EVALUATION OF FOUR SORGHUM HYBRIDS THROUGH THE DEVELOPMENT OF GLUTEN-FREE BEER

by

KIRSTIN NICOLE VEITH

B.S., Kansas State University, 2007

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Food Science

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2009

Approved by:

Major Professor
Dr. Jeffery A. Gwirtz
Department of Grain Science and Industry

Abstract

There is a limited market of gluten-free beer for the 1% of the US population that is diagnosed with an autoimmune response to gluten protein known as celiac disease. Sorghum can be malted and used in the brewing process to replace malted barley, a grain toxic to celiac patients. The objective of the study was to develop an optimum brewing procedure for a gluten-free ale-style beer. Four different sorghum hybrids (82G63, 83G66, RN315, and X303) were malted and used in brewing gluten-free ale and evaluated for physical and chemical property differences. The four sorghum hybrids were characterized first as grain and then as malt using proximate analysis, single kernel characterization system (SKCS), amylose, α-amylase, and β-amylase contents. Isolated starch from unmalted and malted samples was evaluated with differential scanning calorimetry (DSC). Malt was evaluated throughout the malting process and percent nitrogen, percent moisture, 72 hr germinative energy, steep out moisture, germination-end, and malting loss were measured. Malted sorghum hybrid samples were milled into grist, and employed in a double mash, double decoction brewing process. Following the brewing process the wort was evaluated for specific gravity, Brix, pH, color and free α-amino nitrogen (FAN). Wort was also analyzed using HPLC for ethanol and glucose content. The fermented beer was analyzed for specific gravity, Brix, pH, alcohol by volume, and color. HPLC was also used to measure ethanol and glucose content.

Results of analysis found that a significant difference ($p=0.05$) was found for the DSC data onset temperature, which ranged from 61.75 to 65.51, illustrating the difference in starch gelatinization temperature compared to other cereals. A significant difference was found in α-amylase content ($p=0.05$) which ranged from 0.16 to 058 in unmalted sorghum and 71.63 to

96.44 in malted sorghum. In addition, α-amylase and β-amylase contents increased during malting. HPLC analysis of wort indicated a significant difference was found in percent maltose which ranged from 1.27 to 2.81. FAN content of wort was also significantly different and ranged from 65.15 to 151.37. HPLC of beer showed a significant difference in percent ethanol and percent glucose. Percent ethanol in the final beer ranged from 3.28 to 4.17 and percent glucose range from 0.16 to 0.31. Process development evaluation indicated a gluten-free ale style beer could be successfully produced with 100% sorghum malt.

Table of Contents

List of Figures

List of Tables

Acknowledgements

I would like to raise a toast to…

To my professors and committee members, Drs. Jeff Gwritz, Scott Smith, Scott Bean, Jeff Wilson and Tom Herald, thank you for sharing your knowledge, your passion for great beer, and your home brewing experiences with me.

To Chris Martens at the USDA-ARS Cereal Crop Research Unit in Madison, WI for malting grain samples and providing information about malting.

To everyone at the USDA-ARS GMPRC in Manhattan, KS, thank you for the time, knowledge, and resources you donated to my project.

To my professors and co-workers at FSI your friendship, guidance, and support have been tremendous. Thank you.

To my peers, the past two and a half years have been a rollercoaster ride, and through it all, I found myself working with friends. The times we spent brewing, bottling, researching, editing, and "sampling" are some of my fondest memories. Ryan, I'll see you for future August Fests.

To my editors: Chris, Deanna, Kelly, Sharon, and Dad, my deepest gratitude for your patience, time, and input.

To Sarah, sharing an office with you has been one of my greatest blessings at K-State. I can't imagine it without you. You've been my coach, cheerleader, and friend – thank you for it all!

To my family, especially, Mom and Dad – thank you for raising us in a home filled with love, support, and faith; and for instilling in us a stubborn drive to continue on against all odds.

Cheers!

Dedication

This work is dedicated to my family.

CHAPTER 1 - Literature Review

History of Beer

Beer is the most consumed alcoholic beverage in the world, and is the most popular beverage behind water and tea (Nelson 2005). Beer is a beverage of great variety. Most often beer is produced from malted barley, hops, yeast, and water, yet simple changes in the formulation has created 25,000 to 35,000 varieties of beer worldwide. Variations of this simple formula include beer brewed from a variety of grains such as rice, millet, barley, and corn depending on the regional staple, and fermented by wild yeasts. Brewing began at home and was followed by small village breweries that eventually led to the modern day large brewery (Papazain 2003).

Early Beer History

According to historians, beer has been produced for centuries. Early records show beer was produced in ancient Babylon about 8,000 years ago. Beer was an important aspect of both the Egyptian and Mesopotamian cultures where barley was the staple grain (Papazain 2003). Ancient Sumerian literature, which dates to about 1800 BC, provides a hymn to the Sumerian goddess of brewing that included an ancient recipe for beer (Katz and Maytag 1991). Historians have argued about the advent of beer and whether ancient cultures developed beer or bread first. Historians have held that ancient cultures abandoned hunter-gatherer practices to grow grain for beer (Braidwood et al. 1953). Bamforth (2006) reasons that the adoption of grain production, and subsequent production of beer, makes brewing the world's oldest biotechnology.

Ancient beer was subject to wild yeast and bacterial contamination which meant spoilage occurred quite easily. Thus a majority of the beer consumed was probably sour most of the time (Maytag 1992). Without modern packaging to prevent spoilage, beer was consumed in the home or village where brewing had recently occurred, and the beer was not widely distributed. While ancient beer was not of the high quality seen today, the demand was great because the brewing process eliminated pathogenic microorganisms commonly found in drinking water (Papazain 2003).

Throughout Europe beer was initially brewed at home by women. As time progressed, individual communities developed local breweries. In Europe brewing was often done by skilled monks in local monasteries, leading to the development of regional styles. Beer was an important part of many cultures; especially the British and German, which later influenced American beer culture. Many societies wrote legislation to regulate brewing in an effort to routinely produce a quality product. Germany is most famous for beer production legislation passed in 1516, called the Reinheitsgebot. This law stated that only barley malt, hops, and water could be used to produce beer. Yeast was later discovered, and is included as part of current brewing law (Bamforth 2006).

History of Beer in the United States

Beer was first shipped to the New World by the Pilgrims in 1620. Plymouth Rock was chosen as the Pilgrim's' destination due to the *Mayflower*'s low beer supply; the crew wanted to ensure enough beer would be available for the return trip to England (Bamforth 2003).

In the New World, beer production initially occurred at home and in small local breweries due to the lack of packaging and shipment methods. The first brewery

established in the New World was in New Amsterdam in 1613 by a Dutch man (Bamforth 2003). Beer was important to these early settlers and many important figures, including President George Washington, who had a brewery at his Mount Vernon estate. During the War of Independence, each soldier was rationed one quart of beer per day. Up until Prohibition, thousands of breweries existed across America producing an array of beer unique to each region (Papazain 2003).

Prohibition impacted the brewing industry in the United States. Enacted in 1920, the Eighteenth Amendment made the production of intoxicating liquors illegal. The Volstead Act of the same year stipulated that beer could be legally produced with alcohol content no greater than 0.5%. The next thirteen years saw an increase in home brewing, bootlegging, speakeasies, and organized crime. During the Great Depression, many political figures promoted the end of Prohibition as a means to stimulate the economy and bring revenue to the government through taxes. The repeal of Prohibition with the Twenty-Second Amendment to the United States Constitution aided in establishing the foundation for the current alcohol production and distribution system seen today.

