TECHNICAL AND ECONOMIC ASSESSMENTS OF STORAGE TECHNIQUES FOR LONG-TERM RETENTION OF INDUSTRIAL-BEET SUGAR FOR NON-FOOD INDUSTRIAL FERMENTATIONS

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Title

Technical and Economic Assessments of Storage Techniques for Long-term Retention of Industrial-beet Sugar for Non-food Industrial Fermentations

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

DOCTOR OF PHILOSOPHY

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ABSTRACT

Industrial beets may compete against corn grain as an important source of sugars for non-food industrial fermentations. However, dependable and energy-efficient systems for beet sugar storage and processing are necessary to help establish industrial beets as a viable sugar feedstock. Therefore, technical and economic aspects of beet sugar storage and processing were evaluated. First, sugar retention was evaluated in whole beets treated externally with either one of two antimicrobials or a senescence inhibitor and stored for 36 wk at different temperature and atmosphere combinations. Although surface treatment did not improve sugar retention, full retention was enabled by beet dehydration caused by ambient air at 25 °C and with a relative humidity of 37%. This insight led to the evaluation of sugar retention in ground-beet tissue ensiled for 8 wk at different combinations of acidic pH, moisture content (MC), and sugar:solids. Some combinations of pH $\leq$ 4.0 and MC $\leq$ 67.5% enabled retentions of at least 90%. Yeast fermentability was also evaluated in non-purified beet juice acidified to enable long-term storage and partially neutralized before fermentation. None of the salts synthesized through juice acidification and partial neutralization inhibited yeast fermentation at the levels evaluated in that work. Conversely, yeast fermentation rates significantly improved in the presence of ammonium salts, which appeared to compensate for nitrogen deficiencies. Capital and operating costs for production and storage of concentrated beet juice for an ethanol plant with a production capacity of 76×10^6 L y^{-1} were estimated on a dry-sugar basis as U.S. ¢34.0 kg^{-1} and ¢2.2 kg^{-1}, respectively. Storage and processing techniques evaluated thus far prove that industrial beets are a technically-feasible sugar feedstock for ethanol production.

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DEDICATION

To my Parents:

Juan and Guillermina

To my siblings:

Veronica and Pablo

To my niece:

Valentina

To my best friend:

Haley

To my second family:

Rhonda, Mark, Jenny, and Kelli

Thank you for your unconditional love and constant words of encouragement.

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LIST OF ACRONYMS

ABEN.....Agricultural and Biosystems Engineering

GVG.....Green Vision Group

HPLC .....High-performance liquid chromatography

MC .....Moisture content

NDSU.....North Dakota State University

OHTC.....Overall heat transfer coefficient

RDS.....Refractometric dissolved solids

SSR .....Sugar-to-solids ratio

TKN .....Total Kjeldahl nitrogen

USDA.....United States Department of Agriculture

LIST OF UNITS

$M.....Million U.S. dollars

¢.....U.S. cent

μF .....Microfarad

μm .....Micrometer

cm.....Centimeter

d.....Day

g.....Gram

gpm .....Gallons per minute

h.....Hour

hm .....Hectometer

Hz.....Hertz

K.....Degrees Kelvin

kg.....Kilogram

kJ .....Kilojoule

km .....Kilometer

kN.....Kilonewton

kPa.....Kilopascal

kV.....Kilovolt

kW.....Kilowatt

kWh.....Kilowatt-hour

L .....Liter

lb .....Pound

m .....Meter

Mg.....Megagram

mg .....Milligram

Mha .....Megahectare

min .....Minute

mo .....Month

MΩ .....Megaohm

Pg .....Petagrams

ppm .....Parts per million

psia .....Pounds per square inch absolute

s.....Second

Tg.....Teragram

W.....Watt

wk.....Week

y.....Year

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DISSERTATION ORGANIZATION

This dissertation consists of the following sections: general introduction, five chapters, general conclusions, and recommendations for future work. Chapter 1 is a general literature review, which covers topics intended to prepare the reader with background on current hurdles to industrial beet development. Chapter 2, entitled "Effect of storage conditions on industrial sugar retention in energy beets", summarizes the effects of surface treatment, storage atmosphere, and temperature on sugar retention in beet roots stored for up to 36 wk. Chapter 2 also presents treatment conditions that resulted in best sugar retention. Chapter 3, entitled "Beet tissue ensiling: an alternative for long-term storage of sugars in industrial beets for non-food use", describes the effects of acidic pH, moisture content (MC), and sugar:solids on sugar retention in ground-beet tissue ensiled for up to 8 wk. A polynomial model is given to predict sugar retention based on acidic pH and MC combinations. Combinations of acidic pH and MC (within ranges evaluated) that result in successful retention are illustrated. Chapter 4, entitled "Effects of pre-storage concentration and acidification, and post-storage conditioning of non-purified beet juice on yeast (*Saccharomyces cerevisiae*) fermentation" summarizes the effects on yeast of salts synthesized in concentrated, non-purified beet juice acidified to enable long-term storage and partially neutralized to allow fermentation. A recommendation of acids for pre-storage acidification and bases for post-storage neutralization is given based on experimental results. Chapter 5, entitled "Economic analysis of an industrial process for long-term industrial-beet sugar storage", presents capital and operating cost estimates associated with concentrated juice production, storage, and conditioning for yeast fermentation. Equipment design presented in Chapter 5 was based on engineering principles and laboratory experiments. Cost estimates were performed using costs found in the literature or provided by equipment manufacturers, and

adjusted using the *Six Tenths Rule* and/or updated using the *Chemical Engineering Plant Cost Indices*. Chapter 5 is followed by the general conclusions from the overall project and some recommendations for future work. Finally, the appendices at the end of the dissertation contain supplementary material to Chapters 2, 3, and 5.

GENERAL INTRODUCTION

There is currently significant, worldwide interest in renewable sources of energy and chemical precursors to reduce petroleum use. Carbohydrate-rich plant biomass has gained particular attention since it is a source of sugars that may be readily metabolized by microorganisms that secrete products with high industrial value. Some of these metabolic products represent end products and/or chemical precursors to certain non-food industries. For example, ethanol produced by yeast fermentation of sugars may be purified and either directly used as a biofuel or blended with gasoline. Similarly, lactic acid produced through microbial fermentation of sugars is a precursor of polylactic acid, a biodegradable thermoplastic.

Plant cell walls are constituted primarily by polymeric carbohydrates such as cellulose, hemicellulose, and lignin. Some plants also store energy within their cells in the form of polymeric carbohydrates; for example, corn and potatoes synthesize and store large quantities of starch. Although polymeric carbohydrates may be hydrolyzed into their base sugar molecules and then metabolized by microorganisms, the steps involved are significantly energy intensive (Twidell and Weir, 2015). In comparison, plants such as sugarbeet and sugarcane store energy within their cells primarily in the form of sucrose, and in trace amounts of glucose and fructose. Some microorganisms such as yeast are equipped with enzymes that readily hydrolyze sucrose into fermentable glucose and fructose, thus eliminating energy-intensive pretreatment steps.

Sugarbeet is an important crop due to its high sucrose productivity and ability to adapt to different climate and soil conditions (Smith, 1996). This crop is already the primary feedstock for table sugar production in the U.S., contributing to 55% of total domestic production (USDA-ERS, 2012). Its ability to produce and store large quantities of sucrose, which is readily fermentable, has led to the development of varieties tailored for non-food industrial

fermentations. These varieties, referred to simply as *industrial beets*, are primarily bred for dry matter content (McGrath and Townsend, 2015), which is significantly correlated with sucrose content (Hoffman et al., 2004). In contrast, beets for table sugar are bred primarily for sucrose purity.

Industrial beets have many advantages over corn grain, the primary source of sugars for non-food industrial fermentations in the U.S. For example, industrial beets have reached an average sugar productivity of 12.6 Mg ha^{-1} in hexose equivalents (NDSU Carrington REC, 2014). That is 70% greater than the average productivity of corn grain, which was 7.4 Mg ha^{-1} of hexose equivalents in 2014 (assuming a starch weight fraction of 72% and a stoichiometric starch to glucose weight ratio of 0.9:1; Wertz and Bédué, 2013; USDA-ERS, 2014). Additionally, industrial beets may be successfully grown in marginal land under non-irrigated conditions and in saline soils that are less productive for other agricultural crops (NDSU Carrington REC, 2014; McGrath and Townsend, 2015). Furthermore, the long, fibrous roots of beets penetrate deep into the soil, gaining access to moisture and nutrients that other crops cannot reach (Smith, 1996), thus reducing fertilizer requirement.

Despite the significant advantages of beets over corn grain, their high moisture content enables microbial and enzymatic activity under non-freezing conditions, leading to significant sugar losses beginning immediately after harvest (Asadi, 2007a). Although techniques for long-term sugar storage are already well established in the beet sugar industry, these require either freezing temperatures or stringent conditions, and yet entail risks of significant sugar loss (McGinnis et al., 1996; Rorabaugh and Orleans, 1996; Schmalz, 1998; Falsterbo et al., 1998; Asadi, 2007b). Moreover, those techniques focus solely on retention of sucrose, the product of interest to that industry. Therefore, techniques for overall fermentable sugar (sucrose, glucose,

and fructose) retention may be completely different than those followed in the beet sugar industry, and yet have received little attention.

Principles and techniques used in the preservation of perishable commodities may help develop dependable and energy-efficient systems for long-term storage of industrial-beet sugar. External application of antimicrobial agents, storage under modified atmosphere, artificial modifications of matrix moisture content and pH are several of the techniques that can minimize quality loss in perishable commodities. However, the success of these techniques on industrial beets will not only depend on their effectiveness to retain fermentable sugars, but also on their impact on the end use of sugars. Moreover, these techniques should be economically feasible and allow end-product costs competitive to those of similar products derived from alternative sources.

Research objectives

The overall objective of this research project was to evaluate technical and economic aspects of industrial-beet sugar storage and processing. The specific objectives of this research were to:

I. Evaluate effects of surface treatment, temperature, and storage atmosphere on sugar retention in whole industrial-beet roots stored for up to 36 wk.

II. Evaluate effects of acidic pH, moisture content, and sugar:solids on sugar retention in ground beet roots ensiled for up to 8 wk.

III. Evaluate yeast fermentability of concentrated, non-purified beet juice acidified to enable long-term storage and partially neutralized before fermentation.

IV. Estimate capital and operating costs involved in concentrated, non-purified juice production, storage, and conditioning for the purpose of yeast fermentation.

CHAPTER 1. LITERATURE REVIEW

Global petroleum consumption and associated greenhouse gas emissions

Since the Industrial Revolution, human activities have been fueled by non-renewable energy sources such as coal, petroleum (or *crude oil*), and natural gas. Of these fossil fuels, crude oil has been the primary source of energy for many years (Figure 1.1). In 2012, crude oil accounted for the largest portion of the annual global energy consumption (8,979 Tg of oil equivalent), followed consecutively by natural gas and coal (Figure 1.2a). Crude oil is used in several economic sectors, including transportation, non-energy, and industry. The transportation sector dominates crude oil consumption and is followed consecutively by the non-energy and industry sectors (Figure 1.2b). Crude oil is also used in smaller proportions in agriculture, commercial and public services, and residential activities (Figure 1.2b).

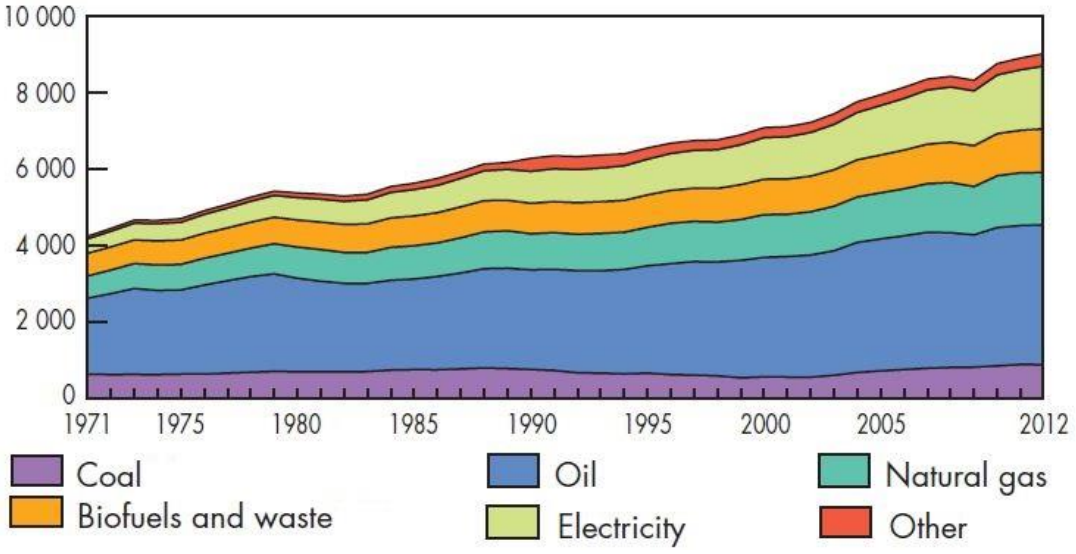

Figure 1.1. Global fuel consumption (in Tg of oil equivalent) from 1971 to 2012 (Source: IEA, 2014)

Although fossil fuels have contributed significantly to worldwide development, their combustion gas emissions have been the primary cause of global warming over the years due to their greenhouse effect (Casper, 2010). Crude oil is the second largest contributor to global

greenhouse gas (GHG) emissions, just after coal (Figure 1.3). The higher GHG emissions from coal (Figure 1.3) are due in part to the fact that 41% of the electricity used in the world is produced in coal-fired power plants (Burnard et al., 2014). Moreover, the global average efficiency of existing power plants is only about 33% (Burnard et al., 2014).

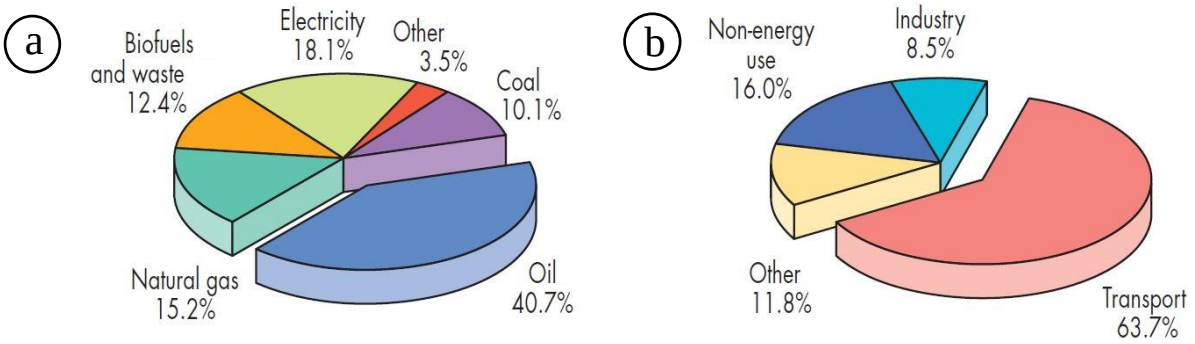

Figure 1.2. Global (a) energy consumption by fuel type as a percent fraction of 8,979 Tg of oil equivalent and (b) crude oil consumption by sector in 2012 (Source: IEA, 2014)

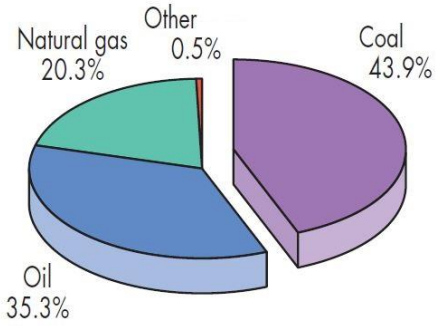

Figure 1.3. Global CO_2 emissions by fuel in 2012 as a percent fraction of 31,734 Tg of CO_2 emitted (Source: IEA, 2014)

Crude oil continues to be the primary source of liquid transportation fuels. Approximately 76% of a barrel of crude oil is used to produce liquid transportation fuels, including gasoline, diesel, and jet fuel (Figure 1.4). However, crude oil is not only a source of fuels; it is also a source of liquefied petroleum gases (or *hydrocarbon gas liquids*), which are building blocks for plastics (Mitchell, 1996).

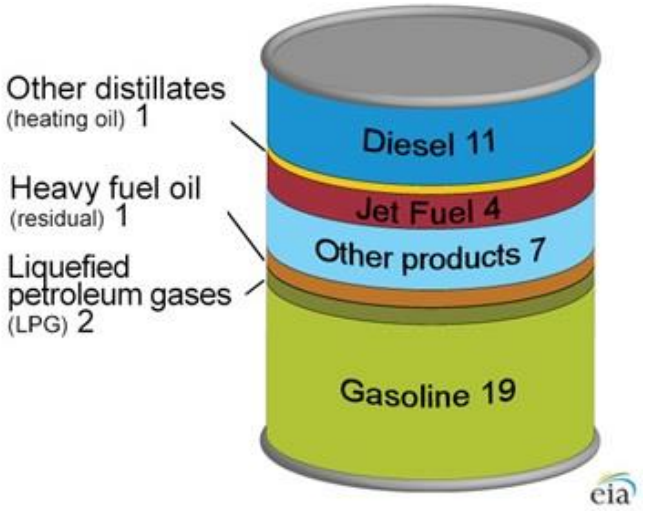

Figure 1.4. Portions (in U.S. gallons) of a barrel of crude oil destined to different products in 2013. Note: A barrel (42 U.S. gallons) of crude oil yields approximately 45 U.S. gallons of petroleum products due to gains during the refining process (Source: U.S. EIA, 2014)

Importance of biofuels and other bioproducts

In an attempt to displace petroleum use, countries around the world have enacted legislation to prompt the development of fuels derived from renewable resources. For example, the 2007 Energy Independency and Security Act enacted in the United States mandates the production and blending of at least 136 hm^3 y^{-1} of biofuels into liquid transportation fuels by 2022. One of the aims envisioned with the displacement of petroleum use is a reduction in net GHG emissions.

The displacement of petroleum use will consequently result in a replacement of a petroleum-based economy with a sustainable economy based on renewable sources. Renewable sources and products produced domestically will contribute to the development of new technologies and industries. Moreover, farming and industrial activities may consequently contribute to the economic development of rural communities. Furthermore, these communities would have a significant potential of becoming environmentally and economically sustainable.

Global biofuel production

In 2012, the global biofuel (ethanol + diesel) production was 110.3 hm^3 y^{-1}. The largest contributors to this production figure were the United States, Brazil, and Germany with 54.5, 26.1, and 3.9 hm^3 y^{-1}, respectively. The most important biofuel in the United States is ethanol derived from corn starch which accounted for 94% of all biofuel produced in 2012 in the nation (USDA-ERS – U.S. Bioenergy Statistics, 2013). In comparison, 90% of the biofuel produced in Brazil in 2012 was ethanol derived from sugar cane (U.S. EIA, 2015). Biodiesel is another renewable fuel which is produced from oil seeds (primarily soy bean) and only accounted for 6% and 10% of the total biofuel production in the U.S. and Brazil, respectively, in 2012 (USDA-ERS – U.S. Bioenergy Statistics, 2013; U.S. EIA, 2015).

Existing and developing biofuel feedstocks

The feasibility of renewable feedstocks for biofuel production has been intensively researched in the United States in an attempt to reduce petroleum use and consequently mitigate the detrimental environmental impacts of petroleum-based fuel combustion. Crops with high cellulosic, starch, and sucrose content are potential renewable feedstocks for the production of biofuels such as ethanol and butanol. Biofuels have an additional benefit of contributing to GHG reductions since the carbon dioxide released during their combustion is captured and utilized by new feedstock generations during their growth.

Corn

Corn is the primary feed grain produced in the United States with approximately 34 Mha planted and 314 Tg produced in 2011-12 (USDA– Foreign Agricultural Service, 2013). For many years, corn has been the primary feedstock for ethanol production in the United States. In 2011-12, 127 Tg of corn were destined to ethanol production; this is equivalent to approximately

40% of the annual corn production (USDA-ERS – Agricultural Baseline Database and U.S. Bioenergy Statistics, 2013).

The process to produce ethanol from corn starch is well developed in the United States. However, despite the popularity of corn as a renewable feedstock for ethanol, ethanol from corn starch reduces GHG emissions by only approximately 20%, relative to gasoline combustion emissions (EPA, 2010). A need for greater GHG reductions has led researchers to develop alternative energy crops that may qualify as feedstocks for biofuels with greater GHG reduction potential.

Sugarcane

Sugarcane is the primary feedstock for ethanol production in Brazil. Mature sugarcane contains between 8% and 17% sucrose (van der Poel, 1998) which may be extracted by the conventional milling process and subsequently fermented into ethanol. Furthermore, the sugar-spent material, known as *bagasse*, is rich in hemicellulose and cellulose which may be broken down into carbohydrate monomers via hydrolytic processes and subsequently fermented into ethanol.

In the United States, sugarcane is the second leading feedstock for granulated sugar production after sugarbeet, accounting for 45% of the nation's production (USDA-ERS, 2012). Sugarcane grows in regions of the U.S. characterized by tropical and sub-tropical climates, and it is harvested at a maturity period between 9 mo and 24 mo (van der Poel, 1998). Sugarcane is grown in the states of Florida, Louisiana, Texas, and Hawaii, which produced 30.4 Tg in the 2013 growing season (USDA-ERS, 2013). Its average cost and high sugar content make it a suitable and potential feedstock for ethanol production in some parts of the U.S. (Rahmani and Hodges, 2009).

Lignocellulosic biomass

Plant material such as that of agricultural residue, forestry residue, perennial switchgrass, among others, is referred to as lignocellulosic biomass. Lignocellulosic biomass is characterized by large contents of hemicellulose and cellulose which are long, polymeric chains of sugar monomers adhered together by a polymer known as lignin.

Lignocellulosic biomass is a potential feedstock for the production of cellulosic biofuel (as classified by the EISA) in the United States. Studies have estimated an availability of 98.9 Tg of corn stover, 15.4 Tg of wheat straw, and 44.9 Tg (on a dry basis) of forest residues for bioenergy use (Walsh, 2008). Perlack et al. (2005) reported an annual U.S.-biomass-potential estimate of approximately 1.3 Pg. Applying a theoretical conversion rate of lignocellulose to ethanol of 0.32 m^3 Mg^{-1}, this would be equivalent to approximately 65% of the U.S. transportation fuel requirement (McLaren, 2008). Technical and handling constraints have been a hurdle for lignocellulosic biomass, which was a non-viable feedstock for commercial biofuel production until 2014. In 2014, the first commercial-scale cellulosic ethanol plant opened in Emmetsburg, IA, USA. The plant processes corn cobs and stover and has an ethanol production capacity of 0.095 hm^3 y^{-1} (POET, 2014).

Industrial beets

Sugarbeet (*Beta vulgaris* L.) is the primary feedstock for granulated sugar production in the U.S, accounting for about 55% of total domestic production (USDA-ERS, 2012). Recently, sugarbeet varieties known as *industrial beets* or *energy beets* have been envisioned as a potential renewable feedstock for ethanol and other bioproducts in the United States. Industrial beets have high harvest yields and also a high impurity fraction, making them inadequate for granulated sugar production. A high impurity fraction can make the juice purification and crystallization

processes difficult (McGinnis, 1996; Van der Poel, 1998; Asadi, 2007). Nevertheless, industrial beets can be grown in marginal land areas characterized by relatively dry and saline soils (Pates, 2011) thereby reducing the food versus biofuel controversy. These beets also require lower nitrogen fertilizer as compared to conventional sugarbeet for granulated sugar production. These differences in growth requirements make industrial beets suitable for biofuel production. Yield trials conducted at the Northern Research Extension Center of North Dakota State University, have resulted in crop yields that range from 69 Mg ha^{-1} to 101 Mg ha^{-1} in dryland and irrigated land, respectively (Roesler, 2012).

Industrial beets as a potential feedstock for ethanol and other bioproducts

The sugars contained in industrial beets (sucrose, glucose, and fructose) can be readily metabolized by microorganisms to produce other high-value added bioproducts such as ethanol or organic acids (Vargas-Ramirez et al., 2013; Visser et al., 2010); for such applications, the sugars may be termed *industrial sugars*. Moreover, some of the beet constituents, such as beet pulp, also have significant value as potential feedstocks for the production of other bioproducts, such as thermoplastics (Liu et al., 2005) and livestock feed (Boucque et al., 1976). Additionally, beets are a commercial source of betaine, a compound used as a dietary supplement in both animal and human nutrition (Craig, 2004).

Industrial beet composition

Industrial beets have a composition similar to that of sugarbeet for the granulated sugar industry, which are constituted by approximately 75% water and 25% dry substance (Asadi, 2007). Approximately 80% of this dry substance is extracted by diffusion and contained in the resulting diffuser juice (or *raw juice*). The remaining 20% are constituents of the spent beet pulp, such as pectin, cellulose, hemicellulose, protein, saponin, and minerals. From the 20% dry

substance contained in the raw beet juice, roughly 17.5% is sucrose and the remaining 2.5% are non-sucrose compounds such as amino acids, betaine, minerals, glucose, and fructose.

Beet sugar extraction

Beets, just like every other plant, are constituted by a complex array of eukaryotic cells which may be classified as phloem-transport or parenchyma-storage cells based on their specific function. These cells contain the organelles that act in conjunction and ensure the proper occurrence of metabolic pathways that maintain the organism alive. The cell contents are suspended in the cytosol, which altogether form the cytoplasm. The cytoplasm in conjunction with the nucleus is generally referred to as the protoplasm of the cell. The protoplasm is bound by a plasma membrane which is immediately followed on the exterior by a primary wall and subsequently the cell wall. Within the plant cell and at a developed stage, the vacuole (Figure 1.5), an organelle that occupies approximately 80% of the total cell volume, is in charge of storing sucrose and other compounds such as proteins.

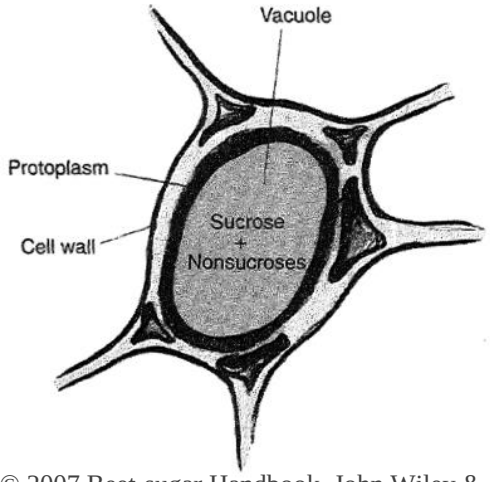

Figure 1.5. Beet tissue cell (Source: Asadi, 2007)

Diffusion is currently the technique followed for sugar extraction in the beet sugar industry, and is described below. Other techniques have been studied in the past to increase the

effectiveness of beet sugar extraction. Among the most significant techniques are: mechanical pressing of beet tissue with assistance of pulsed electric fields and mechanical pressing with sequential washes, which are also described in the following subsections.

Diffusion

The cell wall (Figure 1.5) has a selective characteristic of only allowing the entrance and exit of small particles. Sucrose is securely entrapped within the cell and hence the plasma membrane and cell walls need to be denatured to allow its exit. The plasma membrane and cell walls may be denatured by three mechanisms: heat, freezing, and or coagulating agents. In terms of heat use, denaturation of these cell constituents begins at temperatures above 50 °C and the time for completion depends greatly on the temperature (McGinnis, 1996). The effect of temperature on the time and proportion of cell wall denaturation is presented in Figure 1.6. Temperature values above 70 °C are effective in denaturing the cell wall of about 90% of beet cells in 10 min or less.

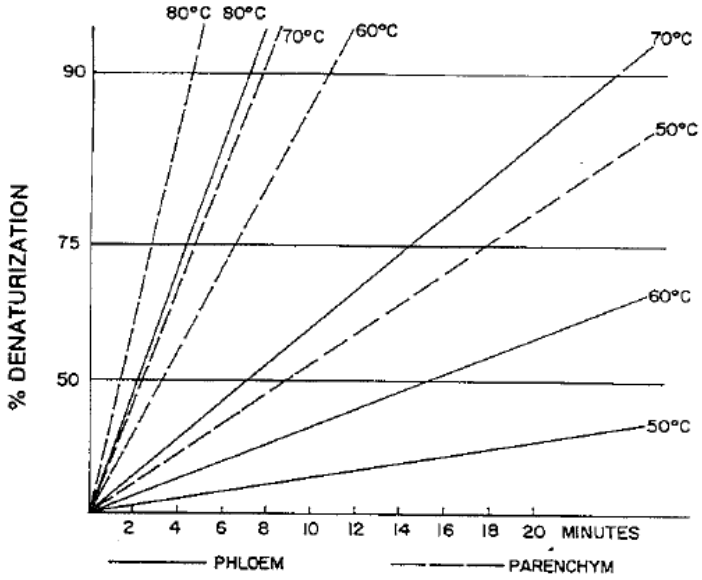

Figure 1.6. Temperature effect on cell wall denaturation of phloem and parenchyma cells in beets (Source: McGinnis, 1996)

The extraction of sucrose from beets in the sugar industry is carried out through the application of diffusion principles (McGinnis, 1996). After storage, beets are washed and conveyed into the factory before entering a slicer that finely cuts the beets into small crinkle-cut pieces known as *cossettes*. This process maximizes the area of contact of the beet cells with the water in the diffuser and consequently increases the sugar extraction efficiency.

Beet-root tissue pressing assisted by pulsed electric fields

Pulsed electric fields have shown to damage the membrane of plant cells which enhances the extraction of their constituents by techniques such as pressing (Vorobiev and Lebovka, 2008). Additionally, pretreatment of plant tissues by pulsed electric fields has shown to inactivate microbes by damaging their cell membrane (Toepfl et al., 2007).

In an attempt to increase sugar extraction yields and reduce process energy requirements, relative to conventional extraction by diffusion, pulsed electric fields have been used in combination with mechanical pressing to assist non-thermal extraction. Jemai and Vorobiev (2006) were able to extract 60% of beet tissue mass in the form of juice, and by incorporating four washing steps they were able to extract up to 97% of the initial sugar content in the tissue. Through their work, they also found that pulsed electric fields were also effective in reducing the amount of non-sugar compounds (potassium, sodium, and amino nitrogen) in expressed beet juice, resulting in purities of up to 97% as compared to 92% in juice from untreated tissue.

Maskooki and Eshtiaghi (2012) found that treating beet tissue with 100 pulsed electric fields at a field strength of 1 kV cm^{-1} and capacitance of 8 μF was effective in disintegrating 87% of the cells in the tissue. They also achieved 88% cell disintegration by vacuum packaging beet tissue samples and submerging them in water at 70 °C. Although both techniques disintegrated very similar cell fractions, beet tissue treated with pulsed electric fields only received 8 kJ kg^{-1} as

compared to 156 kJ kg^{-1} applied to tissue that was vacuum packed and submerged in water at 70 °C.

Beet-root tissue pressing with sequential washes

Pothula et al. (2014) developed a novel front-end processing technique that consists of grinding beet roots by means of a hammer mill before subjecting them to several pressing and washing steps in an attempt to maximize sugar extraction (Figure 1.7). The first step consists of pressing the ground beet-root tissue in a basket press to extract beet sugar dissolved in raw juice; the juice expressed in the first step accounts for about 35% of the fresh tissue weight. Thereafter, pressed tissue is mixed with tap water (weight equal to 1.25 times the weight of first press juice) and the mixture is subjected to a second pressing step. Depending on the initial beet condition, the former step is repeated using tissue from the second pressing step to achieve maximum sugar extraction. Pothula et al. (2014) found that 92% of the sugar in tissue from freshly-harvested beets was extracted after two pressing stages. In comparison, 97% of the sugar in tissue from frozen beets was extracted after three pressing stages.

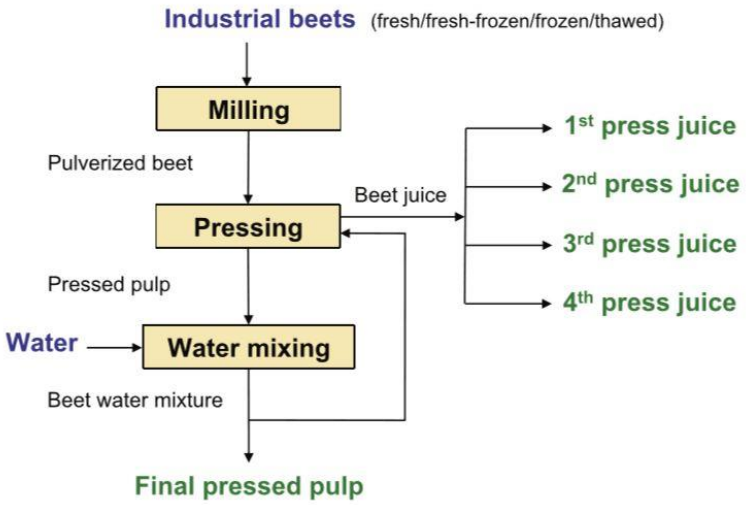

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Figure 1.7. Flow diagram of front-end processing technique consisting of beet-root tissue pressing with sequential washes (Source: Pothula et al., 2014)

Conventional methods for beet sugar storage

Sugarbeet is typically harvested when the root has reached a mass of at least twice the mass of the crown and the ambient air temperature has dropped to below 15 °C (Asadi, 2007). In the Red River Valley of the North Central United States, sugarbeet harvesting typically begins in October and is completed within three to four weeks. After harvesting, the beets are processed through a defoliator which removes their petiole, leaves and a portion of the crown. This defoliating process reduces the amount of impurities that will eventually go into the factory and contributes to an increase in process efficiency.

Two storage techniques are commonly practiced in North America, specifically in regions where climate conditions allow. In regions such as the Red River Valley of the North Central United States, sugarbeet may be stacked to form piles that will be subsequently frozen through forced ventilation with freezing air during the winter season. Subtropical regions with hot to moderately warm climates do not allow extended periods of storage. For example, in the Imperial Valley of California, beets are harvested on demand by the sugar factories. In such regions, a common practice is to extract the sugars from the beets and store them in the form of a concentrated, purified beet juice (also known as *thick juice*) that results from the partial removal of water. Both of these techniques are discussed in detail in the following subsections.

Frozen pile storage

Immediately after harvesting, the beets are transported to receiving stations within an 80-km radius from the factories or storage sites adjacent to them. At this stations or sites, beets are piled by means of a piling system to begin an extended storage phase which typically lasts up to 180 d (McGinnis, 1982). Beet pilers are equipped with a screen that partially removes soil adhered to the beet surface and trash hauled along with the beets. Approximately, 50% of the soil

and trash that is hauled with the beets is removed through screening (Asadi, 2007). Rectangular piles with a base width between 37 m and 49 m, a top width between 8 m and 11 m, and a height between 5 m and 7 m are common practice (McGinnis, 1982).

The fields onto which beets are piled are equipped with arrays of above- or under-ground ventilation ducts through which air at freezing temperatures is forced into the interior of the piles. The air should decrease the temperature of beets to below their freezing point (-7 °C). This forced ventilation mechanism is active during the storage period to ensure a stable temperature within the piles. The removal of soil and trash before piling the beets is therefore essential to allow proper ventilation within the storage piles.

Even though pile storage has been adopted as a conventional storage technique by beet sugar factories in regions where climate conditions allow, there are still risks associated with this storage technique. The ventilation systems used to decrease and maintain the piles under freezing conditions do not always maintain a consistent air flow throughout the piles. This ultimately leads to an increase in the temperature of internal sections of the pile. An increase in temperature causes the reactivation of enzymes involved in beet respiration and thus creates a suitable environment for microbial proliferation. These activities are fueled by sugar, thereby resulting in significant sucrose losses. Figure 1.8 shows a general trend of sucrose loss during pile storage in relation to pile temperature.

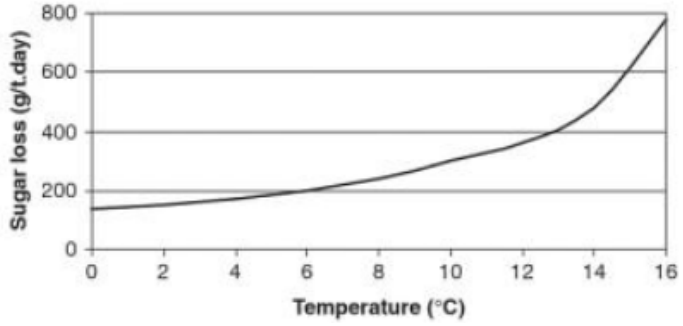

Figure 1.8. Effect of temperature on sucrose loss during pile storage (Source: Asadi, 2007)

Concentrated, purified beet juice storage

Concentrated, purified beet juice (or *thick juice*) storage is discussed in thorough detail by Vargas-Ramirez (2012). After storage, beets are processed to extract their sugars via diffusion. The aqueous sugar solution that results from the diffusion process is known as *raw juice*, which contains about 98% of the sugar in the beet cossettes (Asadi, 2007) and almost all the non-sugar components. This juice is subjected to a purification process that involves the use of lime, which is mixed with the juice in the form of calcium hydroxide and precipitated as calcium carbonate with carbon dioxide. This purification process yields an aqueous sugar solution almost free of impurities, which is known as *thin juice*. The sugars are further concentrated in the thin juice by removing a portion of its water in a multiple-effect evaporation process.

Potential alternative methods for storing industrial-beet sugars

Although the techniques described in the previous subsections have been the most favorable in terms of sugar retention during storage at beet sugar factories, large quantities of energy are required prior to or following their application. For this reason, research has been conducted to evaluate techniques that may help reduce energy requirements and risks associated with conventional techniques. Some of these proposed techniques are described in detail in the following subsections.

Modified and controlled atmosphere storage

As discussed previously, respiration has been identified as the major cause of sucrose loss following beet harvest, accounting for 50% to 80% of the total loss during storage (Klotz-Fugate et al., 2010). Beets maintain metabolic processes through respiration to provide energy for repairing wound tissue imposed during harvest and increase the defense against pathogens (Campbell and Klotz, 2006). Microorganisms present on the beet tissue lead to storage rots

during which heat is dissipated and root respiration rates increase, creating an even more hostile environment for microorganisms to proliferate (Klotz-Fugate et al., 2012). Mumford and Wyse (1976) concluded that fungi can increase root respiration by two fold in beets stored for one month and with 20% of their surface area infected.

Wyse and Peterson (1978) studied the effect of root injury on respiration rates using treatments of 7 kg to 10 kg of beets replicated seven times. They found that inflicted injury on beets, in addition to that caused by the mechanical removal of petioles and leaves, resulted in a 43% increase in respiration rate in comparison to hand-harvested controls, after 10 d of storage. In general, Wyse and Peterson (1978) found that the degree of injury was directly proportional to the increase in respiration rate. Dilley et al. (1968) used samples of 3 beets each, replicated 5 times, and reported a similar finding with injured beets, showing a steady-state respiration rate 4 times greater than that of intact beets during a 10-d storage period. They hypothetically attributed that difference to the increase in surface area available for gas diffusion in injured beets and the possibility of microbial activity in the wounds. Stout and Smith (1950) concluded that beets cut into top and bottom halves prior to storage, showed a higher respiration rate and deteriorated faster than intact, control beets.

Nowadays, many agricultural commodities are stored under modified, controlled atmospheres and low temperatures to reduce their respiration rate and increase quality retention for longer periods (Brody, 1989). A modified storage atmosphere may be generated by purging the air out of a storage container using a combination of gases, typically oxygen, carbon dioxide, and nitrogen, at varying proportions. Many studies that have dealt with the use of modified atmospheres have helped identify storage atmosphere compositions that are unique to the agricultural crop to be stored. The term modified atmosphere is generally used to refer to an

atmosphere that is only initially modified and not disturbed artificially throughout the storage period. In contrast, controlled atmosphere storage consists of initially modifying the storage atmosphere and maintaining its composition throughout storage.

