EVALUATION OF HULLESS BARLEY FOR POTENTIAL ETHANOL PRODUCTION

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POTENTIAL ETHANOL PRODUCTION

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NOMENCLATURE

ANOVA	Analysis of variance
HPLC	High Performance Liquid Chromatograph
SSF	Simultaneous Saccharification and Fermentation
w/w	Weight to weight
v/v	Volume to volume

Units

%	Percent
ºC	Degree Centigrade
g	Gram
hr	Hour
kg	Kilogram
mL	Millliliter
µL	Microliter
min	Minute
s	Second
Ha	Hectare

CHAPTER I

INTRODUCTION

1.1 STATEMENT OF PROBLEM

In the U.S. ethanol is used as a gasoline additive. Bio-ethanol can be produced from sugar, starch and lignocellulosic biomass. The technology to produce ethanol from cellulosic crops has not been commercialized yet. Currently conventional starch-based technologies are utilized for bio-ethanol production because technical aspects, capital and operating costs of these technologies are well understood and documented. Corn is the main feedstock used for fuel ethanol production in the U.S. Corn is preferred because of its availability and high starch content, however, with the high demand for ethanol, corn supplies will not be enough to meet feedstock needs of the transportation fuel industry. Moreover, corn is needed for food production. Hence, it is necessary to examine other feedstocks that have potential for ethanol production.

Wheat, sorghum and barley grains are other sources of starch that can be used for ethanol production. Previous studies on conversion of barley to ethanol were not very encouraging because of barley's physical and chemical properties. Barley has an abrasive hull that causes wear in processing equipment. High viscosity of fermentation mash caused by high β-glucan content in barley grain leads to increased pumping cost and reduced ethanol production yields.

1.2 HYPOTHESIS

Hulless winter barley is a viable local feedstock for ethanol production plants to be built in Oklahoma. Dry milling produces high starch content barley fractions that can be used for ethanol production with similar or better yields than those obtained with corn.

1.3 OBJECTIVES

The main objective of this thesis is to evaluate potential of winter hulless barley as a local feedstock for ethanol production in Oklahoma. The specific objectives are as follows:

i. To screen Oklahoma grown hulless winter barley varieties for their starch and β-glucan contents,
ii. To further characterize chemical properties of winter barley by measuring moisture, protein, ash and oil contents,
iii. To examine the efficacy of dry milling to obtain high starch content barley fractions to be used for ethanol conversion,
iv. To evaluate ethanol conversion yields of whole grain and high starch content barley fractions.

CHAPTER II

REVIEW OF LITERATURE

2.1. ETHANOL

Pure ethanol is a colorless, volatile and flammable liquid with a boiling point of 78.4^{o}C and specific gravity of 0.7851 at 20^{o}C. Ethanol is soluble in water and in most organic liquids (Kosaric and others 2001). Production of ethanol worldwide contributes to 73% fuel ethanol, 17% beverage ethanol and 10% industrial ethanol (Sánchez and Cardona 2008). Interest in ethanol as a gasoline alternative is growing around the world. In 2006 world ethanol production reached about 51,000 million liters, which represents a 10% increase over the previous year (RFA 2007). U.S., Brazil, European Union, India, Thailand, Australia and China have been trying to boost ethanol consumption to reduce their dependence on petroleum imports and to subsidize their agricultural products (Lyons 2003). According to Wheals (Wheals and others 1999) bio-ethanol is renewable and less polluting compared to gasoline and those are the main environmental advantages of ethanol over gasoline.

Ethanol is used as a blending component to add oxygen and octane to gasoline. Today, E10, 10% ethanol and 90% gasoline blend, is available at most of the gas pumps in the U.S. It is expected that E85, a blend of 85% ethanol and 15% gasoline will be the preferred fuel for flexible fuel vehicles in the near future (Lyons 2003). Using ethanol fuel

(E85) in midsize passenger vehicles can reduce greenhouse gas emissions by 41–61% for every km driven, compared to gasoline-fueled vehicles. According to Argonne National Laboratory, E95 (blend of 95% ethanol and 5% gasoline) can reduce fossil energy use by 42 to 44% and greenhouse gases by 19 to 24%, compared to conventional gasoline (RFA 1999). A major disadvantage of ethanol is its lower energy content, 26.8 MJ/kg, than that of gasoline, 43.5 MJ/kg (Kim and Dale 2005). Hence, significantly more ethanol fuel is needed when replacing gasoline.

2.2. FEEDSTOCKS

Bio-ethanol can be produced from sugar, starch and lignocellulosic biomass. Today no cellulosic ethanol industry exists. In the U.S. most of the ethanol production facilities currently use starch-based technologies. This is because of the fact that technical aspects and capital and operating costs of these technologies are well understood and documented. Corn is the main feedstock used for fuel ethanol production in the U.S. Corn is preferred because of its availability and high starch content. An analysis of the Illinois State variety Test results for total and extractable starch content in 708 samples of 402 commercial varieties of corn showed that average extractable starch content was 66%, which was similar to the previous data published in the literature (Patzek 2006; White and Johnson 2003).

The nonrenewable energy requirement for ethanol production varies between 13.4 and 21.5MJ/kg ethanol (based on lower heating value-LHV) depending on corn milling technologies used (Kim and Dale 2005). Since the energy content of ethanol is 26.8 MJ/kg, the net energy value of ethanol is positive. However, with the increasing need for ethanol, the current corn supply will not be enough to meet the demand. Other

cereal grain crops such as wheat, barley, rye, and triticale have been studied for their potential as feedstock for ethanol production via starch-based technologies (Sosulski and Sosulski 1994; Sosulski and others 1997). One prospective raw material in ethanol production is barley (Ingledew and others 1995; Sohn and others 2007; Flores and others 2005; Bhatty 1999).

2.3. BARLEY

2.3.1. INTRODUCTION

Barley is an ancient crop which belongs to the family Poaceae, the tribe Triticeae and the genus *Hordeum* (Newman and Newman 2008). Barley genotypes are classified as spring or winter types, two-row or six-row and hulled or hulless. Categories on barley grain composition are based on the content of amylose (normal, waxy, and high amylose), lysine, β-glucan and proanthocyanidin (Baik and Ullrich 2008).

Barley production in the United States was ranked 10th highest in the world with 4.6 million metric tons in 2007 (FAO 2010). The ten countries producing the most barley are Russia, Spain, Canada, Germany, France, Turkey, Ukraine, Australia, the United Kingdom and the US. In Oklahoma, barley production has fluctuated over the years. Maximum production was 509,464 metric tons in 1970. After a record low in 2002 (2,143 metric tons) barley production in the state has reached to 4,523 metric tons in 5 years (USDA 2007). During the same time period, barley hectares have increased from about 948 hectares to 2039 hectares (USDA 2007). According to Dr. Jeff Edwards, Oklahoma State University grain specialist, "There are several reasons for the fluctuation, but much of it has to do with the price and availability of corn. There are always a few

acres of barley grown for the dairy and horse industries, as some nutritionists prefer barley in their rations. The jump in 2007 is a reflection of increased demand for corn in ethanol production. Oklahoma is a corn-deficit state and a few barley producers took advantage of this." (Personal communication). Currently barley production in the state is well below its optimum production capacity. There is a big potential for expansion of barley production in the state.

Today, about 60% of barley grain is used as feed. Malting is the second largest application for barley grain. Only 2% of barley is used for food production in the U.S. However, in regions with extreme climates, such as Himalayan nations, Ethiopia and Morocco, barley remains to be an important food source (Baik and Ullrich 2008). Interest in incorporating barley in the human diet is increasing because of its high nutritional value (Newman and Newman 2008). The health benefits of barley include blood cholesterol (low density lipoprotein-LDL), glycemic index, and body mass reduction which lead to control of heart disease and type-2 diabetes. The beneficial effects of barley are due to the presence of several bioactive compounds such as β-glucans, tocopherols and tocotrienols in the grain (Baik and Ullrich 2008).

Barley grows well outside the Corn Belt area where there is demand for ethanol, thus making it a potential feedstock for ethanol in these regions (Kim and others 2008; Sohn and others 2007). Corn is not a common feedstock for fuel ethanol production in Europe; therefore, barley has been successfully used to replace corn (Sohn and others 2007). Although the price of hulless barley is higher than that for hulled barley, it is lower than that for wheat (Ingledew and others 1995). Furthermore, production of valuable high protein content DDGS (Distillers Dried Grains with Solubles) as a by-

product improves the feasibility of barley as feedstock for potential fuel alcohol production (Ingledew and others 1995).

2.3.2. GRAIN STRUCTURE AND COMPOSITION

In general, mature barley grain has an elongated oval structure. More spherical kernels also exist among the genotypes. Anatomy of barley grain is shown in Figure 1. Hulless barley generally has lower ash and dietary fiber and higher starch, protein and oil content due to the absence of the hull (Andersson and others 1999). According to Andersson, barley hull comprises about 13% of the kernel and consists mostly of cellulose, hemicelluloses (xylans), lignin and a small amount of protein (Andersson and others 1999). Hulls adhere to the caryopsis of the hulled barley. Hulls are not attached or loosely attached to the grain surface of hulless barley. According to Bhatty (1999) hulless barley would ideally have less than 5% adhering hulls. Thickness of the hulls varies. Thick hulls adhere to the caryopsis less firmly than thin hulls. The pericarp, testa (seed coat), epidermis nucleus, endosperm and embryo (germ) make up the caryopsis. The pericarp is the layer beneath the hull and over testa which covers the entire kernel. The pericarp and testa comprise about 3% of the kernel. The endosperm is the largest section of the grain and includes aleurone, subaleurone and starchy endosperm. Aleurone and starchy endosperm make up 3 and 76% of the grain, respectively. The embryo is the energy source for plant growth. It comprises about 3% of the grain. The rachilla has a brisk like hairy form and located at the base where kernel is attached to the rachis (axis/stalk) (Newman and Newman 2008).

Chemical composition of barley grain varies significantly depending on genotype, agronomic practices used and environmental conditions as pointed out in many studies (Griffey and others 2009; Aman and others 1985 ; Oscarsson and others 1996). A study on the chemical composition of 92 Swedish barley varieties showed that two-rowed barley varieties had slightly higher starch content while six-rowed barleys had higher crude protein and total and crude fiber content (Aman and others 1985). Typical compositions of hulled and hulless barley as comparison to corn are shown in Table 1. As expected, fiber content of hulled barley is higher than that for hulless barley. Starch, lipid and protein contents of hulled barley are lower than those of hulless barley. This is because of the dilution effect of fibrous components on other grain constituents in hulled barley. Depending on the variety, starch and protein contents of hulless barley can be as high as those for corn (Table 1).

Carbohydrates comprise about 80% of the barley grain. Starch which is considered a soluble polysaccharide is the major component in the grain. Barley may contain up to 65% starch (Song and Jane 2000). Chemical composition and properties of barley starch vary depending on the genotype (Song and Jane 2000). Amylopectin and amylose are two structural components of starch. Amylopectin comprises 72-78% of the total starch in barley (Bhatty 1999). Barley is also classified based on its amylose content. Zero or waxy, normal and high amylose barley contain 0-5, 20-30% and up to 45% amylose, respectively (Baik and Ullrich 2008). Waxy barley varieties contain very high levels of amylopectin. Barley varieties with 100% amylopectin have been reported (Bhatty 1997). It is well established that waxy barley varieties contain 5-8% less starch than that of non-waxy/regular barley varieties (Bhatty 1999). Barley starch consists of a

mixture of large (10-25µm) and smaller granules (<10µm). Starch granule size in hulless barley ranges from 2-30 µm (Bhatty 1999; You and Izydorczyk 2002). Among four types of hulless barley (normal, high, waxy and zero amylose), normal amylose type has the greatest amount of large granules (74.7%). Waxy, zero and high amylose starches consist of 66.4%, 43.9% and 19.4% large granules, respectively (You and Izydorczyk 2002).

The major non-starch carbohydrates in barley include (1,3)(1,4)-β-D-glucans and arabinoxylans. β-Glucan consists of high-molecular-weight linear chains of β-glucosyl residues polymerized through both β-(1-3) and β-(1-4) linkages (Newman and Newman 2008). High β-glucan content of barley (2.5% to 11.3%) is notable (Izydorczyk and Dexter 2008). High amylose and waxy barley have been reported to contain higher β-glucan than normal amylose type (You and Izydorczyk 2002). The β-glucans are mainly present in the endosperm cell walls (Oscarsson and others 1996) and they are partially soluble in aqueous solutions due to molecular, structural and solubility differences of its two polysaccharides (Newman and Newman 2008). The water soluble part of β-glucan produces high viscosity starch slurries that can cause problems during industrial processing and ethanol production. High viscosity mash increases pumping costs and complicates production. Low β-glucan content in the grain leads to low viscosity and little need of expensive enzymes to break it down for efficient processing and fermentation. Cellulose (1-4 β-D-glucan), fructans, arabinoxylans, glucommannan, galactomannan, arabinogalactan and a number of simple sugars and oligosaccharides are also present in barley grain in relatively small quantities.

2.4. BARLEY PROCESSING FOR ETHANOL PRODUCTION

Husks and high content of glucans are two major disadvantages of hulled barley in terms of its potential as feedstock for ethanol production (Kosaric and others 2001). Utilization of hulless barley types that require less pretreatment to remove hulls would alleviate the problem caused by abrasive hulls. Hulless barley has more protein, starch, and total and soluble β-glucan compared to hulled barley. Higher β-glucan content in barley can be beneficial for ethanol production as long as starch levels are high (Sohn and others 2007). Prior to fermentation, β-glucans can be hydrolyzed to simple sugars that can be converted to ethanol by yeast. Moreover, high protein content is preferable for food and feed applications of barley (Baik and Ullrich 2008). Starch and consequently ethanol yields can be further increased by pretreatment and milling hulless barley.

2.4.1. PRETREATMENT

In this study the term "pretreatment" refers to a series of processes that barley grain goes through prior to fermentation for ethanol production.

2.4.1.1. TEMPERING

In general grain is tempered or conditioned prior to milling. Tempering which means controlled addition of moisture to the grain results in high starch and flour yields during milling. Moisture addition causes swelling, loosens the outer layers and increases the grain elasticity which is beneficial during grinding grains into flour (Dexter and Wood 1996; Bhatty 1987). Izydorczyk has shown that increasing the moisture content of hulless barley grain from 12.5% to 14.5% prior to roller milling improved the brightness of the flour with only a moderate loss of flour yield (Izydorczyk and others 2003). The yield of fiber free fraction and its β-glucan content increased as the moisture

content of hulless barley was further increased to 16.5% preceding milling. Bhatty (Bhatty 1987) has reported a similar trend. Tempering Argyle and Tupper barley varieties to 11% and 13% moisture content, respectively, resulted in the best flour recovery, 74%. Wang (Wang and others 1997) examined the effect of grain moisture content on starch recovery during sequential abrasion of wheat, two-row barley, rye and triticale. The effect of tempering at 12.5 and 15% moisture levels on the rates of grain mass removal by abrasion was not consistent among the cereals examined in the latter study. However, starch losses to the abraded fines could be minimized by optimization of the cereal moisture content.

2.4.1.2. MILLING

Fractionation of cereal grains into flour (starch and protein) and fiber rich components are essential not only for food applications but also for production of other bio-products such as bio-ethanol. Wet or dry milling, abrasion milling followed by sifting and/or air classification and impact/pin and roller milling are some of the techniques used for cereal grain fractionation. Unlike wheat, barley does not have a long history of conventional roller milling. However, this technique is applicable to barley. In contrast to wheat bran that separates out as large flakes, hulless barley bran shatters and ends up in the fraction called “shorts” which is a mixture of flour and bran during milling. A broad range of flour yields, 51 to 72%, was obtained when a roller mill was used to mill diverse barley cultivars (Bhatty 1987). Genotype, more specifically starch and β-glucan contents, was the most important factor in flour recovery yields. Bhatty (Bhatty 1987) examined the roller milling characteristics of two hulless barley varieties, Tupper and Argyle, and was able to obtain 70 to74% flour yield, which is similar to that from wheat.