Approximately one-half of US breweries survived Prohibition, and these were the larger breweries that produced malt products for the food industry. The surviving breweries began brewing lighter styles of beer to appeal to a broader consumer base, especially women and developing new technologies that revolutionized the brewing industry (Papazain 2003). One innovator was the Anheuser-Busch Brewery which employed the use of refrigerated railcars to transport the beer across the country without spoilage. This development set the stage for beer production to occur in large commercial breweries versus the small local breweries seen in the past.

During World War II, the shortage of food and restrictions on raw materials forced brewers to produce beer with less malt and begin using more abundant ingredients, such as corn and rice. Beer drinkers seeking volume, compromised on strength and alcoholic content (Bamforth 2003). These events along with mass marketing lead to the development of the characteristic American beer, a light-colored, light-bodied lager beer, produced by brewing giants such as Anheuser-Busch and Molson-Coors (Papazain 2003).

Up until the 1980s most beer was brewed by the large brewers. In February 1979, President Jimmy Carter signed a bill that permitted home brewing to be legal again, which allowed Americans to explore different styles of beer brewed at home. A few of these home brewers decided to take their hobby further and in the 1980s the United States saw a large increase in small breweries. These small breweries were called microbreweries in comparison to the giants of American beer production. As time progressed, many of these breweries grew into regional breweries, whose products are now referred to as craft beer (Papazain 2003).

Craft breweries have grown over the last three decades and encompass a greater portion of the industry each year as illustrated in Figure 1.1. According to the Brewers Association (2009), the number of US breweries totaled 1,545 in 2008, of these, 20 were large (non-craft) breweries, 24 were other non-craft breweries and 1,501 were craft breweries. The Brewers Association (2009) estimates the actual dollar sales from craft brewers in 2008 was $6.34 billion and took 4.04% share of the US beer market. The Brewers Association (2009) divides the craft brewing industry into distinct markets, outlined in Table 1.1. The breweries in this industry are defined as small, producing fewer than 2 million barrels per year; independent, with no more than 25% of ownership

by an alcoholic beverage industry member who is not a craft brewer; and traditional, meaning a minimum 50% of the beer must be produced on an all malt basis (Brewers Association 2009).

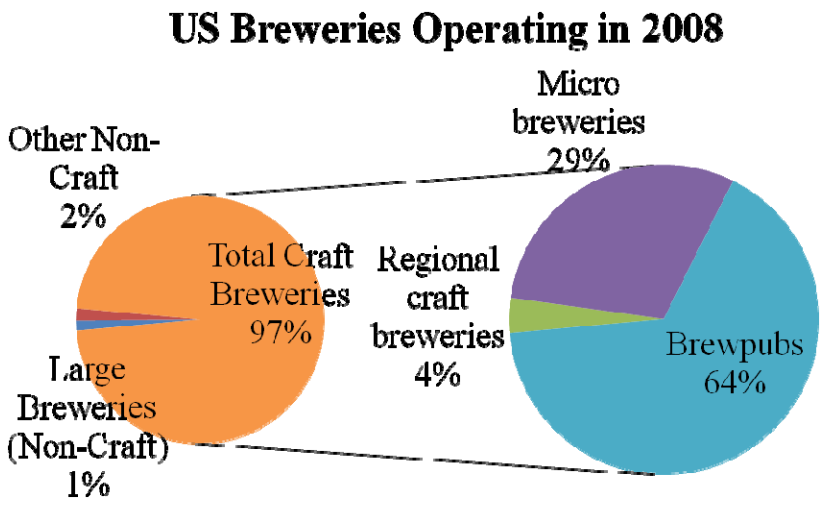


Figure 1.1 Number of US Breweries Operating in 2008.
Source: Adapted from the Brewers Association Craft Beer Statistics (2009).

Table 1.1 Distinct craft brewing markets in the United States.

Market	How beer is sold	Beer production per year
Regional Craft Brewery	Independent regional brewery producing 50%-100% all-malt beer.	15,000 to 2,000,000 barrels
Microbrewery	75% or more of beer produced is sold off site	Fewer than 15,000 barrels (17, 600 hectoliters)
Brewpub	25% or more of beer is sold on site in restaurant and bar	n/a
Contract Brewing Company	Sells beer produced by another brewery	n/a

Source: Adapted from the Brewer's Association Craft Brewing Statistics (2009).

Historical Use of Sorghum in Beer Production

Sorghum beer was first explored during World War II, when sorghum was used as an adjunct in lager beer brewing because of the shortage of malted barley. When the war ended, sorghum use in brewing was reduced (Agu 2005; Odibo et al. 2002; Ogbonna 1992; Owuama 1999). Research that investigated the use of malted sorghum in beer concluded that sorghum wort contained similar levels of glucose when compared to barley wort (Taylor and Dewar 1994). However, barley malt contains several times more maltose than glucose (Table 1.2). Taylor and Dewar (1994) and Palmer et al. (1989) reported that sorghum did not develop an adequate amount of β-amylase (20.0°L Diastatic power (DP)) when compared to barley malt (80.0°L DP); further studies suggested the high level of glucose in sorghum malt worts was due to α-glucosidase activity. Interest in sorghum beer using malted grain sorghum lessened due to the factors described above. The focus then became to use raw sorghum and employ commercial enzymes for starch digestion. Further research indicated that malted sorghum did contain enough enzymes to produce continental-style lagers, following central European formulations, when the brewing process temperatures were adjusted upwards from 55-59°C to 64-68°C for the higher gelatinization temperature of sorghum starch (Agu 2005). The recent emphasis on gluten-free foods has increased interest in using malted sorghum to produce beer (Sweeney 2002).

Table 1.2 Relative Amounts of Fermentable Sugars in Barley and Sorghum Malt Worts

Sugar (%)	Sorghum [a]	Barley [b]
Fructose	3.5+1.42	1.8+0.19
Glucose	29.9+5.34	11.9+0.72
Sucrose	0.41+1.35	4.2+0.43
Maltose	52.5+6.22	70.5+1.23
Maltotriose	13.7+2.42	11.7+1.14

[a]n=50
[b]n=20
Source: Dufour et al. (1992).

Celiac Disease

Celiac disease (CD) is a condition commonly referred to as celiac sprue or gluten-sensitive enteropathy and is generally understood as a wheat or gluten allergy. Celiac disease is in fact an autoimmune disorder in which the consumption of wheat, barley, and/or rye proteins causes damage to the small intestine. The only successful treatment is the lifelong avoidance of foods containing these proteins (Ciacci et al. 2007).

Fasano and Catassi (2001) provide a definition of celiac disease as a syndrome characterized by damage of the small intestinal mucosa caused by the gliadin fraction of wheat gluten and similar alcohol-soluble proteins (prolamines) of barley and rye in genetically susceptible subjects. The consumption of wheat, barley, and rye proteins in these individuals causes an immune system reaction. The small intestine contains villi, or fingerlike projections, that work to absorb nutrients from food into the bloodstream by increasing the surface area of the small intestine. In celiac patients, intestinal villi are damaged or destroyed, inhibiting the ability to absorb necessary nutrients. Symptoms of celiac disease vary greatly, and may include chronic diarrhea, vomiting, and abdominal

distension which lead to malabsorption of nutrients causing numerous other health issues. Both Leeds and Hopper (2008) and Fasano and Catassi (2001) describe atypical forms of the disorder which may include dermatitis herpetiformis, where the consumption of toxic grains causes a blistering skin disease; osteoporosis; iron-deficiency anemia; neurological problems; and other symptoms. Patients may be diagnosed using a variety of methods, including serological testing; however, a better diagnosis may be achieved with a duodenal biopsy (Leeds and Hopper 2008). Other methods, including serological testing using a variety of serological markers, may produce a false positive due to different digestive disorders. Repeat biopsies before and after the removal of gluten from the diet helps insure the correct diagnosis is achieved and the intestine is repairing itself. Currently the only successful long-term treatment of celiac disease is the removal of toxic grains from the diet (Ciacci et al. 2007). This includes any food products made from wheat, barley, rye, or oats. Most often, bakery items are implicated, however, these proteins may be found in sauces, gravies, prepared meats, and beverages as well as cosmetics.