Attempts have been made to reduce post-harvest respiration of beet roots and consequent sucrose loss. Karnik et al. (1970) studied the effects of various modified, controlled atmospheres on respiration and microbial growth among other characteristics. They stored single samples of 10 beet roots each for periods of up to 200 d and at 2 °C or 10 °C. Through their experiments, they found that beets stored for 200 d under a modified atmosphere consisting of 6% CO_2, 5% O_2, and 89% N_2, and at 2 °C, retained 87% of the initial sucrose content. In contrast, beets stored under an aerobic atmosphere at the same temperature, retained 75.5% of the initial sucrose content. Beets stored under the modified atmosphere described above and at 10 °C retained 82% of the initial sucrose whereas beets stored under the same temperature and aerobically retained 73%. In their study, a concentration of 10% CO_2 had a toxic effect on beets resulting in the browning of the tissue after 90 d of storage. Beets stored under this CO_2 concentration, 5% O_2, and 85% N_2, and at either 2 °C or 10 °C retained either 35% or 23% of their initial sucrose content, respectively, after a 165-d storage period.

Karnik et al. (1970) also observed that beets stored at 2 °C at either aerobic or modified, controlled atmosphere did not sprout in general. In addition to this, a low temperature in combination with a modified, controlled atmosphere consisting of either 3% or 4% CO_2, in combination with 5% O_2 and balance N_2, significantly inhibited the fungal growth on the beet surface.

As part of their experimental work, Wu et al. (1970) stored beets in sealed polyethylene bags at 10 °C for up to 90 d. Through respiration, the stored beets depleted the O_2 concentration

within the bag and established an anaerobic atmosphere that contained CO_2 produced through respiration. Although this portion of their work was not the main focus of their experimental work and lacked details, they concluded that a "high CO_2 concentration inhibits respiratory rate". They hypothesized that the low respiration rate may have been the result of enzyme inhibition due to a high CO_2 concentration in the storage atmosphere.

Beet-surface treatments

During the production of sugar beets, chemicals such as herbicides, insecticides, and fungicides, are applied to the soil or sprayed on the beet foliage to prevent or control plagues that may attack the crop during growth. However, after harvest, sugar beets also become susceptible to microbial attack which may result in a significant sugar loss during storage. In an attempt to reduce the respiration rate of stored sugar beets and microbial activity on their surface, research has been conducted to determine the effectiveness of growth regulators and antimicrobial agents on sucrose preservation. Some of this attempts have yielded positive results while others have not been satisfactory.

Dilley et al. (1968) immersed beets for 15 s in solutions of 3.6 g L^{-1} potassium azide (respiration inhibitor), 1.2 g L^{-1} Merck HZ 3456 (growth suppressor), and 1.8 g L^{-1} Botran (fungicide). They also subjected beets to a CO_2-free atmosphere containing 1000 ppm of ethylene for 12 h at 20 °C. After 10 d of storage at 20 °C, they found that potassium azide had increased the respiration rate of beets by 60% in comparison to untreated beets. In general, all of the post-harvest treatments they tested increased the respiration of beets during storage. On the other hand, Wu et al. (1970) reported that beets dipped for 1 h in solutions of N^6-benzyladenine (senescence inhibitor) and Randox (pesticide) at 500 ppm, and stored for up to 182 d at 10 °C, retained 83.9% and 84.6% of their initial sucrose content, respectively. These two chemicals

were the most effective in reducing sucrose loss among a total of six chemicals tested. Mumford and Wyse (1976) found that spraying solutions of benomyl and thiabendazole at 500 ppm on injured beets, inhibited the proliferation of the fungi *Penicillium* and *Botrytis* on the treated surface. The beets in this experiment were stored for 3 wk at 98% humidity and 15 °C, conditions that favor infection fungi proliferation. Similarly, Miles et al. (1978) found that spraying thiabendazole at 1500 ppm on sugarbeet piles, helped control rot during storage.

Concentrated, non-purified beet juice storage

An alternative to whole beet storage is to store the sugars in a concentrated solution after they have been extracted. The extraction of sugars by diffusion using hot water yields a solution, known as raw juice, which contains sugars and non-sugar compounds. Raw juice may be concentrated immediately after production by the removal of a water portion by evaporation. The resulting concentrated, non-purified beet juice, also known as raw, thick juice, has been a topic of research in past years from which limited literature has derived. Results from one study suggest that spraying the surface of raw, thick juice (with a solids weight fraction of approximately 67% and at pH 9) with formalin at 30 g m^{-2} results in microbial inhibition and enables complete sugar retention for up to 300 d at a 15 °C to 20 °C (Fiedler et al., 1993). Although formalin enabled sugar retention in raw, thick juice in the study described above, that chemical may not be a viable option to the ethanol industry as the effective antimicrobial levels may hinder post-storage yeast fermentation.

Vargas-Ramirez et al. (2013) evaluated the effects of acidic (2 to 5) and alkaline (8 to 11) pH in combination with solid weight fractions between 60% and 69% on sugar retention in raw, thick juice stored for 24 wk at 23 °C. Their experimental results indicate that sugar retentions of up to 99% can be achieved in raw, thick juice stored at combinations of $pH \leq 3.5$ or $pH \geq 9.5$

with solid weight fractions ≥ 64.5%. However, post-storage fermentability tests of samples stored under acidic and alkaline conditions resulted in fermentation efficiencies of < 82% and < 54%, respectively, relative to raw juice. For this reason, further experiments are necessary to improve post-storage fermentability of raw, thick juice.

Silage and its potential application in industrial-beet sugar storage

Ensilage is the process of storing a crop in an air tight container to enhance the natural establishment of an anaerobic environment that will result in the partial fermentation of crop sugar constituents. The fermented material is referred to as *silage* and is widely produced to preserve the nutritional value of a crop or its substrates for the subsequent production of biofuels. Literature regarding the ensilage of crops such as grasses, cereals, legumes, among others, is available (e.g., McDonald et al., 1991). KWS SAAT AG developed a patent in which it claims that the use of an ensiling agent containing primarily ammonium tetraformate and ammonium propionate at weight fractions of 60% to 90% and 10% to 30%, respectively, is effective in preserving total sugars in ensiled whole, defoliated beets (von Felde, 2012). Other ensiling techniques are briefly described in the same patent. For example, one technique consists of finely shredding the beets before treating them with sodium benzoate and subsequently ensiling them. As a requirement, the seepage produced during the ensilage process should be contained within the silo.

Cole and Bugbee (1976) suggested that bacteria resided in the internal tissue of beets and was the cause of sucrose inversion and loss during storage. Their results indicate that beets stored under air tight conditions for up to 21 d at 26 °C, depleted oxygen within the storage units within 24 h. After 21 d of storage, the stored beets had only retained from 2% to 5% of their initial sucrose and their invert sugar content had significantly increased. They hypothesized that

the decrease of a portion of sucrose and increase in invert sugars may had been associated with a pH drop throughout the storage period. After 21 d, beets under an oxygen-depleted atmosphere had reached a pH of approximately 3.8 and under acidic pH values sucrose is known to readily hydrolyze (Gabriel, 2008). Cole and Bugbee (1976) also observed a decrease in bacterial colonies in the beet tissue, which they mentioned could have been associated with a decrease in available sugars or pH. Acidic pH has an inhibitory effect on microbial growth starting at pH 5 and this effect is intensified as the pH decreases to more acidic values (Tewari, 2007). The findings by Cole and Bugbee (1976) and theory on pH effect on microbial growth may be used as the basis to establish sugar beet storage experiments based on ensilage techniques.

Parameters influencing silage quality

In order to produce a good-quality silage, the crop to be ensiled should be compacted tightly to reduce the void spaces within the bulk in the silo. Less void spaces will ensure that anaerobic conditions will be established soon after sealing the silo. The method of compaction to be used will depend on the crop to be ensiled. For example, for grasses, which are typically stored in bunker silos, the crop is unloaded within the silo with a front-end loader which is used to drive on top of the crop to achieve compaction. However, this method may not be adequate for crops with a high moisture content since a risk for machinery sinking would exist.

A successful ensilage process may be divided into four distinguishable phases: aerobic, lag, fermentation, and stable phases (Figure 1.9). Immediately after sealing a silo, the oxygen entrapped within it begins to be consumed by aerobic microbes present on the crop surface and also by plant respiration. If the silo is completely hermetic, an anaerobic environment is achieved within 1 d to 2 d in its interior. At this point, dormant anaerobic microbes become activated and a lag phase initiates in which the microbes begin adapting to the medium. Once adapted, microbes

begin reproducing and proliferating throughout the silage and at the same time fermenting available water soluble sugars; this marks the beginning of the fermentation phase. The fermentation process results in the production and accumulation of organic acids, primarily lactic, which decrease the pH of the silage to a value between 3.6 and 4.5, depending on the crop (Kung, 2000). This pH drop becomes a stress factor for the microbes and it eventually inactivates them permanently. At this point, it can be said that the silage has reached a stable phase which will remain throughout the storage period until the content in the silo is disturbed.

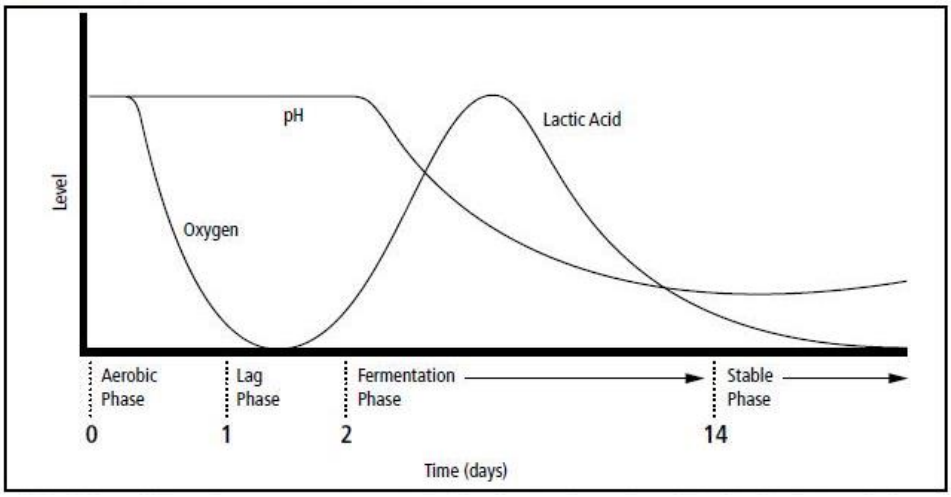

Figure 1.9. Distinguishable phases of a successful ensilage process (Source: Pitt, 1990)

An important parameter that influences the quality of silage is its moisture content. Crops may be harvested at different moisture levels which will result in either benefits or damages depending on the level and the crop. For example, a high-moisture-content silage (between 75% and 85%) will be more likely to become a favorable environment for the proliferation of clostridial bacteria which will result in the production of butyric acid (Schroeder, 2013), a problematic compound when silage is to be used as animal feed. A generalization of optimum moisture levels (and associated dry matter contents) for silage preparation is shown in Figure

1.10. Some of the common advantages and possible disadvantages of forage silage such as hay at different moisture levels is presented in Figure 1.11.

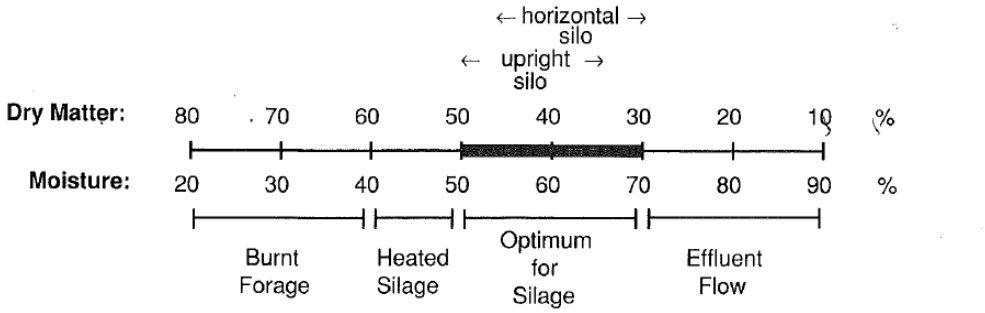

Figure 1.10. Optimum moisture levels and associated dry matter content for well-managed silage. (Source: Pitt, 1990)

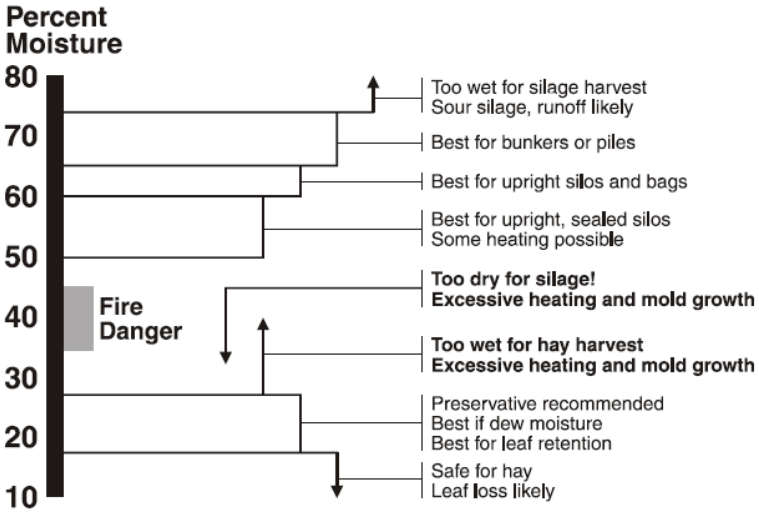

Figure 1.11. Advantage and possible disadvantages of different moisture levels in forage silage such as hay. (Source: Schroeder, 2013)

Silage additives

Crops for silage production may be treated with additives prior to ensiling to ensure a good-quality product. Additives may be divided into two categories based on their function: *stimulants* and *inhibitors*. Stimulants consist of either inoculants, enzymes, or sugars. Inoculants, such as homofermentative lactic acid bacteria, may be used to enhance the dominance of this type of bacteria in the silage. This bacteria would lead to a higher production rate and

accumulation of lactic acid and consequently a pH decrease that will stabilize the silage. Enzymes may also be added to the silage to hydrolyze a portion of the complex carbohydrates (e.g., cellulose) and increase the availability of water soluble carbohydrates as substrate for microbes. Sugars may also be used to directly supplement and enhance microbial metabolism and fast lactic acid production.

Agents that are inhibitory to certain microbes may also be used to reduce quality deterioration in silage. For example, propionic acid, which is known to have a great inhibitory effect on yeast and molds, in comparison to other chemical additives, is used to reduce deterioration of silage under initial aerobic conditions (Kung, 2000). Nevertheless, the high application rates of priopionic acid that are effective for microbial inhibition also hinder the growth of lactic acid bacteria in the silage. Additionally, this inhibitor is very corrosive which results in a high risk associated with its handling. On the other hand, acid salts such as ammonium propionate and ammonium tetraformate are less corrosive and their use has been common as constituents of buffered propionic acid mixes of $5.5 < pH < 6.0$ that may be added to crops before ensilage (Kung, 1995; Kung, 2000).

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CHAPTER 2. EFFECT OF STORAGE CONDITIONS ON INDUSTRIAL SUGAR RETENTION IN ENERGY BEETS[1]

Abstract

Energy beets could compete with corn grain as important industrial-sugar feedstocks for biofuels. However, long-term energy beet storage is necessary to maximize processing equipment use, and storage conditions may entirely differ from those established in the sugar industry. This work evaluated combined effects of surface treatment, temperature, and storage atmosphere on beet sugar retention. Initially, beets were dipped in solutions of either a senescence inhibitor (N^6-benzylaminopurine) or one of two antimicrobial agents (acetic acid and pHresh 10.0®) at weight fractions of 0.05% and 0.1%, and 0.1% and 1%, respectively. Beets were then stored for up to 36 wk either under aerobic conditions or in sealed containers, at 6 °C or 25 °C. Surface treatment did not show a statistically significant effect on sugar retention. Aerobic storage at 25 °C enabled retention of initial beet sugars due to dehydration caused by low relative humidity (37%) in air. In contrast, aerobic storage at 6 °C enabled sugar retention for 24 wk; however, sugar retention decreased sharply thereafter to 56%. This decrease coincided with mold appearance on beet surfaces. Beets stored in sealed containers at both temperatures retained 38% of initial sugars. Increasing surface area to better incorporate preservatives into beet tissue could improve long-term sugar retention.

[1] Chapter 2 consists of a scientific manuscript that was approved for publication in April 2015 by the journal of Biofuels Engineering. Authors: Juan M. Vargas-Ramirez and Dennis P. Wiesenborn. Juan M. Vargas-Ramirez designed and conducted the experiments in this work, and is the first author of the manuscript. Co-author Dennis P. Wiesenborn provided advice throughout the work and assisted in the editing.

Introduction

In recent years, new sugarbeet (*Beta vulgaris* L.) varieties have been envisioned as a potential alternative to corn grain as an important source of industrial sugars. These varieties are referred to as *energy beets* and have been bred primarily for higher sugar yield per unit area as compared to beets for table sugar production, which are bred for higher sucrose content. Since 2010, energy beet yield trials have been conducted in the North Central U.S., where climatic conditions are among the most favorable for sugarbeet growth in North America (Cattanach et al., 1992). Energy beets grown under non-irrigated conditions in North Dakota have yielded approximately 13.3 Mg ha^{-1} of sugars in hexose equivalents (NDSU Carrington REC, 2013). In contrast, sugarbeet for table sugar and corn yielded 11.3 Mg ha^{-1} (USDA-ERS, 2014a) and 6.4 Mg ha^{-1} (Shapouri and Salassi, 2006; USDA-ERS, 2014b) of sugars in hexose equivalents, respectively, in 2014.

Beet industrial sugars are primarily sucrose, glucose, and fructose, with sucrose constituting 15% to 20% of the fresh beet weight, and both glucose and fructose representing only trace amounts of less than 0.1% (Asadi, 2007; PSU, 2010; NDSU Carrington REC, 2013). These sugars may be readily fermented by microorganisms into important bioproducts, including advanced biofuels (Vargas-Ramirez et al., 2013). Stochastic simulations have already shown that sugarbeet may be an economically feasible feedstock for a 0.076 hm^{-3} y^{-1} ethanol plant in North Dakota (Maung and Gustafson, 2011). Furthermore, sugarbeet is a commercial source of betaine (Craig, 2004), while beet pulp has significant value as livestock feed (Boucque et al., 1976) and is a potential feedstock for other bioproducts such as biodegradable thermoplastics (Liu et al., 2005).

Despite the great potential of energy beets to become a competitive industrial-sugar feedstock in the U.S., these have a high moisture content, which could constitute a challenge for long-term storage in beet-growing regions across the nation. Freezing halts sugarbeet-cell and microbial activities, thereby preventing sucrose inversion and accumulation of respiration and fermentation products in the beet tissue. Thus, freezing ensures satisfactory raw juice purification and sucrose crystallization efficiencies in the beet sugar industry (Asadi, 2007). Harsh winter conditions in the Red River Valley of the North Central U.S. allow natural freezing of sugarbeet. Here, defoliated sugarbeet is stored frozen for up to 180 d in piles on remote sites between fields and beet sugar processing facilities (McGinnis, 1996; van der Poel et al., 1998; Asadi, 2007). However, hot spots can occur within the piles creating favorable environments for microbial activity, which can yield significant sugar losses (McGinnis, 1996; Asadi, 2007). Additionally, freezing enhances cell wall rupture leaving cell contents, including sugars, susceptible to leaching during beet washing and fluming into the factory. Other beet-growing regions in the U.S., characterized by warmer climates, can only store freshly-harvested beets in small piles for significantly shorter periods to avoid substantial sugar loss. For example, in southern California (a Mediterranean-climate region) and northeastern Colorado (a temperate-climate region) the recommended maximum storage times for beets are 20 h (McGinnis, 1996) and 6 wk (van der Poel et al., 1998), respectively.

Without freezing, stored sugarbeet maintains metabolic processes, such as respiration, that convert sugars into energy to repair tissue wounded during harvest and handling, and to increase defense against pathogens (Campbell and Klotz, 2006). The major cause of sucrose loss in freshly-harvested beets is respiration (Klotz-Fugate et al., 2010), which may consume up to 80% of initial sucrose content in beets stored for 112 d at 5 °C (Wyse and Dexter, 1971).

Modified, controlled storage atmospheres and application of chemical solutions to the beet surface have been evaluated as alternatives to frozen storage in an attempt to reduce respiration and microbial activity, and consequent sugar loss in freshly-harvested beets. Karnik et al. (1970) reported that a modified, controlled atmosphere composed of 5% oxygen (O_2), 6% carbon dioxide (CO_2), and 89% nitrogen (N_2), helped retain up to 87% of initial sucrose content in sugarbeet stored for 200 d at 2 °C. Fungal growth on beet surface and sprouting were also significantly reduced under the same storage conditions, as compared to control beets. Wu et al. (1970) reported that freshly-harvested beets dipped for 1 h in aqueous solutions of N^6-benzylaminopurine (a senescence inhibitor) and Randox® (a herbicide) at 500 mg kg^{-1}, and stored for up to 182 d at 10 °C, retained 84% and 85% of their initial sucrose content, respectively. These two chemicals were most effective in reducing sucrose loss among six chemicals tested. In the same study, beets dipped in water (control) lost about 23% of their initial sucrose content. Other chemicals, such as acetic acid and acidic calcium sulfate, have been used to prevent postharvest decay of fruit. For example, vapors of aqueous solutions of acetic acid at volume fractions between 4.2% and 6% prevented decay caused by gray and blue mold in apples incubated for 5 d to 7 d at 20 °C (Sholberg et al., 2000). Litchi fruit dipped for 2 min in solutions of acidic calcium sulfate at volume fractions > 2.5% showed no signs of mold after storage for 15 d at either 5 °C or 10 °C (Wang et al., 2010). However, neither of these chemicals has been previously tested on beets; hence, optimum concentrations to prevent microbial growth may be completely different than those found in the studies mentioned above.

Storage techniques with minimal risks of sugar loss (as compared to frozen pile storage), that could also be adopted by regions with warm climates, are necessary to achieve long-term industrial sugar retention in energy beets. These techniques would allow yearlong operation of

industrial facilities to convert energy beets into useful bioproducts such as biofuels. The main objective of this research work was to determine combined effects of surface treatment, temperature, and storage atmosphere on industrial sugar retention in beets stored for up to 36 wk. Combinations of these storage techniques may result in additive effects that could favor sugar retention in beets throughout long-term storage.

Materials and methods

Sugarbeet – collection and preparation

Approximately 10 Mg of sugarbeet (*Beta vulgaris* L.) taproots were obtained from a storage pile adjacent to American Crystal Sugar Co. (Moorhead, MN, USA; 46° 54’ 0.90” N 96° 45’ 44.94” W) towards the end of the 2012 growing season. Beets delivered to these storage piles are harvested within an 80-km radius from the factory. Beets were stored outdoors for less than 5 d between 4 °C and 10 °C before preparation for the storage experiments. Beet preparation consisted of sorting and discarding roots with signs of spoilage and severe tissue wounds caused by harvesting equipment and handling. Beets that met the sorting requirements were washed in Great Western batch washers (McGinnis, 1996) for 2 min to remove soil. Washed beets were immediately packed in perforated, plastic bags (34.3 cm × 71.1 cm) and stored for less than two weeks at 6 ± 1 °C and 89 ± 11% relative humidity (RH) prior to the experimental setup.

Experimental design

Initially, a subset of seven replicates containing eight beets per replicate was analyzed for sugar content according to the sugar extraction and quantification methods described later. The sugar content in these beets was used as a baseline (i.e., initial sugar content in all beets going into storage).

Aqueous solutions of three preservatives were prepared and applied independently to the beet surface, each at two concentrations. Control samples consisted of beets treated with tap water following the application procedure described below. Surface-treated beets were stored for 2, 4, 12, 24, and 36 wk either aerobically or in sealed containers with an initially modified storage atmosphere, and at either 6 ± 1 °C and 89 ± 11% RH or room temperature (25 ± 2 °C) and 37 ± 13% RH. This resulted in 28 independent treatment combinations prepared in triplicate for each of the five storage times. Thus, a total of 420 experimental units were prepared and analyzed throughout the 36-wk period.

Beet surface treatment

The three preservatives evaluated in this study consisted of one senescence inhibitor and two antimicrobial agents, which were applied independently to the beet surface. The senescence inhibitor was N^6-benzylaminopurine (Caisson Laboratories; North Logan, UT, USA) and the antimicrobial agents were acetic acid and pHresh 10.0® (pHresh Technologies; Sabetha, KS, USA). pHresh 10.0® is a commercial product that contains 40% to 50% acidic calcium sulfate as its active ingredient. Solutions of the senescence inhibitor were prepared at weight fractions of 0.05% and 0.1%; whereas, solutions of the antimicrobial agents were prepared at weight fractions of 0.1% and 1%. Each experimental unit consisted of 8 beets which were randomly packed in mesh bags (56 cm × 46 cm, ULINE; Eagan, MN, USA). The mesh bags containing beets were submerged in pairs for 5 min in four 68-L tote boxes, each initially containing 18.2 kg of the solutions described above; the solutions were not replaced throughout the dipping step. Subsequently, dipped beets were placed on wood pallets and allowed to drain, and their surface was dried for 15 min with room air (25 ± 2 °C, 37 ± 13% RH) assisted by box fans. Beets used as the experimental control were submerged for 5 min in tap water and also air-dried.

Storage methods

After air-drying, beets to be stored under aerobic conditions (ambient air) were left in mesh bags and weighed before storage. Beets stored under aerobic conditions at 6 ± 1 °C or 25 ± 2 °C were exposed to a relative humidity of 89 ± 11% or 37 ± 13%, respectively.

Beets to be stored in sealed containers with an initially-modified atmosphere were packaged in modified 19-L high-density polyethylene pails with lids. One of the modifications to the pails consisted of mounting a 0.64-cm barbed polypropylene check valve (Ark-Plas Products, Inc.; Flippin, AR, USA) with a cracking pressure of 3.45 kPa, to the base to relieve any pressure exerted by respiration and fermentation gases produced by beets throughout storage. Also, a 1.27-to-0.95-cm National Pipe Tapered Thread (NPT) polyethylene hose reducer with a cap on the narrow end, and a polyethylene tee fitting with a 1.3-cm NPT and two 0.64-cm barbs, were mounted on the outer side of the lids of the pails. Galvanized hardware cloth, with 1.3-cm mesh and overall dimensions of 21.6 cm × 30.5 cm, was used to hold the beets above any exudate produced during storage. A diagram of a sealed storage unit and an image of an actual unit are presented in Figures 2.1a and 2.1b. The tee fitting was used as an inlet for the purge gas before storage and as a storage-atmosphere sampling port after storage. The low end of the tee fitting was sealed with a Suba-Seal® septum (Sigma-Aldrich Co.; St. Louis, MO, USA) before flushing the storage units. The storage atmosphere was modified by flushing the pails with a gas mixture consisting of CO_2, O_2, and N_2 at volume fractions of 6%, 5%, and 89% (Matheson; New Brighton, MN, USA), respectively, introduced through the high end of the tee fitting. The check valve was removed during the flushing step to allow free gas flow within the pails. Each pail was flushed for 4 min and the check valve was subsequently replaced and the purge-gas inlet sealed with a Suba-Seal® septum. Immediately after flushing, the storage atmosphere was analyzed as

described in the subsection below. The experimental units were weighed and stored at either 6 ± 1 °C or 25 ± 2 °C for up to 36 wk. At the end of each storage period, the storage atmosphere within the units was analyzed and the barbed hose reducer was used to drain exudate released by the beets throughout storage.

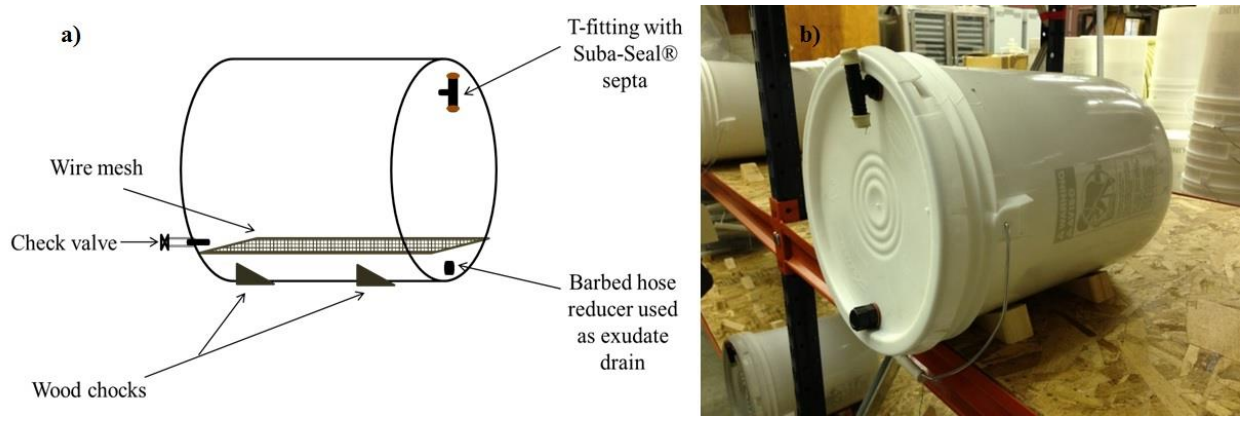

Figure 2.1. (a) Schematic diagram showing the various features of sealed storage units, and (b) an actual sealed storage unit.

Analytical methods

Experimental units were stored in triplicate for up to 36 wk and sacrificed after each storage period for sample analyses. Before collecting beet-tissue samples, each experimental unit was weighed to quantify moisture lost during storage. Also, before tissue sampling, the atmosphere of each sealed storage unit was characterized using a Pac Check® 325 hand-held headspace analyzer capable of quantifying O_2 and CO_2 (MOCON; Minneapolis, MN, USA). The headspace analyzer was calibrated before analysis with ambient air using the Cal-Smart option, according to manufacturer's specifications. Ambient air samples were analyzed after every five experimental units to verify and ensure accurate readings.

Beet-tissue samples were collected at specified time intervals (2, 4, 12, 24, and 36 wk), by drilling each beet with a power drill equipped with a 1.6-cm spade bit. Drilling was initiated below the beet crown and proceeded in transversal direction extending toward the root tip.

Samples were immediately placed in individual Ziploc® bags (S.C. Johnson & Son, Inc., Racine, WI, USA), mixed thoroughly by inverting each bag several times, and frozen overnight in a chest freezer (at about -25 °C) to enhance the rupture of cells and facilitate sugar extraction. Each sample was processed following the cold digestion method for extraction and determination of sugar in cossettes (Asadi, 2007). The pH of resulting extracts was adjusted to a value between 6 and 8, using calcium carbonate, to comply with method recommendations for HPLC analyses. The moisture weight fraction of each sample (on a wet basis) was determined by oven-drying between 9 g and 12 g of beet tissue in aluminum weighing dishes at 105 °C for 24 h. The moisture loss of each sample was calculated as:

$$Moisture\ loss\ (\%) = \left(1 - \frac{x(t) \cdot M(t)}{x_0 M_0}\right) \times 100 \qquad \text{(Eq. 2.1)}$$

where $x(t)$ and x_0 are the moisture fractions in beet tissue as a function of time and at the start of storage, respectively, and $M(t)$ and M_0 are the beet tissue masses as a function of time and at the start of storage, respectively. Moisture content in the tissue versus time was fitted to a first-order kinetic model of the form:

$$x(t) = x_0 \cdot e^{-k \cdot t} \qquad \text{(Eq. 2.2)}$$

where k is the moisture change rate constant in units of wk^{-1} and t is time in wk. This rate constant was determined and used to describe rate of moisture content change.

The concentrations of industrial sugars (defined here as sucrose, glucose, and fructose) extracted from beet-tissue samples were determined by HPLC at the beginning of the experiment and at the specified time intervals, as described by Vargas-Ramirez et al. (2013). Exudate samples were diluted with 18.2-MΩ·cm water in a volume ratio of 1:3 and the pH of the resulting solutions adjusted with a 7 mol L^{-1} NaOH solution to values between 6 and 8, per method recommendations for HPLC analyses. Sugar concentrations in exudate samples were also

determined following the same method. Ethanol concentration in beet tissue was determined directly on extracts used for sugar analyses, following the HPLC method described by Vargas-Ramirez et al. (2013). Ethanol concentration in exudate solutions was determined in the same manner. The industrial sugar and ethanol concentrations in the beet tissue and exudate were translated to weight of hexose equivalents per weight of beet tissue (on a wet basis). This translation was done assuming a complete hydrolysis of sucrose into glucose and fructose, and that ethanol present in the samples resulted from the fermentation of hexoses at a maximum theoretical ethanol yield. Weight fractions of hexose equivalents were then combined to obtain overall sugar concentrations. Initial and final sugar quantities were estimated as the products of initial sugar concentration and initial beet weight (both on a wet basis), and final sugar concentration and final beet weight (both on a wet basis), respectively. Furthermore, sugar retention throughout storage was estimated as:

$$Sugar\ retention\ (\%) = \left(\frac{FHE}{IHE}\right) \times 100 \qquad \text{(Eq. 2.3)}$$

where *FHE* and *IHE* represent final and initial sugar quantities in hexose equivalents (given in consistent weight units), respectively.

Statistical analysis

SAS software (Version 9.4 - SAS Institute Inc.; Cary, NC, USA) was used for all statistical analyses. The destructive nature of the sugar extraction method followed for initial (baseline) and final sugar quantification in beet tissue rendered the use of different beets necessary. Hence, two-sample *t*-tests were conducted to compare means of initial and final sugar quantities in beets stored for up to 36 wk. Sugar retention as a result of storage time and temperature combinations was fitted to a cell means model for beets stored under aerobic conditions and in sealed containers. Furthermore, Tukey's studentized range test (Falk et al.,

2002) was used to compare multiple sugar retention means as a result of storage time and temperature combinations; the significance level for the tests was set to $\alpha = 0.05$. All graphical representations of data were generated in SAS using GPLOT, SGPLOT, and GCHART procedures.

Results and discussion

Storage under aerobic atmosphere

The combined effects of surface treatment, temperature, and storage atmosphere on industrial sugar retention in sugarbeet was evaluated. The goal was to minimize sugar loss during a 36-wk storage period. In general, beets stored aerobically at 6 °C had a significant sugar loss as indicated by $P < 0.05$ from the corresponding two-sample *t*-tests (Table 2.1). On the other hand, sugar quantities in beets stored at 25 °C appeared to have increased, which is difficult to justify since beet sugars cannot increase during storage. However, two-sample *t*-tests indicated no statistically significant differences between initial and final sugar quantities in these beets (Table 2.1).

Table 2.1. Two-sample *t*-test comparisons of initial and final sugar quantities[a] in beets stored 36 wk under aerobic atmosphere.

	6 °C		25 °C	
Surface treatment	Initial (g)	Final (g)	Initial (g)	Final (g)
Control	1110 ± 120	690 ± 90 *	1230 ± 470	1440 ± 360
0.05% BAP[b]	1050 ± 180	490 ± 70 *	690 ± 160	800 ± 170
0.1% BAP[b]	850 ± 100	570 ± 140*	800 ± 70	930 ± 80
0.1% AA[c]	990 ± 250	600 ± 130	1040 ± 100	1290 ± 120
1% AA[c]	1120 ± 190	470 ± 180*	990 ± 110	1140 ± 200
0.1% pHresh[d]	980 ± 30	560 ± 60 *	970 ± 100	1050 ± 140
1% pHresh[d]	1220 ± 150	660 ± 50 *	1020 ± 10	1180 ± 120

* indicates significant difference, relative to initial sugar quantity, at $\alpha = 0.05$.
[a]Sugar quantities are given in hexose equivalents as mean ± standard deviation ($n = 3$) on a dry basis. Initial sugar quantities were calculated using the baseline sugar concentration (dry basis) and dry weight of samples prior to storage.
[b]BAP: N^6-benzylaminopurine; [c]AA: acetic acid; [d]pHresh: pHresh 10.0®

Figure 2.2 shows the industrial sugar retention in the same beets. Sugar retention means that were lowest within each temperature group are encompassed by dashed rectangles in Figure 2.2. Surface treatment effects on sugar retention were not consistent among storage temperatures. Moreover, sugar retentions for beets that received surface treatments were not significantly different than retentions of respective controls when stored at 6 °C (P = 0.174) and 25 °C (P = 0.345). Hence, under the conditions evaluated in this experiment, surface treatments did not have a significant effect on sugar retention during storage. For this reason, subsequent statistical analyses ignored the effect of surface treatments by merging data with respect to temperature and storage periods.

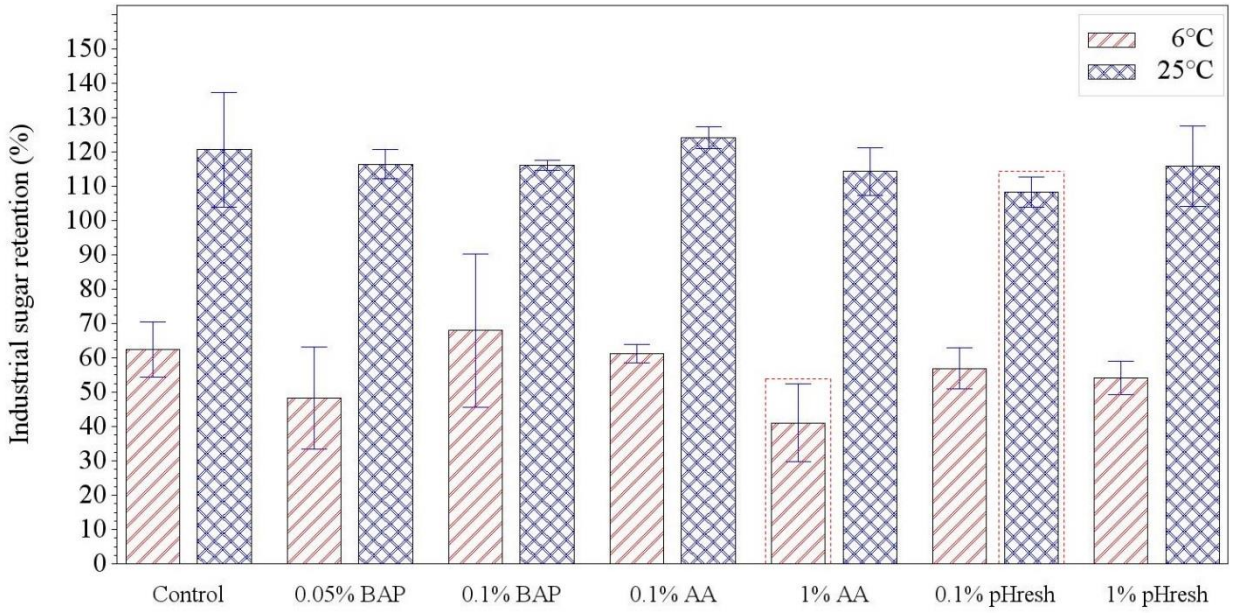

Figure 2.2. Sugar retention (mean ± standard deviation of three replicates) in beets that received a surface treatment and control beets, which were stored 36 wk under aerobic atmosphere. Lowest means within each temperature group are encompassed by dashed rectangles.
NOTE: BAP: N^6-benzylaminopurine; AA: Acetic Acid; pHresh: pHresh 10.0®.

