates an outlier.

NOTE: Fermentable sugar contents are given in grams per kilogram of sugar beet (dry basis).

Table B5. Fermentable sugars in exudates from sugar beets stored anaerobically at 25°C for 14 wk

Week	Samples	Exudate Weight (g)	Sugars in Exudate (g kg^{-1})	Sugars from Exudate (g)	Average	SD	Initial Sugars in Beets (g kg^{-1}; w. b.)	% of In. sugars	SD
4	13A	16.3	48.58	0.79	0.93	0.19	114.78	0.8%	0.2%
	13B	16.4	65.06	1.07					
	13C	missing	missing	missing					
7	14A	0.78	72.99	0.06	0.18	0.17	75.79	0.2%	0.2%
	14B*	122.2	23.14	2.83					
	14C	8.3	36.64	0.30					
10	15A	21.12	5.03	0.11	0.44	0.48	65.56	0.7%	0.7%
	15B	16.36	47.88	0.78					
	15C*	18.46	3.61	0.07					
12	16A	34.45	52.82	1.82	2.01	0.17	167.07	1.2%	0.1%
	16B	33.39	61.51	2.05					
	16C	63.28	34.03	2.15					

* indicates an outlier.

Table B6. Fermentable sugars in exudates from sugar beets stored anaerobically at 4°C for 14 wk

Week	Samples	Exudate Weight (g)	Sugars in Exudate (g kg^{-1})	Sugars from Exudate (g)	Average	SD	Initial Sugars in Beets (g kg^{-1}; w. b.)	% of In. sugars	SD
10	115A	8.87	23.29	0.21	0.93	1.02	122.12	0.8%	0.8%
	115B	52.1	31.55	1.64					
	115C*	15.4	46.04	0.71					
12	116A	37.53	48.10	1.81	2.14	0.47	127.75	1.7%	0.4%
	116B*	8.73	35.22	0.31					
	116C	42.18	58.52	2.47					

* indicates an outlier.

Table B7. Ethanol in exudates from sugar beets stored anaerobically at 25°C for 14 wk

Week	Samples	Exudate Weight (g)	Ethanol in Exudate (g kg^{-1})	Ethanol from Exudate (g)	Average	SD	Glucose Equivalents (g)	% of Initial Sugars	SD
4	13A	16.3	12.53	0.20	0.22	0.03	0.44	0.4%	0.0%
	13B	16.4	14.89	0.24					
	13 C	missing	missing	missing					
7	14A	0.78	11.33	0.01	0.06	0.07	0.11	0.1%	0.2%
	14B*	122.2	12.85	1.57					
	14C	8.3	12.83	0.11					
10	15A	21.12	22.02	0.47	0.32	0.21	0.63	1.0%	0.6%
	15B	16.36	10.56	0.17					
	15C*	18.46	35.64	0.66					
12	16A	34.45	12.57	0.43	0.67	0.39	1.32	0.8%	0.5%
	16B	33.39	13.98	0.47					
	16C	63.28	17.68	1.12					

* indicates an outlier.

Table B8. Ethanol in exudates from sugar beets stored anaerobically at 4°C for 14 wk

Week	Samples	Exudate Weight (g)	Ethanol in Exudate (g kg^{-1})	Ethanol from Exudate (g)	Average	SD	Glucose Equivalents (g)	% of Initial Sugars	SD
10	115A	8.87	8.23	0.07	0.21	0.19	0.41	0.3%	0.3%
	115B	52.1	6.58	0.34					
	115C*	15.4	6.61	0.10					
12	116A	37.53	5.19	0.19	0.21	0.03	0.42	0.3%	0.0%
	116B*	8.73	5.41	0.05					
	116C	42.18	5.53	0.23					

* indicates an outlier.