Symptoms of celiac disease have been documented for centuries but research in the 1950s established wheat as the cause of celiac disease. Further research has shown that the storage proteins of barley and rye are toxic to celiac patients. These storage proteins are referred to as gluten in the field of CD but include the gliadins and glutenins of wheat, secalins of rye, and hordeins of barley (Weiser and Koehler 2008). A study by Vader et al. (2003) found that the composition of these cereal proteins contain high amounts of glutamine and proline which are the basis of toxicity. The taxonomy of plants can provide a pictorial illustration of toxic grain with the grass family *Poaceae*,

thereby illustrating that wheat, barley, and rye are found in a single tribe, the *Triticeae*, displayed in Figure 1.2 (Hughes 2007; Weiser and Koehler 2008).

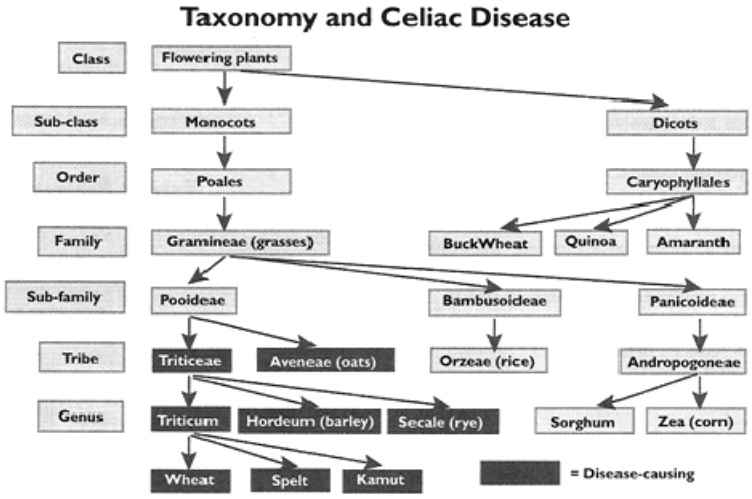


Figure 1.2 Taxonomy of cereals that contribute to Celiac disease.
Source: Hughs (2007).

The National Institutes of Health (NIH) Celiac Awareness Campaign estimates 1% of the US population suffer from celiac disease (National Digestive Diseases Information Clearinghouse (NDDIC) 2009). Celiac disease is most prevalent in Europe and countries to which Europeans have emigrated (Weiser and Koehler 2008; Ciacci et al. 2007).

Physicians are diagnosing more cases of CD, due to better testing methods. In fact, Ciacci et al. (2007) expect to see an exponential increase in the number of CD diagnosis based on epidemiological studies of CD prevalence. As the CD population expands, so does the market for gluten-free foods. Annual sales of gluten-free foods are expected to grow from $700 million, reported in 2006, to $1.7 billion in 2010 (O'Brien 2007). The growth of this niche market has caused the European Union to limit gluten-

free claims to food products containing less than 20 ppm of gluten (Food Standards Agency 2009).

Gluten-free foods being developed for celiac patients include breads, cakes, cookies, tortillas, and crackers. One of the products being produced is gluten-free beer. Beer, a beverage frequently requested by celiac patients, is toxic to them because the primary ingredient is malted barley, and in some beer styles wheat or rye (BurnSilver 2007; Sweeny 2002). The hordein protein of barley (part of the *Triticeae* Tribe, of which wheat is also a member (Figure 1.2) is present in beer and vital to produce the characteristic foam or head; meaning that all traditional styles of beer are toxic to celiac patients.

Gluten-Free Beer

Sorghum, a cereal grain, has been shown to be safe for people with celiac disease (Ciacci et al. 2007). Current gluten-free beer seen on the national market in the US utilize sorghum as an ingredient (BurnSilver 2007). The US beer are lager-style beer; there is no ale-style gluten-free beer available nationally. Bard's Tale Dragon's Gold, an American lager-style beer, was introduced in 2004 (O'Brien 2007). Bard's was developed by home brewing celiac patients, Craig Belser and Kevin Seplowitz, and utilizes 100% malted grain sorghum. The Bard's Tale Beer Company, LLC, website (2009) states that Bard's Beer is, "America's first gluten-free sorghum beer and the only beer brewed with 100% malted sorghum." In December 2006, Anheuser-Busch launched Redbridge, a sorghum-based, amber-colored, lager-style beer available nationally. The Anheuser-Busch website (2009), describes the beer: "Redbridge is a rich, full-bodied lager brewed from sorghum for a well-balanced, moderately hopped taste." In November

2006, Lakefront Brewery in Milwaukee, WI launched New Grist, a gluten-free beer made form sorghum syrup and rice (Kitsock 2007).

Other gluten-free beer have been produced in Europe and Africa. One example is a copper-colored ale, called Toleration Ale, from Hambleton Ales in Melmerby, England (Kitsock 2007). SABMiller developed a clear sorghum beverage called Eagle, which is brewed in Tanzania, Uganda, Zambia, and Zimbabwe in Africa (BurnSilver 2007; INSORTMIL Report 2008).

Table 1.3 Gluten-free beer available commercially in the United States.

Beer	Redbridge	New Grist	Dragon's Gold
Producer	Anheuser-Busch St. Louis, MO Merrimack, NH	Lakefront Brewery Milwaukee, WI	Bard's Tale Lee's Summit, MO Norwalk, CT
Source of fermentables	Sorghum	Sorghum and rice	100% malted sorghum
Description (Kitsock 2007)	"Creamy mouth-feel and a spicy hops character not unlike that of a Samuel Adams Boston Lager. A few gulps reveal a tart fruitiness."	"Bright straw-gold with a thick white foam, sour aroma, a crisp cider-like flavor, notes of vanilla, and a faintly grainy finish."	"It's roughly in the pilsner style, with a dry, earthy, nutty flavor. Although Redbridge has more body and flavor, this one comes closest to a barley-based beer."

Source: Compiled from www.redbridgebeer.com (2009); http://lakefrontbrewery.com/sorghum.html (2009); http://www.bardsbeer.com (2009); Kitsock (2007).

Ingredients and Functionality

The factors that most influence the final sensory characteristics of beer are ingredients and climate. Beer requires only four ingredients: water, fermentable sugars (malted barley), hops, and yeast. Ingredient choice is based on quality, cost, and

accessibility. Agricultural conditions, economics, and world events are reflected in different varieties of barley and hops; these, along with growing location, and crop year, have an impact on the attributes of the final beer. Each climate exhibits unique temperature and humidity fluctuations along with characteristic microflora. In England the climate is more suitable for ale-style yeasts, where as the traditional geographic brewing regions of Germany include caves suitable for lager-style yeast fermentation.