The initial moisture weight fraction in beets (prior to storage) was 73 ± 1%. After 36 wk, beets stored under aerobic atmosphere and at 25 °C had lost 98 ± 1% of their initial moisture fraction (Figure 2.3). On the other hand, beets stored for the same time period under the same

atmosphere and at 6 °C lost only 77 ± 4% of their initial moisture fraction. The contrasting moisture losses were a result of the very different relative humidities in each storage space. The storage space at 25 °C had a relative humidity of 37 ± 13% which enhanced the dehydration of beets throughout the storage period, yielding a first-order moisture change rate constant of 0.066 wk^{-1} ($P < 0.0001$). In contrast, the cold room (at 6 °C) had a relative humidity of 89 ± 11%, which yielded a much smaller first-order moisture change constant of 0.018 wk^{-1} ($P < 0.0001$).

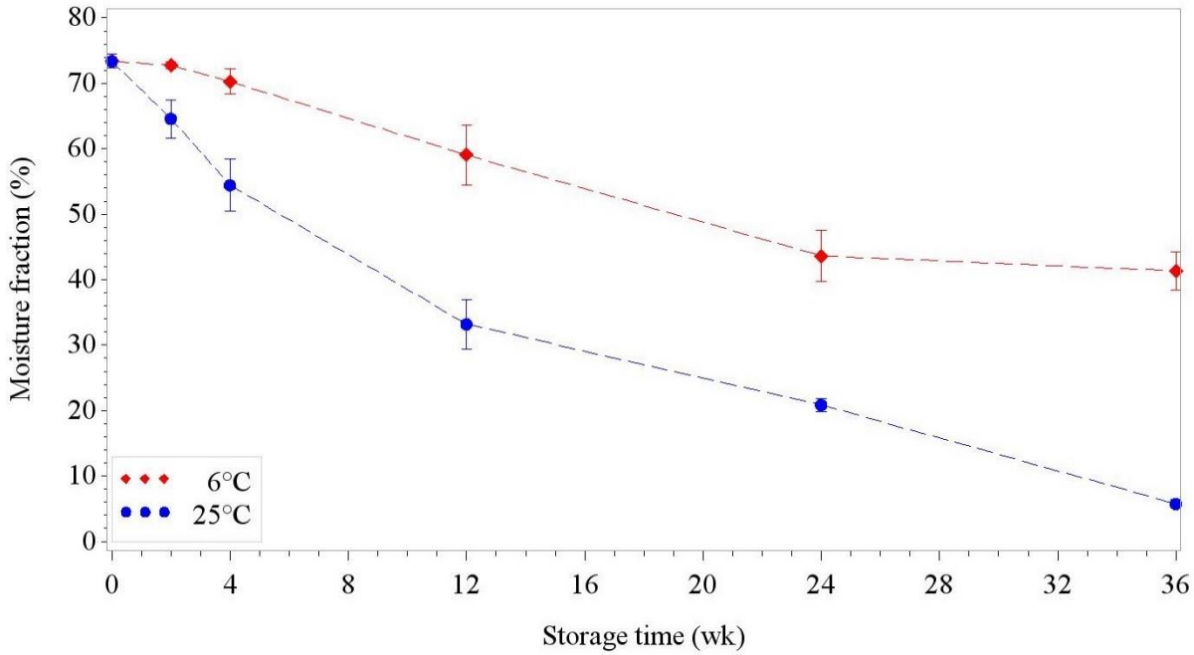

Figure 2.3. Moisture fraction (on a wet basis, and as mean ± standard deviation of 21 replicates) in beets stored for up to 36 wk under aerobic atmosphere.

Beets stored at 6 °C retained up to 100% of their initial sugar content ($P = 0.134$) for up to 24 wk (Figure 2.4). An abrupt sugar drop occurred after 24 wk, which coincided with the appearance of significant mold on beet surfaces, and sugar retention fell to 56 ± 13% of initial sugars by week 36. In contrast, as reported above, beets stored under ambient temperature and an associated relative humidity of 37% did not show a decrease in industrial sugars over the 36-wk storage period (Figure 2.4). Instead, sugar retention appeared to increase to 116 ± 8% after 36 wk

of storage; however, an increase in sugars is not possible. Further statistical analyses using Tukey's studentized range test indicated that the initial and final sugar quantities of these beets was not statistically different (Figure 2.4). Hence, it was concluded that, under these conditions, industrial sugars were adequately retained throughout the storage period. Successful retention may have been a result of beet tissue dehydration and an associated decrease in water activity. However, despite a successful retention of industrial sugars, these dehydration conditions are not economically feasible for conventional storage piles, which typically contain thousands of tons of beets. Although such a technique may not be viable at industrial scale, it may be useful when conducting smaller experiments, such as at a pilot scale.

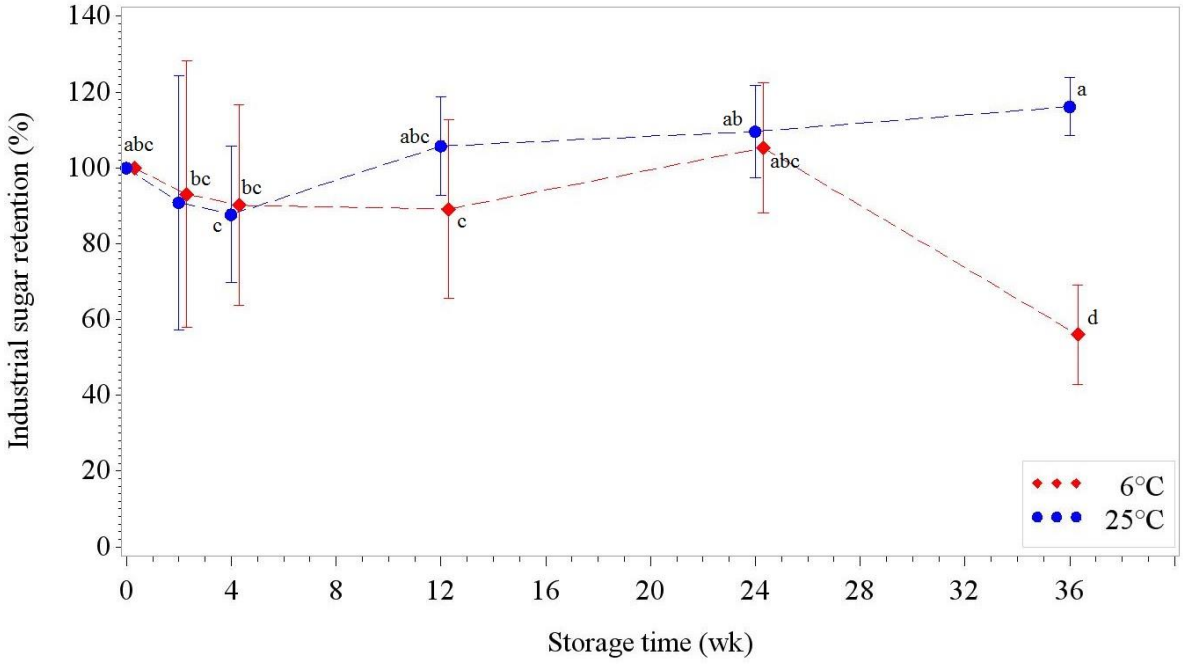

Figure 2.4. Industrial sugar retention (mean ± standard deviation of 21 replicates) in beets stored for up to 36 wk under aerobic atmosphere. Letters represent group means of a Tukey's studentized range test; means with the same letter are not statistically different from each other at $\alpha = 0.05$. NOTE: Jittering was used on the time axis to prevent overlapping standard deviations.

Storage in sealed containers

After flushing the storage units with the gas mixture, the initial storage atmosphere consisted of 5.5 ± 0.1% O_2, 5.1 ± 0.1% CO_2, and the remainder nitrogen. The initial presence of

oxygen and the high moisture in the beet tissue constituted a favorable environment for mold growth observed on the beet surface. Moreover, beets stored in sealed containers were isolated from the effect of relative humidity in air and, hence, had greater surface moisture in comparison to beets stored under aerobic conditions. This intensified mold proliferation on the beet surface early into the storage period. Additionally, unlike beets stored under aerobic conditions, beets stored in sealed containers exuded a liquid substance that was collected for further analysis. This exudate was rich in microbial fermentation products, including ethanol, and also contained some residual sugars. Ethanol and residual sugars in the exudate were translated to hexose equivalents and counted as part of total sugars retained during storage.

Bacteria and yeast colonies have been isolated from fresh internal sugarbeet tissue (Bugbee et al., 1975) and partly associated with a decrease of over 50% of initial sucrose in beets stored anaerobically at 26 °C for 21 d (Cole and Bugbee, 1976). This suggested the presence of fermentation products in the beet tissue and, hence, ethanol was quantified. Besides hexose equivalents from ethanol and residual sugars in exudate, hexose equivalents from ethanol in the beet tissue were also taken into account when estimating final sugar quantities. Even after accounting for residual sugars in exudate, and ethanol in exudate and tissue, nine out of twelve treatment combinations showed a statistically significant sugar loss after 36 wk of storage, by means of two-sample *t*-tests (Table 2.2). The other three treatment combinations showed variances sufficiently large for *t*-tests to indicate that sugar losses were not statistically significant, though all showed a marked decrease.

The ethanol in the beet tissue accounted for 23% to 54% of final sugars in beets stored at 6 °C, and 44% to 80% of final sugars in beets stored at 25 °C (Table 2.3). Thus, this ethanol substantially contributed to hexose equivalents retained during storage. Also, the exudate

collected from the storage containers was equivalent to 3% to 5% and 5% to 9% of initial fresh weight of beets stored at 6 °C and 25 °C, respectively. These exudate fractions represented 3% to 9% and 8% to 22% of hexose equivalents retained in beets stored at 6 °C and 25 °C, respectively (Table 2.3). In general, hexose equivalents from residual sugars and ethanol in the exudate accounted for a smaller portion of final sugars, as compared to those from ethanol in the tissue. Nonetheless, their contribution should not be ignored as it influences overall ethanol potential from beets stored under these conditions.

Table 2.2. Two-sample *t*-test comparisons of initial and final sugar quantities[a] in beets stored 36 wk in sealed containers.

	6 °C		25 °C	
Surface treatment	Initial (g)	Final (g)	Initial (g)	Final (g)
Control	710 ± 250	280 ± 140	770 ± 90	350 ± 110*
0.05% BAP[b]	910 ± 340	290 ± 90 *	810 ± 10	370 ± 50 *
0.1% BAP[b]	720 ± 110	220 ± 30 *	660 ± 40	260 ± 40 *
0.1% AA[c]	930 ± 370	390 ± 140	870 ± 110	270 ± 50 *
1% AA[c]	990 ± 130	350 ± 20 *	990 ± 180	460 ± 60 *
0.1% pHresh[d]	1030 ± 360	520 ± 320	920 ± 370	750 ± 590
1% pHresh[d]	730 ± 120	280 ± 90 *	770 ± 40	300 ± 110*

* indicates a significant difference, relative to initial sugar content, at $\alpha = 0.05$.
[a]Sugar quantities are given in hexose equivalents and as mean ± standard deviation ($n = 3$) on a dry basis. Initial sugar quantities were calculated using the baseline sugar concentration (on dry basis) and dry weight of samples prior to storage. Final sugar quantities include contribution of residual sugars in exudate and ethanol in beet tissue and exudate, given in hexose equivalents.
[b]BAP: N^6-benzylaminopurine; [c]AA: acetic acid; [d]pHresh: pHresh 10.0®.

Sugar retentions in beets stored in sealed containers at 6 °C and 25 °C, typically ranged from 30% to 50% (Figure 2.5). Sugar retention means were not significantly different for beets stored at 6 °C ($P = 0.181$) and at 25°C ($P = 0.212$). In fact, sugar retentions of beets stored at both, 6 °C and 25 °C, were also not significantly different between temperature groups at the end of the 36-wk storage period ($P = 0.054$). Thus, none of the surface treatments showed a significant effect on sugar retention in beets stored at either temperature. Hence, combining data

for all six treatments at both temperatures, beets stored 36 wk in sealed containers retained, on average, 38 ± 13% of initial sugars. Such retention was significantly lower as compared to that of beets stored aerobically at 25 °C, which retained up to 100% of initial sugars.

Table 2.3. Contribution of ethanol in tissue, and sugars and ethanol in exudate, to final sugar quantities in beets stored 36 wk in sealed containers.

	6 °C		25 °C	
Surface	Tissue	Exudate	Tissue	Exudate
Treatment	Ethanol[a] (%)	Sugars + Ethanol[a] (%)	Ethanol[a] (%)	Sugars + Ethanol[a] (%)
Control	35 ± 28	5 ± 5	67 ± 4	10 ± 1
0.05% BAP[b]	35 ± 19	4 ± 2	58 ± 1	19 ± 5
0.1% BAP[b]	30 ± 5	5 ± 3	44 ± 4	17 ± 3
0.1% AA[c]	29 ± 7	5 ± 3	68 ± 5	22 ± 9
1% AA[c]	30 ± 11	9 ± 3	62 ± 5	12 ± 3
0.1% pHresh[d]	54 ± 23	6 ± 1	80 ± 18	8 ± 6
1% pHresh[d]	23 ± 5	3 ± 2	44 ± 10	10 ± 4

[a]Fraction of final sugar quantity in hexose equivalents, given as mean ± standard deviation (n = 3).
[b]BAP: N^6-benzylaminopurine; [c]AA: acetic acid; [d]pHresh: pHresh 10.0®.

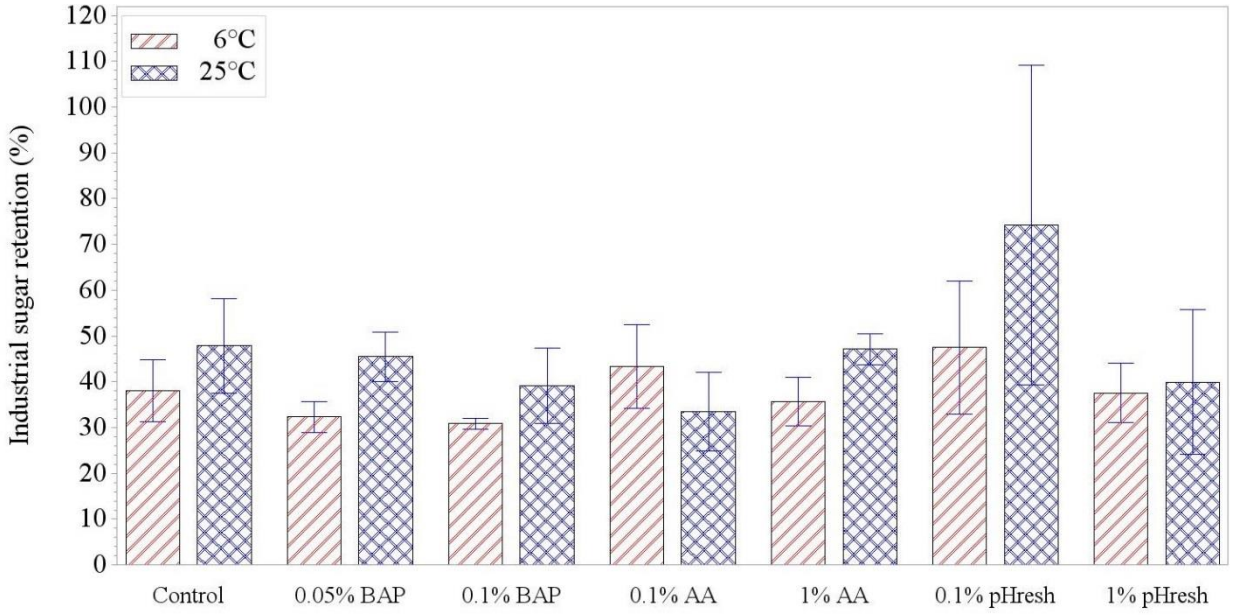

Figure 2.5. Sugar retention (mean ± standard deviation of three replicates) in beets that received a surface treatment and control beets, which were stored 36 wk in sealed containers.
NOTE: BAP: N^6-benzylaminopurine; AA: Acetic Acid; pHresh: pHresh 10.0®.

Since surface treatments did not show a statistically significant effect on sugar retention, subsequent statistical analyses ignored the effect of such treatments by merging data with respect to temperature and storage periods. The O_2 in units at 6 °C dropped slowly and was depleted by week 24. Moreover, the atmosphere in these units showed a steady increase in CO_2 throughout the storage period, reaching a concentration of 36 ± 7% by the end of storage. In contrast, the O_2 concentration in the storage atmosphere in units at 25 °C dropped sharply to 0.3 ± 0.1% by week 2, while CO_2 increased to 64 ± 12% (Figure 2.6). By week 4, this atmosphere had evolved to reach a maximum CO_2 concentration of 82 ± 10%. After this point, the CO_2 concentration unexpectedly began to drop and a gradual increase in oxygen was detected. A thorough inspection of storage units showed that the seal septa placed on the tee fittings in those units had cracked due to dryness caused by the low relative humidity of air, allowing air to leak into the units.

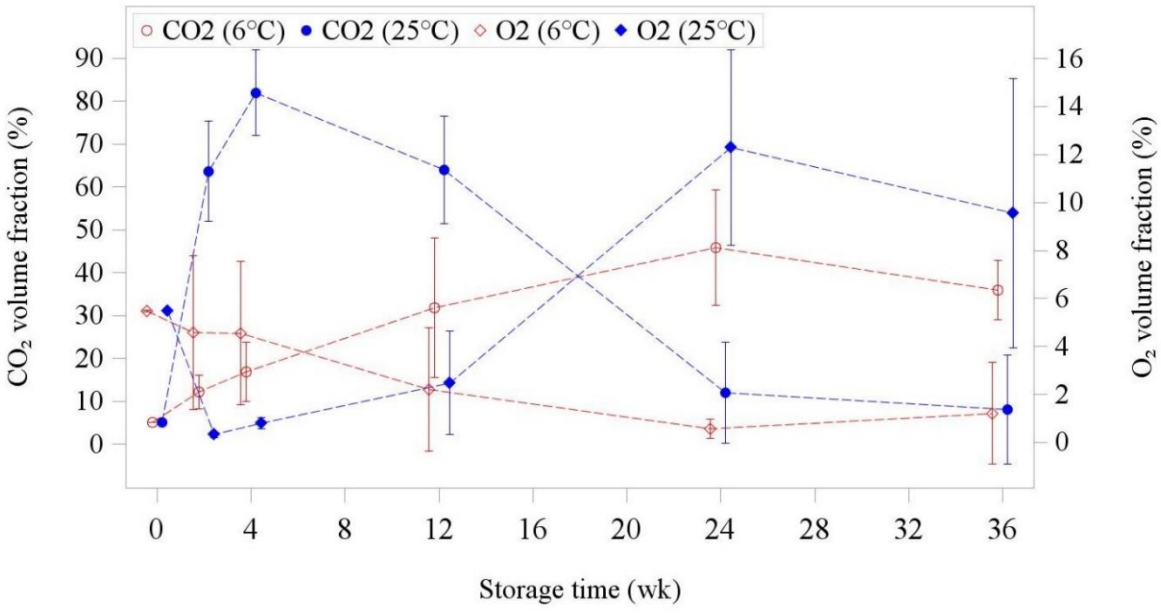

Figure 2.6. Carbon dioxide and oxygen concentrations (given as mean ± standard deviation of 21 replicates) in storage atmospheres in sealed units kept at 6 °C and 25 °C for up to 36 wk. NOTE: Gas volume fractions cannot take negative values in the cases where standard deviations extend below zero. Jittering was used on the time axis to prevent overlapping standard deviations.

In comparison to beets stored aerobically, beets stored in sealed containers showed an increase in moisture content throughout storage (Figure 2.7). Beets stored at 25 °C showed an increase of 9 ± 2% in moisture content by week 12. This increase was adequately described by a first-order kinetic model with a rate constant of 0.015 wk^{-1} ($P < 0.0001$). After 12 wk, the moisture content of these beets remained unchanged ($P = 0.856$). In contrast, beets stored at 6 °C showed an increase of 5 ± 3% in moisture content by the end of the 36-wk storage period. That increase occurred gradually, according to a first-order kinetic model with a rate constant of 0.003 wk^{-1} ($P < 0.0001$), throughout the storage period, and coincided with O_2 depletion and CO_2 appearance in the atmosphere within those storage units. Such changes were most likely caused by beet and microbial respiration (processes that require oxygen), and subsequent microbial fermentation, with all of these processes utilizing sugars and yielding CO_2, water, and other volatile liquid products. Moreover, unlike beets stored aerobically, these beets were isolated from the dehydrating effect of low relative humidity, which allowed for the accumulation of these respiration and fermentation products in the beet tissue.

Beets stored at 6 °C retained > 80% of their sugars during the first 24 wk and sugar retention decreased more rapidly thereafter (Figure 2.8). By week 36, these beets had retained only 37 ± 8% of sugars. On the other hand, beets stored at 25 °C retained > 80% of their sugars for only up to four weeks, decreasing sharply thereafter to reach a plateau by week 12 (Figure 2.8). This stability was likely a result of the accumulation of ethanol and other fermentation products in the beet tissue, which halted microbial activity. By week 36, only 46 ± 16% of sugars remained in beets stored under these conditions. Overall, refrigeration provided an advantage over room temperature for up to 24 wk of storage (Figure 2.8). However, no significant difference in sugar retentions between beets stored at 6 °C and 25 °C was detected at 36 wk.

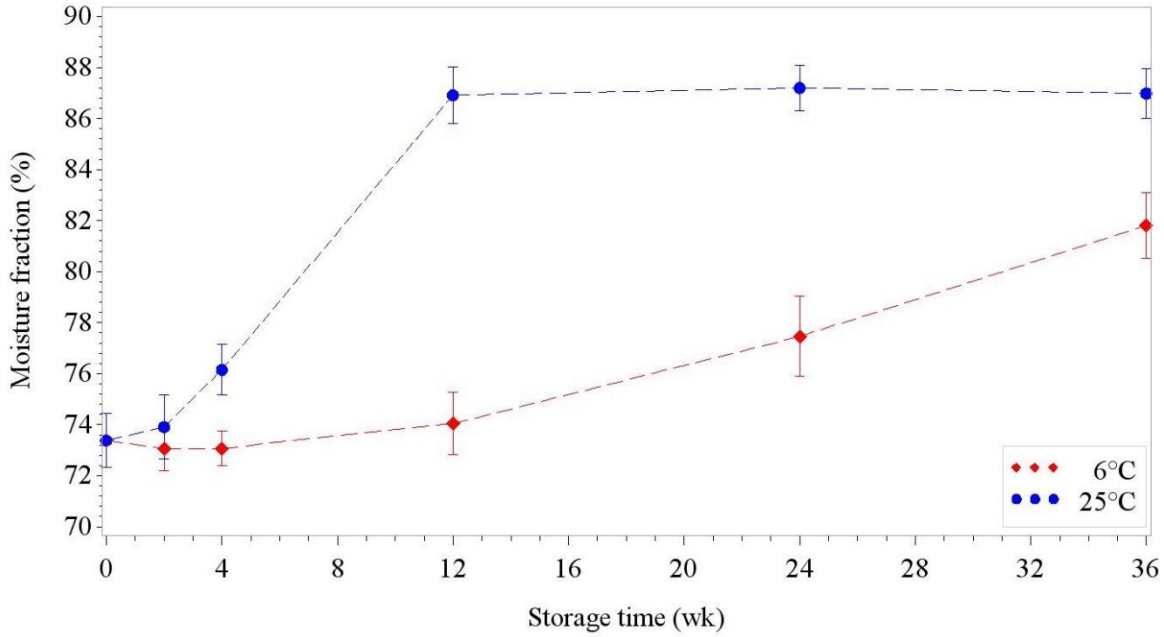

Figure 2.7. Moisture fraction (on a wet basis, and as mean ± standard deviation of 21 replicates) in beets stored for up to 36 wk in sealed containers at 6 °C and 25 °C.

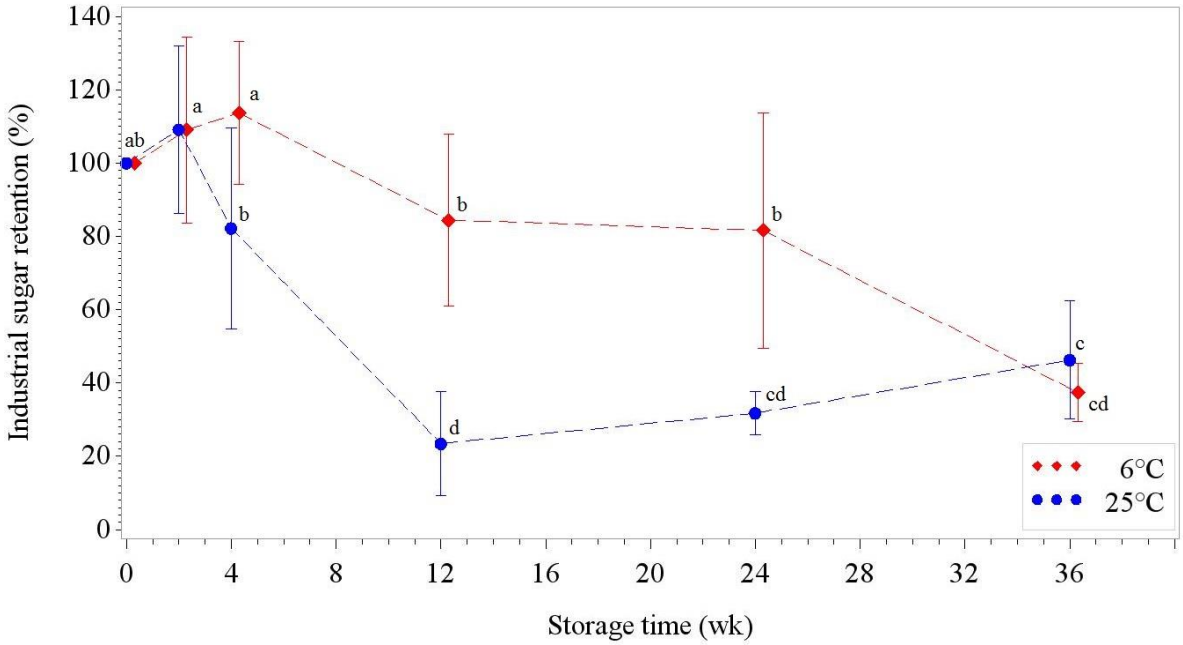

Figure 2.8. Industrial sugar retention (mean ± standard deviation of 21 replicates) in beets stored for up to 36 wk in sealed containers at 6 °C and 25 °C. Letters represent group means of a Tukey's studentized range test; means with the same letter are not statistically different from each other at $\alpha = 0.05$. NOTE: Jittering was used on the time axis to prevent overlapping standard deviations.

Conclusions

The low relative humidity (37%) in ambient air at 25 °C promoted beet tissue dehydration, significantly reducing its water activity and favoring industrial-beet sugar retention. Beets stored under these conditions for up to 36 wk showed a sugar retention of up to 100%; nonetheless, these dehydration conditions may not be economically feasible in conventional storage piles, which hold thousands of tons of beets. On the other hand, storage in sealed containers offered no benefit to industrial sugar retention in beets stored at either 6 °C or 25 °C; these beets showed an average retention of only 38% of initial sugars.

Although, surface treatments showed no statistically significant effect on beet sugar retention, a reduction in sample variability may have shown otherwise. A larger number of beets per treatment may be used in future experiments. Moreover, beets may be sorted by similar initial sugar contents in samples. A non-destructive method (e.g., near-infrared spectroscopy) could also be developed to quantify industrial sugars and ethanol in the same beets before and throughout storage. Alternatively, beet tissue could be ground and mixed thoroughly; this may reduce variability and at the same time increase surface area. Increased surface area would allow better incorporation of preservatives, such as acidulants, which could improve sugar retention in tissue that would essentially be ensiled.

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CHAPTER 3. BEET TISSUE ENSILING: AN ALTERNATIVE FOR LONG-TERM STORAGE OF SUGARS IN INDUSTRIAL BEETS FOR NON-FOOD USE[2]

Abstract

Industrial beets have significant potential to compete against corn grain as an important source of sugars for non-food industrial processes including microbial bioconversions. However, dependable, long-term storage techniques are necessary to extend processing campaigns and meet increasingly-important industry requirements such as carbon footprint reductions. This work evaluated industrial-beet tissue ensiling as an alternative for long-term sugar storage. Industrial-beet tissue was ensiled for 8 wk at 23 °C and various combinations of pH, moisture content (MC), and sugar:solids (SSR). The pH, MC, and SSR values ranged from 2.0 to 6.8, 50% to 85%, and 38% to 76%, respectively, according to a central composite rotatable design. Response surface methodology was used to model and illustrate effects of parameter combinations on beet sugar retention. MC and pH had statistically significant effects on sugar retention in ensiled tissue, whereas SSR had no significant effect. Some combinations of pH $\leq$ 4.0 and MC $\leq$ 67.5% enabled the highest retentions in ensiled tissue ($\geq$ 90%). Moreover, tissue ensiled at pH $\leq$ 3.0 and MC $\leq$ 67.5% showed increases of $\leq$ 7% over initial sugars after 3 d, suggesting that highly-acidic conditions may partially hydrolyze tissue cellulose and/or hemicellulose. In contrast, tissue ensiled at some combinations of pH < 6.5 and MC > 67.5% achieved sugar retentions of < 30% at 8 wk. Sulfuric acid cost estimates (on dry-sugar basis) to achieve effective pH (2.0 to 4.0) for sugar retentions $\geq$ 90% range from \$4.9 Mg^{-1} to \$18.6 Mg^{-1}.

[2] Chapter 3 consists of a scientific manuscript that was submitted for publication in May 2015 to the journal of Biomass and Bioenergy. Authors: Juan M. Vargas-Ramirez, Darrin M. Haagenson, Scott W. Pryor, and Dennis P. Wiesenborn. Juan M. Vargas-Ramirez designed and conducted the experiments in this work, and is the first author of the manuscript. The co-authors provided advice throughout the work and/or assisted in the editing.

Introduction

Renewable feedstocks for nonfood industrial processes are becoming increasingly popular worldwide. Carbohydrate-rich biomass is of particular interest since carbohydrates can be used in bioconversion processes such as fermentations. Carbohydrates, such as sucrose, glucose, and fructose, from renewable sources can be readily fermented by microorganisms into compounds that can be used as biofuels (e.g., ethanol and butanol) or chemical precursors (e.g., ethanol and lactic acid).

Corn grain is the primary carbohydrate source for nonfood industrial processes in the U.S. However, corn grain undergoes energy-intensive and costly pretreatment steps to hydrolyze the starch into glucose (Shigechi et al., 2004). Crops that produce readily-fermentable carbohydrates have gained much attention in recent years since these eliminate the need for energy-intensive pretreatment steps and consequently may reduce the carbon footprint of derived products. In particular, sugarbeet varieties simply known as *industrial and energy beets* are envisioned as alternative carbohydrate sources for nonfood industrial processes in the U.S (McGrath and Townsend, 2015). In comparison to beets processed in sugar factories, which are bred primarily for sucrose purity, beets tailored for nonfood processes are bred for dry matter content (McGrath and Townsend, 2015), which is significantly correlated with sucrose content (Hoffmann et al., 2004). Yield trials conducted in North Dakota have shown that industrial and energy beets have significant advantages over corn. In 2014, these beets produced an average of 12.6 Mg ha^{-1} of sugars in glucose equivalents (NDSU Carrington REC, 2014). That sugar output was almost double than that of corn grain in 2014 (6.4 Mg ha^{-1}) (Shapouri and Salassi, 2006; USDA-ERS, 2014). Besides this significant advantage over corn grain, beets can readily adapt to a variety of climate conditions and successfully grow in saline soils that are less productive for

other agricultural crops (McGinnis, 1996; van der Poel et al., 1998). Moreover, the long, fibrous roots of beets penetrate deep into the soil, gaining access to moisture and nutrients that other crops cannot reach (McGinnis, 1996) and thus reducing fertilizer requirement.

Despite the significant advantages of beets over other crops, their high moisture content can be an impediment to long-term storage since it supports microbial and enzymatic activities. In the Central Valley of California, a Mediterranean-climate region, beets can be stored in small piles for less than 20 h with minimal sugar loss (van der Poel et al., 1998). On the other hand, beets may be stored frozen for up to 180 d with minimal sugar loss in outdoor piles ventilated with frigid winter air in the Red River Valley of the North Central U.S. (McGinnis, 1996; van der Poel et al., 1998; Asadi, 2007). Nonetheless, the success of outdoor pile storage is highly dependent on weather conditions; thus, this technique entails risks of major sugar loss due to hot spot formation in the interior of piles (Cole and Bugbee, 1976).

Several storage techniques have been evaluated to maximize long-term beet sugar retention and extend processing campaigns in sugar factories. Some examples are: treating the beet surface with antimicrobials (Wu et al., 1970; Akeson et al., 1979), applying wax coatings to the beet surface to reduce post-harvest respiration (Wyse and Dilley, 1973), and storing beets under modified atmosphere to reduce respiration and microbial spoilage (Wu et al., 1970; Karnik et al., 1970). A technique that has been most successful, and implemented in beet sugar factories since the 1960s as an alternative to outdoor pile storage, consists of storing beet sugars in the form of a partially purified concentrate known as *thick juice* (McGinnis, 1996; van der Poel et al., 1998; Asadi, 2007). More recently, Vargas-Ramirez et al. (2013) showed that a non-purified beet sugar concentrate (*raw, thick juice*) can be successfully stored at solid fractions between 64.5% and 69.0% and both acidic ($pH \leq 3.5$) and alkaline ($pH \geq 9.5$) conditions. This

concentrate may be more suitable for industrial processes that are not food-oriented. However, raw, thick juice production may entail significant capital investment and operational costs.

Similar to raw, thick juice storage, perishable commodities can be stored with minimal quality loss by artificially adjusting moisture content (MC) and pH in their matrix (Rahman, 2007). In fact, a popular method for long-term forage storage, known as *ensiling*, is based on quality preservation by MC and pH adjustments. Forage crops for silage are wilted, chopped into fine particles, and stored under anaerobic conditions to enhance selective microbial fermentation of readily-fermentable sugars. Under optimal ensiling conditions, naturally-existing lactic acid bacteria thrive and produce primarily lactic acid, which reduces crop pH and enables long-term storage stability (Pitt, 1990).

Artificial pH adjustment is also possible through acid addition to the crop material (Haigh, 1998) and could effectively help retain industrial sugars in beet tissue. Woolford (1978) tested the effects of mineral and organic acids, among other antimicrobial compounds, on quality retention of silage at initial pH of 4, 5, and 6. Results from his experimental work suggested that mineral acids had no antimicrobial effect in silage at the pH values tested. In contrast, organic acids such as formic, acetic, and propionic are effective acidulants and suppressors of spoilage microbes (Woolford, 1975). Although organic acids are more effective than mineral acids in halting microbial activity due to their lipophilic nature and ability to permeate the microbial membrane (Rahman, 2007), organic acids may inhibit desired fermentation processes. Alternatively, mineral acids may be used at high-enough concentrations to halt microbes by creating an imbalance between the microbial cytoplasmic pH and that of the cell surroundings during storage (Rahman, 2007). These acids may be neutralized after storage and prior to intended fermentation processes.

Sucrose in beets not only varies from root to root, but also within each root (Asadi, 2007). Beets with higher sucrose content and purity have shown less deterioration during storage than lower-quality beets (Dexter and Frakes, 1967). Although sucrose is the most important carbon source that sustains respiration and microbial growth in beets, the quality-preserving effect of this sugar on beet tissue may derive from its impact on water activity. Water activity is a measure of the water portion that supports microbial growth (in a directly-related manner) in biological materials (Rahman, 2007). Solutes such as sucrose and salts are known to decrease water activity of biological materials (Pennington and Baker, 1990). Moreover, glucose and fructose, which result from sucrose hydrolysis, cause an even greater reduction in water activity than sucrose (Gabriel, 2008). Application of mineral acids to beet tissue would enhance sucrose hydrolysis (Godshall, 2007), resulting in glucose and fructose that would further decrease tissue water activity and potentially improve sugar retention.

The objective of this research work was to evaluate the combined effect of pH, MC, and sugar content (expressed as "sugars:solids" to indicate the proportion of solids which were sugar) on sugar retention in ground beet tissue ensiled for up to 8 wk. Ensiling may be an alternative to retain sugars in beet tissue, and thus extend processing campaigns. Microbial activity begins within the first hours of ensiling and silage achieves complete stability within 2 wk (Pitt, 1990). Therefore, it was assumed that ensiled beet tissue showing stability within 8 wk would remain stable if undisturbed. Sugar retention in beet tissue silage was fitted to a second-order polynomial model and response surface methodology was used to illustrate effects of parameter combinations on beet sugar retention.

Materials and methods

Sugarbeet collection and preparation

Approximately 50 kg of sugarbeet (*Beta vulgaris* L.) were collected in November of 2014 from a storage pile at American Crystal Sugar, Co. (Moorhead, MN, USA; 46° 54’ 0.90” N 96° 45’ 0.94” W). Beets processed in this factory are harvested within an 80-km radius. Beets were washed by hand under tap water to remove soil, and placed on wood pallets to allow excess water to drain. Additionally, the surface of beets was dried for 20 min with ambient air (25 °C, 40% relative humidity) assisted by box fans. The beets were then stored overnight in a walk-in refrigerator at 4 °C prior to processing.

Sugarbeet grinding, raw juice extraction, and beet tissue drying

Washed beets were sliced longitudinally into fourths and each section was placed into a 152.4-µm industrial polyethylene bag (76 cm × 91 cm). Thereafter, two bags were randomly selected, and beet tissue from those bags was further cut into cubes of approximately 16.4 cm^3. The beet tissue cubes were combined into a single industrial polyethylene bag (described above) and stored at -15 °C for approximately 2 wk. The frozen tissue cubes were ground in multiple batches using a single-speed food processor equipped with a 400-W motor (Ninja® Model QB900 – EURO PRO Operating LLC; Boston, MA, USA). The food processor was filled to half of its capacity and operated for 20 s during each batch. The ground beet tissue was collected in a 68-L high-impact polypropylene tote, thoroughly mixed using a transfer scoop, and frozen again until used.

A portion of the sugars was extracted from the ground tissue using a basket press. The basket consisted of a perforated PVC coupling (Type I, Schedule 40 - LASCO Fittings, Inc.; Brownsville, TN, USA) with a nominal diameter of 15.2 cm and height of 18.6 cm. Perforations

were created with a handheld electrical drill equipped with a 0.48-cm steel drill bit. The perforations were distributed in nine rows with a 1.3-cm separation between rows and a 2.5-cm interval within each row, occupying 11 cm from one end of the fitting (Figure 3.1a). The basket was placed on a circular steel sheet (0.64-cm thick, 21.4-cm radius) mounted on a high-density polyethylene tray used to contain extracted juice. The tray was fabricated using the bottom portion of a 19-L pail and had dimensions of 3.8-cm height and 26.7-cm diameter (Figure 3.1b). A 0.95-cm-to-0.64-cm National Pipe Tapered Thread polyethylene hose reducer with a 0.64-cm radius clear braided PVC tubing attachment was mounted on the bottom of the tray to allow free juice flow into a storage container. A high-density polyethylene circular sheet (0.6-cm thick, 16.7-cm radius) was used as the top plate to minimize any gap across the basket diameter and distribute pressure evenly over the beet tissue samples.