It has been suggested that pearling prior to milling would improve the quality of barley flour obtained from a roller mill (Izydorczyk and others 2003). Wu (Wu and others 1994) used a three step milling process, grinding, sieving and air classification, to obtain barley fractions enriched in protein and β-glucan. Three varieties, Portage (commercial dehulled barley), CI4362 (high-protein hulless barley) and Prowashonupana (high protein-high β-glucan hulless barley) were examined in the study. Barley type had a significant effect on the composition of the fractions obtained from the 3-step milling process. Increase in protein content of fractions obtained from normal-protein dehulled barley was higher than that for hulless high-protein barley variety.

Four different types of experimental mill (Chopin CD1 Auto, Quadrumat Sr, Buhler and Ross roller mill walking flow) were examined for their efficacy to obtain starch enriched fractions from two hulless barley cultivars, Doyce and Merlin, and one commercial hulled barley (Flores and others 2007). It was found that break flour fractions obtained from Merlin and commercial hulled barley by using Chopin CD1 had the highest starch content. Quadrumat Sr produced the highest starch content fraction from Doyce hulless barley. It was noted that experimental mills evaluated in the study were originally designed for wheat milling. Furthermore, in the latter study milling conditions were not optimized for each variety. These results indicate that milling technique used to fractionate grain and variety have significant effect on the composition of fractions, specifically starch content of the fractions. Hence, further research is needed to modify wheat milling equipment and optimize milling conditions for different barley varieties.

The abrasion characteristics of CDC Dolly, a two-row barley, was examined by using a Satake abrasive test mill equipped with a medium abrasive roller stone (Wang

and others 1999a). It was found that two-row barley had to be abraded for 70 s to obtain a barley fraction containing about 70% starch. During this process 33% of the grain mass was removed and 18.5% starch was lost in the abraded fines. Wheat, triticale and rye which were also examined in the same study had significantly lower starch and grain mass loss than barley. It was also reported that abrasion process improved ethanol yields from barley (per ton of fermentation feedstock) by 22.5% indicating that plant throughput can be increased by using abraded barley as feedstock for ethanol production. Utilization of an abrasive process to remove selected kernel layers allows for optimization of the grain fractionation for various applications. However, capital and operating costs of an abrasion system need to be evaluated for ethanol production.

2.5 STARCH CONVERSION TO ETHANOL

2.5.1 STARCH HYDROLYSIS

Starch cannot be utilized directly by yeast for ethanol conversion. Yeast lacks starch-degrading or amylolytic enzymes to release glucose from polysaccharides. The bonds between the glucose subunits in starch must be hydrolyzed to release free glucose molecules that can be utilized by yeast (Power 2003). α-Amylase and glucoamylase are two types of enzymes used in starch hydrolysis prior to fermentation. Starch hydrolysis involves two steps, dextrinization or liquefaction during which starch is partially hydrolyzed to soluble dextrins, and saccharification or conversion step during which the dextrins are hydrolyzed to fermentable sugar. In barley, liquefaction is usually performed at high temperatures (100-120^{o}C) in conjunction with direct steam injection (jet-cooking) (Song and Jane 2000; Lyons 2003; Gibreel and others 2009). The dextrins produced are

further hydrolyzed into glucose in a saccharification process using glucoamylase (Lyons 2003).

2.5.2 FERMENTATION

Fermentation is a process of converting glucose into ethanol and carbon dioxide using yeast.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \qquad (1)$$

Glucose Ethanol Carbon dioxide

Yeast metabolizes sugars to ethanol through the Embdem-Meyerhof-Parnas /Glycolytic pathway under anaerobic conditions (Power 2003). One mole of glucose produces two moles of ethanol and two moles carbon dioxide (Equation.1). *Saccharomyces cerevisiae* is a widely used yeast in industrial ethanol production (Kosaric and others 2001). In general, yeast can grow and efficiently convert glucose into ethanol at pH and temperature range of 3.5-6 and 28-35^{o}C, respectively. Fermentation usually ends when the concentration of the residual sugars is below 2-4 g/L in the fermentation broth (Francisco Pizarro 2007). Rapid sugar conversion and high ethanol levels in fermentation broth are desirable to minimize capital cost and energy needed to distill ethanol.

Fermentation of barley has been studied by several research groups. Ingledew et al. evaluated fermentability of hulless barley as compared to hulled barley and wheat (Ingledew and others 1995). The fermentation process was carried out by using a normal gravity barley mash, 20 g of dissolved solids per 100 mL, which is common for wheat and corn fermentation. Hulless barley mash was more viscous than wheat mash. High viscosity of barley mash was easily controlled by β-glucanase addition. It was also

observed that during the fermentation viscosity decreased over time even without enzyme addition. The fermentation time for hulless barley was slightly shorter when compared to that of wheat. About 10.6% (v/v) ethanol yield, which corresponded to 94% of the theoretical value, was achieved .

Hulless barley fermentation using very high gravity mash (VHG) (>30 g dissolved solids per 100 mL) has been reported (Thomas and others 1995; Wang and others 1999b; Gibreel and others 2009). VHG mash fermentation has also been successfully applied to oats, rye, wheat and triticale fermentation (Wang and others 1999b). The advantages of VHG mash fermentation include greater water savings (Wang and others 1999a), lower capital, processing, and labor costs and reduced bacterial contamination (Thomas and others 1995). It was possible to achieve a maximum ethanol concentration of 17.1% (v/v) when SB90354, hulless barley, was fermented by using VHG mash, 31.1 g/100 mL (Thomas and others 1995). It was noted that VHG mash could not be prepared from barley without using viscosity lowering enzymes. The authors overcame high viscosity by increasing the water-to-grain ratio and hydrolyzing β-glucans before starch gelatinization. When starch from dehulled Bold and Xena barley was hydrolyzed by jet-cooking and then fermented by using a VHG mash, ethanol yields were slightly higher than that for wheat but lower than corn. Treatment of barley mash with enzyme Stargen 001 improved the fermentation yield and ethanol concentrations similar to that of corn were achieved (Gibreel and others 2009).

This literature review on barley fermentation clearly indicates that barley type and processing techniques used for both pretreatment and fermentation have a significant effect on ethanol yield. To the best of our knowledge there is no study published in the

literature examining pretreatment and fermentation of Eve and VA125 barley varieties. Hence, this thesis is a first attempt to fill this knowledge gap.

CHAPTER III

METHODOLOGY

3.1 SAMPLE COLLECTION

Eve and VA125, two-row hulless barley varieties grown in Oklahoma, were examined in this study. Samples were collected from Marshall (97^{o}35'W, 36^{o}07'N), Buffalo (99^{o}38'W, 36^{o}49'N), Perkins (97^{o}02'W, 35^{o}59'N) and Stillwater (97^{o}05'W, 36^{o}07'N), Oklahoma. Two sets of barley samples were received. The first set included samples that were planted in Marshall, Perkins and Stillwater on October 30, 2007, October 31, 2007 and October 30, 2007, respectively. These samples were harvested in June 2008. The second set was collected from barley planted in Marshall and Buffalo on October 20, 2008 and September 17, 2008. The harvest date for these samples was June 2009. Due to the limited amount of sample available for this study, the first set of samples was used for the characterization of winter hulless barley varieties. The second set of samples was used for the pretreatment and ethanol conversion study. Barley samples were received and stored in brown paper bags and kept in a freezer at -20^{o}C until used.

3.2 BARLEY CHEMICAL ANALYSIS

3.2.1 SAMPLE PREPARATION

Approximately 50 g of whole grain hulless barley was ground with a Perten mill (3600, Perten, Sweden) at a setting of 0, which is the finest setting of the instrument. The Perten mill was cleaned between grinding samples to avoid sample carryover. The ground barley samples were then kept in airtight plastic bags in the freezer at -20°C until being used for experiments.

3.2.2 MOISTURE CONTENT

The moisture content of the ground barley samples was determined according to the AACC method 44-19 (AACC 1995). In summary, ground barley samples were brought to room temperature before testing. First, empty aluminum weighing dishes were dried in a forced-air oven (VWR Scientific, Model 1370 FM, Bristol, CT) at 135°C for an hour, cooled to room temperature in a dessicator, and approximately 2 g of sample was weighed in the weighing dishes and dried at 135°C for 2 h. Samples were weighed before and after drying. The difference between the final and initial sample weight as percent of the initial sample weight was reported as the moisture content.

3.2.3 OIL CONTENT

Oil content of the samples was determined as outlined in AOAC method 960.39 (AOAC 1995). Approximately 1 g of ground barley samples was weighed in a cellulose thimble. The thimble was then placed in the Soxtec extraction unit (Tecator, Model 1043 Extraction Unit, Sweden), and 40 mL of petroleum ether (Mallinckrodt, Paris, KE) was

used to extract the oil from the sample. The oil content of the samples was determined as the percentage of oil extracted of initial sample weight.

3.2.4 PROTEIN CONTENT

The protein content of barley samples was determined by Forage Analyses Procedures (1993). Protein was analyzed as nitrogen on a Leco TruSpec carbon-nitrogen analyzer (TruSpec CN, Leco USA, St. Joseph, MI). A factor of 6.25 was used to convert nitrogen to protein.

3.2.5 ASH CONTENT

The ash content of barley was determined according to AOAC method 923.03 (AOAC 1995). Ground barley samples were brought to room temperature prior to the analysis. Crucibles were pre-dried in a furnace (Fisher Science, Model 58 Isotemp Muffle Furnace 600 Series, Fair Lawn, NJ) for 5 h, at 525°C and then cooled down to room temperature in a desiccator. Approximately 2 grams of sample were weighed in the dried crucible, then ashed in the furnace for 5 h at 525°C. The percentage residual weight was reported as the ash content of the sample.

3.2.6 STARCH CONTENT

Starch content of the samples was analyzed using a starch determination kit (Megazyme International Ireland Ltd., Bray, Ireland) according to the AOAC method 996.11(AOAC 2005). Approximately 100 mg of sample was weighed in a glass test tube. Thermostable α-amylase was used to hydrolyze starch to branched and unbranched

maltodextrins at 100^{o}C and pH 7. Then amyloglucosidase (AMG) was utilized to quantitatively hydrolyse maltodextrins to D-glucose. Glucose oxidase was used to oxidize D-glucose to D-gluconate. This reaction releases one mole of hydrogen peroxide (H_2O_2) which was quantitatively measured in a colorimetric reaction employing peroxidase and the production of a quinoneimine dye. The absorbance of the samples and the glucose control was read with a spectrophotometer with absorbance 510 nm against the reagent blank. The starch content (%, on as is basis) was determined as below:

Total starch (%) = A x F x 1000 x 1/1000 x 100/W x 162/180

= A x F/W x 90

Where;

A = absorbance of reaction solutions read against reagent blank

F = factor to convert absorbance values to µ glucose = 100 µg glucose/ absorbance value for 100µg glucose;

1000 = volume correction

1/1000 = conversion from µg to mg

100/W = conversion to 100 mg test portion

162/180 = factor to convert from free glucose, as determined, to anhydroglucose, as occurs in starch

3.2.7 β-GLUCAN CONTENT

The β-Glucan content of the barley samples was determined using a test kit from Megazyme International Ireland Ltd., (Bray, Ireland) according to the AOAC method 995.16 (AOAC 2005). In summary, approximately 100 mg of sample was weighed in a

glass test tube. The sample was hydrated in sodium phosphate buffer at pH 6.5. Then the solution was incubated with lichenase enzyme and filtered. An aliquot of the filtrate is then hydrolysed with β-glucosidase. The D-glucose produced is measured by using a colorimetric assay with glucose oxidase/peroxidase reagent. The absorbance of the samples and the glucose standard was read at 510 nm against the reagent blank. The β-glucan content was calculated as described below:

β-Glucan content $= \Delta E \times F \times 94 \times 1/1000 \times 100/W \times 162/180$

$= \Delta E \times F/W \times 8.46$

Where

ΔE = absorbance of reaction solution (after -glucosidase treatment minus blank absorbance for some sample).

F = factor to convert absorbance values to micrograms of glucose (100 (µg of glucose/ absorbance values for 100 µg of glucose)

94 = volume correction factor of solution from 9.4 ml was analyzed)

1/1000 = conversion from micrograms to milligrams

100/W = conversion to 100 mg of sample

162/180 = factor to convert from free glucose, as determined, to anhydroglucose, as occurs in β-Glucan.

3.3 BARLEY DRY MILLING

Due to the limited amount of sample available to this study, barley samples from Eve and VA125 varieties harvested at different locations in June 2009 were mixed by variety in a Hamilton Kettle (Trinity Industries, Inc, Model DM-US, Fairfield, Ohio, USA) for 30 minutes. Samples were then kept in airtight containers in the refrigerator

until further use. Dry milling of the samples was carried out at two moisture levels, original moisture content and 15% (w/w). Whole grain tempering which raises the moisture content to 15% was carried out according to AACC method 26-95 (AACC 1995). The amount of water required for tempering was calculated by using the following formula:

$$\text{Weight of water to add} = \left[\frac{100 - \text{original moisture}\,(\%)}{100 - \text{desired moisture}\,(\%)} - 1\right] \times \text{weight of sample}$$

The calculated amount of water was sprayed onto the whole grain samples. Bottles containing water and grain samples were sealed with screw caps and shaken frequently until free water was absorbed by the grain. Then samples were allowed to equilibrate for 18 h before milling.

In this study three milling systems, Tangential Abrasive Dehulling Device (TADD), Wiley Mill and Buhler Mill, were examined for their efficacy to fractionate barley grain into starchy endosperm and bran fractions.

3.3.1 TANGENTIAL ABRASIVE DEHULLING DEVICE

A Tangential Abrasive Dehulling Device (TADD) (Venables Tangential Abrasive Dehulling Device, Model no. 4E-10/220, Veenables Machine Works, Ltd, Saskatoon, Canada) was used to remove outer layers of the barley seeds. Details of the equipment design and operation are described elsewhere (Reichert and others 1986). In summary, the TADD with a 5 cup plate which allowed up to 5 samples to be dehulled simultaneously was used for the experiments. The sample cup dimensions were as follows: 6.23 cm inner diameter, 3.49 cm height, 36.6 cm^2 abrasive area, 127.7 cm^3 volume and maximum seed

holding capacity of about 97 g. Dehulling was accomplished by an abrasive mounted on a 10" aluminium backing disc (Coarse disc, Type 4, Perten Instruments, Huddinge, Sweden). A minimum clearance of 0.25-0.38 mm was kept between the abrasive and the bottom edge of the sample cups. The bottomless sample cups containing 40 g of seeds in each cup rested on the abrasive surface. The lid kept the seeds in the cups during operation. The operation cycle was automatically controlled by a timer. During the operation seeds rolled around freely in the sample cups, and were dehulled as they came into contact with the abrasive. Hulls and other fine material (bran) escaped beneath the sample cups into the base where they were sucked through an exhaust and collected in a cloth bag that was attached to the exhaust. Abraded seeds were removed from the sample cups with a vacuum aspiration device. In this study dehulling experiments were carried out at two seed moisture levels, 15% and original moisture, and three cycle times, 50, 70 and 90 s. Yields of bran and dehulled seed fractions were calculated as the percentage of the initial sample weight.

Then dehulled barley seed fraction was ground using a Perten Mill (3600, Perten, Sweden) at the setting of 0. After being milled the sample was further processed by sieve analysis. A stack of five selected U. S. standard sieves (No. 60, 100, 200, 270 and 400, corresponding to sieve opening dimensions of 250, 150, 75, 53 and 38 µm, respectively) and a pan fitted into a sieve shaker (Ro-Tap, Model RX-29, W.S. Tyler, Mentor, Ohio) was used to shake the samples for 60 min. The product which was retained in each sieve was then weighted to calculate the fraction yield.

3.3.2 WILEY MILL

Whole barley grain was ground in a Wiley mill (Thomas-Wiley lab mill, Model 4, Arthur H. Thomas Company, Philadelphia, PA, U.S.A) fitted with 2 mm sieve screen. Approximately 500 g of sample was ground for about 30 minutes until the entire sample passed through the screen. After being milled, the sample was further processed by sieve analysis as described in section 3.3.1. Then each fraction was placed in separate airtight plastic bags and kept in a freezer at -20°C until being used.

3.3.3 BÜHLER MILL

A Bühler Mill (Buhler Brothers, Inc, Number 91727, Uzwil, Switzerland) was used to obtain three fractions, bran, shorts and flour. The AACC Method 26-21A (AACC 1995) was used for the milling process.