As different styles developed from the available ingredients and climate conditions, certain styles were chosen for taste, quality, and cost. For example, many governments tax beer based on alcohol content, therefore beer with higher alcohol content have a higher cost to both the brewer and the consumer. India Pale Ale was developed to provide quality ale for the British troops occupying India and is a style characterized by a high alcohol content of 5-7% and hoppy flavor. The higher alcohol content and increased hop additions created a beer that was suitable to ship great distances without spoiling (Papazain 2003).

Water

Water is the ingredient used in the greatest quantity in brewing. Traditionally, variations in water sources have had a large impact on the characteristics of beer around the world. For example, the soft water in the Pilsen region of the Czech Republic is best suited for light lager production, whereas, the hard water of Dublin, Ireland creates superior dark ales, such as Guinness (Palmer 2006). The hardness of the water influences pH and other factors such as the stability of enzymes, extractability of grist and hop components, and flocculation of yeast (Bamforth 2006).

Palmer (2006) outlines several requirements for brewing water. The important ions that contribute to mash pH are calcium, magnesium, bicarbonate, and sulfate. Sodium, chloride, and sulfate are evaluated for contribution to the taste of the beer. In the United States, water hardness is measured in two ways. Temporary hardness is measured by the amount of bicarbonate, and high levels of bicarbonate, greater than 100 ppm, will cause harsh flavors in the final beer. Permanent hardness is determined by the calcium and magnesium levels. Permanent hardness lowers the pH. At certain levels low pH is desirable in all-grain brewing for enzyme reactions (Papazain 2003). Palmer (2006) reported that the pH of the water is an important parameter. However, for the overall process, the pH of the mash is the more important factor. Papazain (2003) reported the importance of brewing with water at a pH below 8; a pH level above 8 indicates hard water. The water pH affects mash enzyme activity and the extraction of bitter tannins from the grain husks (Palmer 2006).

Table 1.4 Mineral content comparison of brewing waters around world.

Mineral (Ion) (ppm)	City						
	Pilsen	Munich	Dublin	Dortmund	Burton-on-Trent	Milwaukee	Manhattan, KS
Calcium (Ca)	7	70-80	115-120	260	260-253	35	42
Sulfates (SO_4)	5-6	5-10	54	283	630-820	18	100
Magnesium (Mg)	2-8	18-19	4	23	24-60	11	5.4
Sodium (Na)	32	10	12	69	54	NA	61
Chloride (Cl)	5	1-2	19	106	16-36	5	89

Source: Papazain (2003); City of Manhattan (2007).

Hops

In brewing, hops refers to the flower harvested from the cone of the female plant *Humulus lupulus*. The hardy, climbing, herbaceous perennial plant is grown in all the temperate regions of the world. The cone-shaped flowers (Figure 1.3) are valued by brewers for their resins and oils located in the lupulin glands that impart both bitterness and aroma to the beer (Bamforth 2006).

Hops were not used in most ancient beer production; instead, spices and other plants were used to flavor the beer. In 1079, Saint Hildegard of Germany noted the anti-spoilage properties of hops, and brewers began to take note (Bamforth 2006). Cultivation of hops began in central Europe and spread to western Europe and Great Britain in the early 1500s (Palmer 2006). However, hops were not common in beer until the early 1800s. Hops work well as a preserving agent in the brewing process because they eliminate undesirable malt proteins, aid clarification, and stabilize beer flavors. An added benefit is their ease of cultivation, and ability to impart characteristic flavor and aroma (Papazain 2003).

Two components of hop composition are essential to beer production, the essential oils and resins. The oil portion contributes to the aroma characteristic of the final beer (Bamforth 2006). Hop resins contains alpha acids that contribute to bitterness. The level of alpha acid is unique to each variety of hops. Alpha acids are also referred to as humulones and indicate the bitterness imparted to the beer.

Hops are added to the beer during the boiling of the wort. This is necessary to promote the isomerization reaction that renders the alpha and beta acid resins water soluble, as humulinic acid and isohexenoic acid. Once water soluble, these compounds

are released into the sweet wort where bitterness is imparted to the wort (Figure 1.4) (Papazain 2003). Increased boil time increases the bitterness imparted to the final beer. The aroma that hops provide to beer is produced by essential oils, which account for 1%-2% of the total dry weight of the cone. These essential oils are volatile and easily lost during the boil (Palmer 2006). Therefore, hops are added at scheduled intervals during the boil to produce the desired flavor and aroma in the final beer.

Hops are evaluated in two ways, first, by alpha acids in Alpha Acid Units (AAUs), which is the weight of hops (in ounces), multiplied by the percentage of alpha acids, determined by chemical extraction, and second, by International Bittering Units (IBUs) (Equation 1.1). The IBUs estimates how much of the alpha acid is isomerized and dissolved into the beer. The IBUs are calculated from the AAUs, the volume of the boil (V), and utilization (U). Utilization takes into account the time and gravity of the boil to describe the efficiency of the isomerization reaction (Palmer 2006). These equations are stated below.

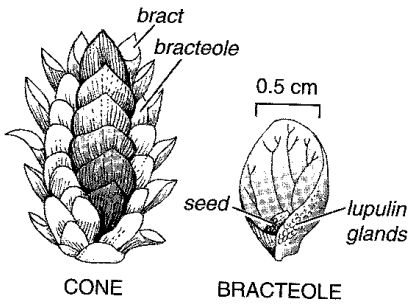


Figure 1.3 Diagram of hop cone and hop bracteole.
Source: Bamforth (2006).

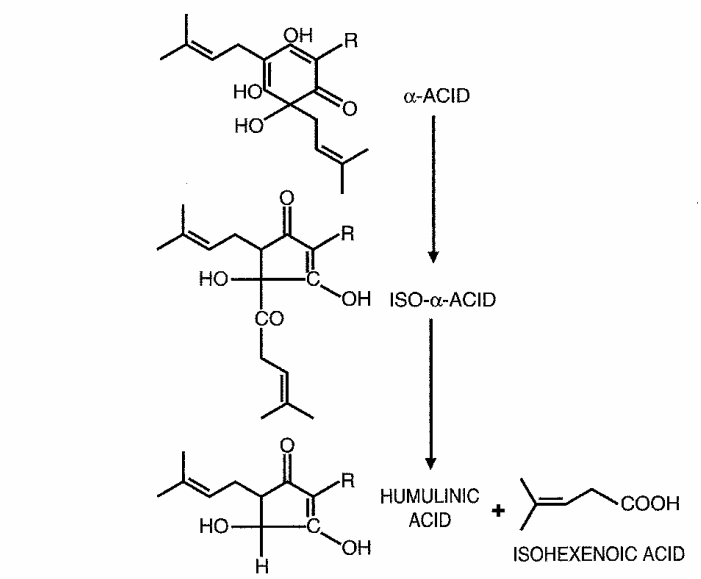


Figure 1.4 Schematic of the isomerization of the degradation reaction of alpha acids during the boil.
Source: Bamforth (2006).

Equation 1.1 Calculation of International Bittering Units

$$IBU = AAU \times U \times 10 \div V$$

AAU = Alpha Acid Units

Utilization (U) = f(G) × f(T)

$f(G) = 1.65 \times 0.000125^{(Gb-1)}$, Gb = boil gravity

$f(T) = [1 - e^{(-0.04 \times T)}] \div 4.15$

10 is constant for the metric units (grams and liters), IBU is calculated in mg/L.

V = Volume of the boil

Source: Papazain (2003).