The basket and its accessories (Figure 3.1c) were readily mounted onto a compression testing machine with a maximum force capacity of 265 kN (Versa-Tester® - Soiltest, Inc.; Evanston, IL, USA). The compression machine was equipped with a fixed bottom plate and a mobile top plate. The top plate was attached to a stem screw with a hand wheel used to adjust its height (Figure 3.1d); this plate exerted direct force on the sample. Before pressing, the frozen, ground tissue was removed from the freezer and left at ambient temperature (25 °C) for 1 h to assist slight thawing to facilitate sugar extraction. Tissue samples (1.5 kg) were packed in 22.9-cm × 40.6-cm cotton bags (ULINE; Pleasant Prairie, WI, USA) and loaded individually into the basket. The basket was then mounted onto the compression machine and the mobile top plate was lowered manually. The machine was operated intermittently at force increments of 4.5 kN, allowing drops to 4.5 kN before reaching 13.4 kN, and drops to 8.9 kN thereafter until finally achieving 53.5 kN. The juice expressed from the tissue (1^{st} press raw juice) was collected in a

9.5-L F-style polyethylene jug and the 1st press tissue in a 68-L high-impact polypropylene tote. The 1st press raw juice was stored at -15 °C and the 1st press tissue was mixed with tap water added at 1.25 times the weight of 1st press juice (Pothula et al., 2014). The mixture was then pressed a second time as described above.

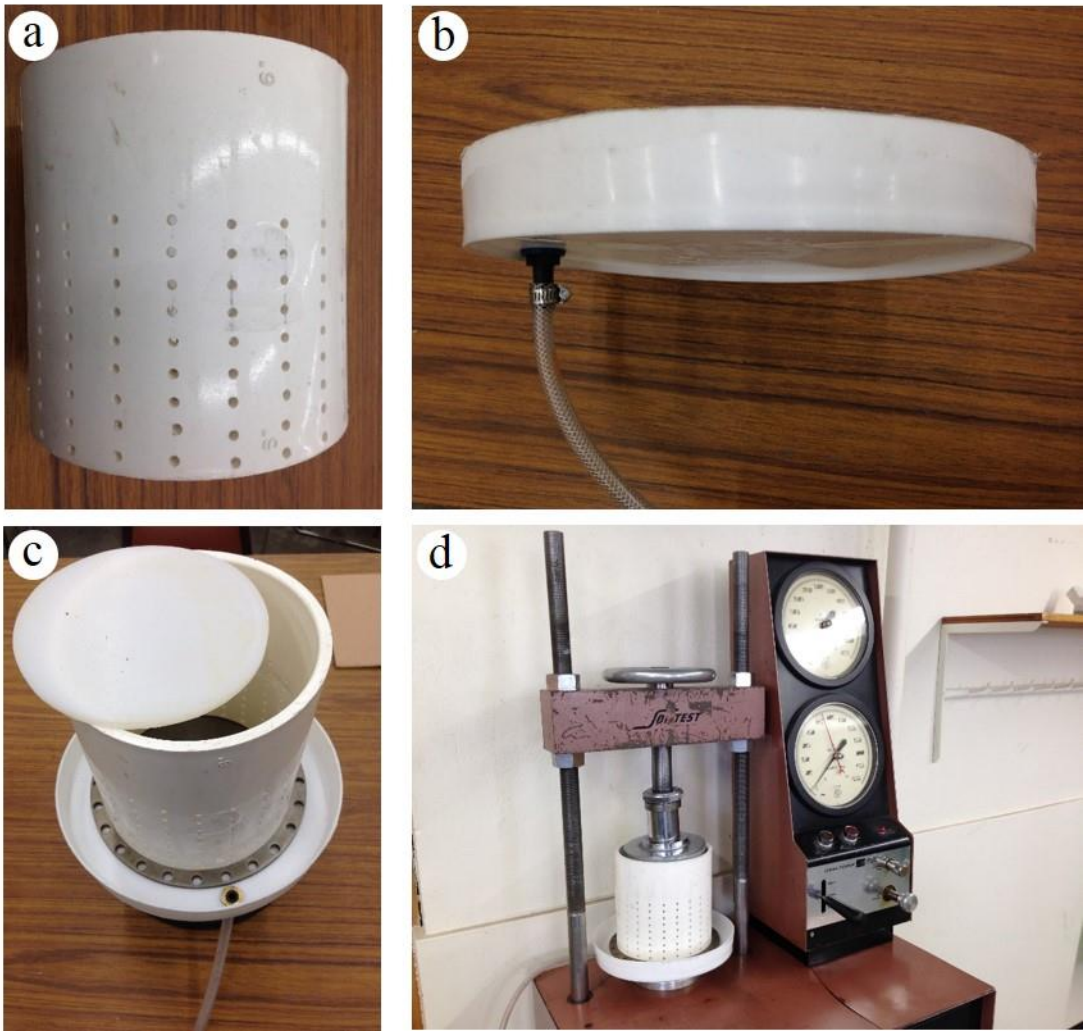

Figure 3.1. (a) Basket fabricated with a PVC coupling fitting (15.2-cm nominal diameter and 18.6-cm height) and perforations occupying about 11 cm of its height, (b) juice collection tray and drain fabricated with the bottom portion of a 19-L high-density polyethylene pail, (c) basket and accessories used to express raw beet juice from fresh, frozen beet tissue, and (d) basket and accessories mounted onto compression machine with a mobile top plate.

The 2nd press tissue was distributed in 100 g portions into 1.1-L disposable aluminum foil pans. The moisture content of the tissue was reduced from 78% to 39% in a vacuum shelf dryer

(Buflovak; Buffalo, NY, USA) operated at 65 °C and an absolute pressure of 10 kPa for 8 h. The partially dried beet tissue (referred to hereafter as *2nd press, dried tissue*) was mixed thoroughly and stored at -15 °C in a 152.4-μm industrial polyethylene bag (76 cm × 91 cm) for 2 d.

Experimental design

A central composite rotatable design (CCRD) with three factors (Myers et al., 2009) was followed to evaluate combined effects of pH, moisture content (MC), and sugars:solids (SSR) on sugar retention in sugarbeet tissue ensiled for 8 wk at 23 ± 2 °C. The design matrix consisted of 20 experimental treatments including 8 factorial points, 6 axial points, and 1 center point replicated 6 times. The coded and uncoded values of the parameters evaluated are presented in Table 3.1. A parallel set of treatments were ensiled in triplicate at conditions similar to those in CCRD treatments 9, 10, and 15. These treatments only varied in initial pH and were representative of intermediate and extreme values, which allowed to monitor the effect of pH on changes in parameter values and sugar contents throughout storage.

Parameter adjustment

The pH, MC, and SSR of all treatments were adjusted by addition of sulfuric acid, water, and 1st press raw juice, respectively. The amount of acid required (in mol g^{-1} on a dry basis) to achieve the target pH values was determined in a beet tissue acidification experiment. This experiment consisted of acidifying 25-g samples of frozen, ground tissue from the same batch with 0.5 mL of aqueous sulfuric acid solutions at concentrations of 0.1, 0.5, 1.0, 2.5, 5.0, and 7.5 mol L^{-1}. A control sample (not acidified) was also prepared to determine the original pH of the frozen, ground tissue. The samples were mixed thoroughly for 2 min with a rubber spatula to incorporate the acid with the tissue. The acidified tissue was stored at 4 °C for 24 h prior to pH analysis, which was carried out in duplicate according to the method described below.

Table 3.1. Central composite rotatable design matrix followed to evaluate combined effects of pH (x_1), moisture content (MC; x_2), and sugars:solids (SSR; x_3) on sugar retention in beet tissue ensiled for 8 wk at 23 °C.

	Coded values			Uncoded values		
Treatments	x_1	x_2	x_3	pH	MC (%)	SSR (%)
1	-1	-1	-1	3.0	57.1	45.9
2	1	-1	-1	5.8	57.1	45.9
3	-1	1	-1	3.0	77.9	45.9
4	1	1	-1	5.8	77.9	45.9
5	-1	-1	1	3.0	57.1	68.3
6	1	-1	1	5.8	57.1	68.3
7	-1	1	1	3.0	77.9	68.3
8	1	1	1	5.8	77.9	68.3
9	-1.682	0	0	2.0	67.5	57.1
10	1.682	0	0	6.8	67.5	57.1
11	0	-1.682	0	4.4	50.0	57.1
12	0	1.682	0	4.4	85.0	57.1
13	0	0	-1.682	4.4	67.5	38.3
14	0	0	1.682	4.4	67.5	76.0
15	0	0	0	4.4	67.5	57.1
16	0	0	0	4.4	67.5	57.1
17	0	0	0	4.4	67.5	57.1
18	0	0	0	4.4	67.5	57.1
19	0	0	0	4.4	67.5	57.1
20	0	0	0	4.4	67.5	57.1

The quantities of beet tissue (either 2nd press, dried or fresh, frozen), 1st press raw juice and water needed for MC and SSR adjustments were determined by means of overall and component mass balances. The mass balance equations used were the following:

Overall mass : $m_a + m_b + m_c = m_d$ (Eq. 3.1)

Total sugars: $m_a \cdot x_a + m_b \cdot x_b + m_c \cdot x_c = m_d \cdot x_d$ (Eq. 3.2)

Total moisture: $m_a \cdot y_a + m_b \cdot y_b + m_c \cdot y_c = m_d \cdot y_d$ (Eq. 3.3)

where: m, x, and y represent mass (in g), wet-basis sugar content (in g g^{-1}), and wet-basis moisture content (in g g^{-1}), respectively, in the beet tissue (a), 1st press raw juice (b), water (c),

and final mixture (*d*). The system of equations above has the same number of equations (three) as variables (three) and, hence, was solved simultaneously using an inverse matrix and Gauss-Jordan elimination (Larson, 2013). The quantities determined for each component were mixed in a 5-L plastic beaker with a rubber spatula (blade size: 7.6 cm × 11.7 cm) for 5 min.

Beet tissue ensiling

After mixing, the samples were distributed equally and packed into 240-cm^3 mason jars (referred to hereafter as *mini-silos*), which were filled to 75% of their capacity, leaving approximately a 60-cm^3 headspace (Figure 3.2). The tissue in the jars was slightly compacted with a spoon to expel air entrapped between tissue particles. An oxygen absorber with absorption capacity of 300 cm^3 (Oxygen Absorbers; Harrisburg, NC, USA) was introduced into each jar to eliminate oxygen in the headspace to halt beet tissue respiration and aerobic microbial growth. The jars were hermetically sealed with steel lids lined with a rubber layer. The lids had been modified by inserting a 5-cm piece of clear PVC tubing with 1 cm outer diameter through the center of each lid. The contact region between the lid and tube was sealed with all-purpose adhesive caulk (Loctite® Polyseamseal® - Henkel Corp.; Westlake, OH, USA), which was left to dry for at least 24 h before the mini-silos were used in the experiment. A 0.64-cm barbed polypropylene check valve (Ark-Plas Products, Inc.; Flippin, AR, USA) with a cracking pressure of 3.45 kPa was mounted on the outer extreme of the tube. The purpose of the check valve was to relieve excessive pressure exerted by any respiration and fermentation gases produced during ensiling.

Figure 3.2. Mini-silo filled to 75 % of its capacity with beet tissue silage.

Sample collection and analysis

Tissue samples from the CCRD treatments were collected after 8 wk of ensiling. In contrast, tissue samples from the parallel set of treatments described in the experimental design were collected at 0.4, 1, 2, and 8 wk. The entire content of the jars was collected and mixed thoroughly to ensure sample homogeneity. A 200-g tissue sample of each treatment combination was collected immediately after preparation to determine baseline sugar quantities. All samples were packed in resealable freezer bags and stored at -15 °C until analyzed.

Ensiled-tissue samples were analyzed for pH, MC, and sugar content. For pH determination, a 5-g silage sample was mixed with 50 mL of 18.2-MΩ·cm water in a 100 mL beaker and refrigerated for 12 h. The samples were allowed to come to room temperature before determining their pH using a benchtop pH meter (Orion Star A111 - Thermo Fisher Scientific, Inc.; Beverly, MA, USA). The MC of each sample (expressed on a wet basis) was determined by oven-drying approximately 10 g of silage in aluminum weighing dishes at 105 °C for 16 h. Sugars in each sample were extracted following the cold digestion method for extraction and

determination of sugars in beet cossettes (Asadi, 2007). Sugars in the extracts were quantified by HPLC following the method described by Vargas-Ramirez et al. (2013).

Statistical analysis

Minitab® Statistical Software 17 (Minitab, Inc.; State College, PA, USA) was used to conduct statistical analyses. Response surface methodology was used to model and illustrate effects of parameter combinations on beet sugar retention. Sugar retention ($\hat{y}$) in tissue ensiled for 8 wk was fitted to a second-order polynomial model:

$$\hat{y} = \beta_0 + \beta_1 \cdot pH + \beta_2 \cdot MC + \beta_3 \cdot SSR + \beta_{11} \cdot pH^2 + \beta_{22} \cdot MC^2 + \beta_{33} \cdot SSR^2 + \beta_{12} \cdot pH \cdot MC + \beta_{13} \cdot pH \cdot SSR + \beta_{23} \cdot MC \cdot SSR + \beta_{123} \cdot pH \cdot MC \cdot SSR \quad \text{(Eq. 3.4)}$$

where *pH*, *MC*, and *SSR* represent uncoded values. The term β_0 is a constant; β_1, β_2, and β_3 are linear coefficients; β_{11}, β_{22}, and β_{33} are quadratic coefficients; and β_{12}, β_{13}, β_{23}, and β_{123} are interaction coefficients. Terms that were not statistically significant ($\alpha > 0.05$) were removed by stepwise backward elimination (Mendenhall and Sincich, 2012).

Sugar retention, MC, and pH of the parallel set of treatments with conditions similar to those of CCRD treatments 9, 10, and 15 were reported as the average of three replicates analyzed at each sampling point. The technique for propagation of errors (Taylor, 1997) was used to determine sample standard deviations associated with sugar retention and MC estimations.

Results and discussion

Beet sugar extraction, tissue drying, and treatment preparation

Preliminary work conducted for the preparation of this experiment uncovered challenges in achieving the treatment combinations defined in the CCRD. For example, the preferred range of SSR values for the CCRD could not be achieved by subjecting fresh, frozen beet tissue to a single pressing step. Therefore, the 1st press tissue was subjected to a washing and pressing step

that helped expand the SSR range. Expanding the SSR range helped precisely attain the SSR targets by simple reconstitution of beet tissue and juice.

The fresh, frozen beet tissue had an SSR of 76%. Approximately 50% of initial sugars were extracted from this tissue in the form of raw juice after a single pressing step, yielding a 1st press tissue with an SSR of 63% (Table 3.2). Subjecting the 1st press tissue to a washing and pressing step increased the fraction of extracted sugars to 84% and yielded a 2nd press tissue with an SSR of 38% (Table 3.2). The 1st press raw juice obtained during sugar extraction had a solids fraction of 32% and an SSR of 92%. This juice was used to adjust beet tissue SSR and achieve the target values defined for each experimental treatment.

Table 3.2. Quantities, moisture content (MC; on a wet basis), and sugars:solids (SSR) of beet tissue pressing products and of 2nd press, dried tissue.

Material	Quantity (kg)	MC (%)	SSR[a] (%)
Fresh, frozen tissue	15.6	70.0 ± 0.4	76.0 ± 0.2
1st press tissue	9.5	72.0 ± 0.2	62.9 ± 0.1
1st press raw juice	6.1	67.7 ± 0.0	91.8 ± 0.3
2nd press tissue	6.7	77.7 ± 0.1	37.6 ± 0.4
2nd press, dried tissue	2.5	39.4 ± 0.6	38.3 ± 0.1

[a]Reported as hexose equivalents.
Note: Values are given as mean ± standard deviation of duplicate analyses on each material.

The SSR, MC, and pH targets were generally achieved by solely combining beet tissue, beet juice, water, and sulfuric acid at different proportions (Table 3.3); however, an additional drying step was needed to achieve the MC targets of several treatments. Upon preparation, treatments 5, 6, 11, and 14 had MCs of about 59%, 59%, 54%, and 70%, respectively, which exceeded the corresponding targets (Table 3.4). In the case of treatments 5, 6, and 11, the off-target MCs were caused by the insufficient solids and sugar contents in the 1st press raw juice and, consequently, the excess amount of juice needed to achieve the SSR targets. In contrast,

treatment 14 was the only treatment prepared with fresh, frozen tissue, which already had an on-target SSR; however, the MC of that tissue exceeded the target defined in the CCRD. The already above-target MCs in those treatments were further elevated during pH adjustment by a predetermined amount of water (Table 3.3) added to assist acid incorporation with the beet tissue. Therefore, treatments 5 and 6, and 11 and 14 were dried after preparation for an additional 45 min and 60 min, respectively. After drying, the MCs of these treatments were within ± 4% of target values.

Table 3.3. Quantities of 2nd press, dried tissue (SPDT) or fresh, frozen tissue (FFT), 1st press raw juice (FPRJ), water, and sulfuric acid needed to achieve parameter targets for each CCRD treatment combination.

Treatments	SPDT (g)	FFT (g)	FPRJ (g)	Water (g)	Sulfuric acid (moles × 10^3)
1	182.0	-	57.0	61.0	25.4
2	182.0	-	57.0	61.0	2.5
3	182.0	-	57.0	343.4	25.4
4	182.0	-	57.0	343.4	2.5
5[a]	93.1	-	224.0	12.0	25.4
6[a]	93.1	-	224.0	12.0	2.5
7	47.9	-	115.4	136.7	13.1
8	47.9	-	115.4	136.7	1.3
9	104.2	-	106.4	89.4	46.4
10	104.2	-	106.4	89.4	0.0
11[b]	160.3	-	163.7	12.0	9.9
12	104.2	-	106.4	439.4	6.4
13	247.5	-	0.0	214.0	9.9
14[b]	-	324.9	0.0	12.0	6.4
15	104.2	-	106.4	89.4	6.4
16	104.2	-	106.4	89.4	6.4
17	104.2	-	106.4	89.4	6.4
18	104.2	-	106.4	89.4	6.4
19	104.2	-	106.4	89.4	6.4
20	104.2	-	106.4	89.4	6.4

[a, b]Treatment dried for 45 min ([a]) or 60 min ([b]) following the vacuum drying method.

Combined effects of pH, MC, and SSR on sugar retention

Sugar retention in beet tissue ensiled for 8 wk at various pH, MC, and SSR combinations ranged widely. Some treatments showed very poor sugar retention (as low as 7%), whereas others showed excellent retention and even net gains (Table 3.4). For example, treatments 5 and 9, ensiled at pH ≤ 3.0 and MC ≤ 67.5%, showed sugar increases of 7% and 4%, respectively, over their initial quantity. This suggests that pH ≤ 3.0 and MC ≤ 67.5% enable successful sugar retention in ensiled beet tissue and that highly acidic conditions (i.e., pH ≤ 3.0) may hydrolyze polymeric carbohydrates, such as cellulose and/or hemicellulose, in the tissue. Concentrated sulfuric acid is known to hydrolyze cellulose at room temperature (25 °C) with hydrolysis levels depending on acid concentration (Xiang et al., 2003). In contrast to the treatment examples above, sugarbeet tissue ensiled at pH 3.0 and MC of 77.9% (i.e., treatments 3 and 7) retained less than 15% of initial sugars (Table 3.4). This comparison shows the detrimental effect of increased MC on sugar retention in ensiled beet tissue. In general, MC is significantly important in ensiling operations and optimal levels range from 50% to 70% depending on crop type (Pitt, 1990).

Sugar retention in ensiled beet tissue was initially fitted to a second-order polynomial model (Equation 3.4). However, only two of the three linear terms (*pH* and *MC*), one interaction term (*pH* × *MC*), and one quadratic term (pH^2) in the model were statistically significant. All SSR terms were not statistically significant, indicating that sugar content did not have a significant effect on sugar retention at the conditions evaluated in this experiment. After omitting terms with no statistical significance, the resulting model was:

$$Sugar\ ret.(\%) = 640 - 125 \cdot pH - 6.68 \cdot MC + 0.952 \cdot pH \cdot MC + 5.80 \cdot pH^2 \qquad \text{(Eq. 3.5)}$$

which had high statistical significance ($P < 0.001$) and coefficient of determination ($R^2 = 0.87$ and $R^2_{Adj} = 0.84$). The R^2_{Adj} of this model exceeds coefficients of determination that are

considered typical and satisfactory for biological system models (0.3 to 0.5; Miller et al., 2001). The agreement between predicted and actual sugar retentions at the various MC and pH combinations evaluated in this experiment are illustrated in Figure 3.3a.

Table 3.4. Actual sugar retention, increase in moisture content (MC), and final pH in beet tissue ensiled at different combinations of pH, MC, and sugars:solids for 8 wk and at 23 °C.

Treatments	Actual sugar retention (%)	Increase in MC (%)	Final pH
1	87	5	3.1
2	48	1	4.2
3	10	10	3.3
4	7	7	3.9
5	107	2	2.9
6	54	4	4.0
7	13	16	3.0
8	39	2	3.5
9	104	0	2.0
10	36	12	3.8
11	84	3	4.1
12	11	8	3.4
13	47	2	3.9
14	40	17	3.8
15	26	16	3.7
16	37	14	3.9
17	25	16	3.8
18	31	16	3.8
19	20	17	3.8
20	31	15	3.8

MC and pH had a combined effect on sugar retention in ensiled tissue, with lower MC and pH values being most effective (Figure 3.3b). Based on the model, only some combinations of pH ≤ 4.0 and MC ≤ 67.5% enabled the highest sugar retentions in ensiled beet tissue (≥ 90%; Figure 3.3b). In contrast, tissue ensiled at some combinations of pH < 6.5 and MC > 67.5% only achieved sugar retentions of < 30% by 8 wk of storage.

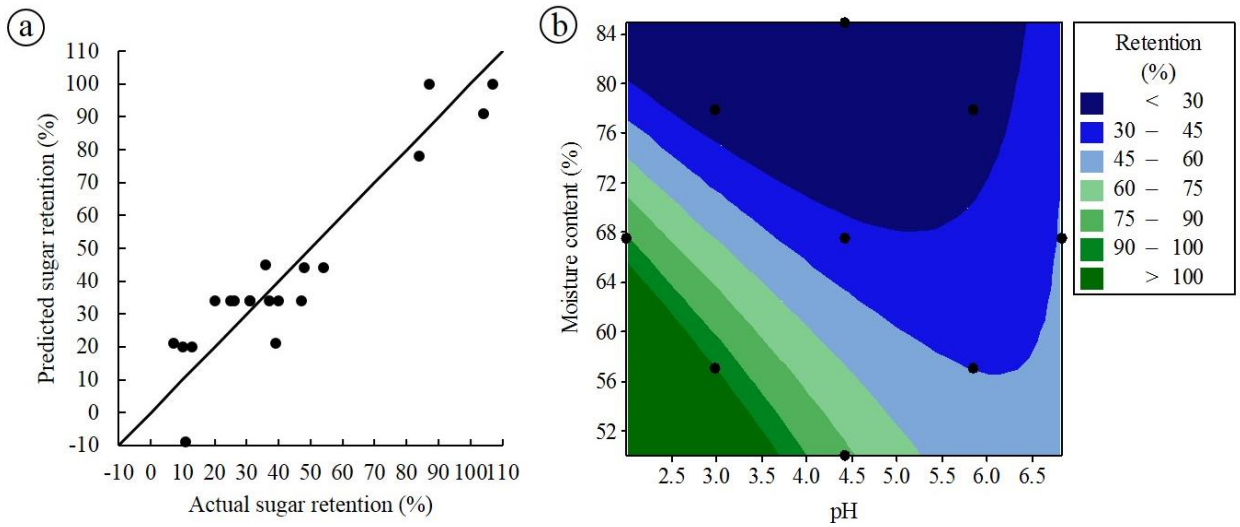

Figure 3.3. (a) Predicted versus actual sugar retentions in reference to a diagonal that represents a perfect fit and, (b) contour plot showing the combined effect of moisture content and pH on sugar retention in beet tissue ensiled for 8 wk at 23 °C. Bold points represent actual data points in (a) and experimental conditions in (b).

Changes in sugar quantity, pH, MC, and total weight of ensiled beet tissue

A parallel set of beet tissue samples, ensiled under conditions similar to those of treatments 9, 10, and 15, was monitored for changes in sugar quantity, pH, MC, and total weight throughout the 8-wk storage period. In particular, tissue ensiled at initial pH of 2.0 and MC of 67.5% showed a statistically significant increase of approximately 7% over its initial sugar quantity (P = 0.001) after 3 d of storage (Figure 3.4a). The highly acidic pH in this tissue hydrolyzed 90% of its sucrose content by 3 d of ensiling and about 100% by 1 wk. However, the observed sugar increase in this tissue cannot be attributed to sucrose hydrolysis since sugars were consistently expressed as total hexose equivalents throughout this work. The sugar quantity in this tissue remained stable after 3 d of storage. This trend confirmed the sugar increase observed in treatment 2 and discussed in the previous subsection. Furthermore, the pH, MC, and total weight of this beet silage remained stable throughout storage (Figures 3.4b, 3.4c, and 3.4d). These trends confirmed that combinations of pH 2.0 and MC < 67.5% enable successful sugar retention in beet tissue ensiled at 23 °C (Figure 3.3b).

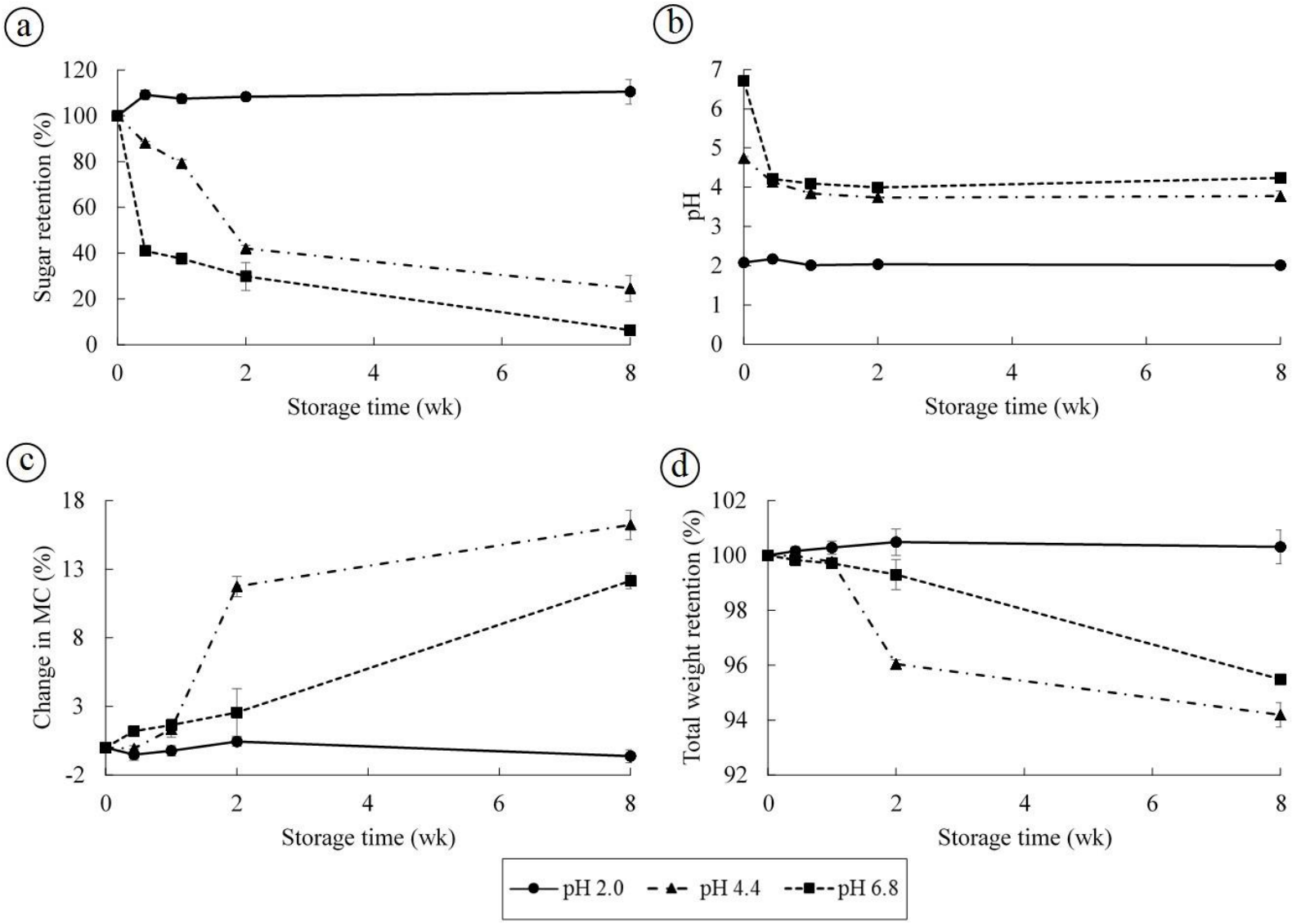

Figure 3.4. (a) Sugar retention, (b) pH, (c) change in moisture content (MC), and (d) total weight retention in beet tissue samples ensiled for 8 wk at 23 °C. The samples had initial pH values of 2.0, 4.4, and 6.8, and fixed wet-basis MC and sugars:solids of 67.5% and 57.1%, respectively.

Tissue ensiled at initial pH of 4.4 reached a stable pH of 3.8 by the end of the first week of storage (Figure 3.4b). This tissue retained 42% of initial sugars after 2 wk and sugars showed a statistically significant drop to 25% by the end of storage (Figure 3.4a). The steep sugar drop during the first two weeks and the gradual drop thereafter suggest that sugar loss in tissue ensiled at pH of 4.4 may have ceased between weeks 2 and 8. In contrast, tissue ensiled at initial pH of 6.8 reached a stable pH of 4.2 by 3 d after storage. This tissue retained the least amount of sugars (6% of initial) among the samples monitored over time. Yet, the sugar quantity in this tissue could decrease further if ensiled for over 8 wk, as suggested by the continuous trend in sugar retention (Figure 3.4a).

Tissue ensiled at initial pH of 4.4 and 6.8 showed increases of 16% and 12%, respectively, in MC after 8 wk of storage (Figure 3.4c). This increase in MC coincided with a decrease in sugars in the same tissue, but both changes were not directly correlated. For example, tissue ensiled at initial pH of 4.4 retained more sugars as compared to tissue ensiled at initial pH of 6.8 (Figure 3.4a). Nevertheless, the former sample showed a larger increase in MC (16%) as compared to the latter sample (12%; Figure 3.4c).

Sugarbeet tissue contains a wide variety of naturally-existing anaerobic microbes (Bugbee et al., 1975). These microbes will ferment sugars to produce new viable microbial cells and, depending on their metabolic pathways, will yield liquid and/or gaseous metabolites. Beet tissue ensiled in this experiment showed decreases in total weight of at most 6% throughout the 8-wk storage period (Figure 3.4d). This suggests that microbial metabolism of sugars throughout storage yielded, aside from viable microbial cells, liquid metabolites that contributed to the total weight of ensiled tissue. Moreover, Figure 3.4b suggests that some of these metabolites were acids that contributed to the abrupt pH drop during the first days of ensiling. Organic acids such

as lactic and acetic are dominant in successful silage operations and are responsible for a pH drop within the first 2 wk of ensiling, which leads to silage stability (Pitt, 1990). In contrast, conditions of MC > 70% (Mueller and Green, 1987; Pitt, 1990) and pH > 5 (Pitt, 1990) lead to unsuccessful silage operations in which *Clostridia* species tend to thrive and ferment sugars and lactic acid into butyric acid. These organic acids have significantly higher boiling points than water due to their increased molecular surface area and tendency to form stable dimers (Nollet and Toldrá, 2013). Hence, any organic acids that resulted throughout the storage period in this experiment were most likely only partially removed during oven-drying at 105 °C.

Economic implications of successful beet tissue ensiling

Among the conditions evaluated in this experiment, some combinations of pH ≤ 4.0 and MC ≤ 67.5% enabled the highest sugar retentions (≥ 90%) in beet tissue ensiled for 8 wk at 23 °C (Figure 3.3b). Although a thorough economic analysis for beet tissue conditioning prior to ensiling is beyond the scope of this work, the costs for acid quantities needed to achieve effective pH values were estimated. To achieve pH values between 2 and 4 in beet tissue, sulfuric acid is needed at dry-basis-solid proportions between 47.5×10^{-5} mol g^{-1} and 82.1×10^{-6} mol g^{-1}, respectively (beet tissue titration data in Appendix B). At a current, representative price for 93% bulk sulfuric acid of \$200 Mg^{-1} FOB (or \$21.1 $kmol^{-1}$ FOB; John White, personal communication on 15 May 2015, Senior Vice President at Brainerd Chemical Company, Inc.), the acid cost estimates to achieve pH values between 2 and 4 would range from \$1.7 Mg^{-1} to \$10.0 Mg^{-1}. Based on the model, in order for an initial pH of 2 to be effective, beet tissue MC would need to be reduced from about 75% (typical in freshly-harvested sugarbeet; Asadi, 2007) to ≤ 67.5% before ensiling. In contrast, an initial pH of 4.0 would only be effective on beet tissue ensiled at an initial MC of 50.0%. Furthermore, since sugars typically represent about 17.5% of

sugarbeet fresh weight (Asadi, 2007), acid cost estimates on dry-sugar basis would range from \$4.9 Mg^{-1} to \$18.6 Mg^{-1}. Capital costs (e.g., for hammer mill, dryer, storage bunkers) and operating costs (e.g., for material handling, drying, pH adjustments) would be required to estimate net costs of effective beet tissue ensiling.

Conclusions

Ensiling is a potential alternative for long-term sugar storage in industrial-beet tissue. MC and pH have statistically significant effects on sugar retention in beet tissue silage, whereas SSR has no significant effect. Beet tissue can retain more than 90% of initial sugars for at least 8 wk when ensiled at some combinations of pH (≤ 4.0) and MC (≤ 67.5%), and at 23 °C. Moreover, beet tissue ensiling at highly acidic conditions (pH ≤ 3.0) results in net sugar gains after 3 d of ensiling. Although beet tissue ensiling under highly acidic conditions is not suitable for the table sugar industry due to significant sucrose hydrolysis (≥ 90% after 3 d), it may provide an opportunity to nonfood industries to extend processing campaigns. Future work should assess the economic feasibility of beet tissue ensiling and the use of sugars retained in beet silage in nonfood industrial processes including microbial bioconversions.

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CHAPTER 4. EFFECTS OF PRE-STORAGE CONCENTRATION AND ACIDIFICATION, AND POST-STORAGE CONDITIONING OF NON-PURIFIED BEET JUICE ON YEAST (*SACCHAROMYCES CEREVISIAE*) FERMENTATION[3]

Abstract

Pre-storage concentration and acidification and post-storage conditioning of non-purified beet juice could impact sugar fermentation by yeast. Ammonium and sodium salts were synthesized in diffuser juice acidified from pH 6.5 to 3.5 with either one of three mineral acids (hydrochloric, sulfuric, or phosphoric) and partially neutralized to pH 4.8 with either one of two bases (sodium hydroxide or ammonium hydroxide). Also, juice was directly supplemented with salts in the same quantities synthesized. A follow-up experiment was conducted to confirm the effects on sugar fermentation by yeast determined in diffuser juice. This experiment involved concentrated, non-purified beet juice acidified from pH 6.4 to 3.5 only with sulfuric acid, and partially neutralized with either sodium hydroxide or ammonium hydroxide, as in the first experiment. The only effects on yeast fermentation were beneficial, and resulted from addition of ammonium ions through ammonium salts. Ammonium ions increased total Kjeldahl nitrogen (TKN) in diffuser juice by 40% to 60%, which almost doubled yeast ethanol production rates between 6 h and 12 h. Although ammonium ions increased TKN in concentrated juice by about 20%, this increase caused only slight improvements in ethanol production rates. Pre-storage acidification and post-storage partial neutralization of concentrated juice can be done strategically to synthesize ammonium salts that can improve yeast sugar-fermentation rates.

[3] Chapter 4 consists of a scientific manuscript that will be submitted for publication consideration after the dissertation defense. Authors: Juan M. Vargas-Ramirez, Scott W. Pryor, and Dennis P. Wiesenborn. Juan M. Vargas-Ramirez designed and conducted the experiments in this work, and is the first author of the manuscript. The co-authors provided advice throughout the work and/or assisted in the editing.

Introduction

Industrial beets (*Beta vulgaris* L.) could soon become a major source of sugar for non-food industrial processes including microbial bioconversions. Industrial beets already have significant advantages over corn grain, the primary source of sugar for non-food industrial processes in the U.S. For example, beets produce readily fermentable sugars, eliminating the need for energy-intensive pretreatment steps that corn grain undergoes to hydrolyze its starch into readily fermentable sugars. Moreover, industrial beets have reached an average productivity of 12.6 Mg ha^{-1} of sugar in hexose equivalents (NDSU Carrington REC, 2014). This is 70% greater than the average productivity of corn, which reached 7.4 Mg ha^{-1} of hexose equivalents in 2014 (assuming a starch weight fraction of 72% and a stoichiometric starch to glucose weight ratio of 0.9:1; Wertz and Bédué, 2013; USDA-ERS, 2014). Despite these advantages, the success of industrial beets as a feedstock for bioproducts will depend largely on the development of energy-efficient systems for storage, transportation, and conversion. The required level of energy efficiency should enable a carbon footprint significantly lower than that of most current systems for corn grain.

The ability to store beet sugar with no or minimal losses dictates the length of processing campaigns in existing beet sugar factories. Systems for long-term sugar storage are already well established in many beet sugar factories worldwide (McGinnis et al., 1996; Rorabaugh and Orleans, 1996; Schmalz, 1998; Falsterbo et al., 1998; Asadi, 2006); however, these systems are intended to maximize purity and retention of sucrose, the most important product of that industry. Furthermore, those techniques need to meet stringent requirements in accordance to the food industry and thus are energy intensive and may far exceed needs for successful storage of sugar for non-food industries.

Concentrated, purified beet juice (or *thick juice*) is already produced and stored as a means of sugar retention in beet sugar factories worldwide (Rorabaugh and Orleans, 1996; Schmalz, 1998, Asadi, 2006). However, before concentration, this juice undergoes energy-intensive purification steps (McGinnis, 1996a) that may be unnecessary for non-food microbial fermentations. In fact, yeast fermentations of beet processing intermediates (diffuser juice, purified juice, and thick juice) have shown no statistical differences in fermentation rates and efficiencies (Grahovac et al., 2011). Moreover, some European beet sugar factories already co-produce variable quantities of ethanol from diffuser juice and molasses as a result of sugar surplus (Jaggard and Townsend, 2014).

Fiedler et al. (1993) evaluated concentrated, non-purified beet juice (or *raw, thick juice*) to establish a simple, dependable technique for long-term sugar storage. However, their experiments focused on retaining sucrose rather than overall sugar (sucrose + glucose and fructose) by storing juice under alkaline conditions ($pH \geq 9.0$) or treating it with formalin. In contrast, Vargas-Ramirez et al. (2013) included acidic conditions (pH between 2.0 and 5.0). Acidic pH is well known to hydrolyze sucrose into glucose and fructose (Edye and Clarke, 1998) and thus is not accepted by the beet sugar industry. However, acidic conditions can effectively enable overall sugar retention in concentrated, non-purified beet juice (Vargas-Ramirez et al., 2013). In fact, their results indicate that juice stored at solid contents between 645 g kg^{-1} and 690 g kg^{-1}, and controlled acidic conditions ($pH \leq 3.5$), can retain at least 99% of initial sugars. Moreover, juice stored under acidic conditions requires less pH control than juice under alkaline conditions, making storage under acidic conditions more dependable. However, post-storage yeast fermentability evaluations showed that juice stored under acidic conditions enabled fermentation efficiencies between 55% and 82%, relative to fresh diffuser juice. Although this

was much better than for juice stored under alkaline conditions (fermentation efficiencies between 9% and 53%), these results suggested a need to further improve yeast fermentation of concentrated, non-purified juice stored under acidic conditions.