3.4 FERMENTATION

3.4.1 STARCH HYDROLISIS

Starch hydrolysis and fermentation procedure are illustrated in Figure. 2. A slurry, 500 g, with approximately 30% (w/w) barley sample was prepared in 0.1 M citrate buffer at pH 5.8. The final pH of the slurry was 5.6. Three enzymes, SPEZYME® ALPHA (α-Amylase), OPTIMASH™ BG (β-Glucanase), and OPTIMASH™ TBG (Thermal β-Glucanase) were added to the slurry at the dose of 0.12, 0.07 and 0.0035 g/kg based on the slurry weight, respectively. All the enzymes were provided by Genencor International, Inc (Rochester, NY, U.S.A). Then the slurry was continuously mixed in a Gyrotony water bath shaker (Model G76, New Brunswick Scientific, Edison, N.J, U.S.A) at 60^{o}C for 45 min at speed setting of 6. The partially hydrolyzed starch slurry was jet

cooked at 107^{o}C for 7 minutes in a Parr Pressure reactor (Parr Instrument Co., Model 4843, Moline, IL) which was connected to a direct steam injection system. The temperature of the slurry was rapidly brought down to 85^{o}C after jet cooking by placing the reactor in an ice bath. Further liquefaction of the barley starch was achieved by adding 0.12, 0.07 and 0.0035 g/kg of α-amylase, β-glucanase and thermal β-glucanase to the slurry, respectively. The temperature of the slurry was maintained at 85^{o}C for 120 min with constant mixing in a reciprocal shaking bath with constant shaking at 85 rpm (Precision Scientific, Model 50, Winchester, VA). Moisture/solid content of the slurry was measured at every step of the liquefaction process. Approximately 2 g of slurry sample was dried in a forced air oven at 102^{o}C for 16 hrs to determine the moisture content. Sugar content of the slurry samples taken at every step of the starch liquefaction process were also determined by using HPLC.

3.4.2 SACCHARIFICATION AND FERMENTATION

Simultaneous saccharification and fermentation was used to convert liquidified barley mash to ethanol. The experiments were carried out in 250 mL flasks containing 100 g of barley slurry. Glucoamylase (GC 650), 0.070 g/kg, and β-glucosidase (GC 151), 1.3 g/kg, were added into the slurry as saccharification enzymes (Genencor International, Inc, Rochester, NY, U.S.A). Approximately 0.4 g/L of Superstart (Lallemand, Rexdale, Ontario), which is a commercial *S. cerevisiae* strain was utilized for fermentation. The flasks containing barley slurry, saccharification enzymes and yeast were fitted with one-way gas release valves and placed in a shaker water bath (C76, New Brunswick Science, Edison, NJ, USA) maintained at 32^{o}C. The shaker speed was set at 150 rpm.

3.4.3 ANALYSES OF FERMENTATION PRODUCTS

Approximately 2 mL of sample was withdrawn from each fermentation flask using a sterile pipette at 0, 6, 12, 24, 36, 48, 60 and 72 h. The samples were centrifuged using a bench top centrifuge (Centrifuge 5424, Eppendorf AG, Hamburg, German) at 13,000 rpm for 8 minutes. The supernatant was filtered through a 0.45 µm syringe filter and injected to HPLC. Samples were diluted 4x with distilled water prior to HPLC injection. An HPLC system equipped with a Bio-Rad Aminex® HPX-87 H ion exclusion column (Hercules, CA) and a Refractive Index Detector (Model G1362A, Agilent Technologies, Inc, Broken Arrow, Oklahoma) was used for sample analysis. The ion exclusion column was maintained at 60^{o}C and sulfuric acid (0.01N) was used as the mobile phase at a flow rate of 0.6 mL/min. Sample injection volume was 20µL. A standard solution containing glucose (25 g/L), ethanol (25 g/L), maltose (25 g/L), succinic acid (2 g/L), glycerol (2 g/L), acetic acid (2 g/L), and lactic acid (2 g/L) was used for peak identification and quantification. The data were processed by ChemStation Software (Rev.A.10.01. Agilent Technologies, Broken Arrow, Oklahoma).

The Fermentation efficiency was calculated as described below:

Max ethanol = solid (liquefaction, g/L) * % db starch * (180/162) * 0.51 **(1)**

Fermentation efficiency (%)=(g/L ethanol observed*0.1)/(max % ethanol)*100 **(2)**

Where solid (liquifaction) is the solid content measured at the end of liquifaction process, % db starch is the initial starch content of the sample on a dry basis, 180 is the molecular weight of glucose, 162 is the molecular weight of starch, 0.51 is the conversion factor for

glucose to ethanol, g/L ethanol observed is the actual ethanol content in the fermentation broth. On the second equation 0.1 is the conversion to %.

3.5 STATISTICAL ANALYSIS

All analytical tests and fermentation runs were carried out in duplicate with the mean values being reported. The Mixed or GLM procedure of SAS (Software Version 9.2., SAS Institute, 2007) were used to analyze the data for chemical composition and milling experiments. In the chemical chemical analysis, variety was a fixed effect and location was included as a random effect. In the milling data both variety and sieve size were fixed effects. Analysis of variance (ANOVA) was used to analyze data for the % solid in barley mash, fermentation efficiency and ethanol observed in this study.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 CHARACTERIZATION OF HULLESS BARLEY

Temperature and water availability are two important environmental factors affecting crop development. Average monthly temperatures and rainfalls at each location during 2008 and 2009 barley growing seasons are shown in the Appendix, Figures A1 to A10. Mesonet data for average temperature and rainfall at all locations are also displayed on the figures for the last 15 years, 1994-2009 (Mesonet 2010). Average daily temperatures at all locations were similar to the long-term averages for the region, except all locations received significantly lower-than-normal precipitation during the month of November. Specifically, the Buffalo location received substantially lower-than-normal precipitation from November 2008 through June 2009 except the month of April.

Chemical characterization of barley varieties were carried out by using the samples harvested in 2008. Starch contents in barley samples varied from 60.3 to 71.1% (w/w, dry basis) (Table 2). These results are within the range reported for hulless barley varieties in the literature (Ingledew and others 1995; Griffey and others 2009). When data was pooled across locations, variety did not have a significant effect on the starch content ($p = 0.1736$). Hulless barley had comparable starch content to that of corn (63.7%) but starch content of hulled barley (56.45%) was significantly lower than that for both hulless

barley and corn (Table 1). Hence, hulless barley has a better potential as feedstock for ethanol production than hulled barley.

The range for the β-glucan content of the samples was 3.5-5.6% (w/w, dry basis) (Table 2). The highest β-glucan content was observed in VA125 grown in Stillwater. This variety had the lowest starch content. Variety had a significant effect on β-glucan content of the samples ($p = 0.0007$). The mean β-glucan content for Eve was significantly lower than that for VA125. Eve variety had more variance in β-glucan content than Virginia. The effect of genotype on β-glucan content of barley has also been reported in the literature (Oscarsson and others 1996; Andersson and others 1999). High β-glucan content in barley is not desirable since β-glucan contributes to high viscosity which leads to problems during industrial processing and ethanol production by increasing pumping costs and complicating production. However, use of β-glucanase to hydrolyze β-glucan during the fermentation process lowers viscosity and increases ethanol yield.

Protein is the second major compound in barley. DDGS with high protein content is a desirable by-product. High protein content barley is also beneficial for food applications. Protein content of barley varieties examined in this study varied from 10.2 to 16.2% (w/w. dry basis) (Table 2). These results are within the range reported in the literature (10.6- 21.9%) (Oscarsson and others 1996). For example, Doyce, a new hulless barley variety that has been developed at Virginia Polytechnic Institute and State University, had 9.5-11.4% protein (Sohn and others 2007). Protein content of hulless barley is comparable to corn (10.9%) (Table1). Variety had a significant effect on protein content ($p = 0.0067$), where Eve had higher average protein content (about 14.5%, w/w, dry basis) than that for VA125 (about 12.2%, w/w, dry basis). This finding is in

agreement with the previous publications indicating that genotype and environmental conditions had significant effect on barley protein content (Oscarsson and others 1996). Ash and oil are minor components in barley (Andersson and others 1999). The range for the ash content of the samples was 1.5-1.9 % (w/w, dry basis) (Table 2). This was similar to the range reported in the literature, 1.3-2.0 % (Oscarsson and others 1996). Variety did not have a significant effect on ash content ($p = 0.0766$). Hulless barley has similar ash content to corn (1.5%) (Table1).

Oil content of the samples varied from 1.0 to 1.9 % (w/w. dry basis) (Table 2). Differences in oil content within varieties were not statistically significant ($p = 0.4424$). Oil content of the samples examined in this study was lower than that for reported for other barley varieties in the literature (2.1-3.7%) (Oscarsson and others 1996). The variations in oil content are due to the differences in growing locations and genotypes which affect chemical composition of barley. Hulless barley has lower oil content than that for corn (4.9%) (Table1).

Moisture content is an important parameter affecting grain storage, processing and milling. Moisture content of barley varieties examined in this study varied from 10.9 to 12.7 % (w/w. dry basis). The effect of variety on moisture content was not statistically significant ($p = 0.6421$)

4.2 MILLING

Milling experiments were carried out using barley grain samples harvested in June 2009 (Table 7). Due to the limited amount of sample available for this study, barley samples harvested at different locations were mixed by variety as described in section 3.4. The chemical composition of the mixture (Table 7) was similar to that of the grain

samples examined in the characterization study discussed in the previous section 4.1(Table 2).

4.2.1 BUHLER MILL

Barley samples were milled under the conditions described in section 3.4.3. Three fractions obtained from the Buhler mill were bran, shorts, and flour. Table 3 shows the yield and starch content of each fraction. Both Eve and VA125 produced more shorts than bran and flour when they were milled at their original moisture content. At 15% moisture level, the highest yields were obtained as flour and shorts from Eve and VA125, respectively. Flour fraction yields obtained in this current study were quite low, 31.2-47.8%, much lower than those reported in the literature (72%) (Bhatty 1993). However, lower flour yields that are similar to that observed in this study were obtained from waxy and low β-glucan content barley types (Bhatty 1999). Lower flour yield resulted in higher shorts yield, 44.3 - 55%, which was much higher than the shorts yield reported in the literature (20%) (Bhatty 1993). VA125 had significantly higher bran yield (6.4% and 21.6% at 11.1% and 15% moisture levels, respectively) than that for Eve (4.02% and 12.1% at 11.1% and 15% moisture levels, respectively) (Table 3). The effects of variety, moisture content and their interaction on flour, bran and shorts yields were all statistically significant ($p < 0.05$). Although tempering significantly increased the bran yield, mass loss in the system and shorts yield decreased with tempering for both varieties. Tempering had a positive effect on flour yield of Eve but not for VA125.

Starch content of flour fractions obtained from both Eve and VA125 was over 75% (Table 3). Bhatty reported slightly lower starch content in flour obtained from

Canadian-registered hulless barley, Scout, (73.1%) (Bhatty 1993). As expected bran had the lowest starch content (33.2-42.6%) among the fractions obtained from the Buhler mill. Bhatty reported even higher starch losses in barley bran fractions (>50%) when a Buhler mill was used to fractionate Scout barley (Bhatty 1993). About 30-40% starch loss in the bran might be too high, adversely affecting the feasibility of using a Buhler mill for barley fractionation. The effects of variety and variety-by- moisture interaction on bran starch content were significant. The p value for moisture effect on bran starch content was 0.0555. Only variety had a significant effect on starch content of shorts. Neither variety nor moisture content had a significant effect on flour starch content. There was a significant variety-by- moisture interaction for the bran starch content ($p = 0.0194$). For Eve variety, tempering significantly reduced starch content in bran ($p = 0.0103$). Tempering had no effect on the starch content of bran obtained from VA125 ($p = 0.4784$).

4.2.2 WILEY MILL

Eve and VA125 were milled as described in section 3.4.2. Table 4 shows the yield and starch content of each fraction obtained by using a Wiley Mill followed by sieving. The particle size of the fraction with the largest yield (84.5% for Eve and 85.5% for VA125) was over 250 µm for both varieties indicating that the Wiley mill was not very effective in grinding barley samples. This is partly due to the large screen size (2 mm) used in the grinder. The second largest yielding fraction (8.9% for Eve and 7.4% for VA125) had particle size of 53-38 µm for both varieties. As expected sieve size had a

significant effect on fraction yield ($p = 0.0001$) while the effect of variety and variety-by-sieve size interaction were not statistically significant.

The fractions with high yield had also high starch content. It was possible to obtain fractions with over 73% starch content using Wiley Mill followed by sieving. As expected highest starch content was found in fractions collected with small sieve size. Variety, sieve size and variety-by-sieve size interaction had significant effect on the starch content of the fractions.

4.2.3 TADD AND PERTEN MILL

Eve and VA125 were milled as described in section 3.4.1. Table 5 shows the yields for bran and grain residue obtained from TADD. The effectiveness of TADD for removing barley bran was evaluated based on the yield of residual grain because of the difficulty in collecting bran fraction and losses in the system. As expected more material was removed from the grain as the processing time increased. All, time ($p < 0.0001$), variety ($p = 0.0104$) and moisture content ($p = 0.0002$) had significant effect on the amount of mass removed from barley during TADD processing. Variety-by-time, variety-by-moisture content and time-by-moisture content interactions were not statistically significant.

In Table 6 fraction yields and starch content of debranned barley grain fractions obtained by grinding (Perten mill) followed by sieving are shown. The particles with >250 μm size had the largest yield. This data was similar to that obtained with Wiley Mill. Sieve size ($p < 0.0001$) and variety-by-sieve size ($p < 0.0001$), moisture content-by-sieve size ($p < 0.0001$), variety-by-moisture content-by-sieve size ($p < 0.0001$), moisture

content-by-time-by-sieve size ($p = 0.0462$) and variety-by-moisture content-by-time-by-sieve size ($p < 0.0238$) interactions had a significant effect on fraction yields.

A significant enrichment in starch content was observed in the fractions with particle size < 53 μm for both Eve and VA125 varieties and all treatments. These results were similar to those obtained with a Wiley mill. It was possible to obtain fractions with starch content over 80% when bran fraction was removed with the TADD followed by grinding and sieving. The highest starch content, 87.3%, was obtained from Eve when whole barley grain was tempered and milled with TADD for 50 sec. At higher processing times, 70 and 90 s, more starch was lost into the bran fraction. All the effects except variety-by-time ($p = 0.0518$) and variety-by-time-by-moisture content ($p = 0.1358$) were statistically significant ($p < 0.0001$).

4.3 FERMENTATION

4.3.1 STARCH HYDROLISIS

Hydrolysis of starch is an essential step prior to fermentation for breaking starch into glucose and maltose which are then converted to ethanol by yeast. Starch hydrolysis was done as described in section 3.5.1. The changes in solid content of the barley mash during starch hydrolysis and liquifaction are shown in Figure 3. A large decrease in solid content of barley mash was observed during jet cooking. This was due to the condensation of steam in the system that caused dilution of the solids in the barley slurry. Slight increase in solid content during hydrolysis is due to moisture loss/evaporation from the system.

4.3.2 SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

Substrate utilization and product formation were monitored throughout the SFF process. Typical HPLC chromatograms of the samples collected at 0 and 36 h and a standard mixture were displayed in Figures 4-6. As expected, the main sugar in all the liquified barley samples (time 0) was glucose (Figure 4). Although flour samples had higher starch content (75.4 and 76.2 % for Eve and VA125, respectively) than that for whole barley samples (60.7 and 58.1% for Eve and VA125, respectively), glucose concentrations in liquified flour samples were lower than for whole barley samples. These results can be explained by the presence of significantly higher amount of maltose in both liquified Eve and VA125 flour samples at time 0. It appears that barley pretreatment prior to fermentation, hydrolysis and liquifaction, was more effective for whole grain samples than that for flour samples. Further research is needed to determine the cause of the differences between flour and whole grain during liquifaction. A sharp decrease in glucose amount and increase in ethanol production was observed within 24 h of SSF in the samples prepared from both whole grain Eve and VA125. It took longer, 36 h, to observe a sharp decline in glucose amount and an increase in ethanol accumulation in flour samples. This is due to the initial increase in glucose accumulation in flour samples that might be due to hydrolysis of maltose during SSF.