Yeast

Yeast is important to beer production to convert the sugars in the wort to alcohol, creating beer. Although beer production is one of the world's oldest crafts it was not until 1836 that C. Cagniard-Latour theorized that fermentation of sugar was due to yeast. The following year T. Schwann recognized the fungal nature of yeast and named the organism *Saccharomyces* (Briggs et al. 1981). Determining the type of yeast is often the

first step in beer classification. Beer is categorized into ales and lagers based on the type of yeast used, and traditionally yeast was classified by where it settled in the fermenting vessel. Historically, most of the world used top-fermenting yeasts up until the nineteenth century. Bottom-fermentation yeast was only used by Bavarian brewers. In 1842 a Bavarian monk smuggled these fermentation techniques into Czechoslovakia and the technology began to spread across the globe (Briggs et al. 1981). Ale yeasts typically floated on the top, whereas lager yeast settled to the bottom. The yeast used in brewing belongs to the genus *Saccharomyces*, (Figure 1.5). The taxonomy of yeast in brewing is classified into ale strains that belong to the species *S. cerevisiae* and typically ferment at warmer temperatures 18-22°C. Lager strains are categorized as *S. pastorianus* and typically ferment at 6°-15°C. *S. pastorianus* most likely evolved from the merging of *S. cerevisiae* with *S. bayanus*, a yeast commonly employed in winemaking (Bamforth 2006). Merging of these yeasts resulted in the larger and more complex genome of lager strains. *S. uvarum* and *S. carlsbergensis* were used to identify lager strains prior to the genetic technology to identify *S. pastorianus* (Lewis and Bamforth 2006). The basic difference between ale and lager strains is the ability to ferment the sugar melibiose; only lager strains can ferment this particular sugar (Bamforth 2006).

When brewing gluten-free products, gluten-free yeast selection is important. Often yeast is propagated in a solution that may contain barley or wheat malt. For gluten-free products, yeast should be propagated using other carbohydrate sources such as molasses.

Barley

Malted barley is the most common source of fermentable carbohydrates in beer worldwide with the exception of African sorghum beer (Hoseney 1994). Barley is a cereal crop grown in cool climates with moderate precipitation and is fourth in worldwide production of cereals (barleyworld.org 2009). The United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS) reported that the United States produced 240 million bushels of barley with an estimated value of $1.2 billion dollars in 2008. North Dakota, Idaho, and Montana are the leading barley producers (USDA NASS 2009). In the United States barley usage is divided into 66% food and industrial use, 12% export use, and 22% feed and residual use (US Grains Council 2008).

Barley belongs to the grass family *Poaceae*, and the tribe *Triticae,* along with wheat and rye. Barley is classified as *Hordeum vulgare*, and occurs in two forms, six-row and two-row, based on spikelet arrangement on the rachis (Bamforth 2006). Barley consists of medium-sized kernels weighing on average 35 mg and is composed of 63-65% starch, 10-12% protein, and a 3.3% lipid level in the kernel with one-third located in the germ (Bamforth 2006; Hoseney 1994). Endosperm cells are packed with starch embedded in a protein matrix; and the starch is similar to wheat and rye. The prolamin protein fraction of barley is referred to as hordein and composes 40% of the protein fraction. A unique feature of barley is that the kernel retains the husk after threshing. Hoseney (2007) describes the tight adherence of the hull to the kernel as "cemented." The cemented hull helps to protect the grain after malting and makes the grain suitable for beer brewing because the husks form a filter bed during the brewing process allowing

the sugars to be extracted from spent grains into the wort (Figure 1.5) (Bamforth 2006; Hoseney 1994).

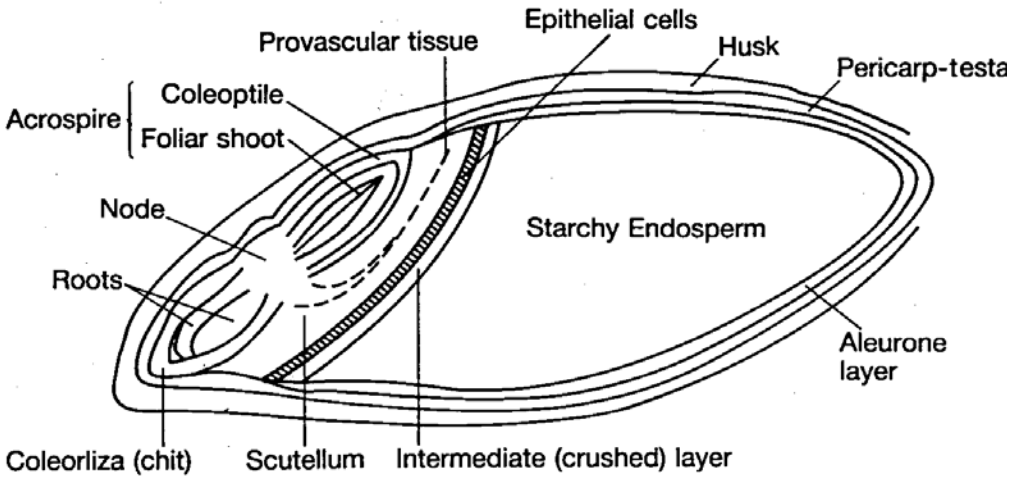


Figure 1.5 Barley kernel diagram.
Source: Palmer et al. (1989).

Sorghum

Sorghum is a grain that grows primarily around the equator in semi-arid climates (Owuama 1997). Two of the best known species are *Sorghum vulgare* and *Sorghum bicolor* L. Moench (Palmer et al. 1989). Sorghum, indigenous to Africa, is a member of the grass family *Poaceae*. Sorghum is ranked as the fifth most important grain in terms of production, preceded by wheat, rice, maize, and barley. While the Food and Agriculture Organization of the United Nations (FAO) estimates that sorghum is less than 5% of world grain production, 40% of sorghum production constitutes a large portion of the nutritional needs of the people in the semi-arid zones of Africa and Asia (Dendy

1995; Taylor and Dewar 2001). In the United States and other Western countries, sorghum is produced primarily for animal feeds (Ciacci et al. 2007).

According to the FAO, the United States produced 12 million tons of sorghum in 2004 (FAO 2006). The USDA NASS (2009) reported sorghum production in the United States in 2007 was 490 million bushel with 35 million bushels were used for food, seed, and industrial use, 180 million bushels were used for feed and industrial, and the remaining 275 million bushels being exported. The USDA NASS Kansas Field Office (2008) reported that in the United States, Kansas was the number one sorghum grain producer for 2007, producing 42% of the U.S. total crop. Kansas produced 212 million bushels of sorghum grain in 2007 with a farm value approximately $500 million dollars. This was up 46% from the 2006 crop of 145 million bushels. Sorghum is growing in popularity for use in the production of gluten-free food products for persons diagnosed with celiac disease (Ciacci et al. 2007).

The physical structure of sorghum kernels are free of hulls or glumes, are oval shaped, weigh 20-30 mg, and may be white, red, yellow, or brown in color. Hand-dissected kernels were found to be 7.9% pericarp, 9.8% germ, and 82.3% endosperm, which is both vitreous and opaque (Hoseney 2004). Some varieties are labeled "bird resistant" due to the bitter tannins that deter birds from consuming the grain prior to harvest (Taylor and Dewar 2001). Sorghum differs from barley in that the aleurone tissue is a single layer of cells as opposed to three cells (Ogbonna 1992). Other varieties are labeled food grade and described by Taylor et al. (2006) as a white sorghum developed to produce bland-tasting flour that is suitable for food products because it does not impart "off" colors or flavors.