Some inorganic salts, including sodium chloride (NaCl), hinder yeast growth in proportion to salt concentration as a result of cell osmotic stress and consequently plasmolysis (Mager and Siderius, 2002; Shelef and Seiter, 2005), and also ion toxicity (Logothetis et al., 2007). In the experiments conducted by Vargas-Ramirez et al. (2013), NaCl was synthesized in the stored concentrated juice upon partial neutralization with either sodium hydroxide or hydrochloric acid prior to fermentation. Hence, NaCl may have been the primary cause of the low fermentation efficiencies and rates detected.

Industrial fermentation media are commonly supplemented with ammonium salts, such as ammonium sulfate or ammonium phosphate, to improve yeast growth and thus fermentation rates (NPCS Board of Consultants & Engineers, 2011). Besides the nitrogen contribution of these salts through the ammonium cation, these salts also contribute non-metal ions (phosphate and sulfate) required by yeast (Taidi et al., 2003). Ammonium salts are readily assimilated by yeast into glutamate and glutamine, key precursors in sugar metabolism (Walker, 1998). Ammonium salts may be strategically synthesized in concentrated, non-purified beet juice for long-term storage. First, the juice can be acidified with mineral acids (such as sulfuric or phosphoric) to enable sugar retention during storage and then partially neutralized with ammonium hydroxide prior to fermentation. The acidification and neutralization steps would yield ammonium salts that can improve yeast fermentation. This strategy was incorporated into this research work.

This work consisted of two major experiments with separate but interrelated objectives. The objectives of the first experiment were to: (1) compare effects on yeast fermentation of sodium and ammonium salts synthesized separately in diffuser beet juice through acidification and partial neutralization, (2) determine if the effects (if any) were solely a result of the presence of salts or were caused by reactions inherent to acidification and partial neutralization, (3) determine if either the ammonium or sodium cations had specific effects on yeast fermentation, and (4) determine if the anion of either one of three mineral acids used to acidify beet juice had specific effects on yeast fermentation. The objectives of the second (follow-up) experiment were to: (1) confirm the results from the first fermentability experiment in concentrated, non-purified beet juice extracted by mechanical pressing and (2) determine if pressed juice concentration was detrimental to yeast fermentability.

Materials and methods

Beet juices and reagents

Two 10-L samples of non-purified beet juice were collected from the diffuser at American Crystal Sugar, Co. (ACSC - Moorhead, MN, USA; 46° 54’ 0.90”N 96° 45’ 44.94” W), one during January 2014 and another during March 2015. Both samples (hereafter referred to as *diffuser juice*) were stored at -15 °C until used in the experimental setups.

Additionally, during March 2015, 100 L of non-purified beet juice were produced using beets collected in November 2013 from an outdoor storage pile adjacent to the ACSC factory. Those beets were harvested in September 2013 in the Red River Valley of the North Central U.S. within an 80-km radius from the factory. The beets were stored at -15 °C until used in sugar extraction trials. Juice from those beets was mechanically expressed following the technique

evaluated by Pothula et al. (2014). Only 1st press juice (hereafter referred to as *pressed juice*) was collected during sugar extraction trials and concentrated within 48 h of collection.

ACS-grade hydrochloric acid, sulfuric acid, sodium chloride, ammonium chloride, and ammonium sulfate were obtained from VWR International (Radnor, PA, USA). ACS-grade phosphoric acid, sodium hydroxide, anhydrous sodium sulfate, anhydrous dibasic sodium phosphate, and potassium hydrogen phthalate were acquired from EMD Chemicals (Gibbstown, NJ, USA). A stock solution of ammonium hydroxide at a volume fraction of 50% was obtained from Alfa Aesar (Ward Hill, MA, USA). Crystal dibasic ammonium phosphate was purchased from Avantor Performance Materials (Center Valley, PA, USA). Ethanol Red *Saccharomyces cerevisiae* pellets were obtained from Phibro Ethanol Performance Group (Teaneck, NJ, USA).

Pressed juice concentration

Pressed juice with a solids weight fraction of 36% was concentrated in two steps in a single-effect, rising-film evaporator (Vargas-Ramirez et. al, 2013). The evaporator was operated at an internal absolute pressure of 54 kPa and juice was fed into the calandria at 0.8 kg min^{-1} and 0.6 kg min^{-1} during the first step and second step, respectively. Saturated steam at 115 °C was used as the heat source in the calandria. Juice exited the evaporator at 83 °C and with solid weight fractions of 40% and 68% after the first step and second step, respectively. *Concentrated, pressed juice* collected after the second step was stored at -15 °C until used in fermentability evaluations.

Experimental design

Two experiments were conducted. The aim of the first experiment was to evaluate effects on yeast fermentability of salts formed by acidification and partial neutralization of diffuser

juice. The second experiment was conducted to confirm results from the first experiment in a subset of treatments prepared with concentrated, pressed juice.

Effects of acidification and partial neutralization of diffuser juice on yeast fermentability

Three acids (hydrochloric, sulfuric, and phosphoric) and two bases (sodium hydroxide and ammonium hydroxide) were used to acidify and partially neutralize diffuser juice samples, giving a total of six acid-base treatment combinations. A parallel set of six treatments was prepared by directly adding salts into acidified diffuser juice samples. Salts were added in the same amounts that would be synthesized during acidification and partial neutralization. This approach was followed to determine if any observed effects were caused either by the salts or by reactions inherent to the acidification and partial-neutralization steps. Additionally, three control treatments were prepared by acidifying diffuser juice with each of the three acids used in this experiment to evaluate potential effects of acid anions. This resulted in a total of 15 experimental treatment combinations. All treatments were fermented in triplicate concurrently.

Effects of acidification and partial neutralization of concentrated, pressed juice on yeast fermentability

One acid (sulfuric) and two bases (sodium hydroxide and ammonium hydroxide) were used to acidify and partially neutralize, respectively, concentrated, pressed juice. This resulted in two acid-base treatment combinations. Analogous to the approach described in the subsection above, a parallel set of two treatments was prepared by directly adding salts to acidified concentrated, pressed juice to confirm if any effects were caused solely by salts. A sample of original pressed juice and a sample of concentrated, pressed juice were acidified with sulfuric acid and used as controls in further fermentability evaluations. Moreover, a sample of diffuser juice collected during March 2015 was also acidified with sulfuric acid and used in

fermentability evaluations to determine if either sugar extraction technique influenced yeast fermentability. In this experiment, a total of seven treatments were concurrently fermented in triplicate.

Treatment preparation

Acid and base preparation

Aqueous solutions of hydrochloric acid (HCl), sulfuric acid (H_2SO_4), and phosphoric acid (H_3PO_4), and of sodium hydroxide (NaOH) and ammonium hydroxide (NH_4OH) were each prepared at a concentration of 5 mol L^{-1}. The acid and base solutions were used to acidify and partially neutralize juice samples, respectively. All solutions were titrated (Clugston and Flemming, 2000) prior to the experimental setup. Initially, a 5-mol L^{-1} sodium hydroxide solution was standardized (Reedy et al., 2003) with 5 g of potassium hydrogen phthalate dissolved in 25 g of water. The standardized sodium hydroxide solution was then used to titrate 10-mL aliquots of each of the three acids. Ammonium hydroxide was reverse-titrated with 5-mol L^{-1} standardized hydrochloric acid.

Acidification and partial neutralization of diffuser juice

Six 350-g samples of diffuser juice (collected in 2014) with a sugar concentration of 159 g L^{-1} were acidified with either HCl, H_2SO_4, or H_3PO_4 solution to pH 3.5. The acidified samples were then partially neutralized with either NaOH or NH_4OH solution to pH 4.8 for fermentation. Acid and base solutions were added dropwise to each diffuser juice sample while stirring continuously at 5 Hz with a magnetic stirrer in a glass beaker. The amounts of acid and base solutions added to the juice were measured and used to determine quantities of salts synthesized according to the corresponding stoichiometric reaction mechanism:

$$HCl + NaOH \rightarrow NaCl + H_2O$$

$$HCl + NH_4OH \rightarrow NH_4Cl + H_2O$$

$$H_2SO_4 + 2 \cdot NaOH \text{ —> } Na_2SO_4 + 2 \cdot H_2O$$

$$H_2SO_4 + 2 \cdot NH_4OH \rightarrow (NH_4)_2SO_4 + 2 \cdot H_2O$$

$$H_3PO_4 + 2 \cdot NaOH \rightarrow Na_2HPO_4 + 2 \cdot H_2O$$

$$H_3PO_4 + 3 \cdot NH_4OH \rightarrow (NH_4)_3PO_4 + 3 \cdot H_2O$$

Either sodium chloride (NaCl), ammonium chloride (NH_4Cl), anhydrous sodium sulfate (Na_2SO_4), ammonium sulfate ($[NH_4]_2SO_4$), anhydrous dibasic sodium phosphate (Na_2HPO_4), or crystal dibasic ammonium phosphate ($[NH_4]_3PO_4$) were directly added to each of six 350-g diffuser juice samples. Treatments with added salts were then acidified to pH 4.8 with either HCl, H_2SO_4, or H_3PO_4 solution.

Acidification and partial neutralization of concentrated, pressed juice

Two concentrated, pressed juice samples of 85 g were acidified to pH 3.5 with H_2SO_4 solution. Subsequently, these samples were partially neutralized to pH 4.8 with either NaOH or NH_4OH solution. Two additional concentrated, pressed juice samples of 85 g were supplemented with either Na_2SO_4 or $(NH_4)_2SO_4$ as explained in the subsection above, and acidified to pH 4.8 with H_2SO_4 solution. Moreover, single samples of diffuser juice (collected in 2015), pressed juice, and concentrated, pressed juice were also acidified to pH 4.8 with H_2SO_4 solution. Acid and base solutions were added dropwise to each concentrated juice sample and each diffuser or pressed juice sample while stirring continuously at 5 Hz with a three-blade, stainless-steel propeller and a magnetic stirrer, respectively. All treatments were subsequently diluted to achieve a sugar concentration of 140 g L^{-1}, consistent with the sugar concentration in diffuser juice.

Yeast fermentability of treatments

Inoculum seed preparation

Three inoculum broths were prepared in 500-mL Erlenmeyer flasks, each containing 18-MΩ·cm water, glucose (30 g L^{-1}), peptone (20 g L^{-1}), and yeast extract (6 g L^{-1}). The pH of each broth was adjusted to 4.8 using 5-mol L^{-1} solutions of either HCl, H_2SO_4, or H_3PO_4 to match juice samples acidified with the same acids. The broths were sterilized in an autoclave at 121 °C and 220 kPa for 20 min, and allowed to reach room temperature in a biosafety cabinet before adding yeast pellets (1.5 g L^{-1}). The inoculum seeds were incubated in a water bath orbital shaker (MaxQ7000; Thermo Scientific - Dubuque, IA, USA) at 30 °C and 3.3 Hz for 18 h prior to inoculating treatments.

Fermentation

A total of 90 mL of each treatment (hereafter referred to as *fermentation media*) were dispensed into 250-mL Erlenmeyer flasks. The fermentation media samples were sterilized in an autoclave as described above and allowed to reach room temperature in a biosafety cabinet before adding inoculum at a volume fraction of 10%. Six-chamber plastic airlocks (Brew PS, Inc.; Moorpark, CA, USA) equipped with No. 6 rubber stoppers and sanitized with a 70% ethanol solution were used to maintain an anaerobic headspace within the fermentation flasks. The flasks were incubated in a water bath orbital shaker for 24 h at the conditions described in the subsection above.

Analytical methods

The pH of treatments was measured during acidification and/or neutralization using a benchtop pH meter (Orion Star A111 - Thermo Fisher Scientific, Inc.; Beverly, MA, USA). Sulfur dioxide was quantified in diffuser juice by iodometric titration (ACS, 2015). Total

nitrogen contents in diffuser, pressed, and concentrated juices were determined by the Kjeldahl method (Rice et al., 2012).

Duplicate fermentation media aliquots of 1.5 mL were collected from each flask in micro-centrifuge tubes at 0, 6, 12, and 24 h. Sample collection was carried out in a biosafety cabinet using 5-mL sterile-polyethylene disposable pipettes. Each sample was used for either total sugar (sucrose, glucose, and fructose) or ethanol quantification. The samples were prepared for analyses as described by Vargas-Ramirez et al. (2013). Sugars and ethanol were quantified following the HPLC methods described by Vargas-Ramirez et al. (2013). Sugars in fermentation media were expressed as hexose-equivalent weight fractions. The ethanol yield in each treatment was expressed as a weight fraction of hexose equivalents fermented. Moreover, fermentation efficiencies were determined as actual ethanol yield divided by maximum theoretical ethanol yield and reported in percentage. Ethanol production rates were determined as the increase in ethanol concentration within a time interval divided by the time interval.

Statistical analyses

Results from both fermentation experiments were reported as the average of triplicate treatments. Tukey's range test was used for multiple pairwise comparisons of means at a significance level $\alpha = 0.05$ in SAS (Version 9.4 - SAS Institute Inc.; Cary, NC, USA).

Results and discussion

Composition of diffuser juices, pressed juice, and concentrated, pressed juice

The beet juices (diffuser, pressed, and concentrated, pressed) used in this study had similar initial pH values (Table 4.1). The two diffuser juices, each collected during a different beet processing campaign, had similar solid contents but slightly different sugar contents (Table 4.1). Nonetheless, the solid and sugar contents of both diffuser juices were in agreement with the

typical composition of diffuser juice in beet processing (Asadi, 2007). In contrast, pressed juice had solid and sugar contents that were approximately double that of either diffuser juice. Pressed juice was further concentrated to achieve a solids content that would enable successful sugar retention in juice subjected to long-term storage (> 645 g kg^{-1}, wet basis; Vargas-Ramirez et al., 2013). Despite the differences in sugar contents noted above, most juices (with the exception of diffuser juice collected in 2014) had similar sugar:solids between 88% and 90% (Table 4.1).

Table 4.1. Initial properties of beet juices (diffuser, pressed, and concentrated, pressed) used in fermentability evaluations.

Properties	Diffuser juice (2014)	Diffuser juice (2015)	Pressed juice	Concentrated, pressed juice
pH	6.5 ± 0.1	6.8 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
Solids fraction[b] (g kg^{-1})	162.9 ± 0.0	158.4 ± 0.1	359.0 ± 0.1	683.8 ± 1.1
Total sugars[b] (g kg^{-1})	159.1 ± 0.3	140.2 ± 0.5	322.4 ± 20.1	601.8 ± 1.5
TKN[a,b] (g kg^{-1})	0.51 ± 0.00	0.55 ± 0.00	2.48 ± 0.01	4.89 ± 0.02

[a]TKN: Total Kjeldahl nitrogen
[b]Values given on a wet basis and as mean ± standard deviation of duplicate analyses.

When expressed on a dry basis, total Kjeldahl nitrogen (TKN; Table 4.1) was similar in both diffuser juices, whether collected in 2014 (3.1 g kg^{-1}) or 2015 (3.5 g kg^{-1}). In contrast, pressed juice and concentrated, pressed juice had dry-basis TKN contents which were similar to one another (6.9 g kg^{-1} and 7.2 g kg^{-1}, respectively), but approximately twice that of the diffuser juices. This contrast was likely related to differences in beet varieties processed and cultivation conditions, both of which influence nitrogenous compound contents in beets (McGinnis, 1996b). Also, the fundamentally different techniques followed for juice production (diffusion versus pressing) may have contributed to differences in TKN. Dry-basis sulfur dioxide contents determined in both diffuser juices were negligible, whether collected in 2014 (0.06 g kg^{-1}) or 2015 (0.05 g kg^{-1}), and were not likely to affect yeast fermentation (Coultate, 2009).

Effects of acidification and partial neutralization of diffuser juice on yeast fermentability

Sugars in some of the diffuser juice treatments were nearly completely fermented by 24 h (Figures 4.1a, 4.1b, and 4.1c). In particular, the six treatments with ammonium salts (whether synthesized *in situ* through acidification and partial neutralization of the juice, or directly added to the juice) had low residual sugar concentrations of 1.2 g L^{-1} to 2.7 g L^{-1} at 24 h. In contrast, the six treatments with sodium salts and the three controls had significantly higher residual sugar concentrations of 28.4 g L^{-1} to 33.7 g L^{-1} at 24 h ($P < 0.0001$; Figures 4.1a, 4.1b, and 4.1c), which accounted for 20% to 24% of the initial sugars in those treatments.

Corresponding ethanol-concentration trends for all treatments (Figures 4.2a, 4.2b, and 4.2c) showed that the six treatments with ammonium salts reached concentrations of 65.4 g L^{-1} to 70.0 g L^{-1} by 24 h. These concentrations were significantly higher than those in the six treatments with sodium salts and the three controls ($P < 0.0001$), all of which reached 52.0 g L^{-1} to 55.4 g L^{-1} by 24 h. This difference agreed with that detected in sugar concentrations at 24 h, discussed above.

The trends in ethanol concentrations (Figures 4.2a, 4.2b, and 4.2c) indicate that treatments with added ammonium cations (NH_4^+) reached a much higher ethanol production rate as compared to treatments with added sodium cations (Na^+). For instance, treatments with NH_4^+ had an average production rate of 4.3 ± 0.2 g $L^{-1}h^{-1}$ between 6 h and 12 h, whereas treatments with Na^+ had a statistically lower average rate of 2.5 ± 0.3 g $L^{-1}h^{-1}$ ($P < 0.0001$). Moreover, the six treatments with Na^+ had production rates similar to those of the three controls, which had no added Na^+ or NH_4^+ ($P = 0.086$; Figures 4.2a, 4.2b, and 4.2c). These results suggest that fermentation rates in treatments with ammonium salts may have been enhanced by NH_4^+. Supplementation of fermentation media with mineral sources of nitrogen is known to

significantly improve yeast fermentability (Dziugan et al., 2013). In this experiment, ammonium salts increased the TKN in diffuser juice by 40% to 60% (Tables 4.1 and 4.2), which almost doubled yeast ethanol production rates between 6 h and 12 h (Figures 4.2a, 4.2b, and 4.2c).

Table 4.2. Synthesized/added salts, nitrogen from salts, and total Kjeldahl nitrogen (TKN) contents in treatments prepared with diffuser juice.

Salt	Synthesized/added ($g\ kg^{-1}$)	Nitrogen from salt ($g\ kg^{-1}$)	TKN[a] ($g\ kg^{-1}$)
HNa_2PO_4	1.28	-	0.46
$(NH_4)_2HPO_4$	0.88	0.19	0.65
NaCl	1.02	-	0.46
NH_4Cl	0.98	0.26	0.72
Na_2SO_4	1.23	-	0.46
$(NH_4)_2SO_4$	1.30	0.27	0.73

[a]After accounting for dilution effect of inoculum volume fraction of 10 %.

Although there were clear differences in sugar and ethanol concentrations between treatments with ammonium and sodium cations, this was generally not true when comparing treatments with the same anion type. For example, ethanol concentrations in treatments acidified with each acid, and either partially neutralized with sodium hydroxide or supplemented with sodium salts, were not statistically different at 24 h ($P = 0.362$). However, treatments either partially neutralized with ammonium hydroxide or supplemented with ammonium salts showed small but statistically significant differences in ethanol concentrations at 24 h ($P = 0.030$). In particular, diffuser juice acidified with hydrochloric acid and partially neutralized with ammonium hydroxide reached a statistically lower concentration (65.4 $g\ L^{-1}$) as compared to the other five treatments with ammonium salts (Figure 4.2b). Nonetheless, since ethanol concentrations in that treatment and the one supplemented directly with ammonium chloride were statistically different, there is no evidence to claim a detrimental effect from the chloride anion on yeast fermentability.

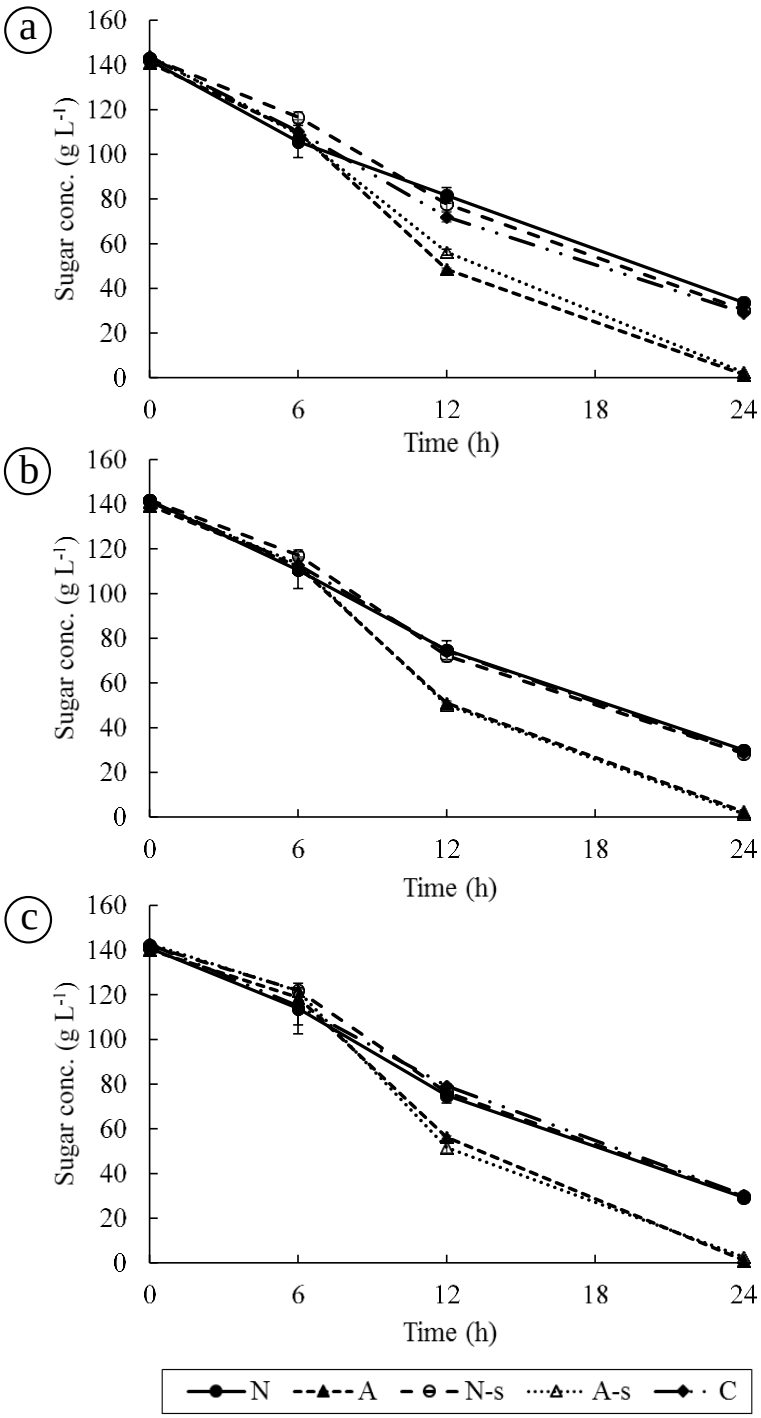

Figure 4.1. Sugar concentrations in diffuser juice acidified with either (a) phosphoric acid, (b) hydrochloric acid, or (c) sulfuric acid. Treatments consisted of either acidification to pH 3.5 followed by partial neutralization to pH 4.8 with sodium hydroxide (N) or ammonium hydroxide (A), or acidification to pH 4.8 and addition of the corresponding sodium salt (N-s) or ammonium salt (A-s) in the same amounts synthesized during acidification and partial neutralization (Table 4.2). The control (C) consisted of diffuser juice acidified to pH 4.8.

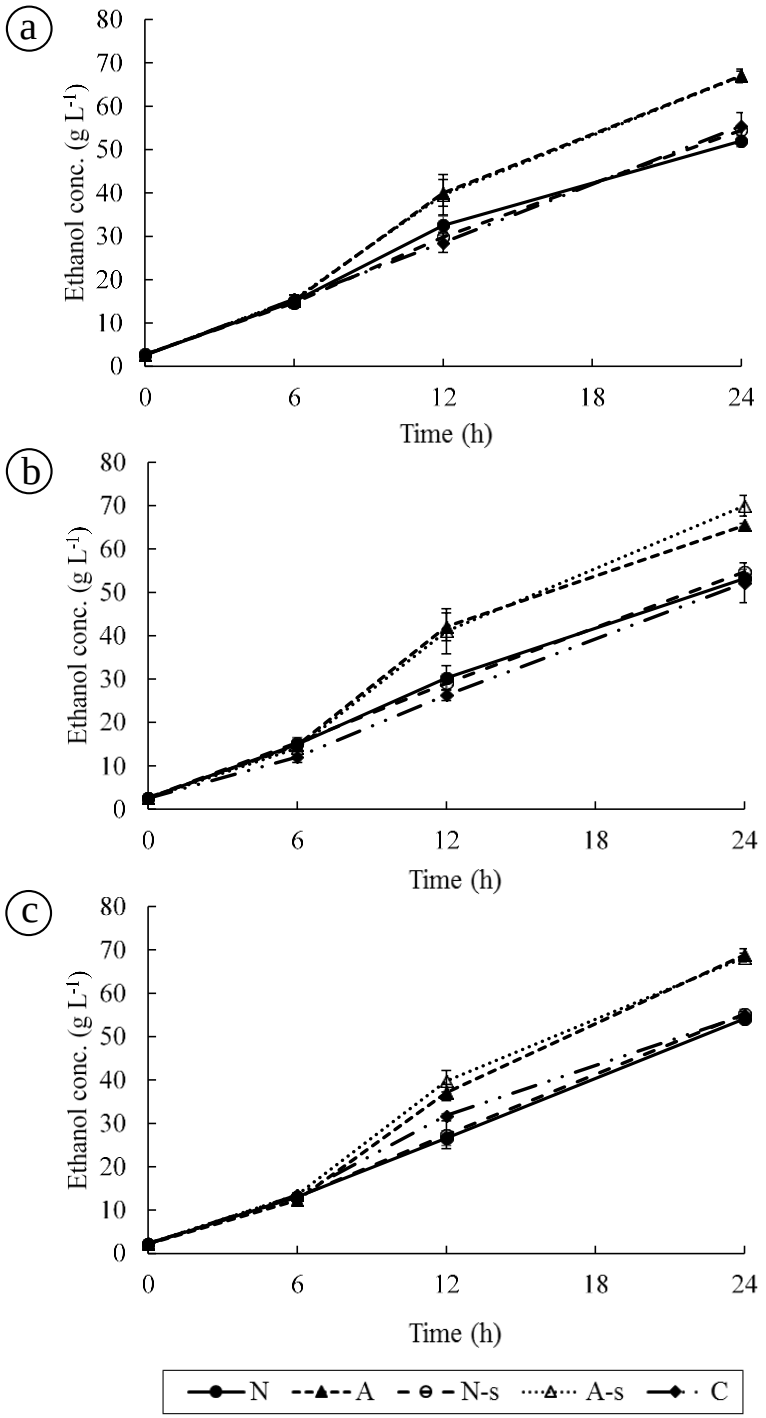

Figure 4.2. Ethanol concentrations in diffuser juice acidified with either (a) phosphoric acid, (b) hydrochloric acid, or (c) sulfuric acid. Treatments consisted of either acidification to pH 3.5 followed by partial neutralization to pH 4.8 with sodium hydroxide (N) or ammonium hydroxide (A), or acidification to pH 4.8 and addition of the corresponding sodium salt (N-s) or ammonium salt (A-s) in the same amounts synthesized during acidification and partial neutralization (Table 4.2). The control (C) consisted of diffuser juice acidified to pH 4.8.

Treatments with ammonium salts achieved fermentation efficiencies between 89.0 ± 1.2% and 94.8 ± 4.1%. Similarly, treatments with sodium salts and the controls achieved efficiencies between 86.8 ± 8.7% and 92.5 ± 0.3%. All of these values were not statistically different from each other ($P = 0.193$) and were larger than typical efficiency of ethanol plants (86.5%; Shapouri and Salassi, 2006). These efficiencies indicate that neither the ammonium nor sodium salts affected ethanol yields at the levels evaluated in this experiment. Furthermore, this suggests that treatments with sodium salts would have reached a final ethanol concentration similar to that of treatments with ammonium salts, if allowed to ferment for a longer period.

Effects of acidification and partial neutralization of concentrated, pressed juice on yeast fermentability

A follow-up experiment was conducted to test if results obtained on yeast fermentability of diffuser juice would also apply using concentrated, pressed juice. Only sulfuric acid was selected for juice acidification in this experiment since neither sulfate, chloride, nor phosphate anions affected yeast fermentability in diffuser juice. Additionally, the price of bulk sulfuric acid is significantly lower than that of phosphoric acid and comparable to that of hydrochloric acid (ICIS Chemical Business, 2006). Therefore, acidification with sulfuric acid may significantly reduce operating costs associated with long-term storage of sugars in concentrated juice.

Sugars in most treatments evaluated in this experiment were nearly completely fermented by 24 h (Figure 4.3a). Only diffuser juice had a significantly higher residual sugar fraction (18%), similar to that of diffuser juice in the preceding experiment (20% to 24%) with a similar TKN content (Table 4.1). The five treatments prepared with concentrated, pressed juice (i.e., those which contained either sodium or ammonium sulfates, and the control) had negligible residual sugar concentrations of less than 1.5 g L^{-1} (Figure 4.3a). Moreover, the corresponding

ethanol concentrations in those treatments at 24 h (63.6 g L^{-1} to 66.3 g L^{-1}) were among the highest as compared to all treatments (Figure 4.3b), resulting in fermentation efficiencies between 97.2 ± 0.6% and 99.7 ± 1.2%.

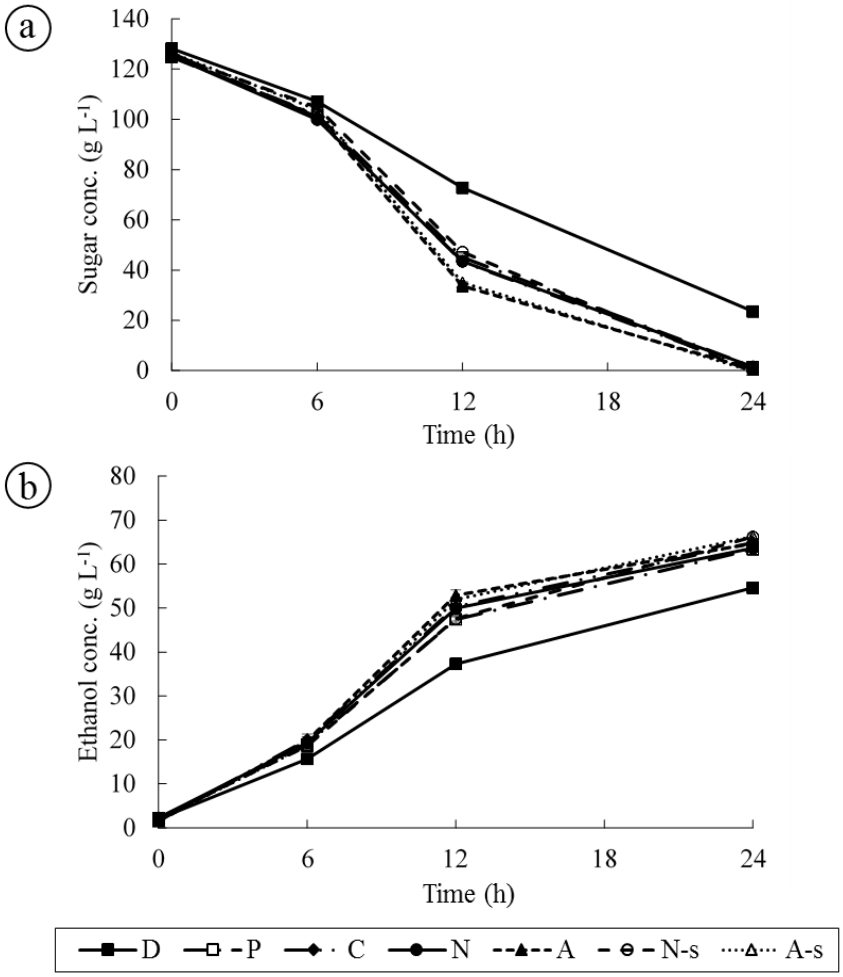

Figure 4.3. (a) Sugar and (b) ethanol concentrations in beet juices acidified with sulfuric acid. Diffuser juice (D) and pressed juice (P) were acidified to pH 4.8. Concentrated juice treatments consisted of either acidification to pH 3.5 and partial neutralization to pH 4.8 with sodium hydroxide (N) or ammonium hydroxide (A), or acidification to pH 4.8 and addition of sodium sulfate (N-s) or ammonium sulfate (A-s) in the same amounts synthesized during acidification and partial neutralization (Table 4.3). The control (C) consisted of concentrated juice acidified to pH 4.8.

The sugar and ethanol trends (Figures 4.3a and 4.3b) confirmed that acidification of concentrated, pressed juice for long-term storage and post-storage partial neutralization does not adversely affect yeast fermentability. Furthermore, neither ammonium nor sodium salts (whether

synthesized *in situ* through acidification and partial neutralization, or added directly) in concentrated, pressed juice are detrimental to yeast fermentability at the levels evaluated in this experiment. However, increased sodium salt concentrations in the juice could hinder yeast growth rates proportionally due to osmotic stress and consequently plasmolysis (Mager and Siderius, 2002, Logothetis et al., 2007), and also to increased cytosolic Na^+ concentrations (Walker, 1998; Andreishcheva and Zviagil'skaia, 1999).

The pressed juice also had a negligible residual sugar concentration (1.4 g L^{-1}), similar to that of the five treatments prepared with concentrated, pressed juice (Figure 4.3a). The ethanol concentration in pressed juice at 24 h (63.4 g L^{-1}) was also similar to concentrations in the treatments prepared with concentrated, pressed juice. These results indicated that concentration of pressed juice was not detrimental to yeast fermentability whatsoever.

The initial dry-basis TKN contents in pressed juice and concentrated, pressed juice were already about double that in diffuser juice used in this experiment (Table 4.1). Ammonium sulfate (whether synthesized *in situ* or added directly) increased the TKN in treatments prepared with concentrated, pressed juice (1.14 g kg^{-1} on a wet basis, after adjusting sugar concentration by dilution) by approximately 20% (Table 4.3). However, unlike yeast fermentability results discussed in the subsection above, NH_4^+ only slightly improved fermentation rates (Figures 4.3a and 4.3b). Specifically, treatments prepared with concentrated, pressed juice, and containing ammonium sulfate, showed slightly enhanced yeast sugar consumption between 6 h and 12 h as compared to treatments with sodium sulfate and the control (Figure 4.3a). The sugar concentrations in treatments with synthesized and added ammonium sulfate were 33.7 g L^{-1} and 35.0 g L^{-1}, respectively, at 12 h. In comparison, concentrations in treatments with sodium sulfate and the control were statistically higher at 12 h (43.3 g L^{-1} to 47.2 g L^{-1}; $P < 0.0001$). Similarly,

ethanol concentrations in treatments with either synthesized or added ammonium sulfate (52.9 g L^{-1} and 51.9 g L^{-1}, respectively) were slightly but statistically higher than those in treatments with either synthesized or added sodium sulfate by 12 h (47.5 g L^{-1} and 49.9 g L^{-1}, respectively; $P = 0.0002$). The ethanol concentration in the control (50.3 g L^{-1}) was not statistically different than concentrations in treatments with ammonium sulfate by 12 h ($P = 0.133$). These results confirmed that TKN in concentrated, pressed juice was sufficient for yeast fermentability. The NH_4^+ supplied to the juice as ammonium sulfate slightly improved yeast ethanol production rates.

Table 4.3. Synthesized/added salts, nitrogen from salts, and total Kjeldahl nitrogen (TKN) contents in treatments prepared with concentrated, pressed juice.

Salt	Synthesized/added (g kg^{-1})	Nitrogen from salt (g kg^{-1})	TKN[a] (g kg^{-1})
Na_2SO_4	1.01	-	1.02
$(NH_4)_2SO_4$	1.08	0.23	1.25

[a]Estimated accounting for dilution effect of inoculum volume fraction of 10%.

Conclusions

Sodium salts do not hinder yeast fermentability of non-purified beet juice at the levels evaluated in this work. Ammonium salts can significantly improve yeast fermentation rates in diffuser juice with total Kjeldahl nitrogen deficiencies. These salts can be synthesized in concentrated juice by acidification to enable long-term storage, and post-storage partial neutralization. No statistical differences were detected in ethanol yields of treatments with either ammonium phosphate, chloride, or sulfate. Hence, the beet ethanol industry could select the least expensive acid and base (among those evaluated in this work) for pre-storage acidification and post-storage partial neutralization, respectively, to yield ammonium salts.

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CHAPTER 5. ECONOMIC ANALYSIS OF AN INDUSTRIAL PROCESS FOR LONG-TERM INDUSTRIAL-BEET SUGAR STORAGE[4]

Executive summary

Raw, thick juice (or concentrated, non-purified beet juice) is a potential alternative for long-term storage of industrial-beet sugars. Experimental results show that combinations of pH ≤ 3.5 or pH ≥ 9.5 with refractometric dissolved solid (RDS) weight fractions ≥ 64.5% enable retention of up to 99% of industrial sugars in raw, thick juice stored for up to 24 wk at 23 ± 1 °C (Vargas-Ramirez et al. 2013). Furthermore, raw, thick juice stored under acidic conditions (pH ≤ 3.5) has shown higher stability throughout storage as compared to juice stored under alkaline conditions. Despite this promising outcome, no information is yet available on the economics to produce and store raw, thick juice under the most favorable conditions described above. This report presents factored estimates of major equipment costs, annual operating and maintenance costs, and total capital investment to establish, operate, and maintain processing equipment to produce and store raw, thick juice.

A quintuple-effect falling-film evaporator is proposed to process 412,000 Mg of raw juice (RDS weight fraction of 15%) and produce 94,000 Mg of raw, thick juice (RDS weight fraction of 65%). This juice will help achieve an industrial sugar inventory that will allow an uninterrupted 48-wk operation of an ethanol plant with a production capacity of 76×10^6 L y^{-1}. Multiple-effect evaporators allow efficient energy use since boiler steam is required only in the

[4] Chapter 5 is the result of a report requested by Green Vision Group on technical and economic assessments of raw, thick juice production. The report was submitted to the North Dakota Industrial Commission as part of an extensive report of accomplishments under a funded project. Authors: Juan M. Vargas-Ramirez and Dennis P. Wiesenborn. Juan M. Vargas-Ramirez developed the process analyzed in this chapter and estimated capital and operating costs in collaboration with equipment manufacturers. Co-author Dennis Wiesenborn provided advice throughout the work and/or assisted in the editing.

first effect and subsequent effects operate with vapor produced in each preceding effect. The raw, thick juice produced will be distributed in equal portions into six high-tensional carbon steel storage tanks with dimensions of 36 m in diameter and 13 m in height. As the raw, thick juice enters the tanks, sulfuric acid will be added at a predetermined proportion to achieve a pH $\leq$ 3.5, which will ensure stable, long-term storage.