Significant amounts of glycerol were produced in all samples. Glycerol is the second most abundant product of fermentation (Radler and Schutz 1982) . The main role of glycerol formation during fermentation is to equilibrate the intracellular redox balance by converting excess NADH to NAD (Wang and others 2001). Lactic, succinic and acetic acids were the other products found in the fermentation broth. Significantly higher amount of lactic acid was produced in flour samples than that in whole grain samples.

Lactic acid was detected in the samples later in the SSF process, after 24 h. Acetic acid is a common by-product of alcohol fermentation (Thoukis and others 1965).

The ethanol conversion efficiency of the samples was calculated based on the liquified barley mash initial starch content (Table 8). The highest conversion efficiency, 88.6%, was attained with the mash prepared from whole grain Eve. For VA125 variety there was no significant difference in ethanol conversion efficiencies between flour and whole grain samples. It is worth noting that higher ethanol concentrations in fermentation broth were achieved with flour samples from both varieties, 59 g/L for Eve and 62.4 g/L for VA125; compared to that attained in the whole grain barley samples, 52.5 g/L for Eve and 41.7 g/L for VA125. Ethanol concentrations observed (5.3 to 8.0 %, v/v) in this study are lower than expected 12%, v/v (du Preez and others 1985; McAloon and others 2000). This is due to the depletion of glucose in the solution. Further research is needed to optimize the SFF process for the varieties examined in this study.

CHAPTER V

CONCLUSIONS

This study examined the potential of two winter hulless barley varieties, Eve and VA125, as a local feedstock for ethanol production in Oklahoma. Barley samples grown under different agronomic conditions and locations were characterized for their chemical composition. Starch content of the grain is one of the most important parameters to evaluate the viability of a crop for ethanol production. Starch content of the samples examined in this study varied from 60.3 to 71.1% (w/w, db) indicating that starch content of hulless barley is comparable to that of corn (about 64%, db) and significantly higher than that of hulled barley (about 56 %, db). Variety did not have a significant effect on the starch content of barley. The range for the β-glucan content in the samples (3.5-5.6% w/w, db) were similar to that for other varieties reported in the literature. The variety had a significant effect on the β-glucan content. The mean β-glucan content for Eve was lower than that for VA125. This can be an advantage for Eve if no β-glucanase is used to convert β-glucan to glucose in the ethanol production system because mash prepared from Eve may have lower viscosity due to its lower β-glucan content. However, further research is needed to determine the effect of β-glucan content on mash viscosity for both Eve and VA125 varieties. Protein content of Eve and VA125 were comparable to corn. Hence, DDGS from barley ethanol can be compatible with that from corn ethanol.

Buhler mill was effective in increasing the starch content in the flour fractions from both Eve and VA125 varieties. Grain tempering to increase the moisture content to 15% did not have a significant effect on the starch recovery when a Buhler mill was used. Flour yields from the Buhler mill were significantly lower than the flour yields commonly obtained with wheat using a similar system (Chen 2007). Wiley mill furnished with a 2 mm sieve was not very effective in grinding barley samples. Hence, substantial amount of starch was lost in large particles (particle size > 250 μm). Processing barley samples with a TADD effectively removed the bran layer. Similar to Wiley mill, Perten mill was not effective in grinding debranned barley. Over 60% of the ground grain mass had particle size > 250 μm causing substantial amount of starch loss in this fraction. It was possible to obtain barley fractions containing >80% starch. However, the yield of this fraction was very low, about 10% of the total grain mass. The highest starch content in a fraction, about 87% starch, was obtained when Eve was debranned for 50 sec at 15% moisture content by using a TADD followed by grinding and sieving. These results clearly indicate that grinding barley grain is challenging and further optimization studies are need to optimize the barley milling conditions to obtain flour fractions with high yield and starch content.

Our initial fermentation tests showed that about 89% ethanol conversion efficiency was possible even without barley grain fractionation to remove bran. Although VA125 flour produced the highest ethanol concentration in the fermentation broth (8%, v/v) among the samples examined in this study, still this concentration was significantly lower than the ethanol concentrations achievable (about 12%). However, this was due to

the lower starch amount in the barley mash used for fermentation experiments and consequently depletion of sugar in the broth.

This study clearly demonstrated that winter barley can be a viable feedstock with similar starch content to corn. Dry milling can be used to obtain high starch content fractions from barley grain. It is possible to produce ethanol from winter barley varieties with acceptable conversion yields. Optimization of dry milling and ethanol conversion process parameters could improve the economic feasibility of barley to ethanol conversion operations.

FUTURE WORK

Our preliminary tests to evaluate the processing characteristics of winter barley varieties indicated that dry milling can be used to obtain barley fractions with >85% starch content. However, further research is needed to improve the flour yields. This can be done by optimizing processing parameters for the milling equipment used in this study. Evaluation and optimization of other milling techniques for winter barley varieties would also be beneficial. Information collected from these tests can be utilized to modify conventional grain milling equipment or design new milling systems which would be used specifically for hulless barley.

This thesis also examined the conversion of barley starch to ethanol. The type of enzymes and the starch hydrolysis protocols used in this study were chosen based on the recommendations of the enzyme supplier. The fermentation tests were designed as screening experiments. Therefore, an optimization study to determine the most effective types of enzymes (both amylase and glucanase) and the starch hydrolysis and SSF conditions is needed to further evaluate the economic feasibility of winter barley varieties for ethanol production. Utilization of VHG mash and high temperature fermentation for barley should also be studied.

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Table 1: Chemical composition of hulless and hulled barley and corn

(%, w/w, dry basis)

	Hulled Barley [1]	**Hulless Barley[1]**	**Corn[2]**
β-glucan	4.17	4.34	-
Starch	56.45	61.45	63.7
Protein	8.95	9.04	10.9
Oil	2.29	2.43	4.9
Ash	2.23	1.81	1.5

All values expressed on a dry matter basis

[1] (Griffey and others 2009)

[2] (Thornton and others 1969)

Table 2: Chemical composition of hulless barley varieties harvested in June 2008 (%, w/w, dry basis).

Sample identifier	(Location-Agronomic practice-Treatment^{N,F})	β-glucan	Starch	Moisture	Protein	Ash	Oil
Eve	Perkins-G-N0-R1	4.2 ± 0.03	69.3 ± 0.5	11.4 ± 0.02	11.0 ± 0.2	1.7 ± 0.04	1.1 ± 0.04
	Perkins-G-N4-R1	4.6 ± 9E-5	60.5 ± 2.1	11.2 ± 0.1	14.1 ± 0.4	1.6 ± 0.01	1.4 ± 0.03
	Perkins-G-N4-R2	4.2 ± 0.2	63.6 ± 0.7	11.3 ± 0.1	14.7 ± 0.4	1.7 ± 0.03	1.1 ±3E-3
	Perkins-G-N3	4.4 ± 0.1	60.7 ± 0.8	11.4 ± 0.1	14.0 ± 0.2	1.7 ± 0.02	1.7 ± 0.01
	Perkins G-N0-R2	4.2 ± 0.1	67.9 ± 0.6	11.2 ± 0.6	10.2 ± 0.1	1.8 ± 0.02	1.4 ± 0.1
	Perkins-G-N1	4.0 ± 0.1	65.5 ± 1.7	11.4 ± 0.1	11.1 ± 0.02	1.8 ± 0.02	1.3 ± 0.02
	Perkins-G-N4-R3	4.6 ± 0.01	62.8 ± 0.4	10.9 ± 0.5	14.1 ± 0.1	1.8 ± 0.1	1.8 ± 0.1
	Perkins-G-N2-R	4.2 ± 0.1	62.1 ± 3.7	11.7 ± 0.1	12.4 ± 0.2	1.9 ± 0.3	1.2 ± 0.1
	Perkins-G-F2	4.4 ± 0.1	71.1 ± 1.2	11.6 ± 0.1	12.1 ± 0.2	1.6 ± 0.01	1.3 ± 0.1
	Perkins-G-F1-R1	4.2 ± 0.04	68.7 ± 0.4	12.1 ± 0.6	11.7 ± 0.1	1.7 ± 5E-3	1.4 ± 0.4
	Perkins-G-F0-R1	4.2 ± 0.1	67.2 ± 0.5	11.8 ± 0.05	12.3 ± 0.1	1.8 ± 0.02	1.9 ± 0.1
	Perkins-G-F1-R2	4.3 ± 3E-3	61.7 ± 0.5	11.8 ± 0.02	11.5 ± 0.1	1.7 ± 0.03	1.0 ± 0.1
	Perkins-G-F1-R3	4.2 ± 0.1	67.7 ± 0.6	11.7 ± 2E-3	13.3 ± 0.3	1.7 ± 0.01	1.5 ± 0.1
	Perkins-G-F0-R2	4.3 ± 0.1	64.0 ± 1.4	12.0 ± 0.1	12.1 ± 0.2	1.8 ± 0.02	1.7 ± 0.01
	Perkins-G-F0-R3	4.2 ± 0.2	65.1 ± 1.5	11.9 ± 0.1	11.8 ± 0.04	1.8 ± 0.02	1.7 ± 0.03
	Marshall-G-R1	4.6 ± 0.2	69.1 ± 1.0	12.5 ± 0.02	16.1 ± 0.2	1.6 ± 0.1	1.6 ± 0.1
	Marshall-G-R2	4.4 ± 0.1	66.3 ± 0.6	12.7 ± 0.1	15.9 ± 0.4	1.6 ± 0.02	1.3 ± 0.03
	Marshall-G-R3	4.6 ± 0.1	64.0 ± 1.4	12.5 ± 0.02	15.7 ± 0.1	1.6 ± 0.1	1.4 ± 0.1
	Marshall-G-R4	4.8 ± 0.01	61.5 ± 1.9	12.7 ± 0.3	16.1 ± 5E-3	1.7 ± 0.1	1.1 ± 0.05
	Marshall (G+G)-R1	3.5 ± 0.04	66.0 ± 4E-3	12.5 ± 0.2	16.2 ± 0.2	1.7 ± 0.01	1.7 ± 0.2
	Marshall (G+G)-R2	3.5 ± 0.1	65.6 ± 0.7	11.9 ± 0.3	16.0 ± 0.1	1.6 ± 0.2	1.3 ± 0.1
	STWT-G-N0,F0	4.0 ± 0.1	60.6 ± 1.9	11.8 ± 0.1	15.3 ± 0.3	1.5 ± 0.01	1.2 ± 0.1

Table 2: continue from the previous page

Sample identifier	(Location-agronomic contions-treatment (N,F))	β-glucan	Starch	Moisture	Protein	Ash	Oil
VA125	Marshall (G+G)-R1	5.0 ± 0.1	63.7 ± 2.1	12.7 ± 0.7	13.5 ± 0.04	1.8 ± 0.04	1.5 ±3E-3
	Marshall (G+G)-R2	4.6 ± 0.1	63.0 ± 2.2	12.2 ± 0.05	13.8 ± 2E-3	1.8 ± 0.03	1.0 ± 0.1
	STWT-G-N0,F0	5.6 ± 0.03	60.3 ± 0.6	11.4 ± 0.03	12.6 ± 0.05	1.5 ± 0.2	1.3 ± 0.1

R means replication

Agronomic practice:

G means “Grain only”

G + G stand for dual purpose crop “Grain and Grazed”

Treatments:

N = Nitrogen treatment

N0 = No additional Nitrogen added

N1 = 30 pounds per Acre of Nitrogen added

N2 = 60 pounds of Acre of Nitrogen added

N3 = 90 pounds of Acre of Nitrogen added

N4 = 120 pounds of Acre of Nitrogen added

F = Fungicide Treatment

F0 = No Fungicide

F1 = Stratego applied at Flag Leaf

F2 = Stratego applied at Heading

Table 3: Yield and starch content of barley grain fractions obtained by using a Bühler Mill.

Variety*	Fraction	Fraction Yield (%, w/w, as is)	Starch (%, w/w, dry basis)
Eve-1	Bran	4.02 ± 0.4	38.2 ± 1.1
	Shorts	44.3 ± 0.8	46.9 ± 0.3
	Flour	39.8 ± 2.0	76.9 ± 0.9
	Loss	11.9 ± 1.6	
Eve-2	Bran	12.7 ± 0.3	33.2 ± 0.6
	Shorts	39.4 ± 0.1	46.8 ± 0.7
	Flour	47.8 ± 0.6	75.4 ± 2.7
	Loss	0.3 ± 0.2	
VA125-1	Bran	6.4 ± 0.8	41.7 ± 0.3
	Shorts	55.0 ± 0.9	48.2 ± 0.7
	Flour	31.2 ± 2.0	76.2 ± 1.2
	Loss	7.4 ± 3.7	
VA125-2	Bran	21.6 ± 0.3	42.6 ± 1.8
	Shorts	45.6 ± 0.5	49.6 ± 0.04
	Flour	31.5 ± 1.3	76.2 ± 0.3
	Loss	1.2 ± 1.1	

*****Varieties labeled as 1 and 2 represent grain samples milled as is and after tempering at 15% moisture content, respectively.

Table 4: Yield and starch content of barley grain fractions obtained by using a Wiley Mill followed by sieve analysis

Variety	Fraction	Fraction Yield (%, w/w, as is)	Starch (%, w/w, dry basis)
EVE	250	84.5 ± 0.4	60.5 ± 1.6
	150	3.1 ± 0.1	37.6 ± 0.7
	75	2.1 ± 0.1	25.2 ± 0.01
	53	0.9 ± 0.02	24.2 ± 0.2
	38	8.9 ± 0.4	73.04 ± 0.4
	Pan	0.2 ± 0.01	71.5 ± 2.4
	Loss	0.3 ± 0.3	
VA125	250	85.5 ± 0.7	47.1 ± 2.2
	150	2.5 ± 1.1	32.7 ± 0.1
	75	2.01 ± 0.004	20.5 ± 0.4
	53	0.9 ± 0.07	21.4 ± 0.7
	38	7.4 ± 0.02	69.4 ± 0.2
	Pan	0.03 ± 0.01	n/a
	Loss	1.6 ± 0.3	

n/a = starch data not available due to small sample size

Table 5: Yield of barley grain fractions obtained by using a TADD.

Variety	Time (s)	Treatment*	Yield (%, w/w, as is)		Loss (%, w/w, as is)
			Bran	Residue	
Eve	50	1	6.6 ± 0.2	92.9 ± 0.1	0.5 ± 0.2
		2	5.2 ± 0.4	94.2 ± 0.2	0.7 ± 0.2
	70	1	8.5 ± 0.2	90.8 ± 0.2	0.7 ± 0.1
		2	7.3 ± 0.2	92.2 ± 0.1	0.6 ± 0.3
	90	1	10.0 ± 0.1	89.2 ± 0.2	0.8 ± 0.1
		2	7.3 ± 3.3	87.6 ± 3.3	5.2 ± 0.1
VA125	50	1	6.0 ± 0.5	94.0 ± 0.5	0 ± 0.1
		2	5.3 ± 0.2	94.4 ± 0.2	0.3 ± 0.3
	70	1	8.0 ± 0.4	90.8 ± 0.1	1.2 ± 0.5
		2	6.8 ± 0.2	92.2 ± 0.6	1.0 ± 0.4
	90	1	9.4 ± 0.4	90.3 ± 0.7	0.2 ± 0.2
		2	8.3 ± 0.1	90.7 ± 0.3	1.1 ± 0.3

*Treatment labeled as 1 and 2 represent grain samples milled as is and after tempering at 15% moisture content, respectively.

Table 6: Yield and starch content of barley grain fractions **after TADD treatment followed by grinding and sieving.**

TADD	Time (s)	Treatment*	Sieves (µm)	Fraction Yield (%, w/w, as is)	Starch (%, w/w, dry basis)
Eve	50	1	250	65.8 ± 0.6	61.9 ± 1.2
			150	13.9 ± 0.6	59.9 ± 0.7
			75	4.9 ± 0.07	48.5 ± 0.5
			53	1.5 ± 0.01	46.4 ± 1.3
			38	2.2 ± 1.5	72.6 ± 1.1
			Pan	10.8 ± 1.2	83.2 ± 0.8
			Loss	0.9 ± 0.4	
			Bran	6.6 ± 0.2	19.2 ± 0.5
			Loss	0.5 ± 0.2	
		2	250	65.0 ± 0.2	61.6 ± 1.6
			150	14.8 ± 0.2	61.3 ± 0.7
			75	4.9 ± 0.1	52.9 ± 1.8
			53	2.5 ± 0.6	61.3 ± 0.1
			38	11.2 ± 0.6	84.3 ± 1.2
			Pan	1.3 ± 1.4	87.3 ± 0.5
			Loss	0.3 ±0.1	
			Bran	5.2 ± 0.4	16.5 ± 2.8
			Loss	0.7 ± 0.2	

*Treatment labeled as 1 and 2 represent grain samples milled as is and after tempering at 15% moisture content, respectively.