Sorghum starch is chemically similar to maize in size and shape. The starch granules vary in shape from almost polygonal, near the outside of the kernel, to almost spherical, towards the center of the kernel (Hoseney 2004). Starch composes the greatest portion of the sorghum grain by weight (Daiber and Taylor 1995). Comparable to barley, starch granules and storage proteins are enclosed in the endosperm cells. However, sorghum starch granules are tightly packed at the peripheral region, giving a steely, vitreous texture, while the inner part is floury (Ogbonna 1992). Starch gelatinization temperature is 68°-78°C. The prolamin protein portion of sorghum is referred to as kafirin and resembles the maize protein, zein, in amino acid composition (Hoseney 2004). Lipid composition of sorghum is 2.1%-5.0%, and 75% of the lipids are contained in the germ with the remainder split evenly between the bran and the endosperm (Hoseney 2004).

Currently, sorghum is widely used in beer brewing in Africa due to the greater availability of sorghum versus barley (Igyor et al. 2001). In the continent of Africa sorghum has been malted for centuries to be used in products such as baby food and traditional alcoholic and nonalcoholic beverages (Beta et al. 1995). Estimates for Southern Africa alone indicate 200,000 tons of sorghum are malted annually and some 3 billion liters of sorghum beer are brewed annually (Taylor and Dewar 2001). In fact, the importation of cereal grains, including barley malt, to Nigeria was banned in 1988. This forced brewers to utilize the sorghum that was locally available and has caused an increased interest in brewing lager-style beer from malted sorghum (Dewar et al. 1997; Taylor and Dewar 2001).

Sorghum has been used as an adjunct in beer in the United States as a response to the popularity of paler and more mildly flavored beer (Hoseney 1994). Sorghum use has been considered in the Mexican brewing industry as an adjunct to replace corn and rice due to lower price and greater availability (Table 1.4) (Orsorio-Morales et al. 2000).

Table 1.5 Cost comparison among five US grains produced in 2008.

Commodity	Price per unit	Value of production (thousand dollars)
Sorghum (for grain)	5.7 dols / cwt	1,681,558
Rice (all)	16.5 dols / cwt	3,390,666
Corn (for grain)	3.9 dols / bu	47,377,576
Barley (all)	5.15 dols / bu	1,208,173
Wheat (all)	6.8 dols / bu	16,568,211

Source: USDA NASS (2009).

Sorghum has been used to produce gluten-free beer in several studies (Pozo-Insfran et al. 2004; Owuama 1999; Okafor 1980; Igyor et al. 2001; Taylor 1992; Owuama and Okafor 1987; Okafor and Aniche 1986). Sorghum malt varies from barley malt in several ways. Physically sorghum does not contain a husk like barley does (Figure 1.6), has a higher starch gelatinization temperature, and has less diastatic, β-amylase, and glucanase activities. Therefore the traditional brewing procedures for barley have to be altered to account for the differences between the grains (Pozo-Insfran et al. 2004).

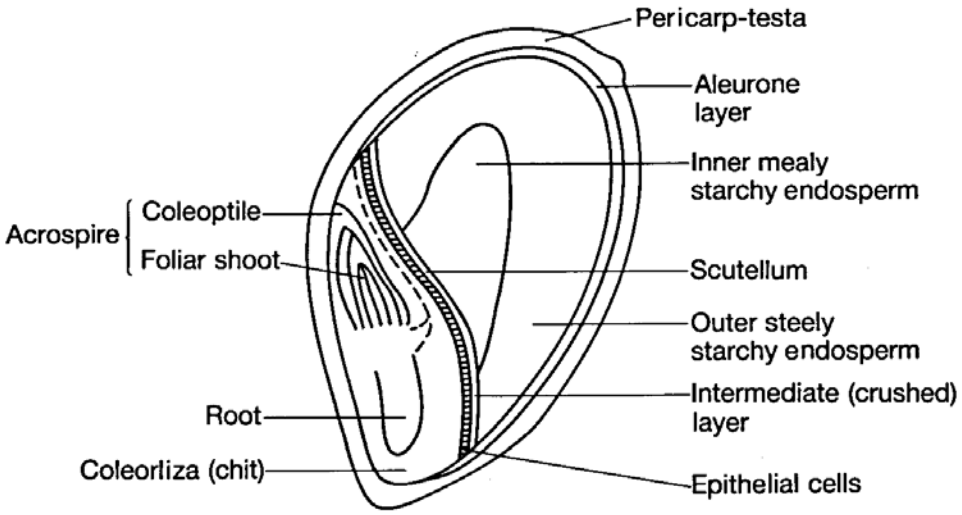


Figure 1.6 Diagram of a sorghum kernel.
Source: Palmer et al. (1989).

Rice Hulls

Rice hulls are a by-product of processing paddy or rough rice into brown rice and are used as a filter aid in the production of gluten-free beer. Hoseney (1994) described rice hulls as tough, fibrous, and abrasive. Ogbonna (1992) reported the use of artificial husks from plant fibers can help with wort filtration problems. Processing of paddy rice generally produces 20% hulls and begins when rough rice is fed through a rubber-roll sheller consisting of two rubber-coated rolls that turn in opposite directions at a differential. This action frees the brown rice from the hull that is removed by aspiration. Rice hulls consist of approximately 20% ash, 30% cellulose, 20% pentosans, 20% lignin, 3% protein, and 2% fat.

Beer Styles

Beer is most often classified according to style. Bamforth (2006) classifies beer by overall style, strength, color, principal grist ingredient, region of (original) production, and technological influence. Palmer (2006) classifies beer by naming all ingredients and fermentation particulars. Modification of any one ingredient changes the style. Table 1.5 outlines the organization of different beer styles.

Table 1.6 Categories for classifying beer styles.

Category	Characteristics			
Overall style	Ale	Lager		
Strength	Original extract	Alcohol content		
Color	Ales can be classified as pale, brown, porters, and stouts.	Lagers are generally pale but may be dark		
Principal grist ingredient	Barley malt	Malted wheat	Malted sorghum	Rye
Region of (original) production	Pilsen	Burton ale	Irish stout	San Francisco Steam Beer
Technological influence	Light beer	Dry beer	Ice beer	Flavor ingredient

Source: Adapted from Bamforth (2006).

Overall Style

Most beer style category systems begin by differentiating between the type of yeast utilized (Palmer 2006). Overall style categorizes the beer into ales versus lagers based on whether the yeast floats to the top or stays on the bottom. Ales are traditionally fermented from dark grist (featuring well-modified, quite highly kilned malts) using yeast

that floats to the surface. These ales are served at warmer temperatures (10°-20°C) and contain low levels of carbon dioxide. Lagers are traditionally fermented at lower temperature ranges from lighter grist (relatively under-modified, gently kilned malts) using yeasts that settled to the bottom of fermenting vessels. These beer are most often stored for extended periods of time before sale and consumption which leads to higher carbon dioxide levels. Lagers are served at cooler temperatures (0°-10°C). In modern brewing, yeast is generally the guideline for ale versus lager, but ale yeasts can be used in lagers and ales can be brewed with pale malts and vice versa (Bamforth 2006). Beer styles can change with the modification of any one of the ingredients – for example, increasing the amount of hops ale can changes category from pale ale to an India Pale Ale (Palmer 2006). Historical significance also plays a role in style characterization based on traditional brewing region (Figure 1.9) (Papazain 2003).