APPENDIX C. PRELIMINARY EXPERIMENTS ON RAW THICK JUICE AND WHOLE BEET STORAGE

Executive Summary

A preliminary study was conducted to characterize stored raw thick beet juice. 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 anaerobically at acidic and alkaline pH. The stable RDS, pH, and fermentable sugar content in raw thick juice stored under acidic pH for up to 12 wk, indicate a high probability of successful long-term storage. Highly acidic conditions (pH 2 or 3) showed an increase (<6.3%) in fermentable sugar content due to a possible hydrolysis of complex carbohydrates. Under alkaline conditions, abrupt drops in pH are a concern in the sugar industry during thick juice storage. However, in this preliminary study, a pH decline did not indicate a significant loss of total fermentable sugars during storage for 12 wk.

An additional preliminary study was carried out to quantify the change in total fermentable sugars in beets stored anaerobically at 4°C for 10 weeks. Results indicated a fermentable sugar loss of <10% under the storage conditions used. However, techniques such as modified and controlled atmosphere storage have been successfully applied to preserve perishable crops. A study combining modified atmosphere with temperature adjustment will assist in determining the viability of such technique in terms of fermentable sugar preservation in stored sugar beets.

Introduction

Methods for feedstock storage must be developed to allow yearlong operation of ethanol plants based on sugar beets. The storage of thick clarified beet juice to preserve sucrose has been successful in sugar factories around the world. This storage method focused on the preservation of sucrose was first implemented at full scale in 1960 in the United States. Thick juice storage is currently a common practice in most sugar factories around the Unites States and some European countries. For the table sugar industry, storing thick juice is more economical than storing sucrose in its crystal form since the equipment required for the latter method is more expensive than that required for thick juice storage.

Refractometric dissolved solids (RDS) content (°Brix), pH, and temperature are determinant factors for sugar preservation in stored thick juice. Recent studies suggest that these factors have a combined preservative effect and values of 69°Brix, pH 9, 10°C<T<15°C are sufficient for sucrose preservation in stored thick juice. Nevertheless, these values could be somewhat conservative if analyzed from the perspective of food preservation principles and may not coincide with the preservation of fermentable sugars in raw thick juice.

Raw beet juice has proved to be an excellent media for ethanol production. This juice has not undergone the typical purification process followed by the sugar industry, which requires significant amounts of CaO (lime). The production of lime is energy intensive and high process energy requirements could restrict the qualification of this crop as an advanced biofuel feedstock for ethanol. Following conventional thick juice storage requirements along with food preservation principles, a method and optimum conditions to store raw beet juice can be determined. This report presents results from preliminary experiments carried out to define optimum storage conditions for raw thick juice for ethanol production.

Materials and Methods

Characterization of Raw Thick Juice Stored Anaerobically Without pH Control

Raw beet juice was obtained from American Crystal Sugar Co. (Moorhead, MN, USA). The raw beet juice had an RDS content of 16.5°Brix, pH 6, and total fermentable sugars content of 160 g kg juice^{-1}. Two concentration systems were tested to increase the RDS content of the juice. 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°Brix using a rising-film evaporator (Fig. C1) operated with steam at 35 kPa (gauge), vacuum of 60 kPa (gauge) inside the evaporating tube, input juice temperature of 20°C, input juice flow rate of 16 L hr^{-1}, and 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.

Figure C1. Rising-film evaporator used for juice concentration

Experiments were conducted to characterize the raw thick juice stored anaerobically at different combinations of pH and RDS content and a temperature of 23±1°C. A factorial design was followed to determine the storability of raw thick juice samples with an RDS content adjusted to 40, 50, 60, 65, and 69°Brix in combination with pH values in the acidic (2 to 6) and alkaline (8 to 11) ranges.

Treated juice samples were stored for 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.

The pH of the stored samples was measured using a Thermo Scientific Orion 2-Star benchtop pH meter (Thermo Fisher Scientific Inc.; Beverly, MA, USA) equipped with automatic temperature compensation. The pH meter was calibrated with buffer solutions of pH 1.7, 7, and 12, prior to analyses to ensure accurate readings. The RDS content was measured with a Pocket Digital Refractometer Mod. 300053 (SPER Scientific; Scottsdale, AZ, USA).