Table 6: continued from the previous page

TADD	Time	Treatment*	Sieves (µm)	Fraction Yield (%, w/w, as is)	Starch (%, w/w, dry basis)
Eve	70	1	250	66.4 ± 0.2	63.7 ± 0.4
			150	13.4 ± 0.2	61.3 ± 0.5
			75	4.8 ± 0.01	49.2 ± 0.7
			53	1.5 ± 0.1	48.2 ± 0.4
			38	1.9 ± 1.5	61.6 ± 0.04
			Pan	11.5 ± 1.8	82.2 ± 0.1
			Loss	0.5 ± 0.4	
			Bran	8.5 ± 0.2	21.22 ± 2.0
			Loss	0.7 ± 0.1	
		2	250	62.9 ± 0.7	63.3 ± 0.1
			150	15.3 ± 0.2	63.05 ± 1.7
			75	5.0 ± 0.1	52.3 ± 1.0
			53	2.0 ± 0.1	59.3 ± 0.7
			38	11.3 ± 0.6	81.6 ± 0.7
			Pan	2.4 ± 0.6	82.5 ± 1.0
			Loss	1.2 ± 0.8	
			Bran	7.3 ± 0.2	19.3 ± 1.3
			Loss	0.6 ± 0.3	

*Treatments labeled as 1 and 2 represent grain samples milled as is and after tempering at 15% moisture content, respectively.

Table 6: continued from the previous page

TADD	Time (s)	Treatment*	Sieves, (µm)	Fraction Yield (%, w/w, as is)	Starch (%, w/w, dry basis)
Eve	90	1	250	66.5 ± 0.04	62.0 ± 3.9
			150	13.5 ± 0.1	61.3 ± 1.3
			75	4.8 ± 0.04	50.7 ± 0.3
			53	1.3± 0.1	48.3 ± 0.2
			38	2.8 ± 0.6	62.4 ± 0.1
			Pan	9.6 ± 1.3	82.7 ± 1.1
			Loss	1.5 ± 0.7	
			Bran	10.01 ± 0.1	23.5 ± 0.2
			Loss	0.8 ± 0.1	
		2	250	64.2 ± 1.1	66.3 ± 1.7
			150	14.8 ± 0.1	63.1 ± 0.6
			75	5.0 ± 0.1	53.3 ± 0.2
			53	1.7 ± 0.4	51.8 ± 0.3
			38	6.6 ± 4.6	70.4 ± 0.1
			Pan	7.5± 4.2	82.8 ± 0.9
			Loss	0.2 ± 0.2	
			Bran	7.3 ± 3.3	23.5 ± 1.5
			Loss	5.2 ± 0.1	

*Treatments labeled as 1 and 2 represent grain samples milled as is and after tempering at 15% moisture content, respectively.

Table 6: continued from the previous page

TADD	Time (s)	Treatment*	Sieves, (µm)	Fraction Yield (%, w/w, as is)	Starch content (%, w/w, dry basis)
VA125	50	1	250	68.6 ± 0.03	60.9 ± 1.713
			150	12.9 ± 0.1	57.4 ± 2.9
			75	4.5 ± 0.1	44.1 ± 0.3
			53	1.5 ± 0.1	44.3 ± 0.04
			38	9.2 ± 0.1	77.1 ± 0.4
			Pan	2.3 ± 0.1	79.8 ± 1.7
			Loss	1.1 ± 0.1	
			Bran	6.007 ± 0.5	11.3 ± 1.2
			Loss	0.044 ± 0.1	
		2	250	72.51 ± 1.9	55.3 ± 0.3
			150	13.3 ± 1.4	53.5 ± 1.1
			75	5.0 ± 1.04	44.3 ± 1.5
			53	2.6 ± 0.02	62.6 ± 0.4
			38	5.01 ± 5.5	80.9 ± 0.9
			Pan	0.9 ± 0.5	83.6 ± 0.3
			Loss	0.7 ± 0.6	
			Bran	5.3 ± 0.2	8.5 ± 0.9
			Loss	0.3 ± 0.3	

*Treatment labeled as 1 and 2 represent grain samples milled as is and after tempering at 15% moisture content, respectively.

Table 6: continued from the previous page

TADD	Time (s)	Treatment*	Sieves (µm)	Fraction Yield (%, w/w, as is)	Starch (%, w/w, dry basis)
VA125	70	1	250	66.5 ± 0.04	62.3 ± 0.2
			150	11.8 ± 0.1	55.7 ± 1.5
			75	4.2 ± 0.2	44.03 ± 0.3
			53	1.3 ± 0.03	44.7 ± 0.3
			38	11.7 ± 0.2	76.9 ± 1.0
			Pan	1.5 ± 0.1	80.3 ± 1.7
			Loss	2.9 ± 0.6	
			Bran	8.001 ± 0.4	12.01 ± 1.7
			Loss	1.2 ± 0.5	
		2	250	70.7 ± 0.2	51.6 ± 1.3
			150	12.3 ± 0.4	48.03 ± 0.5
			75	4.3 ± 0.1	45.7 ± 0.7
			53	2.2 ± 0.4	56.1 ± 0.3
			38	9.6 ± 0.1	83.6 ± 3.2
			Pan	0.7 ± 0.3	84.0 ± 1.1
			Loss	0.2 ± 0.3	
			Bran	6.8 ± 0.2	10.6 ± 0.104
			Loss	1.04 ± 0.4	

*Treatment labeled as 1 and 2 represent grain samples milled as is and after tempering at 15% moisture content, respectively.

Table 6: continued from the previous page

TADD	Time (s)	Treatment*	Sieves µm	Fraction Yield (%, w/w, as is)	Starch (%, w/w, dry basis)
VA125	90	1	250	69.4 ± 0.2	62.4 ± 0.2
			150	12.8 ± 0.2	57.3 ± 1.8
			75	4.9 ± 0.3	43.8 ± 0.3
			53	1.4 ± 0.1	45.9 ± 0.5
			38	7.9 ± 0.2	78.5 ± 1.4
			Pan	2.01 ± 0.3	81.9 ± 1.0
			Loss	1.6 ± 0.8	
			Bran	9.4 ± 0.4	14.4 ± 0.3
			Loss	0.2 ± 0.2	
		2	250	67.1 ± 3.0	55.4 ± 0.6
			150	12.1 ± 0.5	52.01 ± 0.2
			75	5.3 ± 1.1	40.8 ± 0.6
			53	2.2 ± 0.3	52.2 ± 0.3
			38	9.7± 0.04	79.0 ± 2.6
			Pan	0.9 ± 0.6	81.6 ± 1.04
			Loss	2.7 ± 2.7	
			Bran	8.3 ± 0.1	13.04 ± 0.8
			Loss	1.0 ± 0.3	

*Treatment labeled as 1 and 2 represent grain samples milled as is and after tempering at 15% moisture content, respectively.

Table 7: Chemical composition of hulless barley varieties harvested in June 2009 (%, w/w, dry basis).

Variety	β-glucan	Starch	Moisture	Protein	Ash	Oil
EVE	3.9 ± 0.1	60.7 ± 2.8	10.9 ± 0.2	14.8 ± 0.1	1.9 ± 0.03	1.6 ± 0.1
VA125	4.6 ± 0.1	58.1 ± 0.95	11.1 ± 8E3	13.9 ± 0.03	2.0 ± 5E3	1.5 ± 0.3

Table 8: Fermentation efficiency of the barley whole grain and flour.

Sample		Starch (%, w/w, dry basis)	Actual Ethanol (g/L)	Actual Ethanol (%, v/v)	Theoretical Ethanol (g/L)	Fermentation Efficiency (%)
Eve	Whole	60.7	52.5 ± 0.2^{c}	6.7 ± 0.02	59.2	88.6 ± 0.3^{a}
	Flour	75.4	59.0 ± 0.4^{b}	7.6 ± 0.1	68.8	85.8 ± 0.6^{b}
VA125	Whole	58.1	41.7 ± 0.2^{d}	5.3 ± 0.02	49.7	83.9 ± 0.4^{c}
	Flour	76.2	62.4 ± 0.2^{a}	8.0 ± 0.02	75.1	83.0 ± 0.2^{c}

Figure 1: Barley structure (Jadhav and others 1998).

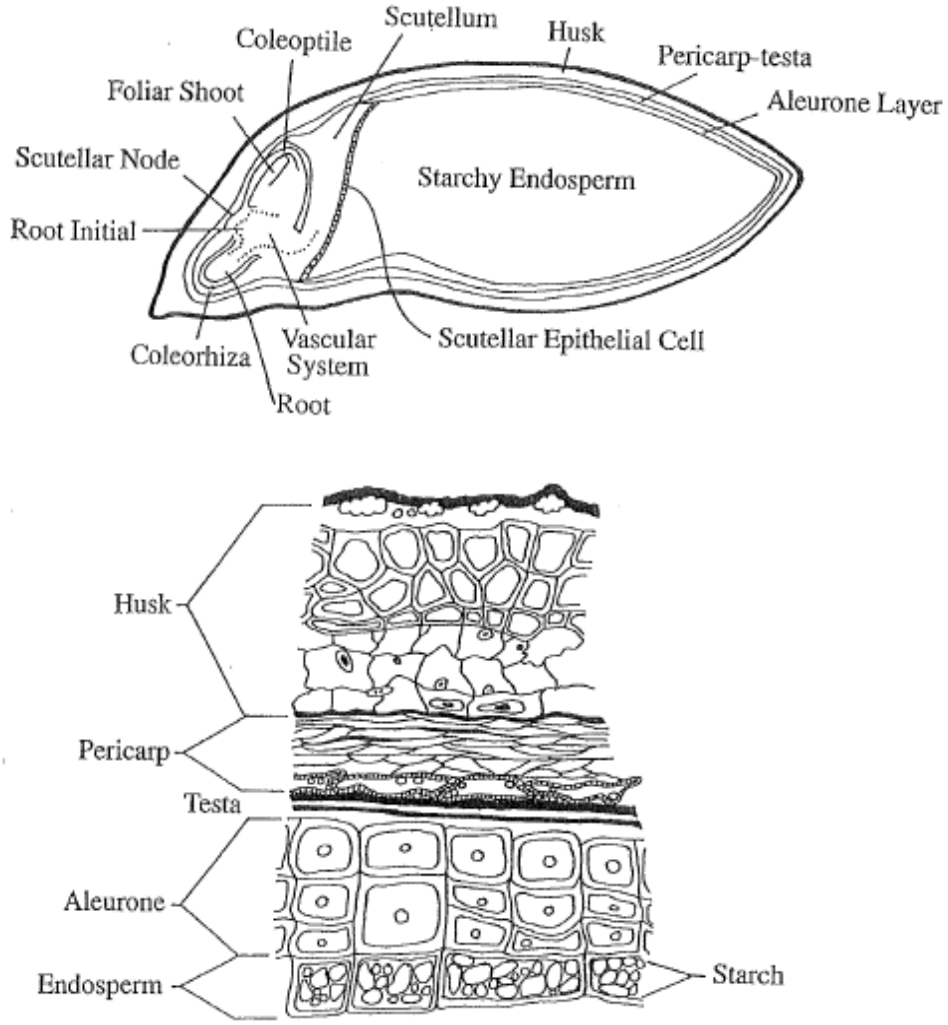


Figure 2: A schematic of the barley fermentation procedure.

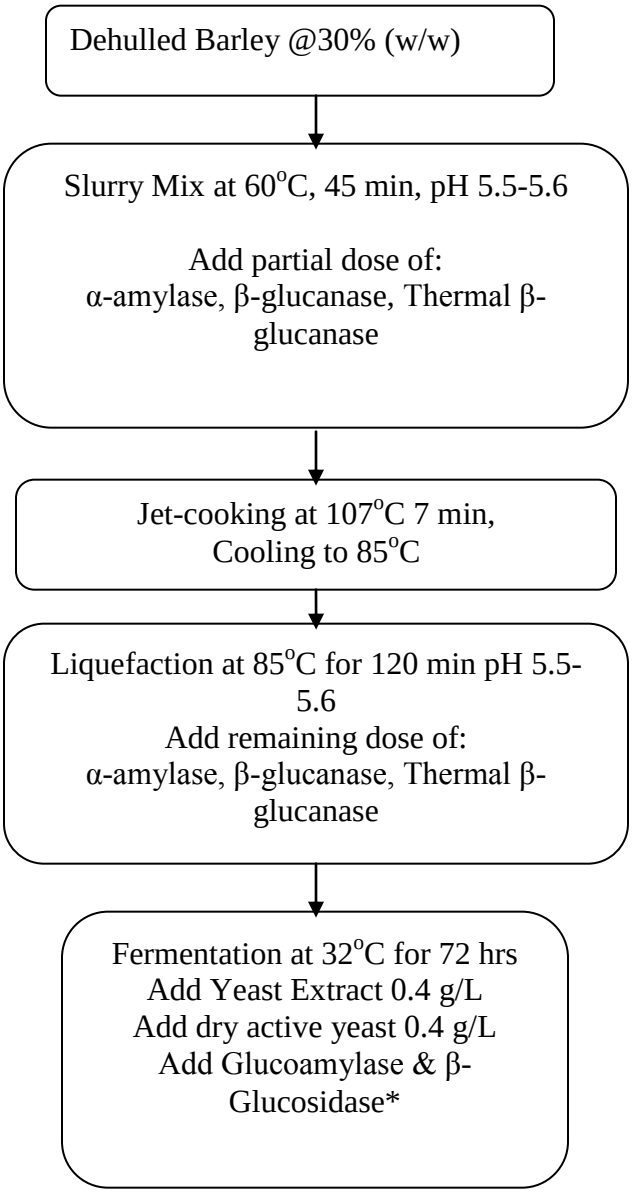
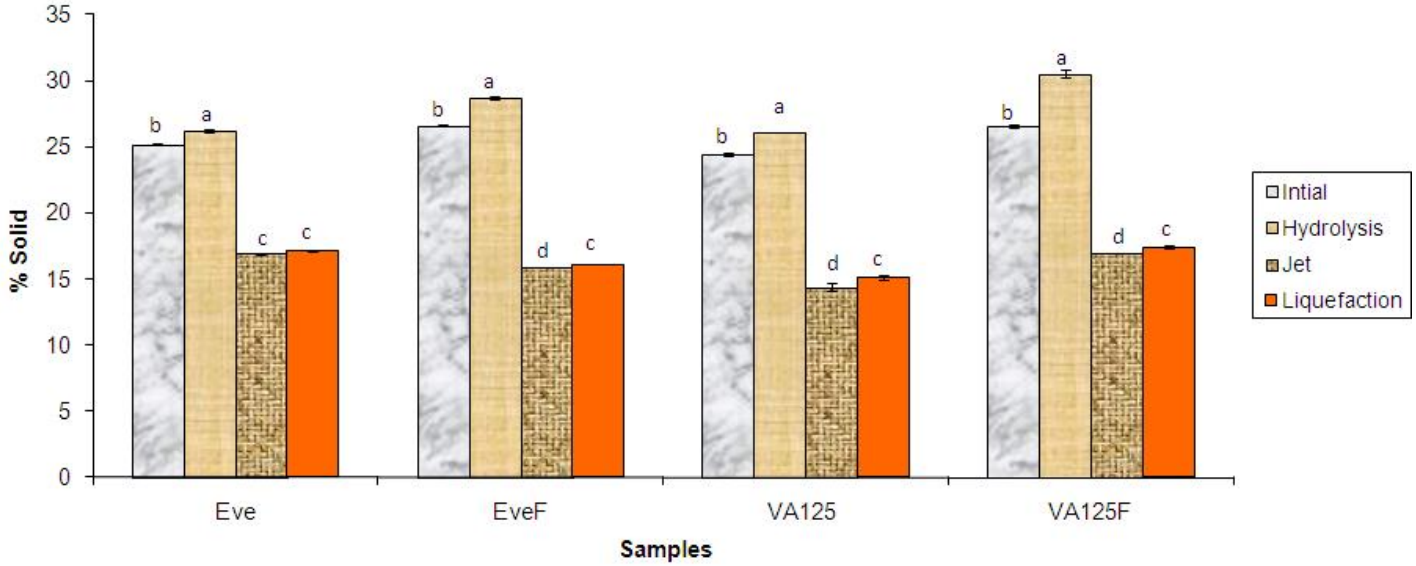


Figure 3: Solid content of barley mash during starch hydrolysis.