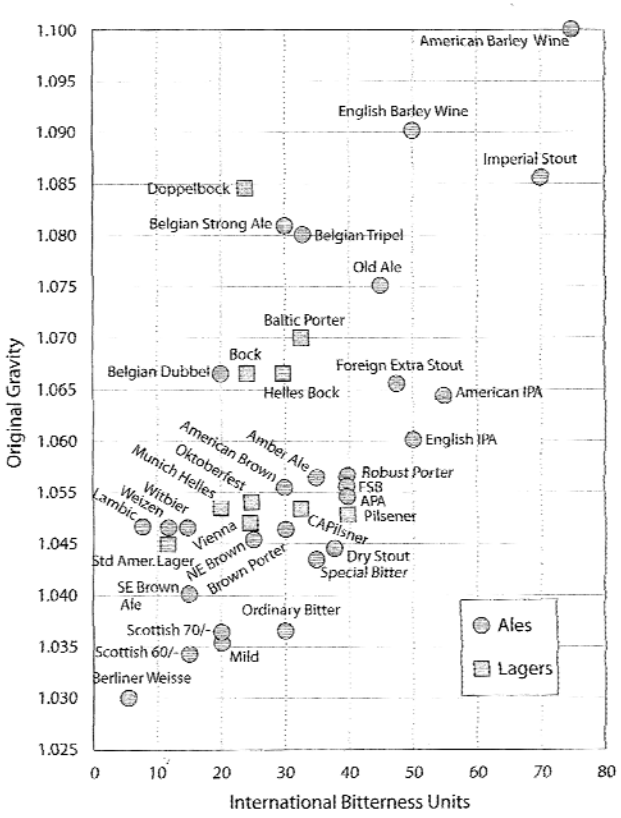


Figure 1.7 Relative flavors of beer styles.
Source: Palmer (2006).

Alcohol Content

Alcohol content is also referred to as strength and is measured by the difference in the specific gravity over time. Specific gravity is measured at the start of fermentation, labeled original gravity (OG). Specific gravity is then measured again at the end of fermentation, and labeled final gravity (FG), and these numbers are used to calculate the final alcohol content or strength (Equation 1.2). Table 1.6 outlines various OGs of different beer styles.

Equation 1.2 Calculation of alcohol by weight and alcohol by volume.

$$\text{Alcohol by weight} = (\text{Original gravity} - \text{Final gravity}) \times 105$$

$$\text{Alcohol by volume} = (\text{Alcohol by weight}) \times 1.25$$

Source: Papazain (2003).

Table 1.7 Overview of original gravity and percent alcohol.

	Original gravity	Final gravity
ALES:		
Ordinary Bitter	1.032-1.040	1.007-1.011
English India Pale Ale	1.050-1.075	1.010-1.018
Brown Porter	1.040-1.052	1.008-1.014
Sweet Stout	1.042-1.056	1.010-1.023
LAGERS:		
Czech Pilsner	1.044-1.056	1.013-1.017
Traditional Bock	1.064-1.072	1.013-1.020
Oktoberfest/Märzen	1.050-1.056	1.012-1.016
Vienn	1.046-1.052	1.010-1.014

Source: Adapted from Palmer (2006) based on Beer Judge Certification Program (BJCP) Style Guidelines.

Color

There are several factors that contribute to beer color, including malt choice and processing parameters. Malt is the predominant factor in differentiating color, which is affected by a process called kilning. Kilning is the roasting of the malt following drying which produces the color in the malt through Maillard reactions that occur between reducing sugars and amino acids. Dark, highly kilned malts contribute more color due to the increase in browning reactions during kilning. Processing conditions, such as decoction mashing, can affect the beer color through caramelization and Maillard

reactions. (Bamforth 2006; Papazain 2003). Figure 1.10 illustrates different barleys of various degrees of kilning.

Commonly the color of beer can be identified as light or dark by visual appearance. However, in modern beer production there are several methods that can be used to identify beer color. Traditionally beer color is measured in degrees Lovibond (°L) (Papazain 2003). Created by J.W. Lovibond in 1883, this method consisted of glass slides of various shades that could be combined to produce a range of colors (Palmer 2006). The method was later modified to the Series 52 Lovibond scale; however, use has faded due to inconsistencies (Palmer 2006). The American Society of Brewing Chemists (ASBC) incorporated the use of optical spectrophotometers in 1950 to develop a more consistent measurement system, which led to the development of the Standard Reference Method (°SRM) for determining color (Palmer 2006). European brewers have their own color scale, referred to as European Brewers Convention (°EBC) (Papazain 2003).

In ales the color varies from pale ales, to brown ales, to porters and stouts. Most lagers have a pale light color; however, there are few darker lagers such as the German Dunkel (Bamforth 2006). Table 1.7 provides an overview of variations in the color of commercial beer.

Figure 1.8 Malted barley; different colors reflects degree of kilning.
Source: Palmer (2006).

Table 1.8 Commercial beer color in degrees SRM.

Commercial beer	Color in degrees SRM	Color description
Budweiser	2.0	Yellow/straw/gold
German Pils	3.0 (average)	Yellow/straw/gold
Pilsner Urquell	4.2	Yellow/straw/gold
Bass Pale Ale (export)	10	Amber
Michelob Classic Dark	17	Brown
Stout	35 and higher	Black

Source: Papazain (2003).

Principle Grist Ingredient

Grist usually refers to malted barley that may be supplemented with various adjuncts including corn, rice, and sorghum. Adjuncts are added to the beer formula to provide an alternate carbohydrate source and reduce costs. Traditional German *Weizenbier* or *Weissbier* is made from malted wheat, and beer in Africa is traditionally produced from sorghum (Bamforth 2006).

Region of Initial Production

The region of original production can have a great effect on a beer style (Table 1.8). Climate and geography cause variation in ingredients as well as the water source. For example, the traditional Pilsner comes from the Pilsen region of old Bohemia known for very soft water (Papazain 2003).

Technological Influence

Technological influence refers to modern technologies applied to beer production. This includes the production of light beer using enzymes to convert long chain dextrins into fermentable carbohydrates; this decreases the amount of carbohydrates, thereby reducing the caloric content. Other technologies include items such as ice beer, which freezes the beer to increase alcohol content.

Table 1.9 Major beer styles of the world[a].

Style	Origin	Notes
ALES & STOUTS		
Bitter (pale) ale	England	Dry hop, bitter, estery, malty, low carbonation (on draught), copper color
India Pale Ale	England	Similar to bitter ale, but substantially more bitter
Alt[b]	Germany	Estery, bitter, copper color
Mild (brown) ale	England	Darker than pale ale, malty, slightly sweeter, lower in alcohol
Porter	England	Dark brown/black, less "roast" character than stout, malty
Stout	Ireland	Black, roast, coffee-like, bitter
Sweet stout	England	Carmel-like, brown, full bodied
Imperial Stout	England	Brown/black, malty, alcoholic
Barley wine	England	Tawny/brown, malty, alcoholic, warming
Kölsch	Germany	Straw/golden color, caramel-like, medium bitterness, low hop aroma
Weizenbier[c]	Germany	Hefeweissens retain yeast (i.e., turbid). Kristalweissens are filtered. Very fruity, clove-like, high carbonation
Lambie	Belgium	Estery, sour, "wet horse-blanket," turbid. Lambie may be mixed with cherry (kriek), peach (peche), raspberry (framboise), etc. Old lambie blended with freshly fermenting lambie is called *gueuze*
Saison	Belgium	Golden, fruity, phenolic, mildly hoppy
LAGERS		
Pilsner	Czech Republic	Golden/amber, malty, late hop aroma
Bock	Germany	Golden/brown, malty, moderately bitter
Helles	Germany	Straw/golden, low bitterness, malty, sulfury
Märzen[d]	Germany	Diverse colors, sweet malt flavor, crisp bitterness
Vienna	Austro-Hungary	Red-brown, malty, toasty, crisply bitter
Dunkel	Germany	Brown, malty, roast-chocolate
Schwarbier	Germany	Brown/black, roast malt, bitter
Rauchbier	Germany	Smokey
MALT LIQUOR	United States	Pale color, alcoholic, slightly sweet, low bitterness

[a]From Bamforth, C. (2005) Food, Fermentation and Micro-organisms, Blackwell, Oxford, UK.
[b]Meaning "old."
[c]Wheat beer.
[d]Meaning "March," for when it is traditionally brewed.
Source: Bamforth (2006).