Sucrose, glucose, and fructose were quantified by HPLC (Waters Corp.; Milford, MA, USA) using an Aminex HPX-87P (300x7.8 mm) carbohydrate column (Bio-Rad Laboratories; Hercules, CA, USA). The HPLC system was equipped with an isocratic pump, autosampler, and refractive index detector (RID, Model 2414 – Waters Corp.). The injection volume into the column was 20µL and the samples were eluted with 18.2-mΩ nano-pure water at flow rate of 0.6 mL min^{-1} and elution time of 25 min. The column and detector temperatures were 85°C and 50°C, respectively.

Change in Fermentable Sugars in Sugar Beets Stored Anaerobically at 4°C for 12 wk

An additional preliminary study was conducted to determine if anaerobic storage of beets at low temperature (4°C) was viable for fermentable sugar preservation. Sugar beet halves were weighed, vacuum-packed and stored for a period of 10 wk. Analyses of stored beet halves were performed biweekly. Sucrose, glucose, and fructose were quantified by HPLC following the method described above.

Results and Discussion

Characterization of Raw Thick Juice Stored Anaerobically Without pH Control

Storage at Acidic pH

A set of results from experiments in which raw thick juice was stored at acidic pH was useful to understand the behavior of the fermentable sugars in the juice throughout storage. Table C1 presents the change in fermentable sugars of stored raw thick juice treatments. The loss of sugars suggested microbial activity in the stored juice, whereas the net sugar gain suggested the hydrolysis of complex carbohydrates.

Table C1. Change in fermentable sugars in raw thick juice stored 12 wk at acidic pH and RDS of 60 and 65°Brix

	Initial RDS content (°Brix)	
Initial pH	60	65
5.0	-1.6%	-0.5%
4.1	-2.6%	-0.6%
3.1	+1.5%	+6.3%
2.1	+5.7%	+4.2%

An increase in invert sugars (glucose and fructose) was detected after storage in samples stored at pH<3.0 after the storage period (Fig. C2). These sugars were a result of sucrose

hydrolysis which was catalyzed by the hydrogen ions of HCl. The increase in glucose and fructose contents during storage is not a concern to the ethanol industry as these sugars are also readily fermented by yeast.

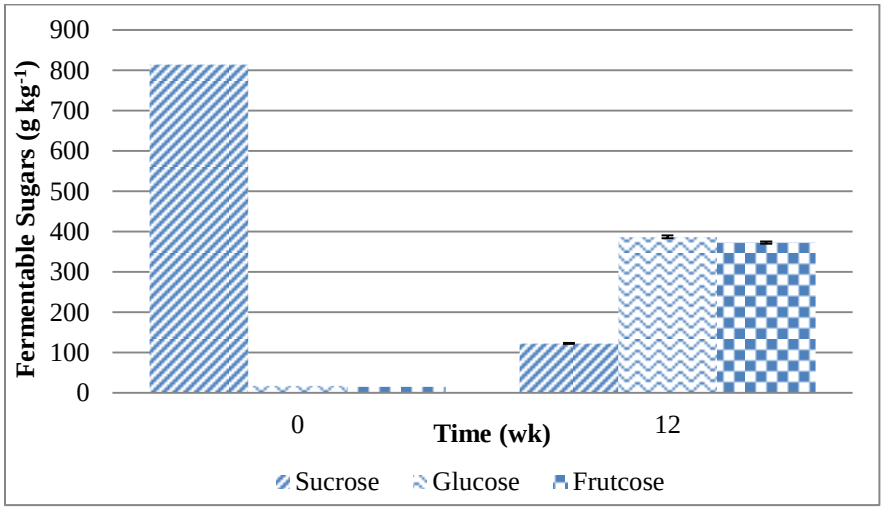

Figure C2. Increase of invert sugars as a result of acidic inversion of sucrose in raw thick juice stored 12 wk at pH 2 and 65°Brix

The RDS content is closely related to the water activity of a biological material. The term water activity makes reference to the water that is not chemically bound to the constituents of a material and thus is available to support the growth of microorganisms. The storage life of a biological material is increased if the water activity is decreased through methods such as evaporation. Measuring water activity is a time consuming task; therefore, RDS measurement is preferred as it is quick and its value is inversely proportional to that of water activity.