User-friendly Excel spreadsheet models were prepared to allow modification of key input parameters to calculate equipment dimensions, utility requirements, and factored estimates of fixed capital and operating costs. Mass and energy balances were conducted to determine steam and vapor requirements in the first and subsequent evaporator effects, respectively, and to estimate the heating areas needed in each effect. Equipment prices were either obtained from the literature or provided by manufacturers through formal quotes. When necessary, prices were updated using the Chemical Engineering Plant Cost Index for chemical process industries. The overall fixed capital and operating costs for the production of stable raw, thick juice at conditions specified in this report are summarized in Table 5.1.

Table 5.1. Fixed capital and operating costs for raw, thick juice production.

Item		(U.S. $M)	
Quintuple-effect falling-film evaporator	Fixed capital cost	8.258	
Storage tanks		12.423	
		(U.S. $M)	(U.S. ¢ kg^{-1} of dry sugar)
Steam	Annual operating cost	0.853	1.4
Electrical power		0.073	0.12
Evaporator maintenance		0.496	0.81
Sulfuric acid		0.088	0.14
Sodium hydroxide		0.339	0.55

Introduction

Industrial sugars are carbohydrates that can be used to produce value-added products that are commonly produced from non-renewable sources such as petroleum. Industrial beets, a

developing source of industrial sugars, have significant potential to compete against corn grain, the current major source of industrial sugars in the U.S. These beets can grow successfully in salty soils, which are detrimental to most food crops, and can withstand non-irrigated conditions (NDSU Carrington REC, 2013). These are two major advantages of industrial beets over crops that are grown in active agricultural land and are current feedstocks for non-food products. Despite these advantages, techniques for efficient conversion of industrial beets into desired products are yet necessary. Moreover, their high moisture content significantly restricts storage length at non-freezing temperatures since it favors microbial proliferation, which can result in significant sugar and economic losses (McGinnis, 1996; van der Poel et al., 1998; Asadi, 2007).

Many storage techniques have been evaluated to minimize sugar losses and extend processing campaigns in beet sugar factories. These techniques include modified atmosphere storage, application of wax coatings and antimicrobial agents to beet surface prior to storage, among others. The most successful, thus far, has been thick juice storage, which consists of storing concentrated, purified beet juice in large storage tanks. Thick juice storage has been practiced in many beet sugar factories across the U.S. since 1960 (van der Poel et al., 1998).

At first glance, thick juice may appear suitable for long-term industrial sugar storage and processing campaign extension in developing industrial beet processing plants. However, thick juice production, as practiced in the beet sugar industry, entails energy-intensive steps such as raw juice purification. Moreover, thick juice storage involves large amounts of alkali to achieve highly alkaline conditions (pH $\approx$ 9) that halt microbial activity and prevent sucrose inversion (i.e., breakdown of sucrose into glucose and fructose) in the juice. Raw juice purification and highly alkaline pH values for feedstock storage may not be necessary in a conversion platform such as that for ethanol production by fermentation. In fact, the omission of the purification step

in the production of a concentrated sugar extract and reduction of chemical use for storage may yield significant operating cost and greenhouse gas reductions. Moreover, highly alkaline pH values are not the only option to achieve successful quality retention in biological materials; likewise, acidic pH values offer an important antimicrobial effect (Rahman, 2007). In contrast to alkaline pH values, acidic values are known to enhance sucrose inversion (Gabriel, 2008); yet, sucrose inversion should not be a concern to an industry such as the ethanol one since invert sugars are more readily fermented by yeast as compared to sucrose.

Based on the reasoning described above, Vargas-Ramirez et al. (2013) evaluated raw, thick juice as an alternative for industrial sugar storage. The juice was stored for up to 24 wk at various pH and refractometric dissolved solid (RDS) weight fraction combinations. Raw, thick juice stored at combinations of pH $\leq$ 3.5 or pH $\geq$ 9.5 with RDS weight fractions $\geq$ 64.5% retained up to 99% of initial industrial sugars. Furthermore, raw, thick juice stored under acidic conditions (pH $\leq$ 3.5) showed more stability throughout storage and provided the highest ethanol fermentation efficiencies (< 82% of theoretical ethanol yield) as compared to juice stored under alkaline conditions (< 54%).

In this report, raw, thick juice storage is proposed as a complementing storage technique to extend the yearly processing campaign of an ethanol plant to 333 d. A longer campaign will help maximize the use of processing equipment and consequently the return on assets. Design calculations for processing equipment to produce and store raw, thick juice, along with fixed capital investments and annual operating costs, are also presented in detail in this report.

Overall processing scheme and timeline

A processing scheme and timeline proposed to convert sugars from industrial beets into ethanol in an uninterrupted 333-d period are summarized in Figures 5.1 and 5.2. Harvesting and

plant start-up will begin simultaneously in early October; harvest will be completed within a month. Two long-term storage techniques are suggested to retain industrial sugars throughout the plant-operation period and maximize use of equipment and processing capacity. The first technique is conventional pile storage under frigid ambient conditions, which will begin in parallel with harvest and continue for approximately 165 d (until the middle of March). The second technique is raw, thick juice storage, which will begin in conjunction with front-end beet processing (i.e., beet-sugar extraction) and continue throughout the plant operation period. All beets must be processed within the length of pile storage (165 d) to secure sufficient industrial sugars to continue ethanol production during the warm months. During the front-end processing period, fermentation and raw, thick juice production will take place in parallel. Raw, thick juice will be stored in 6 storage tanks and processed immediately after piled beets have been consumed. This juice will help ensure that the plant's ethanol production goal is met during the subsequent five (warm) months. Prior to fermentation, stored raw, thick juice will be conditioned by means of water addition and pH neutralization to achieve optimum fermentation parameters. The conditioned juice will then be fed into the fermenters to achieve the uninterrupted production of ethanol.

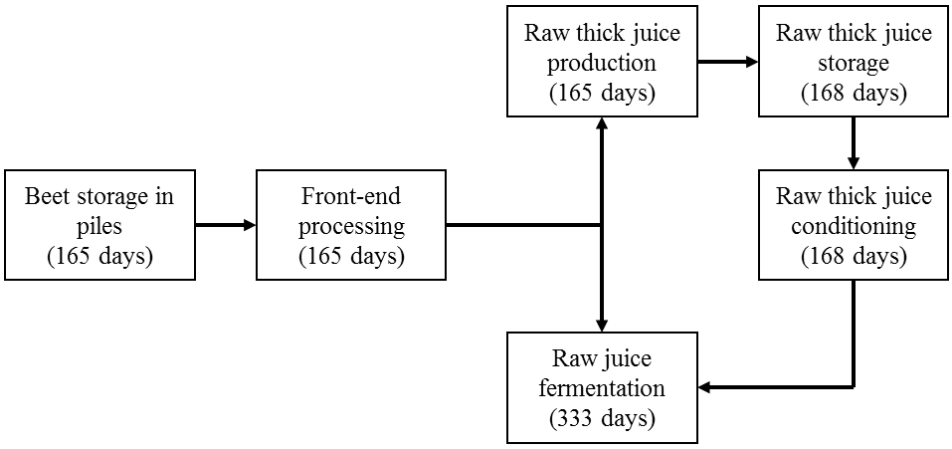

Figure 5.1. Flowchart of a process proposed to convert industrial beets into ethanol in an uninterrupted 333-d processing period.

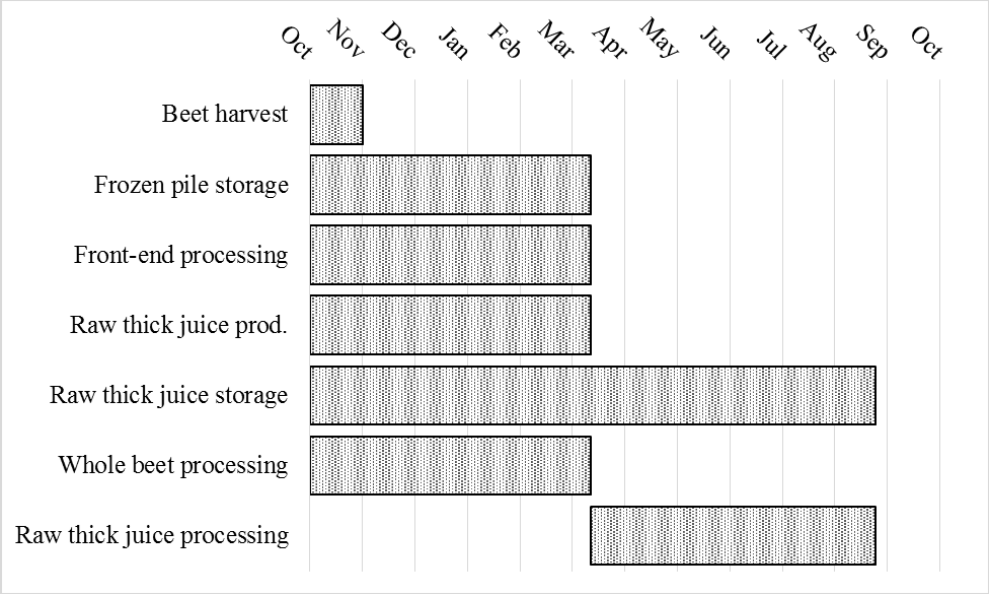

Figure 5.2. Timeline to convert sugars from industrial beets into ethanol in an uninterrupted 333-d processing period.

Raw, thick juice production

Evaporator selection

Continuous-flow evaporators are used in the sugar industry to produce thick juice prior to sucrose crystallization. Thin-film tube evaporators were introduced into sugar factories in the 1980s with significant advantages over Robert evaporators, the most common type in sugar factories built before then (Asadi, 2007). Some of these advantages are: lower retention time and higher overall heat transfer coefficient. Thin-film tube evaporators are designed to work in vertical position and consist of two major components: (1) a heat exchanger (also referred to as *calandria*), and (2) an entrainment separator (Figure 5.3). The heat exchanger is constituted by an arrangement of parallel tubes distributed strategically within a metal shell. The feed is introduced at the bottom or top of the evaporator (depending on type) and flows in equal proportions across the parallel tubes. Steam flows within the shell and in between the tubes that carry the feed. According to evaporation principles, and analogous to what occurs in other types of evaporators, the energy necessary to evaporate the most volatile component in the feed

(typically water) is provided by the steam. The vapor formed within the tubes carrying the feed tends to localize in the (radial) center promoting the formation of a thin layer of feed material, which travels along the inner circumference of each tube. The entrainment separator restricts the exit of product droplets that travel with the vapor as this is produced, minimizing product loss during evaporation.

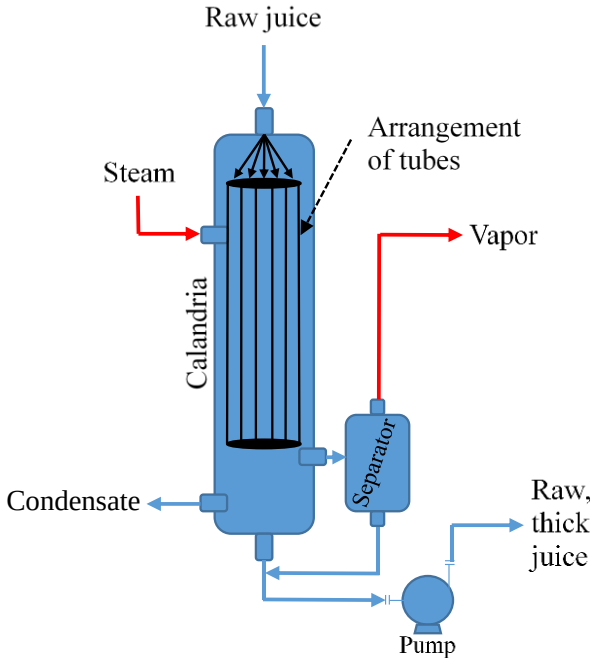

Figure 5.3. Sections and components of a falling-film tube evaporator. Flow directions of feed, steam, vapor, and product are indicated by continuous lines with arrows.

Thin-film tube evaporators can be classified into two types: rising-film and falling-film. Some modern sugar factories use falling-film evaporators (Asadi, 2007) and potential energy savings have been demonstrated when considering the retrofit of this type of evaporator into existing cane sugar plants (Ogden et al., 1990). In fact, falling-film evaporators have several advantages over other types of continuous-flow evaporators (Asadi, 2007). For example, falling-film evaporators can operate at a lower temperature difference between the steam and boiling feed (6 °C) as compared to rising-film evaporators (14 °C). Moreover, when compared to rising-

film evaporators, falling-film evaporators can handle more viscous liquids and have lower retention times (typically ¼ of the retention time of a rising-film evaporator).

Multiple-effect evaporators are arrangements with two or more evaporators connected in series. This allows efficient use of energy contained in the steam, which is only used in the first effect. Under ideal conditions, the amount of steam entering the first-effect of the evaporator should evaporate an equal amount of water from the feed (in this case, raw juice). The vapor produced in the first effect is then directed to the second effect where it will evaporate an amount of water equal to the amount of vapor entering that effect. This mechanism continues until the final effect. The feed will be partially concentrated in each effect and should reach the desired final concentration before exiting the last effect. A diagram of a quintuple-effect falling-film evaporator is presented in Figure 5.4. Practices to increase energy efficiency may be put in place and linked directly to a multiple-effect evaporator. In fact, sugar factories use vapors exiting the different effects to increase the temperature of various process streams that should be at determined temperatures before entering other processing equipment (Ogden et al., 1990; McGinnis, 1996; Van der Poel et al., 1998; Asadi, 2007).

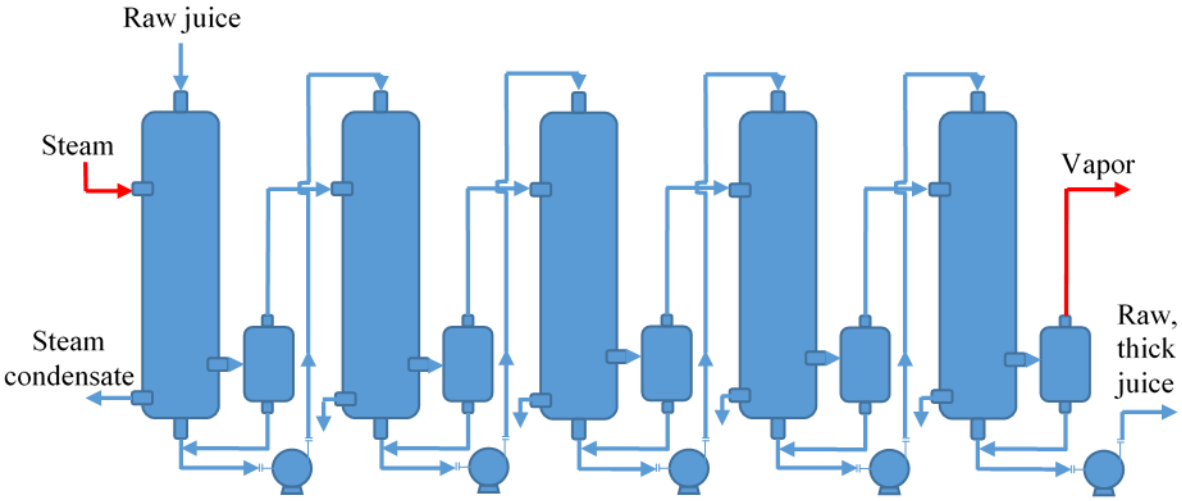

Figure 5.4. Diagram of a quintuple-effect falling-film evaporator that operates with energy re-use.

In the proposed processing scheme, raw, thick juice is assumed to be produced in a quintuple-effect falling-film evaporator. The evaporator was designed following mass and heat transfer principles (as detailed in the following subsection). The results were compared to those of a formal design provided by the engineering firm Rosenblad Design Group, Inc., which specializes in evaporator design.

Evaporator design

The mass and energy balances for the complete evaporator were conducted under the following assumptions, which are typically used to simplify evaporator analyses:

1) The evaporator operates at steady conditions at all times

2) No heat is lost to the surroundings due to perfect insulation

3) No boiling point elevation caused by the increase of solids fraction in the feed

4) Vapor and product exit each effect at equal temperatures

5) Input steam/vapor and output condensate in each effect are at equal temperatures.

Spreadsheet models were prepared in Microsoft Excel (Appendix C) to perform all necessary calculations for the evaporator design. Input parameters used in the calculations for this report are summarized in Table 5.2. Yet, the models were formulated in a way such that parameters like *raw juice draft (kg of juice per 100 kg of beets)*, *RDS fractions of raw and raw, thick juices*, and *temperatures of feed, steam,* and *product* could be readily modified to evaluate the sensitivity of the design with respect to each parameter. The thermodynamic properties of saturated steam (including enthalpies of liquid and steam/vapor, and latent heat of vaporization) were linked to the models and used in the energy calculations. When the temperatures of steam entering the first effect and vapor exiting the last effect are specified, the corresponding absolute pressures are automatically interpolated.

Table 5.2. Assumptions used in calculations for a quintuple-effect falling-film evaporator design.

	Draft (%)
Raw juice (RJ)	110
	Dry solids (%)
RJ	15
Raw, thick juice (RTJ)	65
	Temperature (°C)
Steam	130
Feed	90
Last-effect RTJ (and vapor)	90

Initially, it was assumed that steam/vapor pressures dropped in equivalent proportions across the evaporator effects. In this case, the steam/vapor pressures corresponding to the first and final effects were used to determine the pressure of the vapor exiting each effect. The corresponding temperatures were then interpolated from the thermodynamic properties of saturated steam.

The mass flow rate of raw juice entering the evaporator was determined based on the mass of beets that would be processed in the 165-d period and the raw juice draft as follows:

$$M_{RJ} = M_{Beet} \times \frac{Draft}{100} \quad \text{(Eq. 5.1)}$$

where M_{RJ} and M_{Beet} are the masses of raw juice and total beets, respectively, and *Draft* is the weight ratio (in percent) of raw juice to beets. Moreover, the mass flow rate of raw, thick juice exiting the last effect was determined as follows:

$$M_{RTJ} = M_{RJ} \times \frac{DS_{RJ}}{DS_{RTJ}} \quad \text{(Eq. 5.2)}$$

where M_{RTJ} is the mass of raw, thick juice exiting the last effect, and DS_{RJ} and DS_{RTJ} are the dry solids fractions in the raw juice and raw, thick juice, respectively. The mass of water removed by

evaporation was simply calculated as the difference of the raw juice and last-effect raw, thick juice masses.

The enthalpies of juice entering each effect were determined using the following equation:

$$H_i = C_{p,i} \cdot (T_i - T_R) \quad \text{(Eq. 5.3)}$$

where H_i, $C_{p,i}$, and T_i are the enthalpy, specific heat capacity, and temperature of juice entering the i^{th} evaporator effect, and T_R is the reference temperature (generally 0 °C). The specific heat capacity of the material of interest is influenced by its dry solids fraction according to the following expression:

$$C_{p,i} = 4.187 \cdot (1 - 0.006 \cdot DS_i) \quad \text{(Eq. 5.4)}$$

where $C_{p,i}$ is in units of kJ kg^{-1} °C^{-1} (or kJ kg^{-1} K^{-1}) and DS_i is the dry solids fraction of juice in the i^{th} effect.

In a first attempt, the dry solids fraction of the juice was assumed to increase exponentially across the evaporator effects according to the following expression:

$$DS_i = DS_0 \cdot (1 + r)^i \quad \text{(Eq. 5.5)}$$

where DS_0 is the dry solids fraction in the original raw juice, r is the rate of increase (determined to be 32.4 for this particular case), and i is the evaporator effect number. Once the dry solid fractions for juice exiting each effect were determined, the mass flow rates of the corresponding juice outputs were determined using Equation 5.2.

The enthalpy of vapor condensate formed in each evaporator effect is in essence the enthalpy of liquid at the temperature of steam/vapor entering the same effect. The values corresponding to these enthalpies are also automatically interpolated from the saturated steam

properties. Moreover, the enthalpies of the steam or vapor entering the first effect or subsequent steps, respectively, are interpolated in the same manner.

The overall heat transfer coefficient (*U*) for each evaporator effect was estimated using the *Swedish formula* (Asadi, 2007), which is representative of Robert evaporators, but was used here for practical purposes:

$$U_i = 503 \cdot \frac{T_{P,i}}{DS_{P,i}} \qquad \text{(Eq. 5.6)}$$

where U_i is the overall heat transfer coefficient of the i^{th} evaporator effect in units of W m^{-2} °C^{-1}, and $T_{P,i}$ and $DS_{P,i}$ are the temperature (in °C) and dry solids fraction, respectively, of the product leaving the same evaporator effect.

Once the overall heat transfer coefficients for each effect were estimated, the method described by Earle (1983) was followed to determine steam requirement, vapor outputs in each effect, steam economy, and heat transfer area for each evaporator effect. This method was used under the assumptions that there was equal heat transfer in each evaporator effect and that sensible heat effects and boiling point elevation were negligible. The complexity of the design calculations increases when the aspects neglected in this case are taken into consideration and numerical methods may be required. In this particular case, the simplified calculations were readily performed in Excel and two iterations were required.

The heating areas were assumed to be the same for every effect in the evaporator and the total heating area of the first evaporator effect was calculated using the following equation:

$$U \cdot A \cdot (T_S - T_V) = M_V \cdot (H_V - H_C) \qquad \text{(Eq. 5.7)}$$

where *U* and *A* are the overall heat transfer coefficient and total heating area, respectively, of the effect; T_S and T_V are the temperatures of the input steam (or vapor) and output vapor in the effect, respectively; M_V is the mass flow rate of vapor entering the effect; and H_V and H_C are the

enthalpies of vapor and condensate exiting the effect, respectively. Equation 5.7 shows that total heat transfer is equal to the latent heat contained in the steam/vapor entering each effect. In other words, the equation shows an idealization that all of the energy contained in the steam is efficiently used to evaporate a corresponding portion of water from the feed.

Raw, thick juice acidification

Raw, thick juice produced will be acidified with sulfuric acid prior to storage. The amount of acid required to achieve a pH of 3.5, which will ensure juice stability throughout storage, was determined by means of an acidification experiment. The experiment was carried out using raw juice with an RDS weight fraction of 16% and 5-mol L^{-1} sulfuric acid added at different volumes. The pH of the raw juice and acid mixture was measure after each addition. The resulting acidification curve (Figure 5.5) was used to interpolate the acid concentration (moles g^{-1}; on a dry basis) needed to adjust the pH of juice to 3.5. The total amount of acid required to adjust the pH of all raw, thick juice (produced at an RDS fraction of 65%) to the desired value was calculated using Equation 5.8.

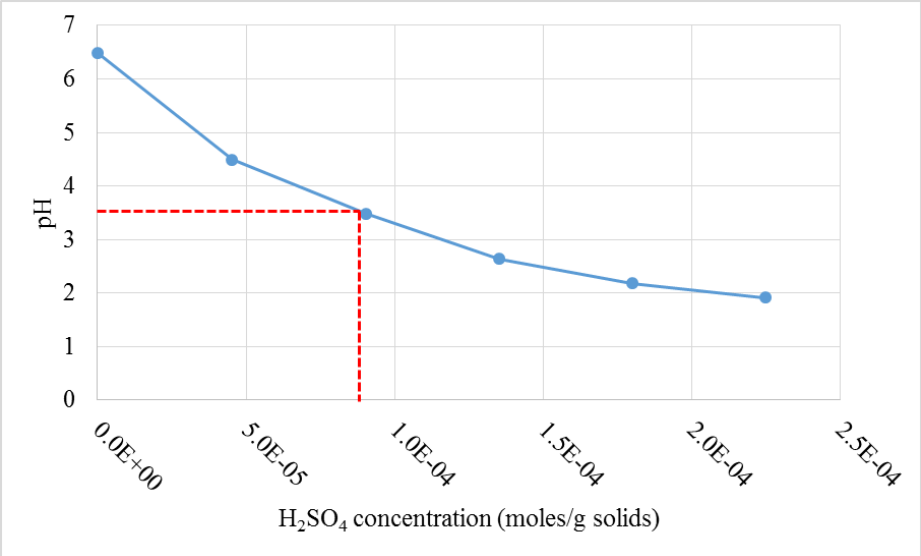

Figure 5.5. Acidification curve of raw juice acidified with 5-mol L^{-1} sulfuric acid. The red, dashed line shows the interpolation of H_2SO_4 concentration needed to achieve a pH of 3.5 to ensure juice stability during storage.

$$Acid\ [moles] = Acid\ conc.\left[\frac{moles}{kg\ solids}\right] \times Total\ RTJ\ [kg] \times RDS\ fraction\ \left[\frac{kg\ solids}{kg\ juice}\right] \quad \text{(Eq. 5.8)}$$

Raw, thick juice neutralization

Following storage and prior to fermentation, the stored raw, thick juice will be conditioned in a mixing tank by means of water addition and pH neutralization. The sulfuric acid in the juice will be neutralized with sodium hydroxide to achieve a pH value of 4.8, which is appropriate for yeast fermentation. The amount of sodium hydroxide needed to achieve the target pH was determined in a laboratory-scale neutralization experiment. The experiment consisted of neutralizing the acidified raw, thick juice using a 5-mol L^{-1} sodium hydroxide solution added at different volumes. The neutralization curve (Figure 5.6) was used to interpolate the concentration of sodium hydroxide needed to adjust the pH of the juice to 4.8. The actual amount of sodium hydroxide required to adjust the pH of the acidified raw, thick juice was calculated as follows:

$$Base\ [moles] = Base\ conc.\left[\frac{moles}{kg\ solids}\right] \times Acidified\ RTJ\ [kg] \times RDS\ fraction\ \left[\frac{kg\ solids}{kg\ juice}\right] \quad \text{(Eq. 5.9)}$$

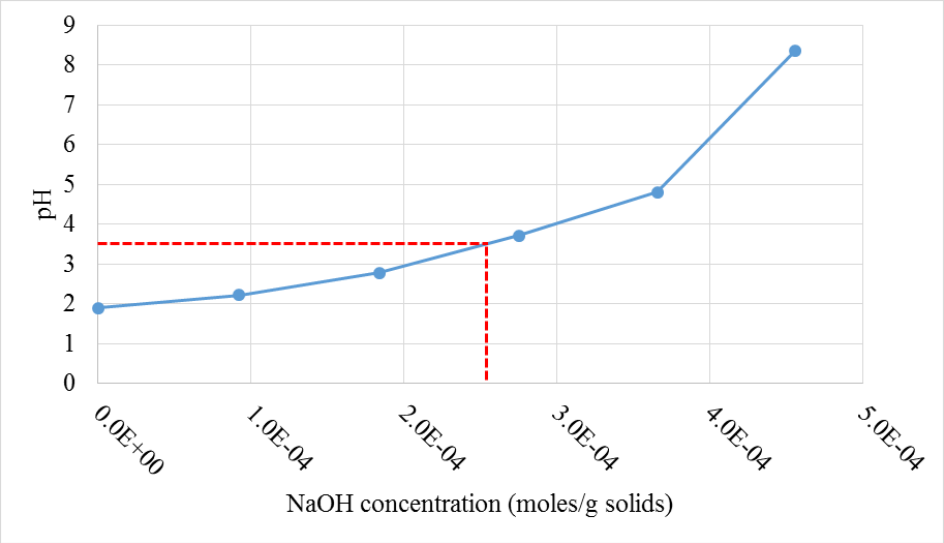

Figure 5.6. Neutralization curve of acidified raw juice using 5-mol L^{-1} sodium hydroxide. The red, dashed line shows the interpolation of NaOH concentration needed to achieve a pH of 4.8, which is appropriate for yeast fermentation.

Fixed capital investment estimations

The fixed capital investment for major equipment needed for raw, thick juice production and storage was determined on the basis of a factored estimate. This type of estimate is typically prepared at early stages of an economic assessment and yields results with ± 30% accuracy (Peters et al., 2003). The determinant items in the estimation of direct and indirect costs of equipment, and factors associated with them, are defined in the literature (Peters et al., 2003). Specific factors for a wet processing plant, such as an ethanol plant, are presented in Table 5.3. These factors correspond to a fraction of the delivered equipment cost, namely, a fraction of the sum of purchased equipment and equipment delivery costs.

Table 5.3. Costs associated with a wet processing plant expressed as a fraction of the delivered equipment cost.

Direct costs	*Fraction of delivered equipment*
Purchased equipment	1.00
Equipment delivery	0.10
Purchased equipment installation	0.47
Instrumentation and controls (installed)	0.36
Piping (installed)	0.68
Electrical systems (installed)	0.11
Buildings (including services)	0.18
Yard improvements	0.10
Service facilities (installed)	0.70
Total direct costs	3.70
Indirect costs	
Engineering and supervision	0.33
Construction expenses	0.41
Legal expenses	0.04
Contractor's fee	0.22
Contingency	0.44
Total indirect costs	1.44
Fixed capital investment	
Direct + Indirect costs	5.14

The two major pieces of equipment involved in the production and storage of raw, thick juice are the quintuple-effect falling-film evaporator and the storage tanks. The installed equipment cost for the evaporator was determined on the basis of total heating area and using the installed cost per unit heating area reported by Ogden et al. (1990). Outdated costs, such as the one used to estimate the total cost of the evaporator, should be treated with care. This original cost was adjusted to current cost using Equation 5.10 and the Chemical Engineering Plant Cost Index for chemical process industries available in the *Chemical Engineering Magazine*:

$$Current\ cost = Original\ cost \times \left(\frac{Index\ value\ at\ present}{Index\ value\ at\ time\ of\ original\ cost}\right) \quad \text{(Eq. 5.10)}$$

A formal design and budgetary quote for a quintuple-effect falling-film plate evaporation plant with surface condenser was requested from Rosenblad Design Group, Inc. (Yulee, FL). The design and quote were prepared for an evaporator with a raw juice processing capacity of 75 Mg h^{-1}, which corresponded to preliminary processing calculations. However, final processing calculations showed that an actual processing rate of 104 Mg h^{-1} was required to achieve the raw, thick juice and ethanol production targets set for the proposed ethanol plant. The price of an evaporator with larger processing capacity was estimated following the *six-tenths factor rule* (Peters et al., 2003) which gives satisfactory results for cost approximations within ± 20%:

$$Cost\ of\ equipment\ A = Cost\ of\ equipment\ B \cdot \left(\frac{Capacity\ of\ A}{Capacity\ of\ B}\right)^{0.6} \quad \text{(Eq. 5.11)}$$

The delivered equipment cost for storage tanks was requested in the form of a formal quote from Brown-Minneapolis Tank (BMT; Albuquerque, NM). The cost estimated by BMT included tank construction (with A516-70 high-tensional carbon steel), interior lining for protection against acid corrosion, exterior paint for protection against outdoor conditions, and freight from Albuquerque, NM to Bismarck, ND. An estimate for exterior insulation was

provided by BMT, but it was not included in the total tank cost. The volume of each tank was determined by dividing the total volume of raw, thick juice to be produced plus a 10% contingency by the number of tanks required. As a starting point, two tanks of equal dimensions were assumed. The budgetary quote for each tank was based on a volume capacity of 37.7×10^6 L, which corresponded to preliminary calculations. Nonetheless, final calculations showed that, if two storage tanks were to be used, each tank should have a volume capacity of 38.9×10^6 L. The cost for each tank was adjusted based on its final capacity using equation 5.11. For comparison purposes, additional analysis were performed to estimate the costs of 6 and 10 storage tanks with the same total volume capacity.

Annual operating cost estimations

The annual operating costs estimated were those directly associated to raw, thick juice production, storage, and conditioning for fermentation. The components considered for these estimations were: energy, maintenance, operation supplies, and acid and base for juice pH adjustment. Labor cost was not estimated, but it may be easily incorporated into the analysis once the number of qualified operators required is defined.

The major energy demand in the process comes from the evaporator, which requires large quantities of steam to concentrate the feed. Additionally, electric power is needed in this system to operate six centrifugal pumps that feed each evaporating effect with the material to concentrate. Steam cost was estimated from the steam requirement calculated in the evaporator design and assuming a steam cost of \$5 $MMBTU^{-1}$ (Spiritwoord Energy Park Association; Jamestown, ND). On the other hand, total electric power cost was estimated from the cost for the industrial sector in North Dakota, which is \$0.0802 kWh^{-1} (U.S. Energy Information Administration, 2014). The evaporator's electric power demand was estimated based on the

demand of the quintuple-effect falling-film evaporator designed by Rosenblad Design Group, Inc. with a processing capacity of 75 Mg h^{-1}. The demand of that evaporator was 165 kWh, which was adjusted to the capacity of the evaporator designed and described in this report assuming a linear relation between capacity and electric power requirement. Total energy requirements and costs were estimated for a continuous processing period of 3,960 h (165 d × 24 h/d).

Annual maintenance costs in process industries typically account for between 2% to 10% of the fixed capital investment (Peters et al., 2003). The annual maintenance cost for the evaporation station was assumed to be 6% of the fixed capital investment. Moreover, the annual cost of operation supplies was estimated as 15% of the annual maintenance cost (Peters et al., 2003). The total costs for acid and base required to adjust raw, thick juice pH to 3.5 and 4.8 before storage and fermentation, respectively, were determined assuming costs of \$0.16 kg^{-1} of acid (Brainerd Chemical Company, Inc.) and \$0.38 kg^{-1} of base.

Results

Fixed capital investment estimates

The initial overall heat transfer coefficient (OHTC) values estimated assuming an equivalent pressure drop among evaporator effects and an exponential increase in dry solids fraction are given in Table 5.4. This table also presents the OHTC values obtained from two iteration steps for the method described by Earle (1983). The quintuple-effect falling-film tube evaporator designed following the method described under the *Evaporator design* subsection is summarized in Figure 5.7.

Table 5.4. Initial overall heat transfer coefficients (OHTC) used in evaporator design calculations and obtained in first and second method iterations.

	OHTC (U; W m^{-2} °C^{-1})		
Effect No.	Initial	1st iteration	2nd iteration
1	3231	3609	3617
2	2351	2825	2847
3	1689	2063	2099
4	1186	1343	1378
5	798	697	697

The quintuple-effect falling-film plate evaporator designed by Rosenblad Design Group, Inc. had a total heating area of 2,460 m^2, which was determined based on a preliminary raw juice processing rate of 75 Mg h^{-1}. The budgetary cost for this evaporator was estimated at $6.8M and included: fabrication of evaporators and condenser vessels, all small condensate and liquor flash tanks, vacuum system, pumps, instruments, control valves, hand valves, process and utility piping and supports inside battery limit. In addition to this, equipment platforms, platform ladders, and support legs for all vessels were included in the quote. The final processing rate was estimated to be 104 Mg h^{-1} and the price of the evaporation station adjusted using the *six-tenths factor rule* was estimated to be $8.26M.

The direct and indirect costs associated with the evaporator designed by Rosenblad Design Group, Inc. are broken down in Table 5.5. The factors accounted for in these cost estimations were selected from Table 5.3 taking into consideration the scope of work defined for the budgetary proposal and total cost (see Appendix D). The annual maintenance and operation supplies costs for the evaporation station were estimated to be about $496,000 and $74,500, respectively.

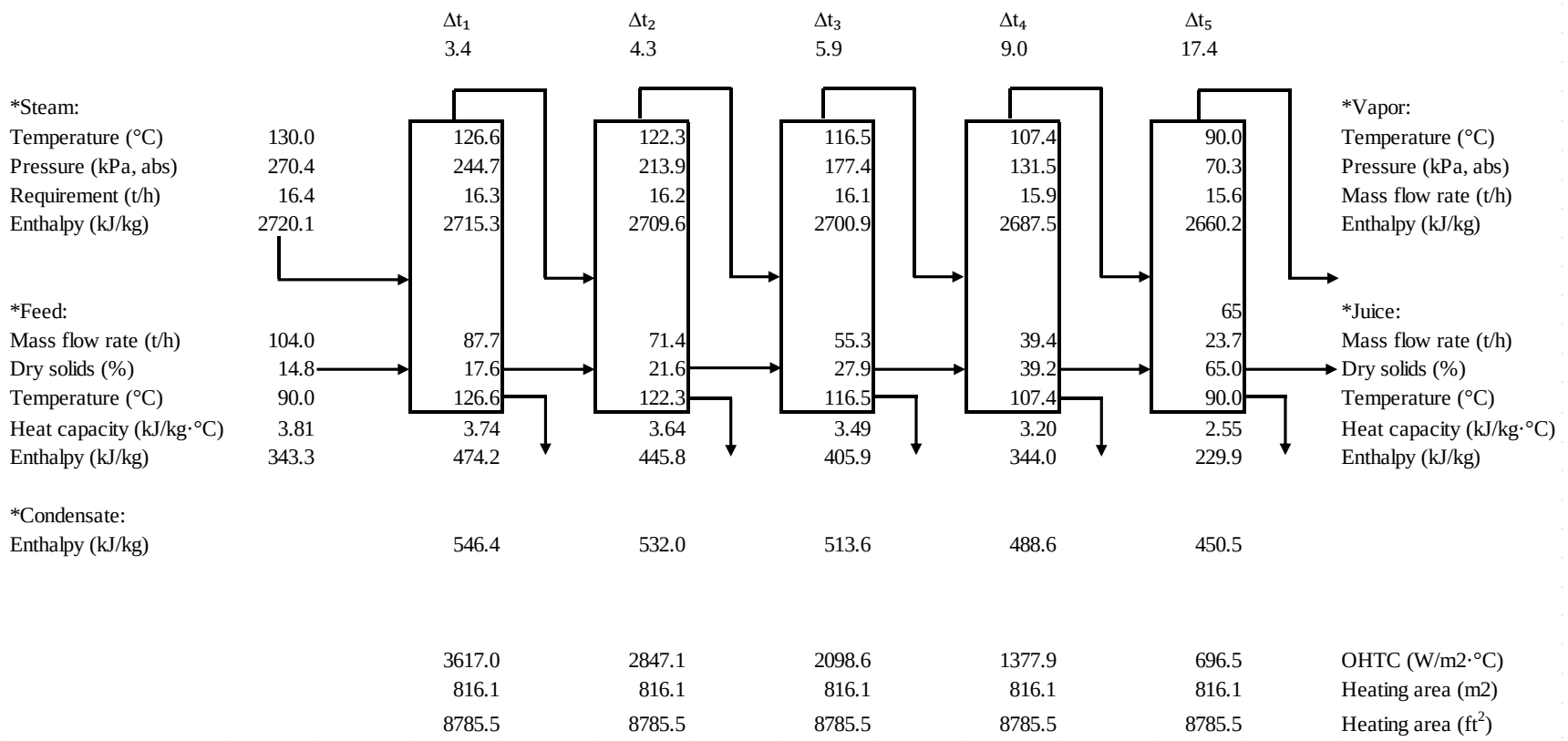

Figure 5.7. Design and operation parameters for a quintuple-effect falling-film tube evaporator proposed for raw, thick juice production.

Table 5.5. Costs associated with a quintuple-effect falling-film tube evaporator proposed for raw, thick juice production.

Direct costs	*Fraction of delivered equipment*	*Cost ($M)*
Purchased equipment	1.00	2.708
Equipment delivery	0.10	0.271
Purchased equipment installation	0.47	1.273
Instrumentation and controls (installed)	0.36	0.975
Piping (installed)	0.68	1.842
Electrical systems (installed)	0.11	0.298
Total direct costs	2.72	7.366
Indirect costs		
Engineering and supervision	0.33	0.894
Total indirect costs	0.33	0.894
Fixed capital investment		
Direct + Indirect costs	3.05	8.260

It may be noted that the area of the falling-film plate evaporator designed by Rosenblad Design Group, Inc. is significantly smaller as compared to that of the falling-film tube evaporator described in detail in this report (4,081 m^2). Even if adjusted, assuming a linear relationship between processing capacity and heating area, the area of a falling-plate evaporator with a capacity of 104 Mg h^{-1} would be approximately 3,410 m^2. One of the major causes of this difference may originate from the technique used to estimate the OHTC values for each evaporator effect. As mentioned earlier in this report, initial OHTC values were determined using Equation 5.6, which is an empirical expression valid for Robert evaporators. Even though an empirical equation for OHTC values representative of falling-film evaporators is available in the literature (van der Poel et al., 1998), it requires important knowledge of the behavior of viscosity of the material concentrated. And, moreover, although expressions have been

developed to estimate the viscosity of technical solutions (van der Poel et al., 1998), these are only valid within narrow temperature ranges that do not encompass values typical of an evaporation process. In contrast, the vast experience of evaporator manufacturers allows them to accurately predict values of important design parameters such as OHTC. This experience also enables them to strategically adjust the specific loading on evaporator plates for functional reasons such as controlling steam/vapor velocities and/or reducing the risk of fouling (Rosenblad Design Group, Inc.; personal communication). Predictions and adjustments such as these have significant impacts on evaporator active heating area.