Error bars represent standard deviation

Means with the same letter are not significantly different from each other ($p < 0.05$) within the same group

Figure 4: A typical HPLC chromatogram of whole barley mash at 0 h.

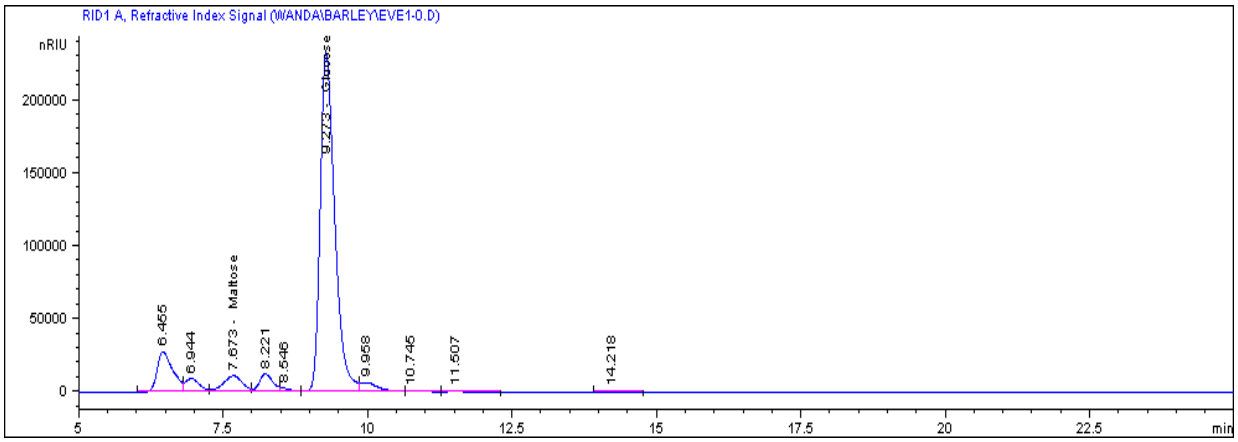


Figure 5: A typical HPLC chromatogram of whole barley mash at 36 h.

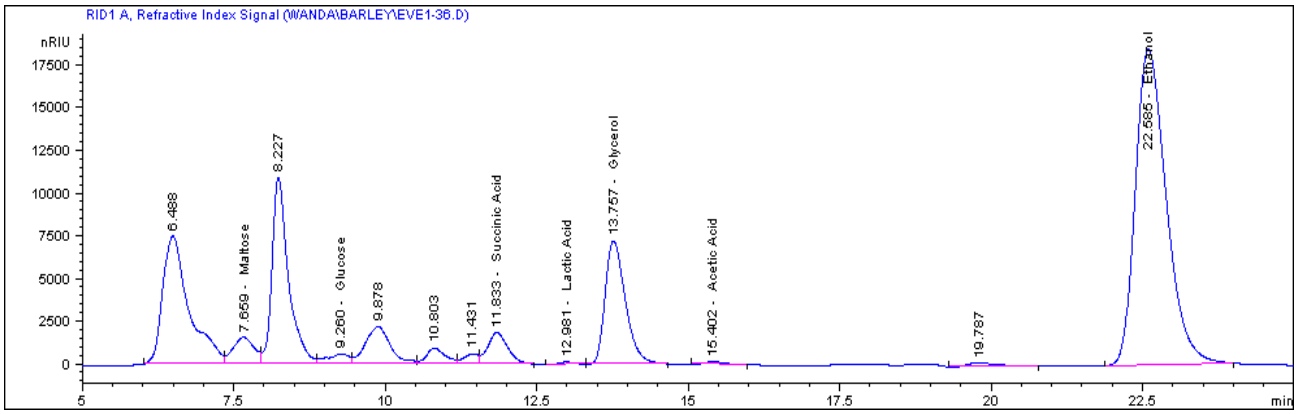


Figure 6: A typical HPLC chromatogram of a mixture of glucose, maltose, ethanol, acetic acid, lactic acid, succinic acid and glycerol standards.

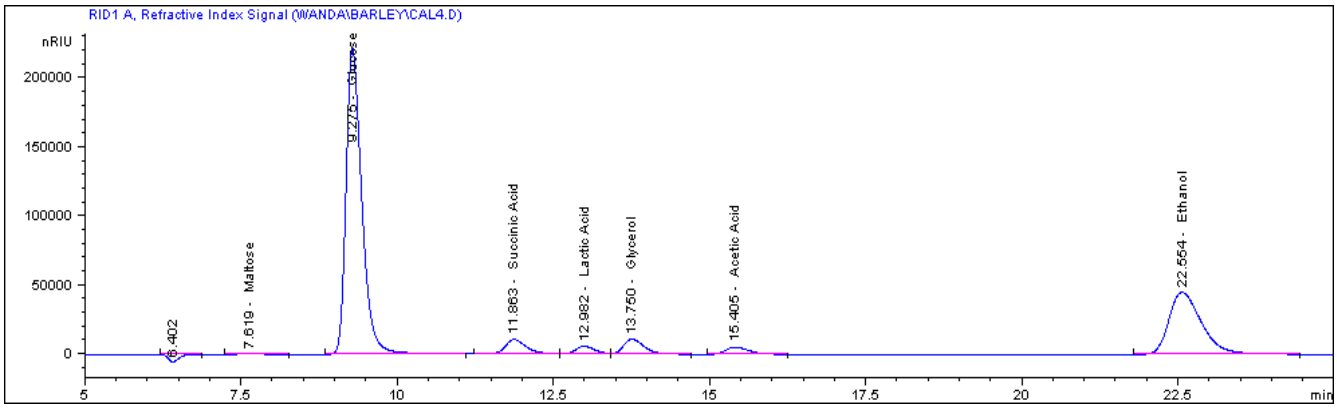


Figure 7: Change in maltose, glucose and ethanol concentrations in the medium during fermentation of whole grain Eve.

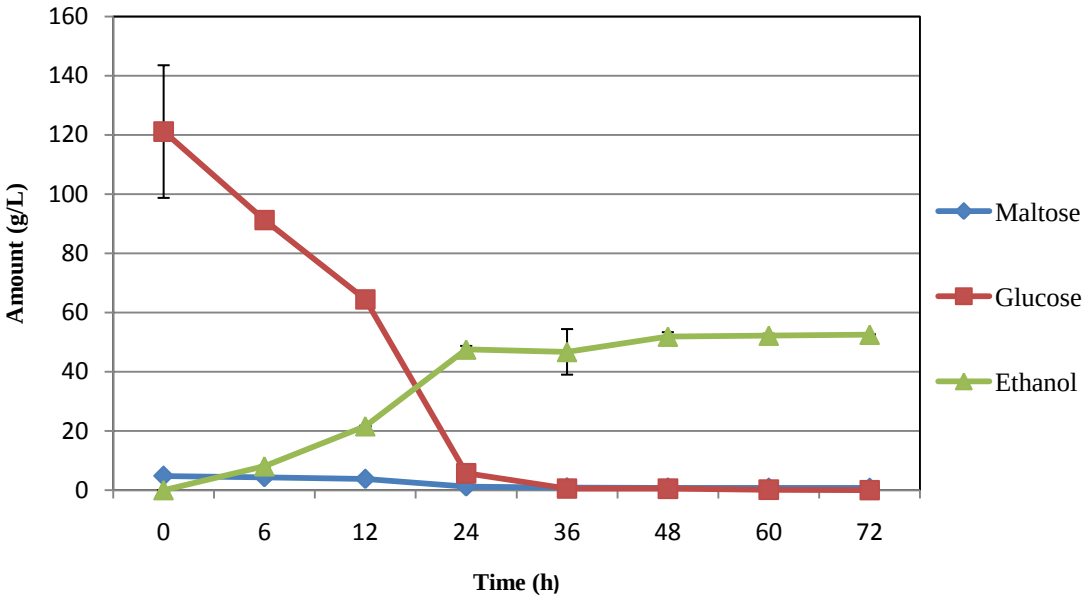


Figure 8: Change in succinic acid, lactic acid, glycerol and acetic acid concentrations in the medium during fermentation of whole grain Eve.

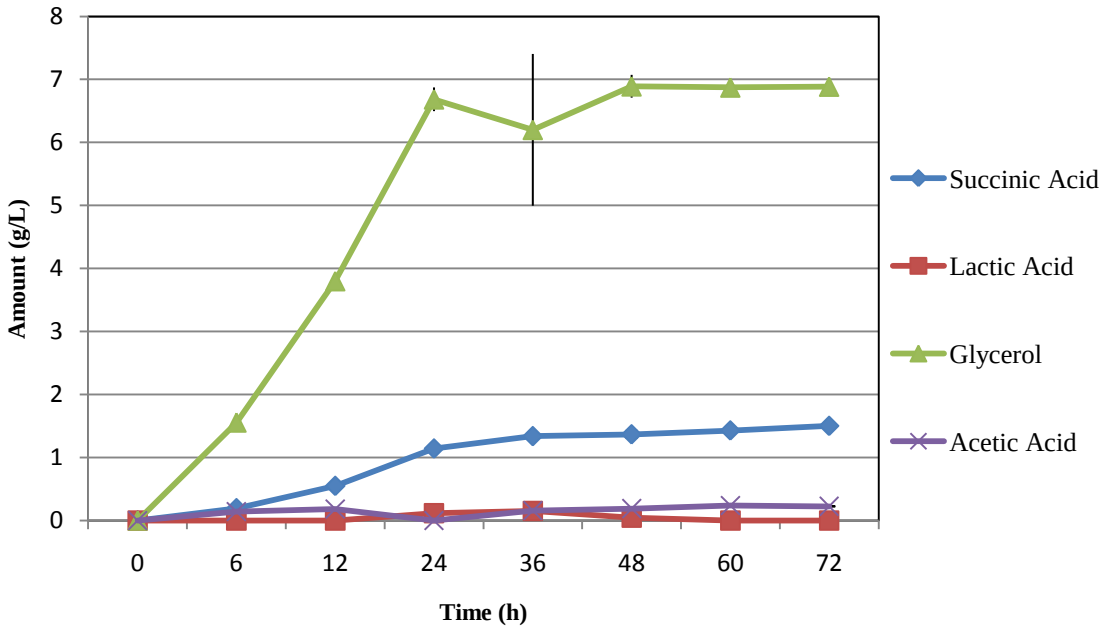


Figure 9: Change in maltose, glucose and ethanol concentrations in the medium during fermentation of Eve flour.

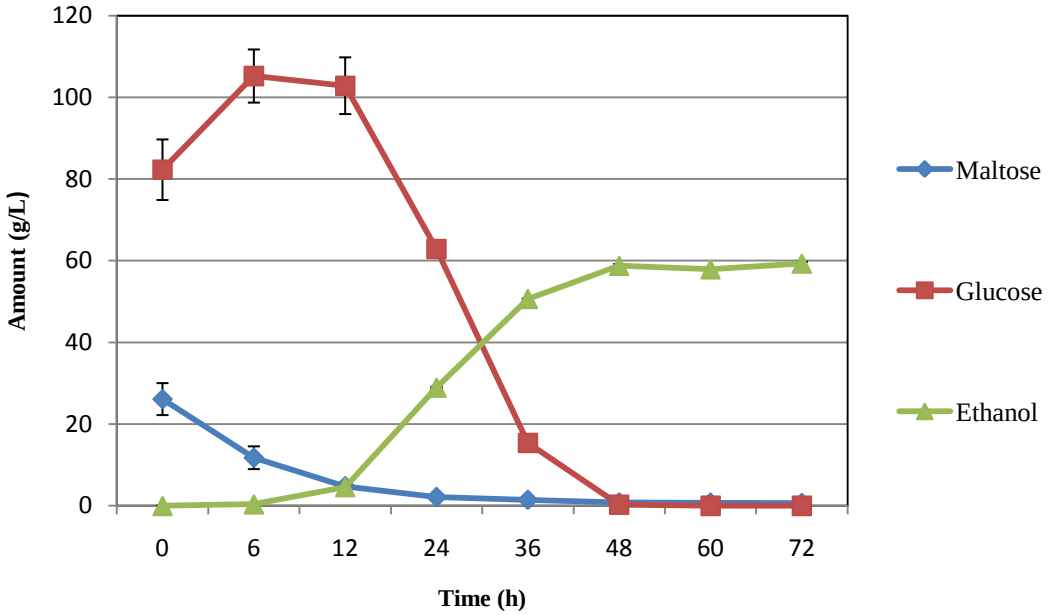


Figure 10: Change in succinic acid, lactic acid, glycerol and acetic acid concentrations in the medium during fermentation of Eve flour.

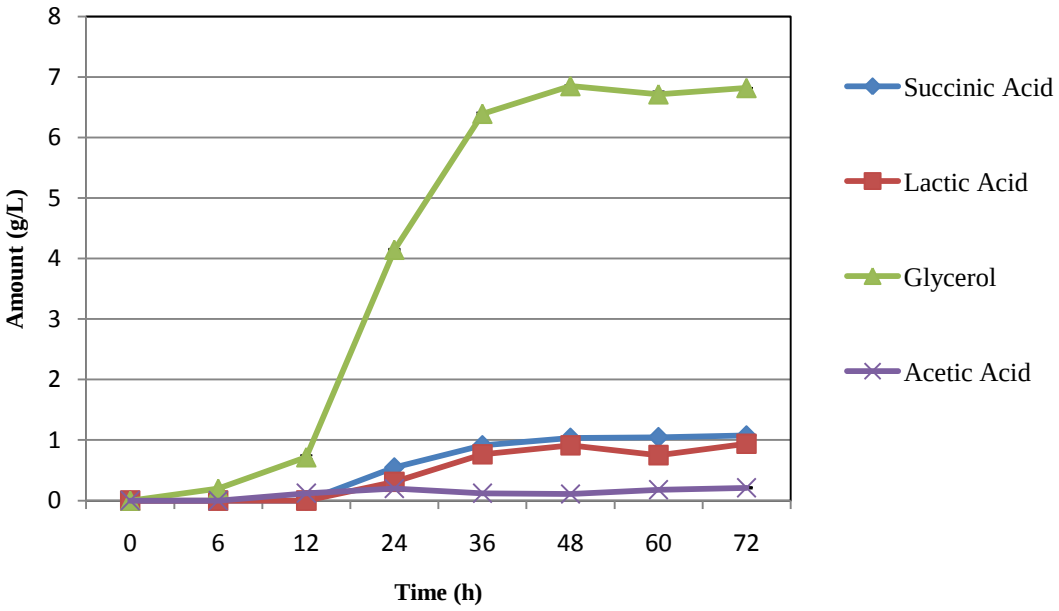


Figure 11: Change in maltose, glucose and ethanol concentrations in the medium during fermentation of VA125 whole grain.