Figure C3(a) shows the stable trend of pH in raw thick juice samples stored at acidic pH values. The pH is a sensitive and simple indicator of spoilage and it is preferred due to its ease of measurement. A stable pH indicates the absence of microbial activity which could cause a rapid spoilage of the stored juice. Figure C3(b) shows a slight but considerable increase in the RDS readings of the juice. This increase is more significant in samples stored at pH 2. These samples

presented an inversion of sucrose that led to the increase of glucose and fructose. These monosaccharides are known to be hygroscopic, namely, they have the ability to easily bind with free water and thus reduce the water activity of the stored juice. Also, the hydrolysis of complex sugars in the beet pulp within the juice was a phenomenon that possibly contributed to the increase of the RDS readings in the samples.

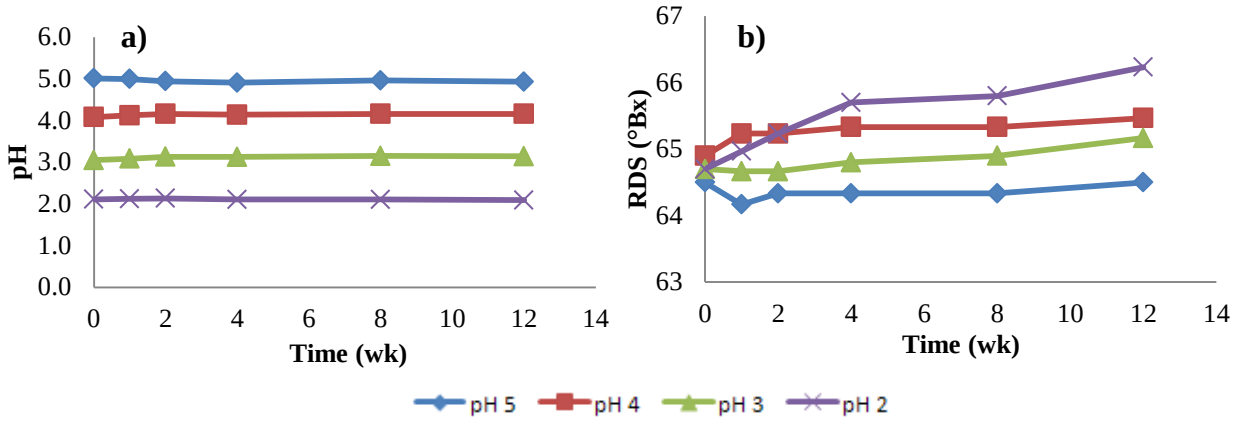

Figure C3. Change in a) pH and b) RDS content of raw thick juice stored for 12 wk at initial RDS of 65°Brix

Storage at Alkaline pH

The storage of pure thick juice under alkaline pH is an accepted practice within the table 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 and optimal storage conditions were determined (Fiedler et al., 1993). Raw thick juice used in the studies conducted by Fiedler *et al.* 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. In most research 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. C4). The fermentable sugars content of this particular sample decreased by 2.2% after 8 wk of storage, but organic acids produced by microbial activity may have contributed to the stability of the RDS readings during storage.

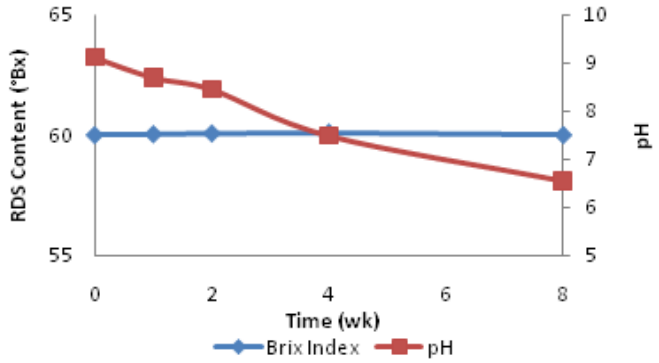

Figure C4. Change in pH and RDS content of raw thick juice stored for 8 wk at initial pH 9 and RDS of 60°Brix

The RDS content and pH trends in four different samples stored for 12 wk at pH 10 is illustrated in Figure C5. The stability of the RDS content in the juice suggests that most fermentable sugars were preserved even when an abrupt pH decline occurred.