Table 5.6. Dimensions and costs of storage tanks in scenarios involving different number of tanks.

		Number of tanks		
		2	6	10
Dimensions (m):	Diameter	52	36	30
	Height	19	13	11
		Costs ($M)		
Tanks		7.27	11.29	13.84
Exterior insulation		1.14	1.14	1.14
Total		8.41	12.43	14.98

Two storage tanks were assumed as a starting point to estimate the capital cost for raw, thick juice storage. BMT estimated a total cost of $3.565M for a single tank with a volume capacity of 37.7×10^6 L; two tanks of the same capacity were required. As mentioned earlier in this report, this total did not include the cost for exterior insulation, which was estimated to be approximately $550,000 for a tank of the same volume capacity. The dimensions and costs of the storage tanks for each of the scenarios analyzed (2, 6, and 10 tanks) are summarized in Table 5.6. Although the same juice volume was assumed to be distributed among the number of tanks

selected for each scenario, the total cost of the tanks increased with an increasing number of tanks. In order to reduce risks of major sugar loss due to potential unexpected problems in the storage farm, the scenario that involved 6 tanks was selected for the overall economic estimate.

Annual operating cost estimations

The steam requirement for the evaporator designed by Rosenblad Design Group, Inc. was of about 16.0 Mg h^{-1}; whereas, the requirement for the evaporator designed following the method described in this report was of about 16.1 Mg h^{-1}. Both evaporators are capable of achieving a steam economy of approximately 4.9 kg of evaporated water per kg of steam introduced in the first effect. A steam economy of this magnitude helps, without a doubt, to minimize overall operation costs. Due to the similarity in energy requirements, and given that the evaporator designed by Rosenblad Design Group, Inc. had the lowest active heating area, the costs for its operation are summarized in Table 5.7.

Table 5.7. Annual energy costs associated with the operation of a quintuple-effect falling-film plate evaporator for raw, thick juice production.

Steam	16.4	Mg h^{-1}
	4.74×10^{-6}	\$ kJ^{-1}
	0.013	\$ kg^{-1}
	853,000	**\$ y^{-1}**
Electricity	228.1	kWh
	903,100	kW
	0.0802	\$ kW^{-1}
	72,500	**\$ y^{-1}**
Total	**925,500**	**\$ y^{-1}**

The required amounts of sulfuric acid to acidify the raw, thick juice to a pH of 3.5 prior to storage, and of sodium hydroxide to adjust the pH of the acidified juice to 4.8 after storage and prior to fermentation, are presented in Table 5.8. Additionally, the unit and total annual costs of sulfuric acid and sodium hydroxide are also presented in that table.

Table 5.8. Annual costs associated with the adjustment of raw, thick juice pH to 3.5 prior to storage and to 4.8 after storage and before fermentation.

Sulfuric acid	5.47×10^5	kg
	0.16	$ kg^{-1}
	87,500	**$ y^{-1}**
Sodium hydroxide	9.03×10^5	kg
	0.38	$ kg^{-1}
	338,500	**$ y^{-1}**

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GENERAL CONCLUSIONS

In recent years, industrial beets have gained notable attention as these continue to show significant potential to compete against corn grain as an important source of sugars for non-food industrial fermentations. Yet, the ability to efficiently and inexpensively store industrial beets and achieve high sugar retention will be crucial to their success. Sugar storage will dictate the length of beet processing campaigns in non-food industries. Therefore, technical and economic aspects of beet sugar storage and processing were the focus of this dissertation.

Some of the principles that govern quality retention in perishable products can be applied to industrial beets to enable sugar retention during long-term storage. Techniques that involve moisture content (MC) and pH adjustments to $\leq 67.5\%$ and ≤ 4.0, respectively, were of particular success in this research work. Successful sugar retention was achieved in whole beet roots stored under ambient air (25 °C) with a low relative humidity (37%). The low relative humidity enhanced beet dehydration enabling complete sugar retention for up to 36 wk. Nevertheless, adequate beet dehydration will not occur naturally in the interior of beet piles, which are currently used in some factories for long-term storage. Although artificial dehydration may not be economically feasible at an industrial scale, it represents a means to retain sugars at a laboratory or pilot scale.

A polynomial model was developed to predict sugar retention in acidified and partially dehydrated ground beet-root tissue ensiled at different combinations of pH and MC. Beet tissue ensiling at some combinations of $pH \leq 4.0$ and $MC \leq 67.5\%$ enables sugar retentions of $\geq 90\%$, thereby representing a potential alternative for long-term sugar storage. Moreover, highly acidic conditions ($pH \leq 3.0$) cause net sugar gains of up to 7% after 3 d of ensiling. Net sugar gains may result from acid hydrolysis of polymeric carbohydrates in the beet tissue. Although this

technique enables successful sugar retention for microbial fermentation, it is not suitable for the table sugar industry as it results in significant sucrose hydrolysis (≥ 90% after 3 d).

One hypothesis evaluated in this dissertation was that salts synthesized in concentrated, non-purified beet juice acidified to enable long-term storage and partially neutralized to allow fermentation, would inhibit yeast fermentability. Contrary to that hypothesis, neither sodium nor ammonium salts inhibited yeast fermentability at the levels evaluated. Conversely, ammonium salts can compensate for nitrogen deficiencies in beet juice and consequently improve yeast fermentation rates. Additionally, neither the acidification nor neutralization steps result in chemical products that affect yeast. Furthermore, none of the specific anions from three mineral acids used in this research affect yeast. Therefore, ammonium salts may be synthesized strategically in concentrated juice through pre-storage acidification with the least expensive acid followed by post-storage partial neutralization with ammonium hydroxide.

Principles of equipment design and economics were applied to estimate capital and operating costs associated with raw, thick juice production, conditioning, and storage. A quintuple-effect falling-film evaporator and six storage tanks (the major and only pieces of equipment considered for the estimates) represent 40% ($8.26M) and 60% ($12.43M), respectively, of fixed capital costs. Moreover, steam and sodium hydroxide contribute 46% ($0.853M) and 18% ($0.339M), respectively, to the annual operating costs. These estimates were based on cost information from a limited number of sources and thus are only intended to provide a broad indication of capital and operating costs. Therefore, opportunities exist to improve the accuracy of cost estimates as well as to reduce costs through improvements in processing and storage methods.

Storage and processing techniques evaluated thus far prove that industrial beets are a technically-feasible sugar feedstock for ethanol production. Sucrose, glucose, and fructose are readily available in industrial beets and are already an important source of carbon for many microbial fermentations that yield high-value products. Therefore, a vast array of opportunities exist for industrial beets to become a feasible sugar feedstock for industrial microbial fermentations besides that for ethanol production.

RECOMMENDATIONS FOR FUTURE WORK

Although the storage techniques evaluated thus far have yielded conditions for successful industrial-beet sugar retention, post-storage sugar fermentation should continue to be evaluated. New fermentation evaluations could involve microorganisms other than yeast that secrete byproducts of interest such as ethanol, butanol, or lactic acid. If post-storage sugar fermentations are unaffected by storage conditions, capital and operating costs associated with the storage techniques should then be estimated.

Capital and operating cost estimates will help determine the viability of ground beet root ensiling in an industrial setting. The costs of sulfuric acid quantities to achieve pH values that enable successful sugar retention were already estimated and reported in Paper 2. However, industrial-scale technology for grinding, partial dehydration, and acidification of root tissue should yet be identified. Moreover, information on equipment size, energy consumption, and storage bunkers is still needed to estimate capital and operating costs associated with ensiling.

An energy-efficient process that involves energy re-use will be crucial for the successful development of the industrial beet industry. In the case of concentrated juice production, the vapor exiting the final evaporator effect contains significant heat that can be used in other process steps, potentially reducing overall process energy consumption and operating costs. Therefore, the storage techniques that have thus far shown technical viability (i.e., concentrated, non-purified beet juice storage and ground beet root ensiling) should be analyzed as part of an integrated process rather than separately.

Logistics systems are essential to achieve efficient beet sugar harvest, storage, and transportation to processing facilities. Two schemes can be assumed in the development of these systems. For example, sugar extraction, concentration, and storage may take place at satellite

facilities; stored sugars may be transported to processing facilities on demand. Alternatively, beets may be conventionally transported to piling stations or directly to processing facilities. The overall carbon footprint of sugar harvest, storage, and transportation systems should be determined through a lifecycle assessment. These systems should enable a carbon footprint significantly lower than that of most current systems for corn grain.

Finally, in an attempt to find alternative lower-cost and less-energy-intensive storage techniques, organic acids such as lactic, propionic, or acetic could be used to artificially adjust the pH of ground beet-root tissue before ensiling. If these acids enable effective sugar retention, evaluations could then be conducted to determine impacts of effective acid levels on intended microbial fermentations. Additionally, yearlong storage of concentrated juice and ground beet-root tissue acidified with sulfuric acid should be evaluated to confirm sugar retention. Aspects associated with concentrated juice production, such as foam and scale formation in the evaporating system should also be assessed. These aspects may help determine and prevent potential unexpected sugar losses and energy efficiency drops. This knowledge may also help determine required maintenance frequency and also life span of processing equipment.

APPENDIX A. SAS CODES FOR FIGURES IN CHAPTER 2

SAS code for Figure 2.2

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%let num=1;
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analyses\Statistical Analyses of Results\SugarRetention.txt'
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      if Trt='Control' then No=1;
      else if Trt='0.05_BAP' then No=2;
      else if Trt='0.1_BAP' then No=3;
      else if Trt='0.1_Acetic' then No=4;
      else if Trt='1_Acetic' then No=5;
      else if Trt='0.1_ACS' then No=6;
      else if Trt='1_ACS' then No=7;
      if Temp=4 then Tp=1;
      else if Temp=25 then Tp=2;
      run;
proc sort;
      by No Trt Atm Tp;
      run;
proc means mean std data=PII noprint;
      by No Trt Atm Tp;
      var SugRet;
      output out=meansugret mean=mean std=std;
      run;
data anno;
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output;
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output;

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      function='draw';         x=-2; width=2; output;

      function='move'; y=mean+(&num*&stat); xsys='2'; group=No;
midpoint=Tp; color='blue'; width=2; output;
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                              5='1% AA'
                              6='0.1% pHresh'
                              7='1% pHresh';
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to 160 by 10);
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axis3 label=none value=(f='Times New Roman' h=10pt);
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pattern2 c=blue v=X3;
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SAS code for Figure 2.3

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run;
proc print;
      run;
```

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      by Time Temp;
      run;
proc print;
      run;
proc means noprint;
      by Time Temp;
      var AeMoisp;
      output out=meanaemois mean=mean std=std;
      run;
data reshape1(keep=Time Temp AeMoisp mean std);
      set meanaemois;
      AeMoisp=mean;
      output;

      AeMoisp=mean - std;
      output;

      AeMoisp=mean + std;
      output;

      run;
proc print data=reshape1;
      run;
proc format;
      value Temp  4='6°C'
                           25='25°C';
      run;
filename aerobic 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of Results\aerobic.jpg';
goptions gsfname=aerobic dev=jpeg xpixels=1500 xmax=7.5in ypixels=826
ymax=4.13in;
legend1 position=(inside bottom left) label=none value=(f='Times New
Roman' h=12pt c=black)
      down=2
      frame;
proc gplot data=reshape1;
      plot AeMoisp*Time=Temp / haxis=axis1 vaxis=axis2 nolegend;
      plot2 mean*Time=Temp / vaxis=axis2 noaxis legend=legend1;
      axis1 v=(f='Times New Roman' h=12pt c=black)
                  order=(0 to 36 by 4)
                  major=(c=black)
                  minor=(c=black n=3)
                  label=(f='Times New Roman' h=12 pt 'Storage time
(wk)');
      axis2 v=(f='Times New Roman' h=12pt c=black)
                  order=(0 to 80 by 10)
                  major=(c=black)
                  minor=(c=black)
                  label=(f='Times New Roman' h=12pt a=90 c=black
'Moisture fraction (%)');
      symbol1 v=none interpol=hilocjt color=red l=3;
```

```
      symbol2 v=none interpol=hilocjt color=blue l=3;
      symbol3 interpol=none v=diamondfilled color=red h=1.5;
      symbol4 interpol=none v=dot color=blue h=1.2;
      format Temp Temp.;
      run;
```

SAS code for Figure 2.4

```
ods html close; ods html;

*title 'Juan Phase II';
data PII;
infile 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of Results\SugRetentionAll.txt'
firstobs=2 dlm='09'x;
length Trt $11;
input  Trt $ Atm $ Temp Time Rep InSug FinSug;
if Atm='Modified' then delete;
Ret=100-((InSug-FinSug)*100/InSug);
run;
proc print;
      run;
proc sort;
      by Time Temp;
      run;
proc means;
      by Time Temp;
      var Ret;
      output out=meansugret mean=mean std=std;
      run;
data reshape1(keep=Trt Atm Temp Time Ret mean std);
      set meansugret;
      Ret=mean;
      output;

      Ret=mean - std;
      output;

      Ret=mean + std;
      output;

      run;
proc print data=reshape1;
      run;
data jitter;
      set reshape1;
      if Temp=4 then Time=Time+.3;
      run;
proc format;
      value Temp  4='6°C'
                          25='25°C';
```

```
      run;
filename aerobic 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of Results\aerobic.jpg';
goptions gsfname=aerobic dev=jpeg xpixels=1500 xmax=7.5in ypixels=826
ymax=4.13in;
legend1 position=(inside bottom right) label=none value=(f='Times New
Roman' h=12pt c=black)
      down=2
      frame;
proc gplot data=jitter;
      plot Ret*Time=Temp  / haxis=axis1 vaxis=axis2 nolegend;
      plot2 mean*Time=Temp / vaxis=axis2 noaxis legend=legend1;
      axis1 v=(f='Times New Roman' h=12pt c=black t=11 ' ')
                  order=(0 to 40 by 4)
                  major=(c=black)
                  minor=(c=black n=3)
                  label=(f='Times New Roman' h=12pt 'Storage time
(wk)');
      axis2 v=(f='Times New Roman' h=12pt c=black)
                  order=(0 to 150 by 20)
                  major=(c=black)
                  minor=(c=black)
                  label=(f='Times New Roman' h=12pt a=90 'Industrial
sugar retention (%)');
      symbol1 v=none interpol=hilocjt color=red l=3;
      symbol2 v=none interpol=hilocjt color=blue l=3;
      symbol3 interpol=none v=diamondfilled color=red h=1.5;
      symbol4 interpol=none v=dot color=blue h=1.2;
      format      Temp Temp.;
      *title1 f='Times New Roman' 'Storage under Aerobic Atmosphere';
      run;
```

SAS code for Figure 2.5

```
ods html close; ods html;
%let stat=std;
%let num=1;
*title 'Juan Phase II';
data PII;
infile 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of
Results\SugarRetentionIncludingExudate.txt' firstobs=2 dlm='09'x;
length Trt $11;
input  Trt $ Atm $ Temp Rep SugRet;
      if Atm^='Modified' then delete;
      if Trt='Control' then No=1;
      else if Trt='0.05_BAP' then No=2;
      else if Trt='0.1_BAP' then No=3;
      else if Trt='0.1_Acetic' then No=4;
      else if Trt='1_Acetic' then No=5;
      else if Trt='0.1_ACS' then No=6;
```

```
      else if Trt='1_ACS' then No=7;
      if Temp=4 then Tp=1;
      else if Temp=25 then Tp=2;
      run;
proc sort;
      by No Trt Atm Tp;
      run;
proc means mean std data=PII noprint;
      by No Trt Atm Tp;
      var SugRet;
      output out=meansugret mean=mean std=std;
      run;
data anno;
      length color function $8;
      retain xsys ysys '2' when 'PII';
      set meansugret;
      function='move'; xsys='2'; ysys='2'; group=No; midpoint=Tp;
y=mean; color='blue'; output;
      function='draw'; y=mean-(&num*&stat); color='blue'; width=2;
output;
      function='draw'; y=mean+(&num*&stat); color='blue'; width=2;
output;

      function='move'; y=mean-(&num*&stat); xsys='2'; group=No;
midpoint=Tp; color='blue'; width=2; output;
      function='draw'; xsys='9'; x=+1; width=2; output;
      function='draw';          x=-2; width=2; output;

      function='move'; y=mean+(&num*&stat); xsys='2'; group=No;
midpoint=Tp; color='blue'; width=2; output;
      function='draw'; xsys='9'; x=+1; width=2; color='blue'; output;
      function='draw';          x=-2; width=2; color='blue'; output;
      run;
proc format;
      value No          1='Control'
                        2='0.05% BAP'
                        3='0.1% BAP'
                        4='0.1% AA'
                        5='1% AA'
                        6='0.1% pHresh'
                        7='1% pHresh';
      value Tp          1='6°C'
                        2='25°C';
      run;
filename modified 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of Results\modified.jpg';
goptions gsfname=modified dev=jpeg xpixels=1500 xmax=7.5in ypixels=826
ymax=4.13in;
legend1 position=(inside top left)     label=none value=(f='Times New
Roman' h=12pt c=black)
      down=2
      frame;
```

```
axis1 label=(a=90 f='Times New Roman' h=12pt "Industrial sugar
retention (%)") value=(f='Times New Roman' h=12pt) order=(0 to 120 by
10);
axis2 label=none value=none;
axis3 label=none value=(f='Times New Roman' h=10pt);
pattern1 c=red v=R3;
pattern2 c=blue v=X3;
proc gchart data=meansugret;
      vbar Tp / discrete annotate=anno sumvar=mean /*type=mean*/
group=No subgroup=Tp legend=legend1 raxis=axis1 maxis=axis2
gaxis=axis3;
      format No No. Tp Tp.;
      run;
```

SAS code for Figure 2.6

```
ods html close; ods escapechar='^'; ods html;

title ' ';
data PII;
infile 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of Results\sealedcont_mv.txt' firstobs=2
dlm='09'x;
length Trt $11;
input  Trt $ Atm $ Temp Time Rep O2 CO2;
run;
proc print;
      run;
proc sort;
      by Temp Time;
      run;
proc means data=PII;
      by Temp Time;
      var O2;
      output out=meancomp mean=O2mean std=O2std;
      run;
proc means data=PII;
      by Temp Time;
      var CO2;
      output out=meancomp1 mean=CO2mean std=CO2std;
      run;
data combined;
      merge meancomp meancomp1;
      by Temp Time;
      O2high=O2mean + O2std;
      O2low=O2mean - O2std;
      CO2high=CO2mean + CO2std;
      CO2low=CO2mean - CO2std;
      if Temp=4 then Temps=5;
      if Temp=25 then Temps=26;
      run;
```

```
proc print data=combined;
      run;
proc format;
      value Temp  4     ='CO2 (6°C)'
                        25    ='CO2 (25°C)';
      value Temps 5     ='O2 (6°C)'
                        26    ='O2 (25°C)';
      run;
data Attrs;
length Value $20 MarkerColor $20 MarkerSymbol $20;
ID = "Jobs";
Value = putn(4,"Temp."); MarkerColor = "Red"; MarkerSymbol="Circle";
LineColor="Red"; output;
Value = putn(25,"Temp."); MarkerColor = "Blue";
MarkerSymbol="CircleFilled"; LineColor="Blue"; output;
Value = putn(5,"Temps."); MarkerColor = "Red"; MarkerSymbol="Diamond";
LineColor="Red"; output;
Value = putn(26,"Temps."); MarkerColor = "Blue";
MarkerSymbol="DiamondFilled"; LineColor="Blue"; output;
run;
ods graphics / reset imagename = 'Sealed containers' imagefmt=jpeg
      width=1500px height=826px border=off;
ods listing gpath = 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of Results';
proc template;
      define style styles.NewStyle;
      parent=styles.htmlblue;
            style GraphBackground / backgroundcolor=white;
            style GraphData1 from GraphData1 / ContrastColor=red
Color=red;
            style GraphData2 from GraphData2 / ContrastColor=blue
Color=blue;
            style GraphFonts from GraphFonts /
             'GraphDataFont' = ("Times New Roman",12pt)
             'GraphValueFont' = ("Times New Roman",12pt)
             'GraphLabelFont' = ("Times New Roman Uni",12pt)
             'GraphFootnoteFont' = ("Times New Roman",12pt)
             'GraphTitleFont' = ("Times New Roman",12pt);
            end;
      run;
ods html style=NewStyle gpath='E:\NDSU\Phase II - Research\Phase
II\Sample analyses\Statistical Analyses of Results';
proc sgplot data=combined DATTRMAP=Attrs noautolegend;
      xaxis label='Storage time (wk)' labelattrs=(size=22pt) values=(0
to 36 by 4) valueattrs=(size=24pt);
      yaxis label="CO^{unicode '2082'x} volume fraction (%)"
labelattrs=(size=22pt) values=(0 to 110 by 10) valueattrs=(size=22pt)
valueshint offsetmin=0.05 offsetmax=0.09;
      y2axis label="O^{unicode '2082'x} volume fraction (%)"
labelattrs=(size=22pt) values=(0 to 22 by 2) valueattrs=(size=22pt)
valueshint offsetmin=0.05 offsetmax=0.09;
```

```
      scatter x=Time y=CO2mean / name='lineA' yerrorupper=CO2high
yerrorlower=CO2low group=Temp groupdisplay=cluster clusterwidth=0.4
markerattrs=(size=12) ATTRID=Jobs;
      series x=Time y=CO2mean / group=Temp groupdisplay=cluster
clusterwidth=0.4 lineattrs=(pattern=4);
      scatter x=Time y=O2mean / y2axis name='lineB' yerrorupper=O2high
yerrorlower=O2low group=Temps groupdisplay=cluster clusterwidth=0.9
legendlabel='O2' markerattrs=(size=12) ATTRID=Jobs;
      series x=Time y=O2mean / y2axis group=Temps groupdisplay=cluster
clusterwidth=0.9 lineattrs=(pattern=4);
      keylegend 'lineA' 'lineB' / location=inside position=topleft
valueattrs=(size=22pt);
      format Temp Temp. Temps Temps.;
      run;
```

SAS code for Figure 2.7

```
ods html close; ods html;

*title 'Juan Phase II - Moisture analyses';
data PII;
infile 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of Results\Moisture.txt' firstobs=4
dlm='09'x;
length Trt $11;
input  Time Trt $ Temp AeMois ModMois;
ModMoisp=ModMois*100;
run;
proc print;
      run;
proc sort;
      by Time Temp;
      run;
proc means data=PII;
      by Time Temp;
      var ModMoisp;
      output out=meanmodmois mean=mean std=std;
      run;
data reshape2(keep=Time Temp ModMoisp mean std);
      set meanmodmois;
      ModMoisp=mean;
      output;

      ModMoisp=mean - std;
      output;

      ModMoisp=mean + std;
      output;

      run;
      proc format;
```

```
      value Temp  4='6±1°C'
                            25='25±2°C';
      run;
filename modified 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of Results\modified.jpg';
goptions gsfname=modified dev=jpeg xpixels=1500 xmax=7.5in ypixels=826
ymax=4.13in;
legend1 position=(inside bottom right) label=none value=(f='Times New
Roman' h=12pt c=black)
      down=2
      frame;
proc gplot data=reshape2;
      plot ModMoisp*Time=Temp / haxis=axis1 vaxis=axis2 nolegend;
      plot2 mean*Time=Temp / vaxis=axis2 noaxis legend=legend1;
      axis1 v=(f='Times New Roman' h=12pt c=black)
                  order=(0 to 36 by 4)
                  major=(c=black)
                  minor=(c=black n=3)
                  label=(f='Times New Roman' h=12 pt 'Storage time
(wk)');
      axis2 v=(f='Times New Roman' h=12pt c=black)
                  order=(70 to 90 by 2)
                  major=(c=black)
                  minor=(c=black)
                  label=(f='Times New Roman' h=12pt a=90 c=black
'Moisture fraction (%)');
      symbol1 v=none interpol=hilocjt color=red l=3;
      symbol2 v=none interpol=hilocjt color=blue l=3;
      symbol3 interpol=none v=diamondfilled color=red h=1.5;
      symbol4 interpol=none v=dot color=blue h=1.2;
      format Temp Temp.;
      run;
```

SAS code for Figure 2.8

```
ods html close; ods html;

*title 'Juan Phase II';
data PII;
infile 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of
Results\SugRetentionAllWithExudate.txt' firstobs=2 dlm='09'x;
length Trt $11;
input  Trt $ Atm $ Temp Time Rep InSug FinSug;
if Atm='Aerobic' then delete;
Ret=100-((InSug-FinSug)*100/InSug);
run;
proc print;
      run;
proc sort;
      by Time Temp;
```

```
      run;
proc means;
      by Time Temp;
      var Ret;
      output out=meansugret mean=mean std=std;
      run;
proc print;
run;
data reshape1(keep=Trt Atm Temp Time Ret mean std);
      set meansugret;
      Ret=mean;
      output;

      Ret=mean - std;
      output;

      Ret=mean + std;
      output;

      run;
proc print data=reshape1;
      run;
data jitter;
      set reshape1;
      if Temp=4 then Time=Time+.3;
      run;
proc format;
      value Temp  4='6°C'
                              25='25°C';
      run;
filename modified 'E:\NDSU\Phase II - Research\Phase II\Sample
analyses\Statistical Analyses of Results\modified.jpg';
goptions gsfname=modified dev=jpeg xpixels=1500 xmax=7.5in ypixels=826
ymax=4.13in;
legend1 position=(inside bottom right) label=none value=(f='Times New
Roman' h=12pt c=black)
      down=2
      frame;
proc gplot data=jitter;
      plot Ret*Time=Temp  / haxis=axis1 vaxis=axis2 nolegend;
      plot2 mean*Time=Temp / vaxis=axis2 noaxis legend=legend1;
      axis1 v=(f='Times New Roman' h=12pt c=black t=11 ' ')
                  order=(0 to 40 by 4)
                  major=(c=black)
                  minor=(c=black n=3)
                  label=(f='Times New Roman' h=12pt 'Storage time
(wk)');
      axis2 v=(f='Times New Roman' h=12pt c=black)
                  order=(0 to 140 by 20)
                  major=(c=black)
                  minor=(c=black)
```

```
                label=(f='Times New Roman' h=12pt a=90 'Industrial
sugar retention (%)');
     symbol1 v=none interpol=hilocjt color=red l=3;
     symbol2 v=none interpol=hilocjt color=blue l=3;
     symbol3 interpol=none v=diamondfilled color=red h=1.5;
     symbol4 interpol=none v=dot color=blue h=1.2;
     format     Temp Temp.;
     run;
```

APPENDIX B. BEET TISSUE TITRATION DATA

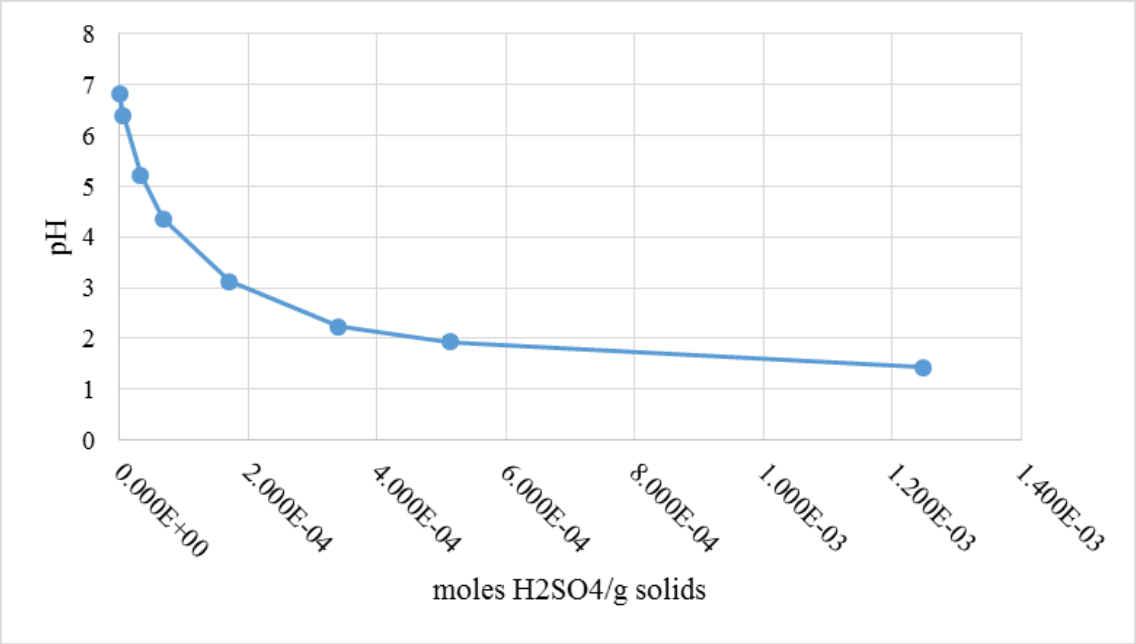
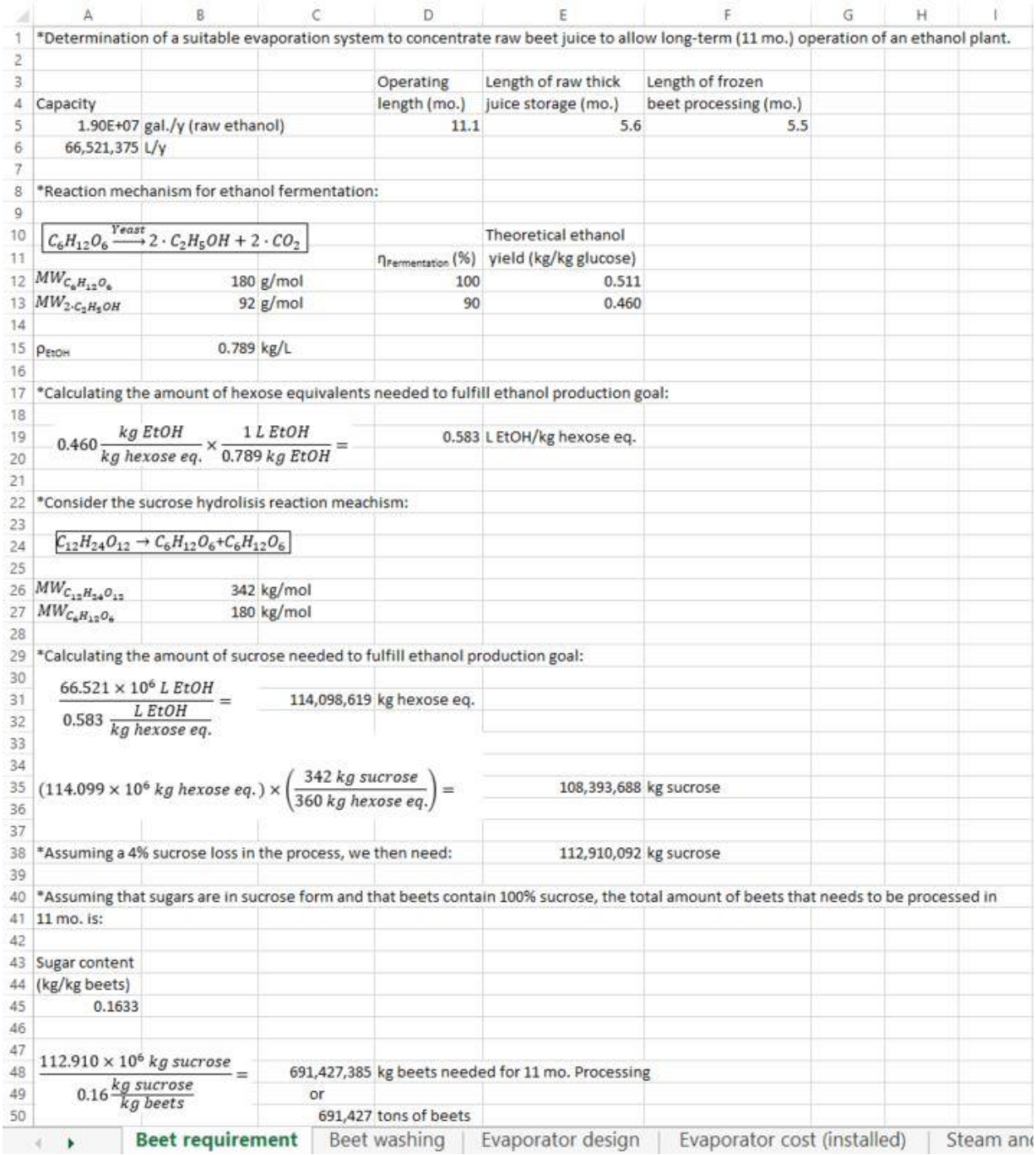


Figure B1. Curve associated with the titration of fresh, frozen beet tissue with a wet-basis solids fraction of 0.30.

Table B1. Raw data associated with the titration of fresh, frozen beet tissue with a wet-basis solids fraction of 0.30.

Tissue (g)	H2SO4 Conc. (M)	Volume (mL)	Moles H2SO4 added	Moles H2SO4/g mix	Moles H2SO4/g solids	pH
25.17	7.92	1.25	0.0099	3.747E-04	1.249E-03	1.43
25.19	7.92	0.5	0.00396	1.541E-04	5.137E-04	1.94
25.22	5.25	0.5	0.002625	1.021E-04	3.401E-04	2.23
25.17	2.63	0.5	0.001315	5.123E-05	1.707E-04	3.12
25.26	1.05	0.5	0.000525	2.038E-05	6.792E-05	4.36
25.21	0.51	0.5	0.000255	9.918E-06	3.305E-05	5.23
25.20	0.10	0.5	0.00005	1.946E-06	6.483E-06	6.40
25.25	-	0.0	0.0	0.000E+00	0.000E+00	6.82

APPENDIX C. SCREENSHOTS OF SPREADSHEET MODELS TO CALCULATE EQUIPMENT DIMENSIONS AND ESTIMATE CAPITAL AND OPERATING COSTS



*Determination of a suitable evaporation system to concentrate raw beet juice to allow long-term (11 mo.) operation of an ethanol plant.