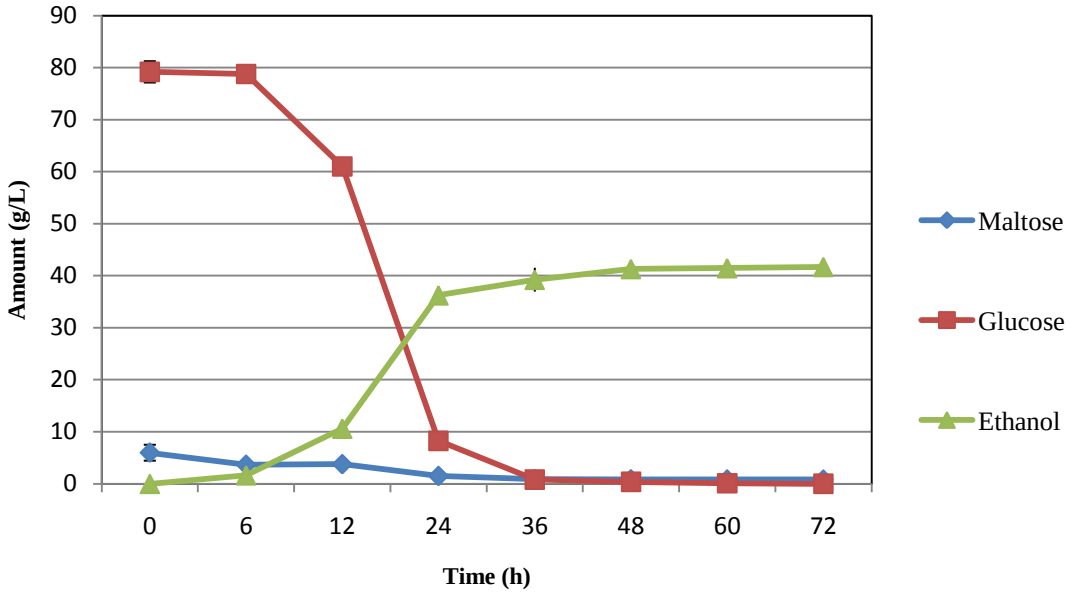


Figure 12: Change in succinic acid, lactic acid, glycerol and acetic acid concentrations in the medium during fermentation of VA125 whole grain.

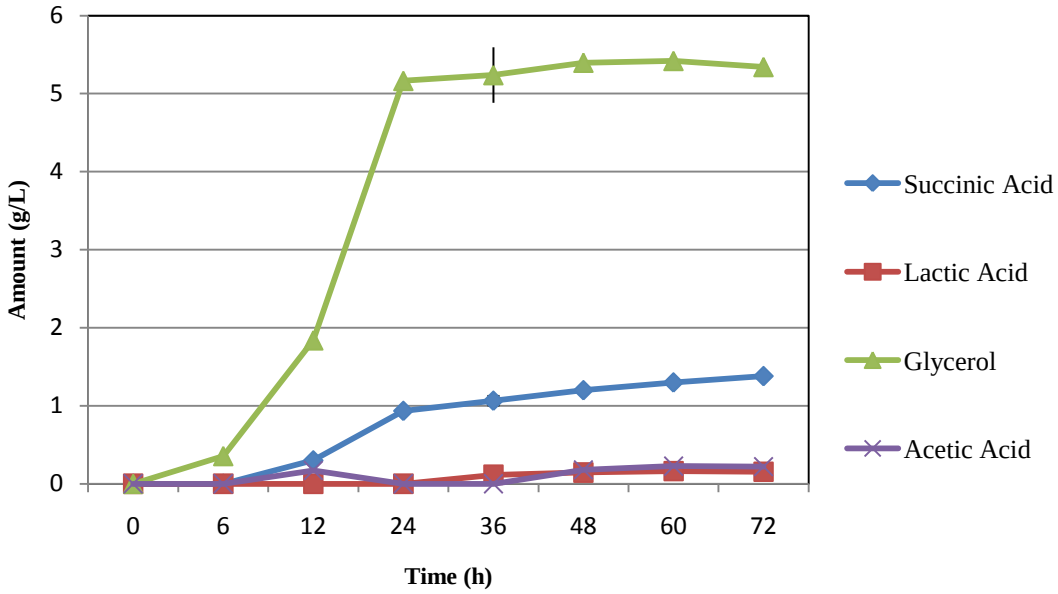


Figure 13: Change in maltose, glucose and ethanol concentrations in the medium during fermentation of VA125 flour.

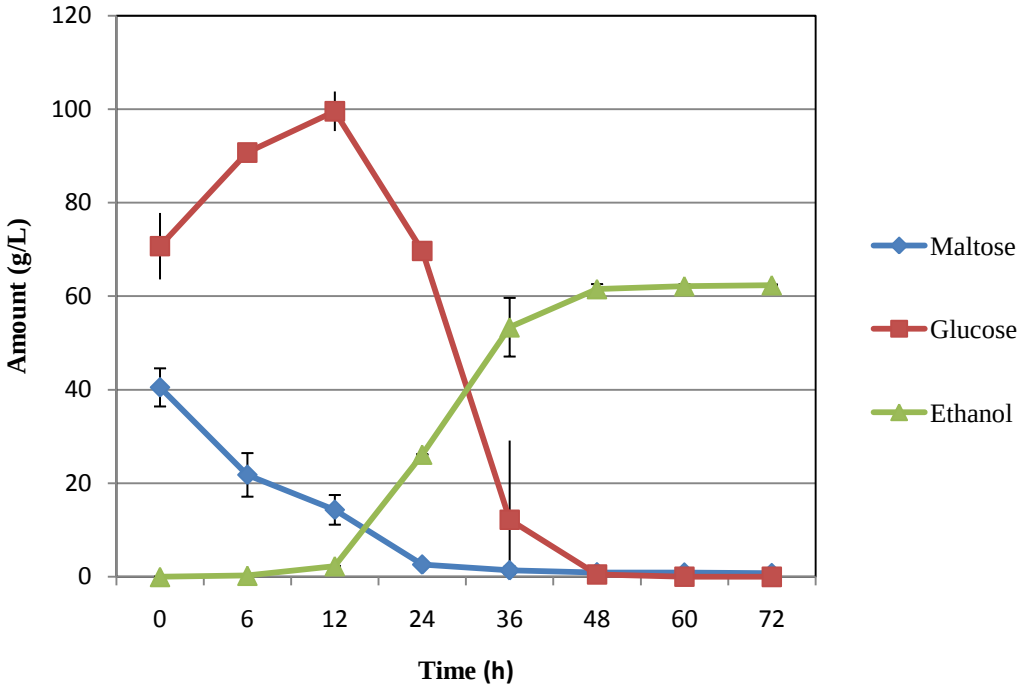
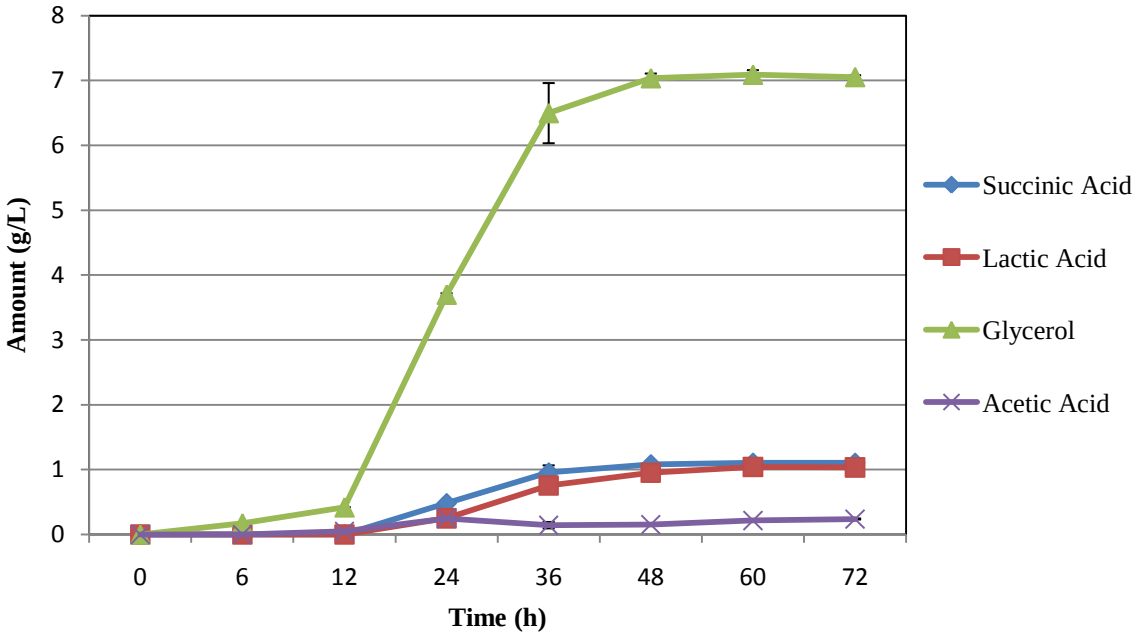


Figure 14: Change in succinic acid, lactic acid, glycerol and acetic acid concentrations in the medium during fermentation of VA125 flour.



APPENDIX

APPENDIX

Figure A1: Average monthly temperature (^{o}C) in Marshall.

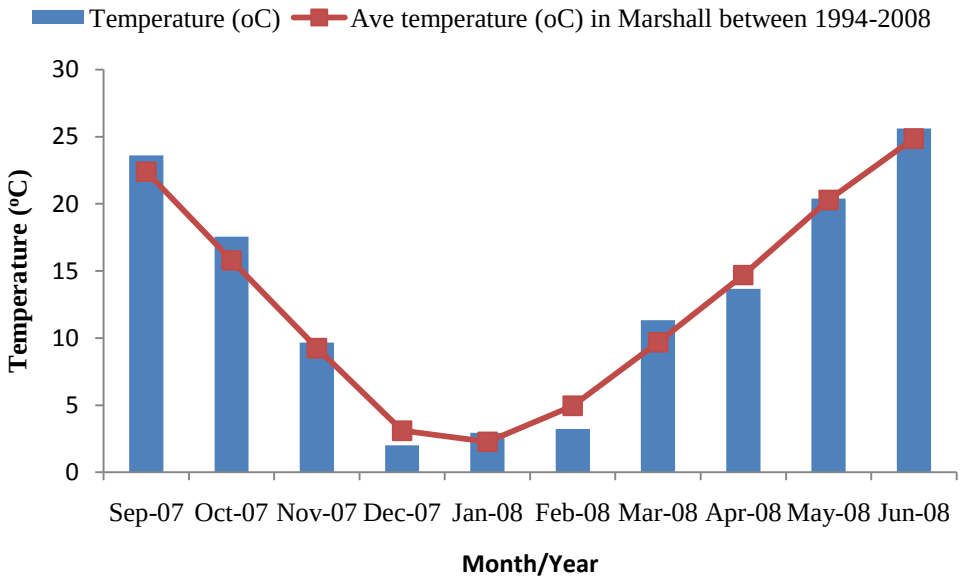


Figure A2: Total monthly rainfall (mm) in Marshall.

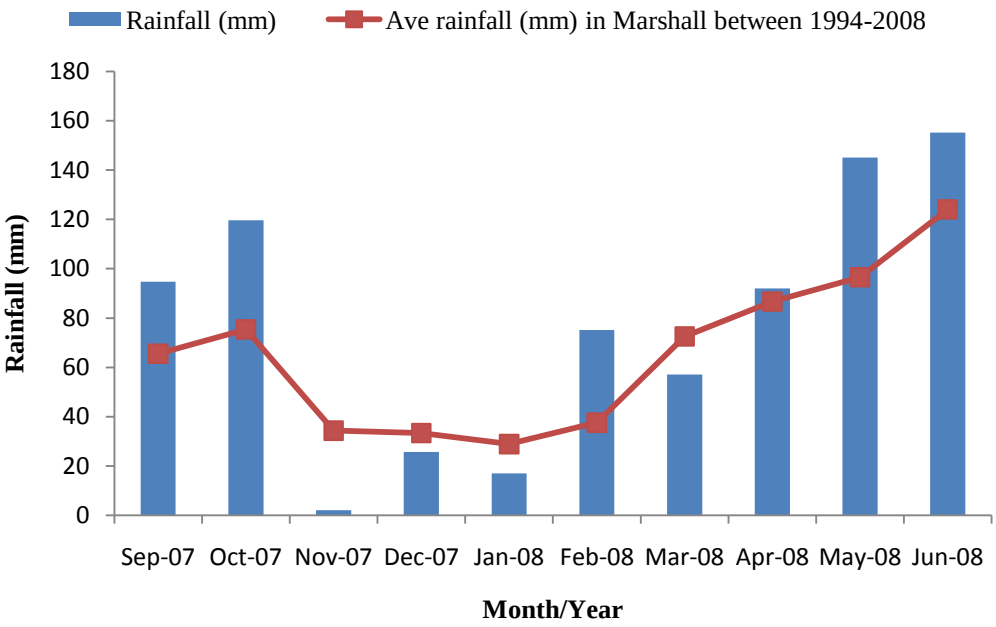


Figure A3: Average monthly temperature (°C) in Perkins.

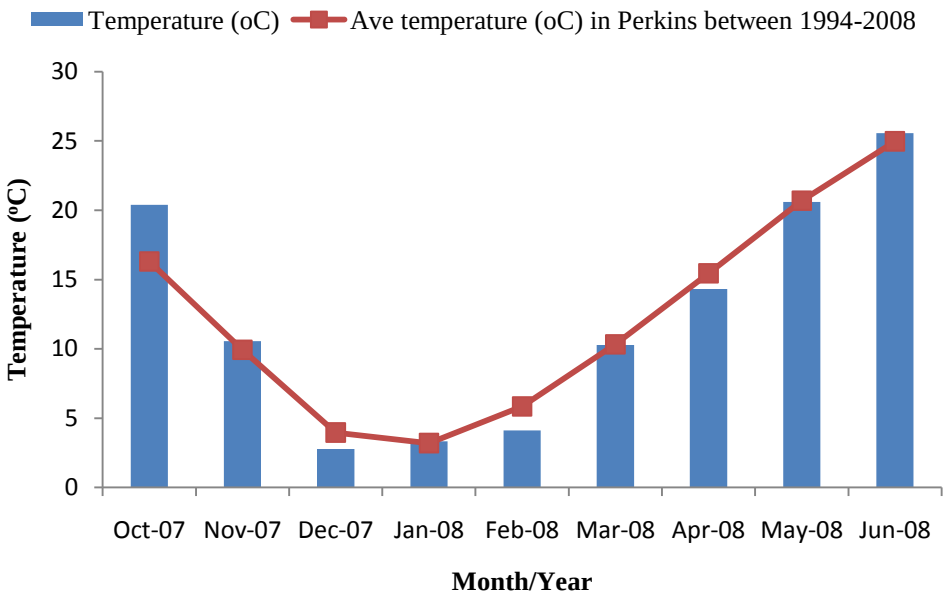


Figure A4: Total monthly rainfall (mm) in Perkins.

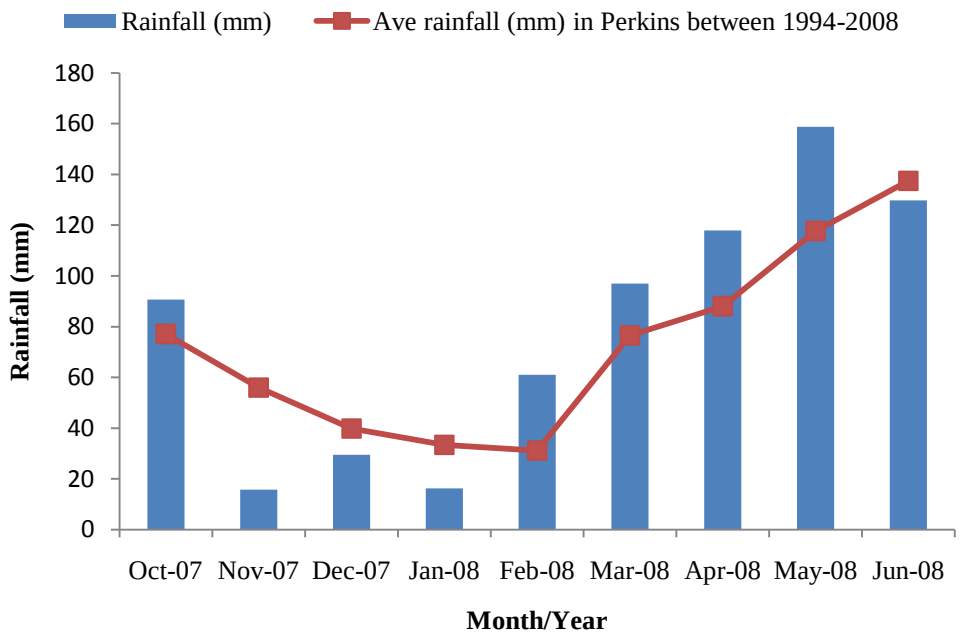


Figure A5: Average monthly temperature (^{o}C) in Stillwater.