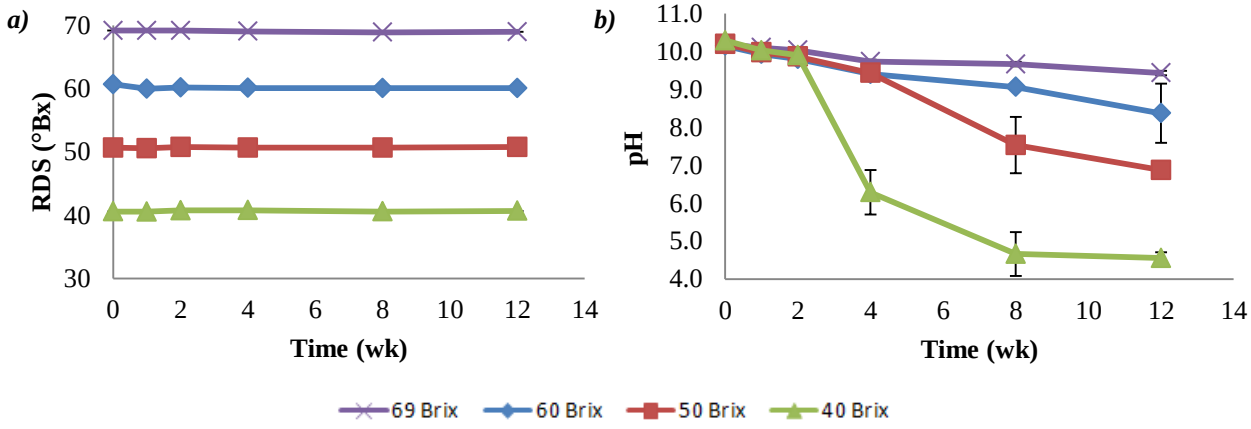

Figure C5. Change in a) RDS content and b) pH of raw thick juice stored for 12 wk at initial pH 10

Change in Fermentable Sugars in Sugar Beets Stored Anaerobically at 4°C for 12 wk

A preliminary study was conducted to evaluate anaerobic storage of sugar beets at 4°C for 10 wk. A sugar loss of <10% was detected at all the storage times considered in this experiment (Table C2).

Table C2. Fermentable sugar loss in beets stored anaerobically at 4°C

Storage Time (Weeks)	Sugar Loss (%)
2	4.2
4	8.9
6	6.4
8	8.7
10	9.2

The beets used in this study were vacuum packed (Fig. C6) and analyzed individually at the specified storage times. From the limited number of beets that were available, some appeared to be in good condition and at least two beet roots showed bruises caused by handling. Exposed beet tissue is more susceptible to microbial contamination than tissue covered by the beet skin. This helps explain the inconsistency of the percent sugar loss reported at week 6 of storage.

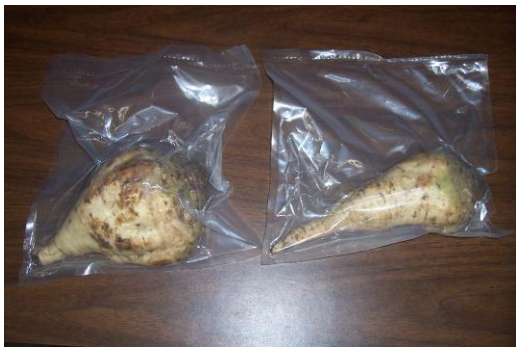

Figure C6. Beet halves vacuum packed individually

The effect of a naturally modified atmosphere on the quality preservation of sugar beets has been studied during storage periods of 28 days (Cole, 1976). The sucrose content was stable

in sugar beets stored in 27.2-L pails for 28 days at 5°C, even after oxygen within the containers was depleted after 5 days of initiating the experiment.

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