Capacity		Operating length (mo.)	Length of raw thick juice storage (mo.)	Length of frozen beet processing (mo.)
1.90E+07	gal./y (raw ethanol)	11.1	5.6	5.5
66,521,375	L/y			

*Reaction mechanism for ethanol fermentation:

$C_6H_{12}O_6 \xrightarrow{Yeast} 2 \cdot C_2H_5OH + 2 \cdot CO_2$

			$\eta_{Fermentation}$ (%)	Theoretical ethanol yield (kg/kg glucose)
$MW_{C_6H_{12}O_6}$	180	g/mol	100	0.511
$MW_{2 \cdot C_2H_5OH}$	92	g/mol	90	0.460
ρ_{EtOH}	0.789	kg/L		

*Calculating the amount of hexose equivalents needed to fulfill ethanol production goal:

$$0.460 \frac{kg\ EtOH}{kg\ hexose\ eq.} \times \frac{1\ L\ EtOH}{0.789\ kg\ EtOH} = 0.583\ \text{L EtOH/kg hexose eq.}$$

*Consider the sucrose hydrolisis reaction meachism:

$C_{12}H_{24}O_{12} \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$

$MW_{C_{12}H_{24}O_{12}}$	342	kg/mol
$MW_{C_6H_{12}O_6}$	180	kg/mol

*Calculating the amount of sucrose needed to fulfill ethanol production goal:

$$\frac{66.521 \times 10^6\ L\ EtOH}{0.583 \frac{L\ EtOH}{kg\ hexose\ eq.}} = 114{,}098{,}619\ \text{kg hexose eq.}$$

$$(114.099 \times 10^6\ kg\ hexose\ eq.) \times \left(\frac{342\ kg\ sucrose}{360\ kg\ hexose\ eq.}\right) = 108{,}393{,}688\ \text{kg sucrose}$$

*Assuming a 4% sucrose loss in the process, we then need: 112,910,092 kg sucrose

*Assuming that sugars are in sucrose form and that beets contain 100% sucrose, the total amount of beets that needs to be processed in 11 mo. is:

Sugar content (kg/kg beets)
0.1633

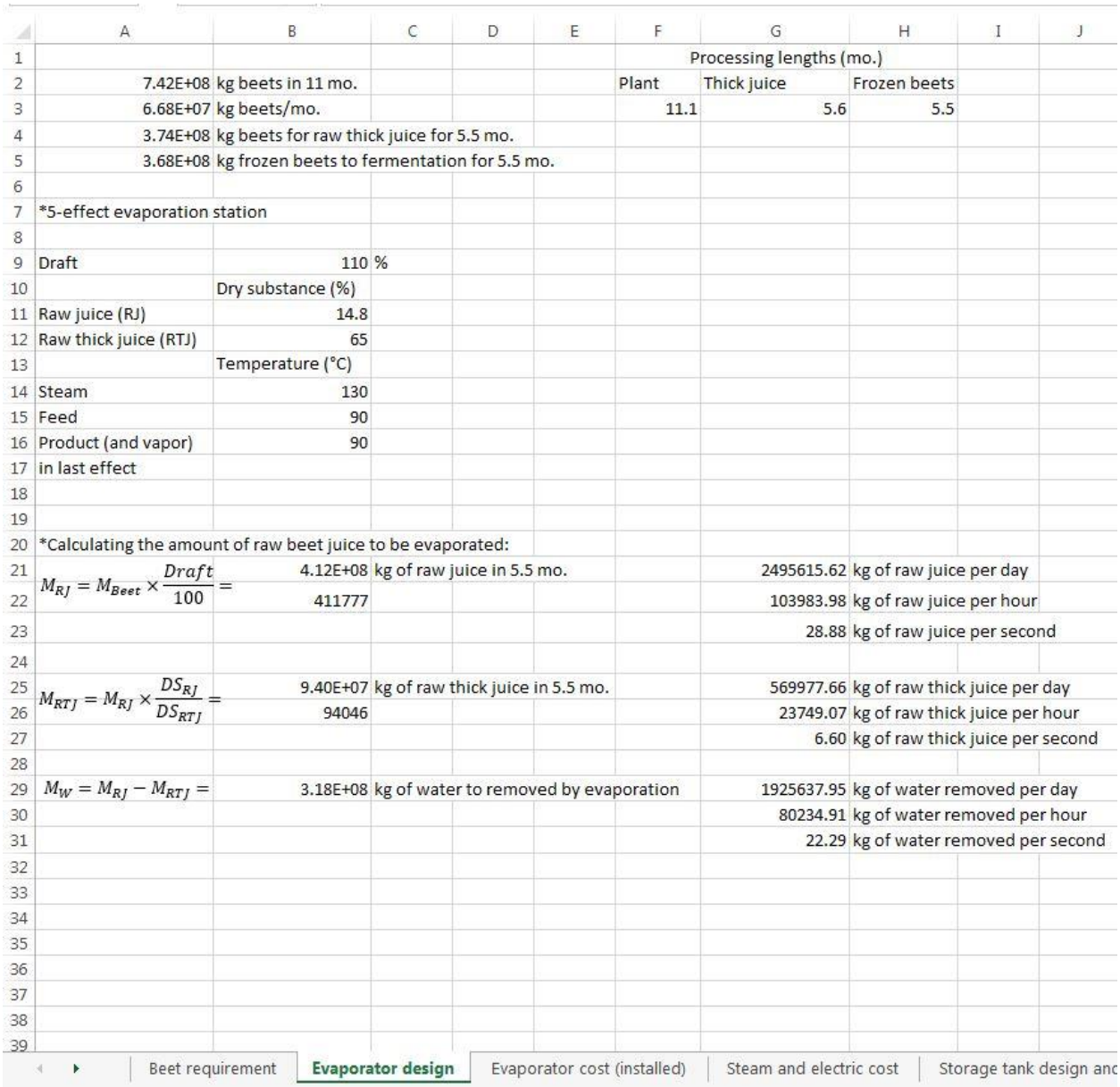
$$\frac{112.910 \times 10^6\ kg\ sucrose}{0.16 \frac{kg\ sucrose}{kg\ beets}} = 691{,}427{,}385\ \text{kg beets needed for 11 mo. Processing}$$

or

691,427 tons of beets

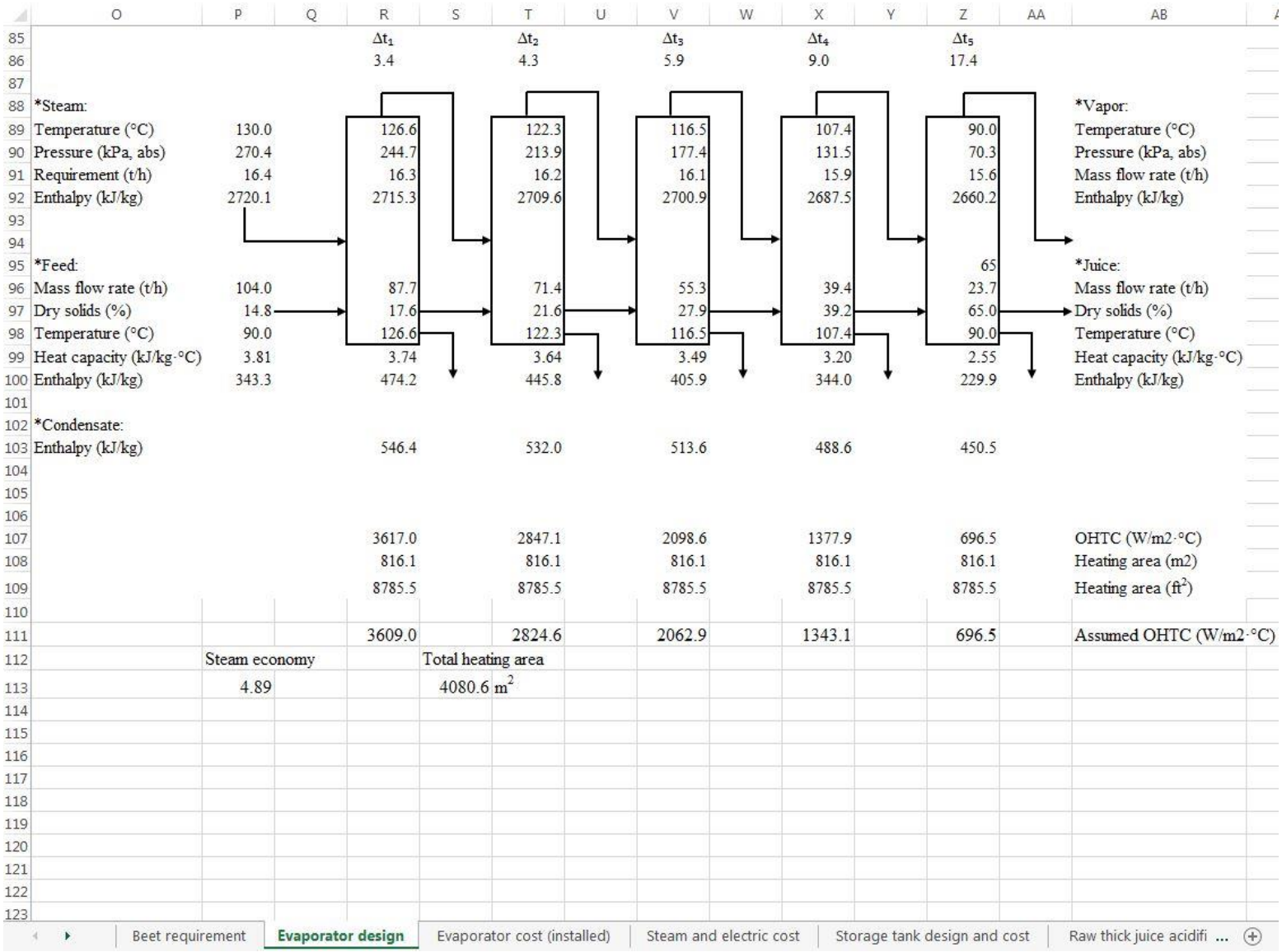
Beet requirement | Beet washing | Evaporator design | Evaporator cost (installed) | Steam an

Figure C1. Screenshot of spreadsheet developed to calculate the industrial-beet requirement for an ethanol plant with a denatured-ethanol production capacity of 76×10^6 L y^{-1}.



	A	B	C	D	E	F	G	H	I	J
1						Processing lengths (mo.)				
2	7.42E+08	kg beets in 11 mo.				Plant	Thick juice	Frozen beets		
3	6.68E+07	kg beets/mo.				11.1	5.6	5.5		
4	3.74E+08	kg beets for raw thick juice for 5.5 mo.								
5	3.68E+08	kg frozen beets to fermentation for 5.5 mo.								
6										
7	*5-effect evaporation station									
8										
9	Draft	110	%							
10		Dry substance (%)								
11	Raw juice (RJ)	14.8								
12	Raw thick juice (RTJ)	65								
13		Temperature (°C)								
14	Steam	130								
15	Feed	90								
16	Product (and vapor)	90								
17	in last effect									
18										
19										
20	*Calculating the amount of raw beet juice to be evaporated:									
21	$M_{RJ} = M_{Beet} \times \frac{Draft}{100} =$	4.12E+08	kg of raw juice in 5.5 mo.				2495615.62	kg of raw juice per day		
22		411777					103983.98	kg of raw juice per hour		
23							28.88	kg of raw juice per second		
24										
25	$M_{RTJ} = M_{RJ} \times \frac{DS_{RJ}}{DS_{RTJ}} =$	9.40E+07	kg of raw thick juice in 5.5 mo.				569977.66	kg of raw thick juice per day		
26		94046					23749.07	kg of raw thick juice per hour		
27							6.60	kg of raw thick juice per second		
28										
29	$M_W = M_{RJ} - M_{RTJ} =$	3.18E+08	kg of water to removed by evaporation				1925637.95	kg of water removed per day		
30							80234.91	kg of water removed per hour		
31							22.29	kg of water removed per second		
32										
33										
34										
35										
36										
37										
38										
39										

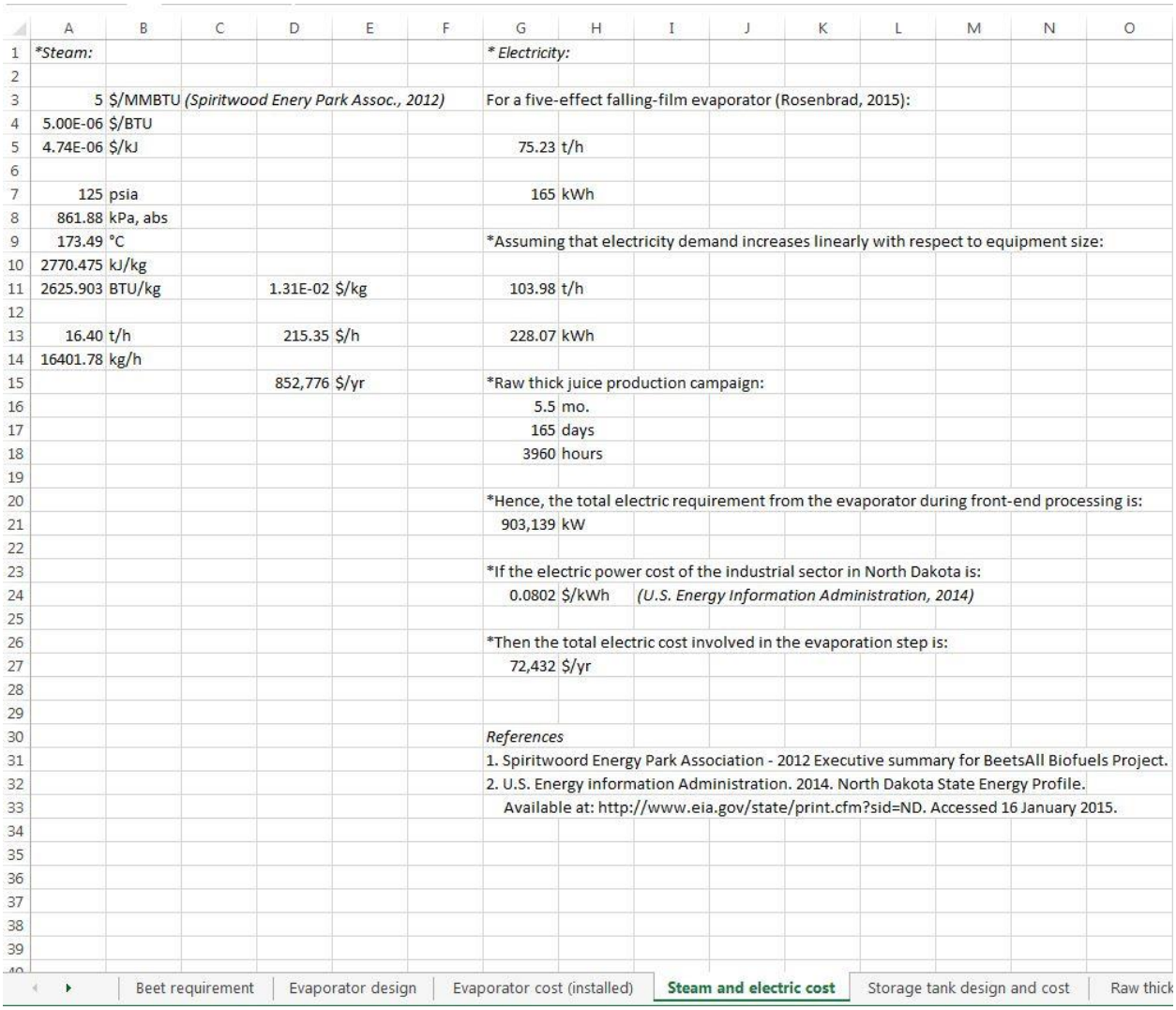
Figure C2. Screenshot of spreadsheet section developed to calculate the quantities of raw juice processed, water evaporated, and raw, thick juice produced using a quintuple-effect falling-film evaporator throughout 5.5 months.



			Δt_1		Δt_2		Δt_3		Δt_4		Δt_5		
			3.4		4.3		5.9		9.0		17.4		
*Steam:												*Vapor:	
Temperature (°C)	130.0		126.6		122.3		116.5		107.4		90.0	Temperature (°C)	
Pressure (kPa, abs)	270.4		244.7		213.9		177.4		131.5		70.3	Pressure (kPa, abs)	
Requirement (t/h)	16.4		16.3		16.2		16.1		15.9		15.6	Mass flow rate (t/h)	
Enthalpy (kJ/kg)	2720.1		2715.3		2709.6		2700.9		2687.5		2660.2	Enthalpy (kJ/kg)	
*Feed:											65	*Juice:	
Mass flow rate (t/h)	104.0		87.7		71.4		55.3		39.4		23.7	Mass flow rate (t/h)	
Dry solids (%)	14.8		17.6		21.6		27.9		39.2		65.0	Dry solids (%)	
Temperature (°C)	90.0		126.6		122.3		116.5		107.4		90.0	Temperature (°C)	
Heat capacity (kJ/kg·°C)	3.81		3.74		3.64		3.49		3.20		2.55	Heat capacity (kJ/kg·°C)	
Enthalpy (kJ/kg)	343.3		474.2		445.8		405.9		344.0		229.9	Enthalpy (kJ/kg)	
*Condensate:													
Enthalpy (kJ/kg)			546.4		532.0		513.6		488.6		450.5		
			3617.0		2847.1		2098.6		1377.9		696.5	OHTC (W/m2·°C)	
			816.1		816.1		816.1		816.1		816.1	Heating area (m2)	
			8785.5		8785.5		8785.5		8785.5		8785.5	Heating area (ft^2)	
			3609.0		2824.6		2062.9		1343.1		696.5	Assumed OHTC (W/m2·°C)	
	Steam economy			Total heating area									
	4.89			4080.6 m^2									

Beet requirement | **Evaporator design** | Evaporator cost (installed) | Steam and electric cost | Storage tank design and cost | Raw thick juice acidifi ...

Figure C3. Screenshot of spreadsheet section developed to calculate operation parameters and heating area of each effect in a quintuple-effect falling-film evaporator used to produce raw, thick juice throughout 5.5 months.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	*Steam:						* Electricity:								
2															
3	5	$/MMBTU	(Spiritwood Enery Park Assoc., 2012)				For a five-effect falling-film evaporator (Rosenbrad, 2015):								
4	5.00E-06	$/BTU													
5	4.74E-06	$/kJ					75.23	t/h							
6															
7	125	psia					165	kWh							
8	861.88	kPa, abs													
9	173.49	°C					*Assuming that electricity demand increases linearly with respect to equipment size:								
10	2770.475	kJ/kg													
11	2625.903	BTU/kg		1.31E-02	$/kg		103.98	t/h							
12															
13	16.40	t/h		215.35	$/h		228.07	kWh							
14	16401.78	kg/h													
15				852,776	$/yr		*Raw thick juice production campaign:								
16							5.5	mo.							
17							165	days							
18							3960	hours							
19															
20							*Hence, the total electric requirement from the evaporator during front-end processing is:								
21							903,139	kW							
22															
23							*If the electric power cost of the industrial sector in North Dakota is:								
24							0.0802	$/kWh	(U.S. Energy Information Administration, 2014)						
25															
26							*Then the total electric cost involved in the evaporation step is:								
27							72,432	$/yr							
28															
29															
30							References								
31							1. Spiritwoord Energy Park Association - 2012 Executive summary for BeetsAll Biofuels Project.								
32							2. U.S. Energy information Administration. 2014. North Dakota State Energy Profile.								
33							Available at: http://www.eia.gov/state/print.cfm?sid=ND. Accessed 16 January 2015.								
34															
35															
36															
37															
38															
39															

Figure C4. Screenshot of spreadsheet developed to calculate steam and electricity consumptions in a quintuple-effect falling-film evaporator used to produce raw, thick juice throughout 5.5 months. The costs of steam and electricity consumed are also calculated in this spreadsheet.

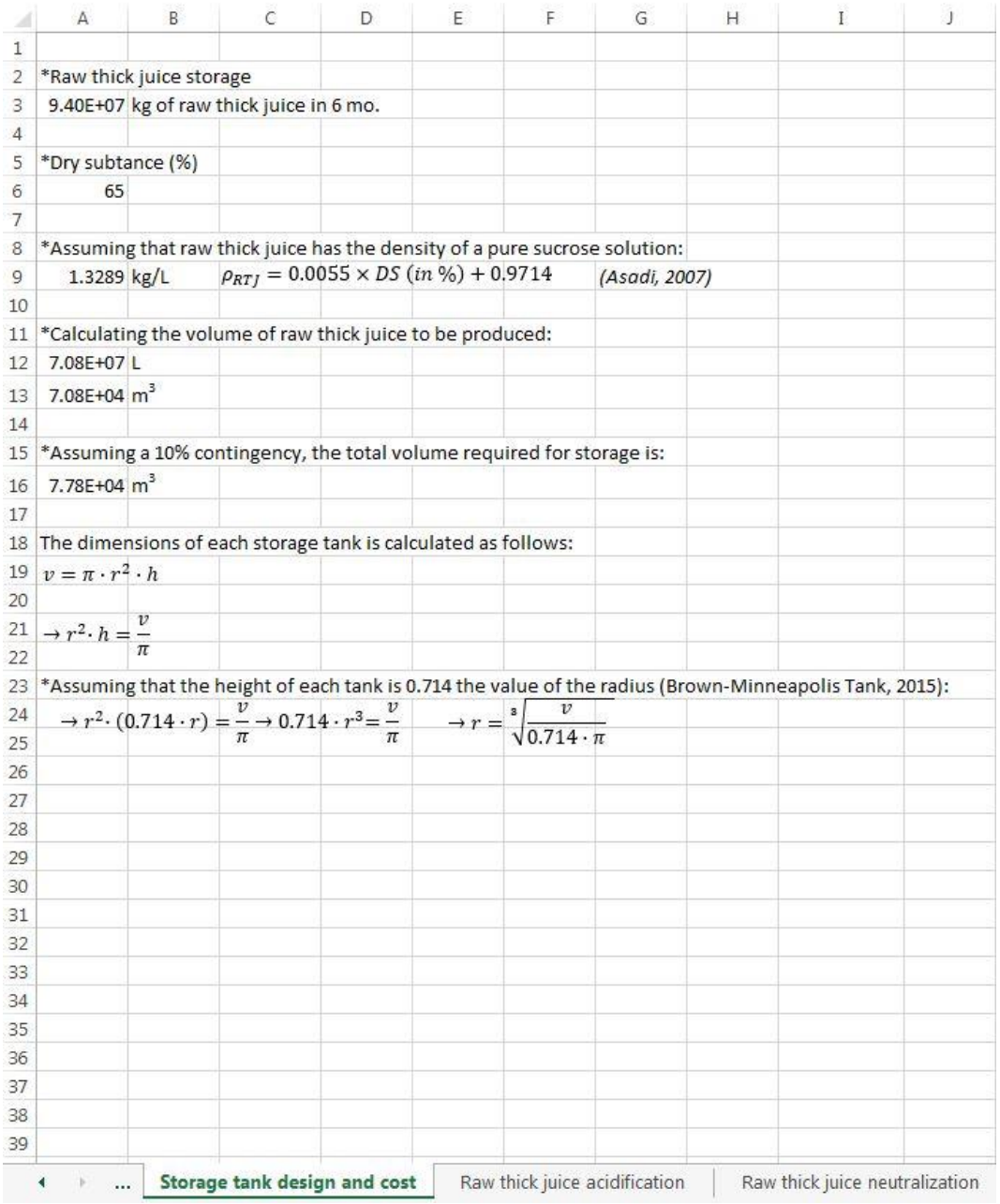


Figure C5. Sectional screenshot of spreadsheet developed to calculate the dimensions of storage tanks based on the total volume of raw, thick juice produced throughout 5.5 months.

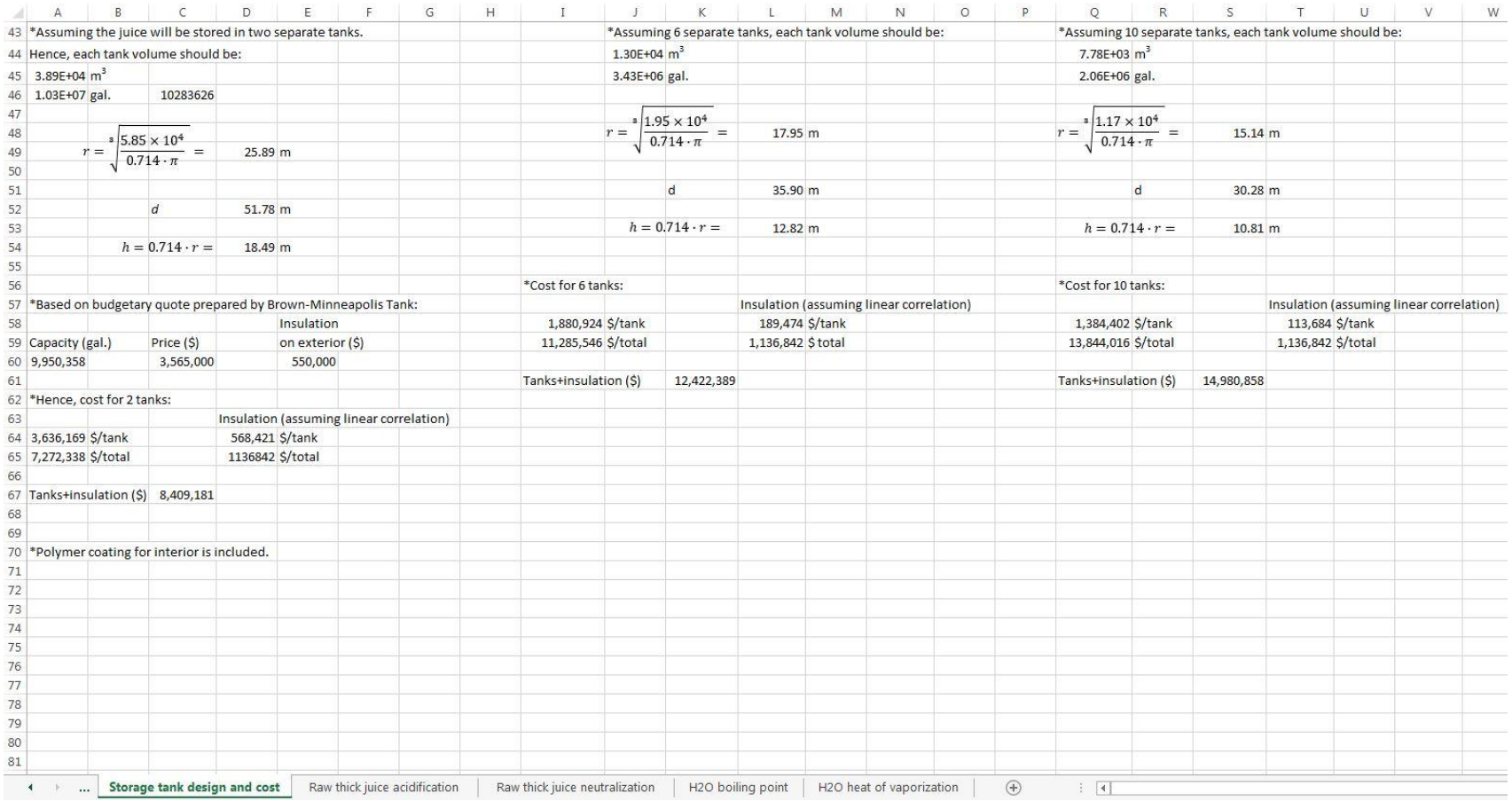
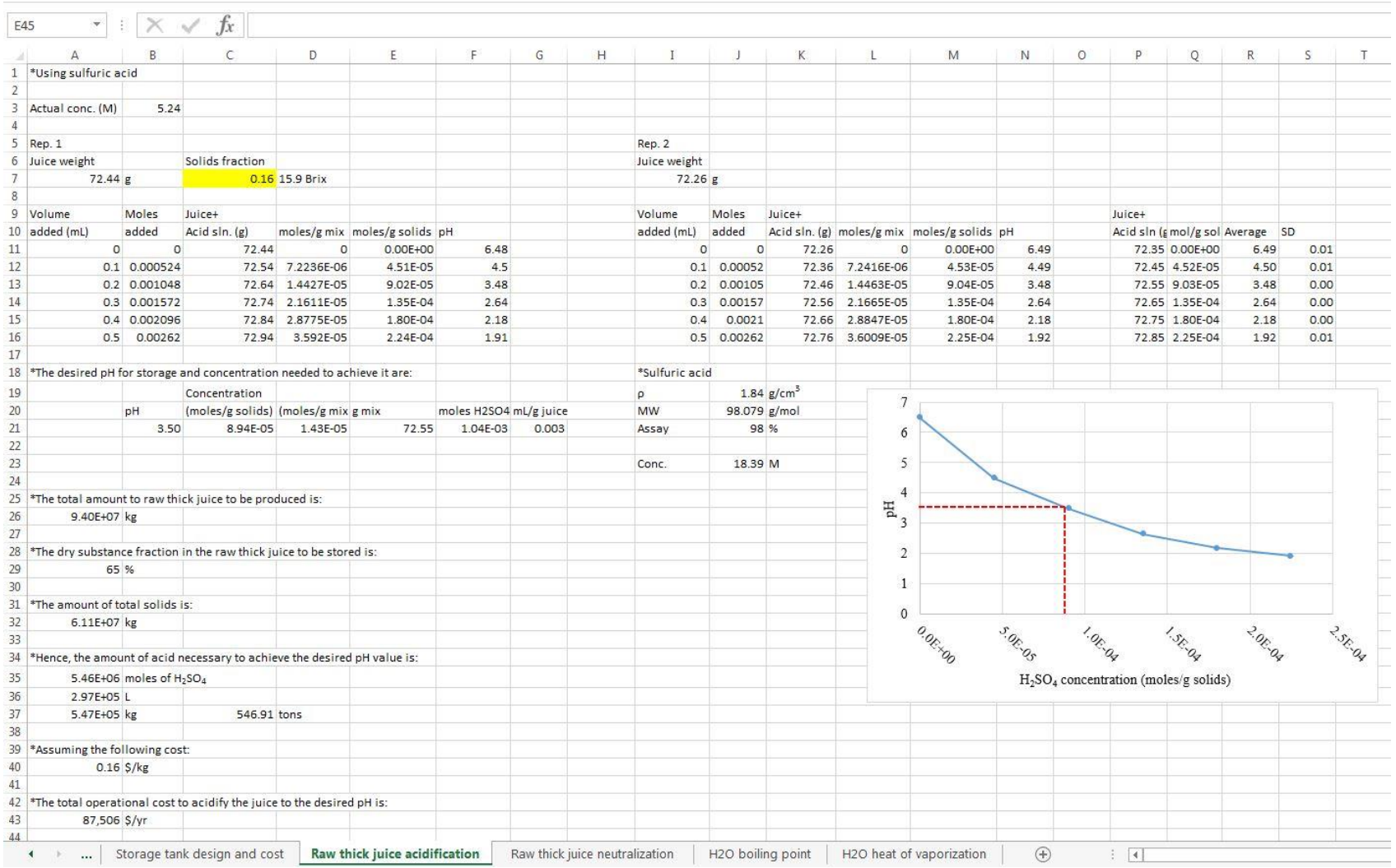
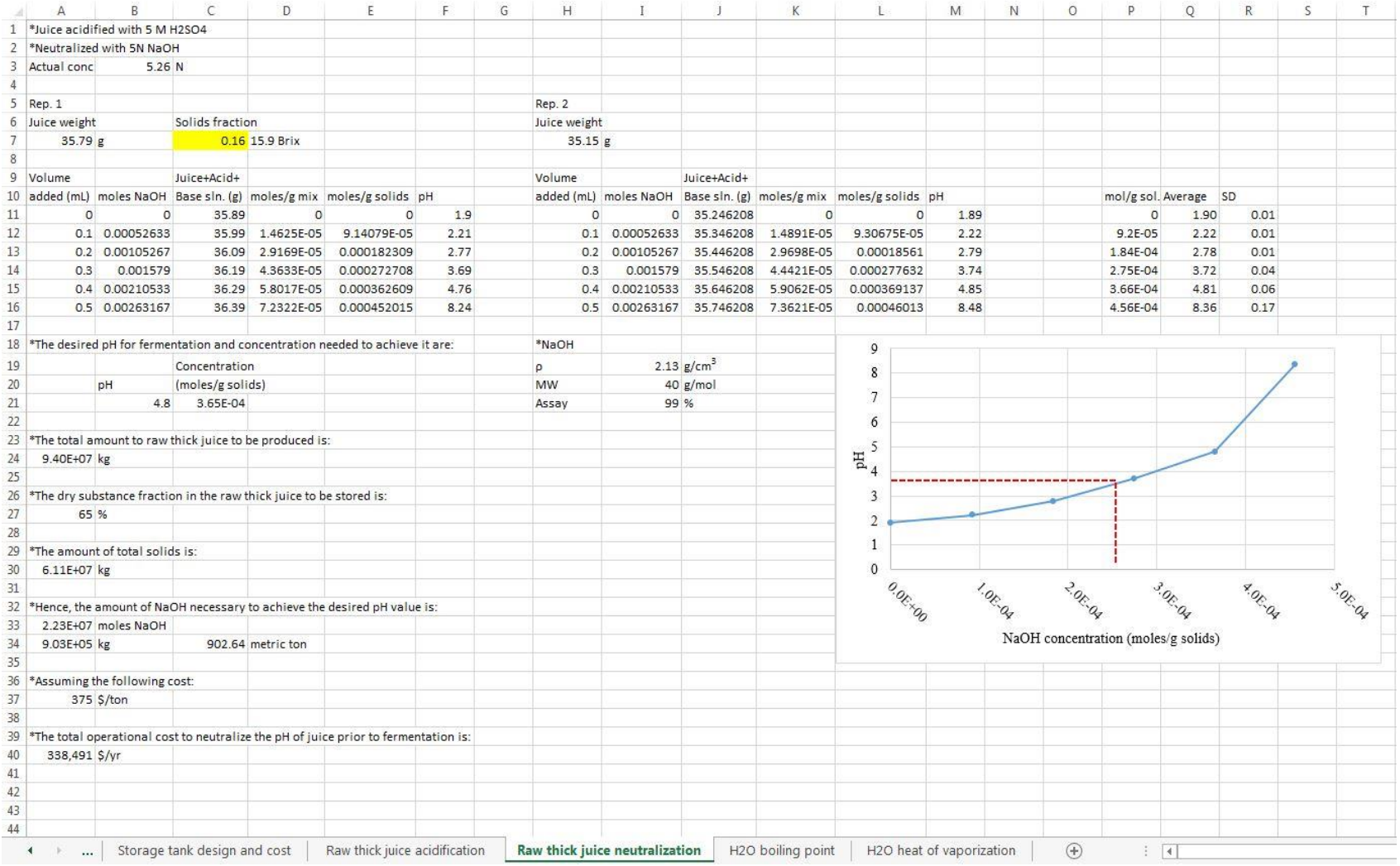


Figure C6. Sectional screenshot of spreadsheet developed to calculate the dimensions of two, six, and ten storage tanks based on the total volume of raw, thick juice produced throughout 5.5 months.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	*Using sulfuric acid																			
2																				
3	Actual conc. (M)	5.24																		
4																				
5	Rep. 1								Rep. 2											
6	Juice weight		Solids fraction						Juice weight											
7	72.44	g	0.16	15.9 Brix					72.26	g										
8																				
9	Volume	Moles	Juice+						Volume	Moles	Juice+					Juice+				
10	added (mL)	added	Acid sln. (g)	moles/g mix	moles/g solids	pH			added (mL)	added	Acid sln. (g)	moles/g mix	moles/g solids	pH		Acid sln (g	mol/g sol	Average	SD	
11	0	0	72.44	0	0.00E+00	6.48			0	0	72.26	0	0.00E+00	6.49		72.35	0.00E+00	6.49	0.01	
12	0.1	0.000524	72.54	7.2236E-06	4.51E-05	4.5			0.1	0.00052	72.36	7.2416E-06	4.53E-05	4.49		72.45	4.52E-05	4.50	0.01	
13	0.2	0.001048	72.64	1.4427E-05	9.02E-05	3.48			0.2	0.00105	72.46	1.4463E-05	9.04E-05	3.48		72.55	9.03E-05	3.48	0.00	
14	0.3	0.001572	72.74	2.1611E-05	1.35E-04	2.64			0.3	0.00157	72.56	2.1665E-05	1.35E-04	2.64		72.65	1.35E-04	2.64	0.00	
15	0.4	0.002096	72.84	2.8775E-05	1.80E-04	2.18			0.4	0.0021	72.66	2.8847E-05	1.80E-04	2.18		72.75	1.80E-04	2.18	0.00	
16	0.5	0.00262	72.94	3.592E-05	2.24E-04	1.91			0.5	0.00262	72.76	3.6009E-05	2.25E-04	1.92		72.85	2.25E-04	1.92	0.01	
17																				
18	*The desired pH for storage and concentration needed to achieve it are:								*Sulfuric acid											
19			Concentration						ρ	1.84	g/cm^3									
20		pH	(moles/g solids)	(moles/g mix	g mix	moles H2SO4	mL/g juice		MW	98.079	g/mol									
21		3.50	8.94E-05	1.43E-05	72.55	1.04E-03	0.003		Assay	98	%									
22																				
23									Conc.	18.39	M									
24																				
25	*The total amount to raw thick juice to be produced is:																			
26	9.40E+07	kg																		
27																				
28	*The dry substance fraction in the raw thick juice to be stored is:																			
29	65	%																		
30																				
31	*The amount of total solids is:																			
32	6.11E+07	kg																		
33																				
34	*Hence, the amount of acid necessary to achieve the desired pH value is:																			
35	5.46E+06	moles of H_2SO_4																		
36	2.97E+05	L																		
37	5.47E+05	kg	546.91	tons																
38																				
39	*Assuming the following cost:																			
40	0.16	$/kg																		
41																				
42	*The total operational cost to acidify the juice to the desired pH is:																			
43	87,506	$/yr																		
44																				

Figure C7. Screenshot of spreadsheet developed to calculate the amount and cost of sulfuric acid needed to adjust the pH of raw, thick juice to 3.5 to enable long-term storage. The amount of acid needed is based on a juice acidification curve and the solids fraction of the juice.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
1	*Juice acidified with 5 M H2SO4																			
2	*Neutralized with 5N NaOH																			
3	Actual conc	5.26	N																	
4																				
5	Rep. 1							Rep. 2												
6	Juice weight		Solids fraction					Juice weight												
7	35.79	g	0.16	15.9 Brix				35.15	g											
8																				
9	Volume		Juice+Acid+					Volume		Juice+Acid+										
10	added (mL)	moles NaOH	Base sln. (g)	moles/g mix	moles/g solids	pH		added (mL)	moles NaOH	Base sln. (g)	moles/g mix	moles/g solids	pH			mol/g sol.	Average	SD		
11	0	0	35.89	0	0	1.9		0	0	35.246208	0	0	1.89			0	1.90	0.01		
12	0.1	0.00052633	35.99	1.4625E-05	9.14079E-05	2.21		0.1	0.00052633	35.346208	1.4891E-05	9.30675E-05	2.22			9.2E-05	2.22	0.01		
13	0.2	0.00105267	36.09	2.9169E-05	0.000182309	2.77		0.2	0.00105267	35.446208	2.9698E-05	0.00018561	2.79			1.84E-04	2.78	0.01		
14	0.3	0.001579	36.19	4.3633E-05	0.000272708	3.69		0.3	0.001579	35.546208	4.4421E-05	0.000277632	3.74			2.75E-04	3.72	0.04		
15	0.4	0.00210533	36.29	5.8017E-05	0.000362609	4.76		0.4	0.00210533	35.646208	5.9062E-05	0.000369137	4.85			3.66E-04	4.81	0.06		
16	0.5	0.00263167	36.39	7.2322E-05	0.000452015	8.24		0.5	0.00263167	35.746208	7.3621E-05	0.00046013	8.48			4.56E-04	8.36	0.17		
17																				
18	*The desired pH for fermentation and concentration needed to achieve it are:							*NaOH												
19			Concentration					ρ	2.13	g/cm^3										
20		pH	(moles/g solids)					MW	40	g/mol										
21		4.8	3.65E-04					Assay	99	%										
22																				
23	*The total amount to raw thick juice to be produced is:																			
24	9.40E+07	kg																		
25																				
26	*The dry substance fraction in the raw thick juice to be stored is:																			
27	65	%																		
28																				
29	*The amount of total solids is:																			
30	6.11E+07	kg																		
31																				
32	*Hence, the amount of NaOH necessary to achieve the desired pH value is:																			
33	2.23E+07	moles NaOH																		
34	9.03E+05	kg	902.64	metric ton																
35																				
36	*Assuming the following cost:																			
37	375	$/ton																		
38																				
39	*The total operational cost to neutralize the pH of juice prior to fermentation is:																			
40	338,491	$/yr																		
41																				
42																				
43																				
44																				

Figure C8. Screenshot of spreadsheet developed to calculate the amount and cost of sodium hydroxide needed to adjust the pH of raw, thick juice to 4.8 to enable juice fermentability (after adequate dilution with water). The amount of sodium hydroxide needed is based on a juice neutralization curve and the solids fraction of the juice.

APPENDIX D. BUDGETARY PROPOSAL FROM ROSENBLAD DESIGN GROUP INC.

Scope of Work

The scope of the project consists of a new MEE evaporation plant with surface condenser. The new system will do 124,928 lbs hr^{-1} of evaporation of 16.0% TDS solution to achieve a final concentration discharge of 65% TDS. This evaporation plant will consist of (5) effects using falling film plate type evaporators.

The evaporation plant supply includes fabrication of evaporators and condenser vessels, all small condensate and liquor flash tanks, vacuum system, pumps, instruments, control valves, hand valves, process and utility piping and associated supports inside the battery limit of the new plant. Evaporator equipment platforms, platform ladders, and support legs for the vessels are included. Also included are equipment & piping installation and vessel & piping insulation.

Commissioning and 1 week of start-up assistance is included in the scope of supply. Rosenblad's engineering services will include P&ID drawings, lamella package drawings, general equipment layout drawings, vessel outline drawings, pump list, conceptual piping layouts, instrument lists, interlock and control logic information.

All civil work, electrical supply, service media connections, lighting, external control systems, and any building work is excluded from the scope of RDG's supply. All storage tanks, piping, instruments, and all other project work outside the immediate evaporator battery limit are excluded from RDG's scope of work.

Basic System Configuration

Evaporators (5ea.), condensate flash tanks (3ea.), liquor flash tank (1ea.), liquor heat exchanger (1ea.), and surface condenser (1ea.).

Table D1. Specifications of a quintuple-effect falling-film evaporator designed to produce raw, thick juice at a rate of 18,500 kg h^{-1}.

Effect	Plates	Active Area
1	33	6,029 ft²
2	28	5,116 ft²
3	28	5,116 ft²
4	28	5,116 ft²
5	28	5,116 ft²

Table D2. Specifications of surface condenser in a quintuple-effect falling-film evaporator designed to produce raw, thick juice at a rate of 18,500 kg h^{-1}.

Effect	Plates	Active Area
1	22	3,345 ft²

Table D3. Performance data of a quintuple-effect falling-film evaporator designed to produce raw, thick juice at a rate of 18,500 kg h^{-1}.

Item	**Data**
Capacity	
Evaporation Rate	125,000 lbs hr^{-1}
Feed Liquor (Beet Sugar Extract)	
Feed Liquor Flow Rate	165,700 lbs hr^{-1}
Feed Liquor Concentration	16.0%
Feed Liquor Temperature	194.0 ^{o}F
Product Liquor	
Product Liquor Flow Rate	40,800 lbs hr^{-1}
Product Liquor Concentration	65%
Product Liquor Temperature	212.0 ^{o}F
Steam Consumption and Economy	
Steam to the First Effect	25,400 lbs hr^{-1}
Steam pressure (Minimum)	28.4 PSIA
Steam Temperature	247.6 ^{o}F
Steam Economy	4.92
Cooling Water Supply and Return	
Cooling Water Flow Rate	1,800 gpm
Cooling Water Inlet Temperature	80 ^{o}F
Cooling Water Outlet Temperature	113 ^{o}F
Power Consumption	
Power Consumption for Pumps	165.0 kW

Total (Budgetary) cost: $6,800,000.00 USD

Lead-time: 12-13 months (Equipment supply, installation, and start-up)

Contact: Phillip Kraft

Rosenblad Design Group Inc.

Phone: 904-962-9803

Main office: 904-225-1011

Web: www.rdgevaporators.com