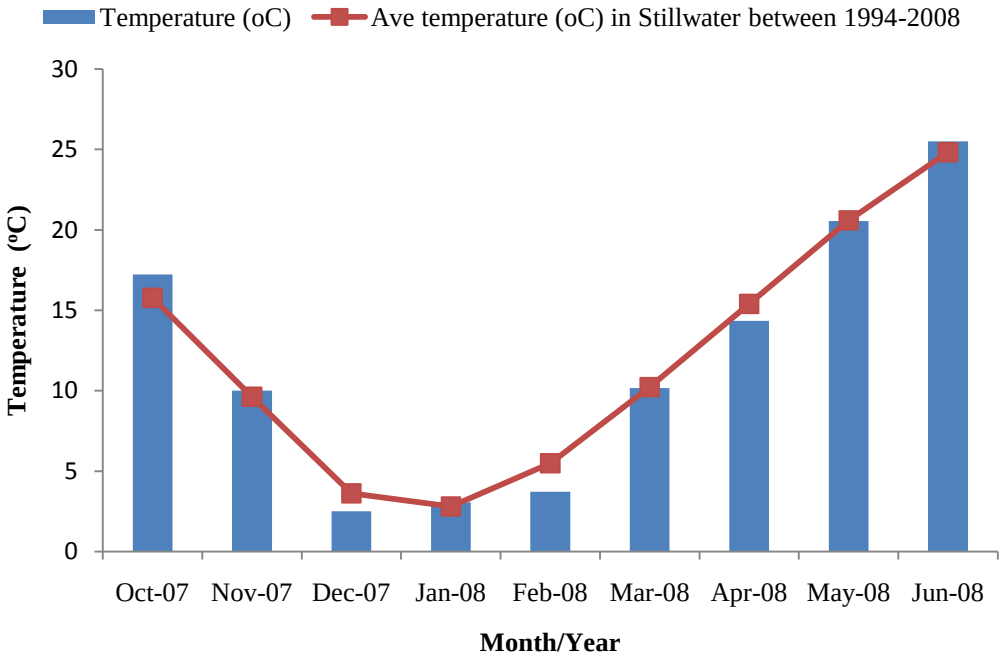


Figure A6: Total monthly rainfall (mm) in Stillwater.

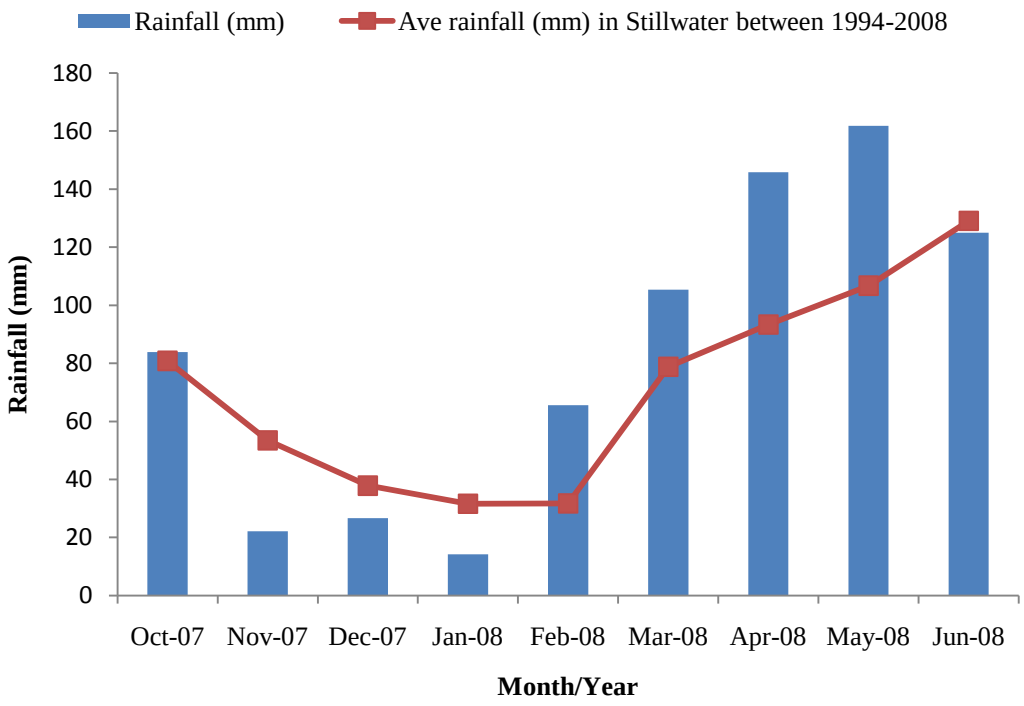


Figure A7: Average monthly temperature (^{o}C) in Marshall.

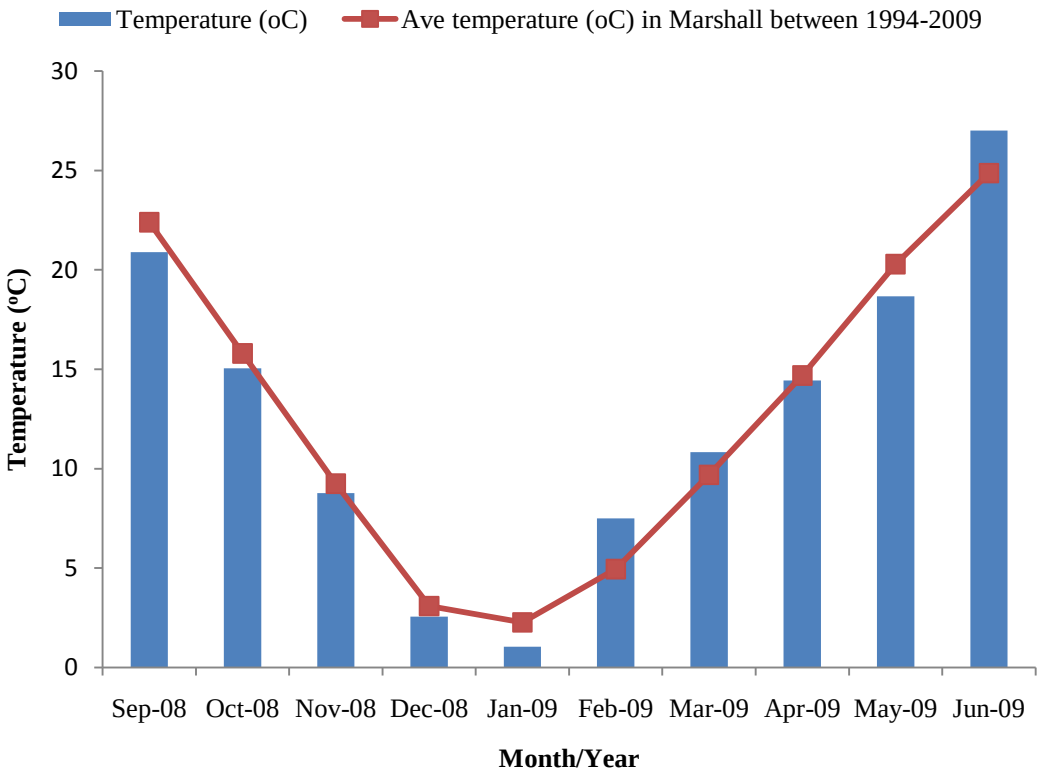


Figure A8: Total monthly rainfall (mm) in Marshall.

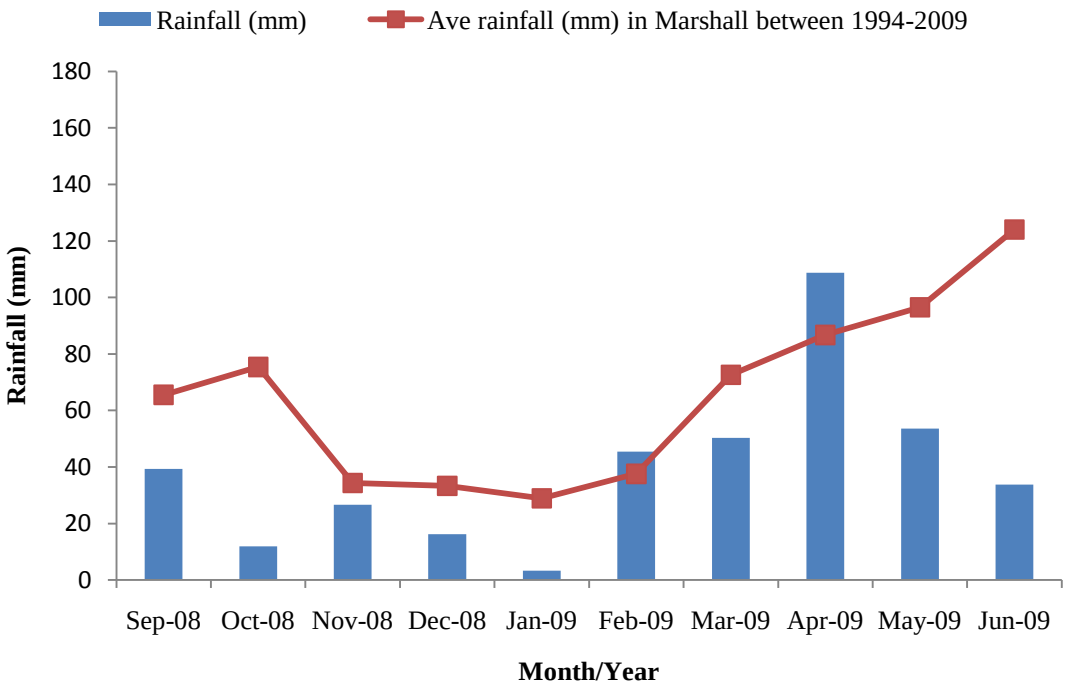


Figure A9: Average monthly temperature (°C) in Buffalo.

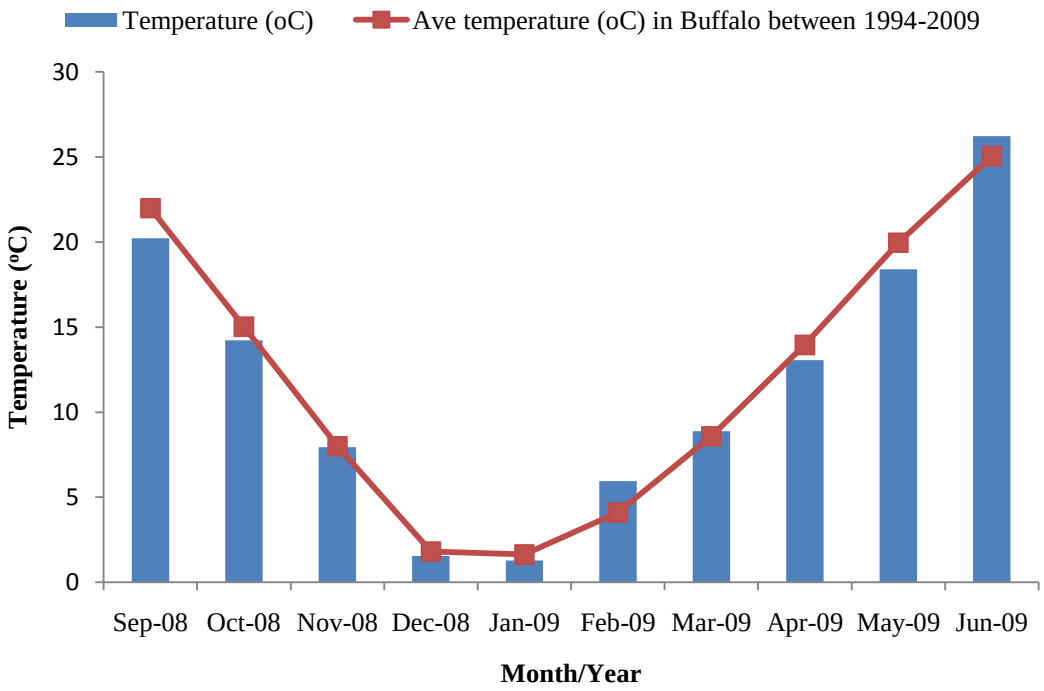
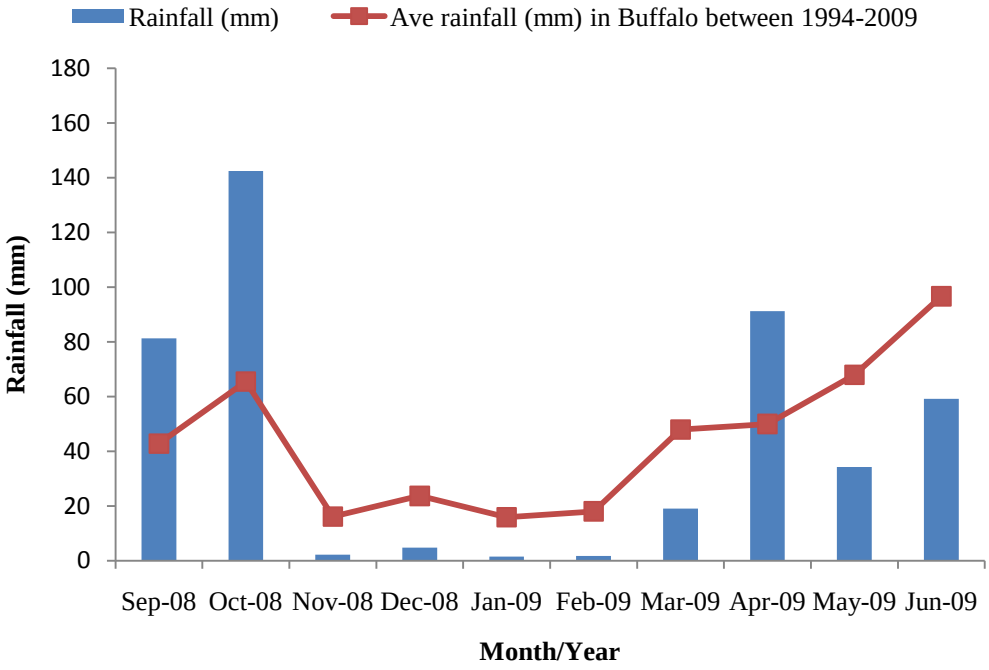


Figure A10: Total monthly rainfall (mm) in Buffalo.



VITA

WANDA PRADJANATA SEPTIANO

Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF HULLESS BARLEY FOR POTENTIAL ETHANOL PRODUCTION

Major Field: Food Science

Biographical:

Personal Data: Born in Jakarta, Indonesia, April 12th, 1984. First daughter of Mr & Mrs. James Basuki.

Education: Received Bachelor of Science degree in Technology from University of Pelita Harapan, Karawaci, Indonesia in April 2006.
Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in May, 2010.

Experience: Employed by Oklahoma State University, Department of Biosystem and Engineering as a graduate research assistant; Oklahoma State University, Department of Biosystem and Engineering, 2008 to 2009.

Professional Memberships: Institute of Food Technologists

Name: Wanda Pradjanata Septiano Date of Degree: May, 2010

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EVALUATION OF HULLESS BARLEY FOR POTENTIAL ETHANOL PRODUCTION

Pages in Study: 87 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study:

The main objective of this study was to evaluate potential of winter hulless barley as a local feedstock for ethanol production in Oklahoma. Two hulless barley varieties, Eve and VA125 from Marshall, Perkins, Stillwater and Buffalo locations were analyzed in this research. Milling characteristics of the samples were evaluated. Chemical composition such as starch, β-glucan, protein, ash, moisture, and oil contents of the samples were also analyzed. Ethanol conversion efficiency of whole grain and flour samples were examined.

Findings and Conclusions:

This study demonstrated that winter hulless barley has similar starch content to corn. Genotype and environmental conditions had significant effects on the barley grain composition. Dry milling produced high starch content fractions from barley grain. Winter hulless barley could be converted to ethanol from with acceptable yields. Optimization of dry milling and ethanol conversion process parameters could further improve the economic feasibility of barley to ethanol conversion operations. It was concluded that winter hulless barley can be a viable feedstock for ethanol production plants to be built in Oklahoma.

ADVISER'S APPROVAL: Dr. Nurhan Dunford