Malting and Brewing Processes

Malting

Malting is the controlled germination of the grain followed by the controlled drying of a seed. The goal of malting is to produce high enzyme activity, endosperm modification, and a characteristic flavor with a minimum loss of dry weight (Hoseney 1994). When the kernel is moistened, the embryo and endosperm become hydrated switching on embryo metabolism. Subsequently, a hormonal signal triggers the synthesis of enzymes responsible for digestion of starch endosperm, as a source of energy for the developing embryo. As the growth process proceeds, enzymes break down cell walls and some of the protein in the starchy endosperm, the grain's food reserve, causing the grain to become more friable. The enzymes produced, especially amylases, are important for breaking down the starch during the mashing process in the brewery (Bamforth 2006).

Owuama (1999) reported that important starch degrading enzymes are α- and β-amylase, limit dextrinase and α-glucosidase. α-glucosidase is present in germinating grains and causes the hydrolysis of terminal, non-reducing α-(1→4) glucosidic linkages in both oligosaccharides and α-glucans yielding glucose.

Commercial malting consists of three stages: steeping, germination, and kilning (Figure 1.10 and 1.11). Steeping, considered the most important stage of the malting process, occurs when water is introduced to the kernel and allowed to penetrate the center of the kernel, increasing moisture content of the grain to a sufficient level to allow metabolism to be triggered in the grain (Dewar et al. 1997). During steeping, the grain requires sufficient oxygen for respiration; therefore, the grain is not submerged

constantly. "Air rest" refers to this period of time when the grain is removed from the steeping solution (Bamforth 2006). The air rest helps to remove carbon dioxide and ethanol, which decreases respiration and deters bacteria and mold growth. Air rests are important because oxygen is necessary to the formation of α-amylase and peptidases that can be inhibited by excessive carbon dioxide, even in the presence of oxygen (Dewar et al. 1997). Important factors in successful initiation of germination are adequate moisture and temperature, and the presence of oxygen (Dewar et al. 1997). At the germination stage, grain is removed from water and placed in germination beds (Hoseney 1994). Germination is visible when rootlets emerge. When sufficient germination has occurred, the grain is dried in a process labeled kilning. The goal of kilning is to slowly heat grain to lower the moisture content stopping the growth process (Bamforth 2003). Time and temperature control of the kilning process is important to preserve enzymes present. When the moisture has been sufficiently reduced, the temperature is raised, causing Maillard and caramelization reactions to occur in the grain. The grain not only darkens in color but also develops unique flavors especially evident in ale-style beer. In the final beer, this contributes greatly to color and flavor (Bamforth 2003). A milder kilning regime is used for lager malts. For both ale and lager malts, kilning is sufficient to eliminate the unpleasant raw, grassy, and beany characteristics associated with green malt (Bamforth 2006; Owuama 1999). When kilning is complete, the grain is allowed to cool, then the malt is "dressed," which is the mechanical removal of the rootlets, dust, broken kernels, and contaminants (Bamforth 2006).

An important factor to consider when malting is to choose varieties of grain that are suitable to the process (Ogu et al. 2004).

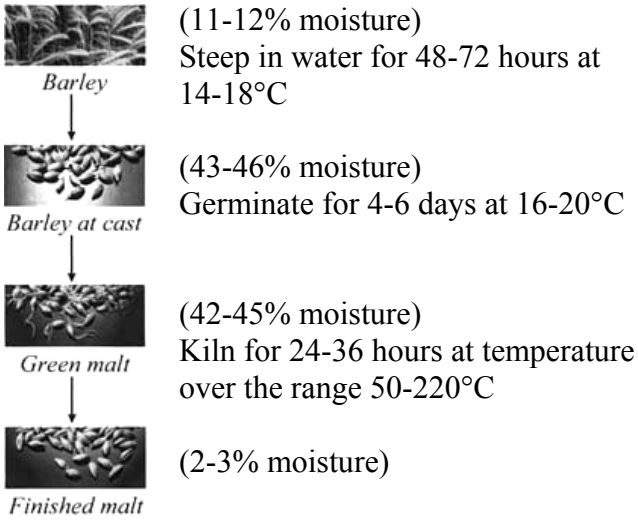


Figure 1.9 Diagram of barley malting process.
Source: Bamforth (2003).

Barley Malting

Malting barley makes the grain suitable for beer production because malting improves the grain for brewing by breaking down the cell walls and proteins that enclose the starches.

During germination, the plant hormone, gibberellic acid, signals the aleurone layer to produce endosperm-degrading enzymes such as α-amylase, protease, pentosans, and endo-β-glucanase (Ogbonna 1992). During malting, barley develops several amylolytic enzymes (α-amylase and β-amylase). Development of these enzymes and the ratio to one another determines the amounts of glucose and maltose produced during mashing. A study by Agu (2005) indicates that there is a direct relationship between the amounts of α-amylase enzymes developed in malted barley to the level of extract recovered from the malt. The study found a relationship between the level of β-amylase

released in the malted barley and the ratio of glucose to maltose sugars present in the wort; this relationship was found to be similar to that in other malted grains (Agu 2005).

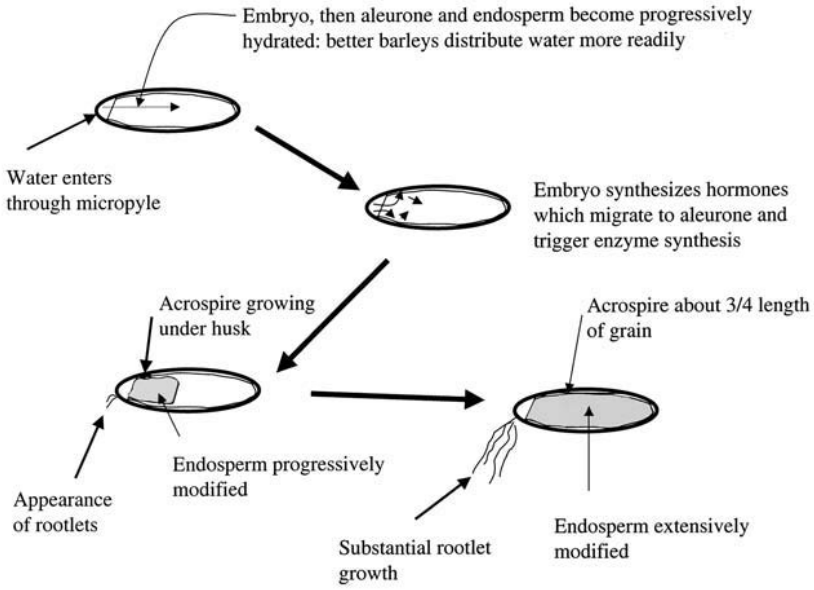


Figure 1.10 Diagram of barley malting process.
Source: Bamforth (2003).

Sorghum Malting

Sorghum malting is best performed with viable grain, that is not of the tannin containing variety and that has been placed in storage (Daiber and Taylor 1995). Storage of sorghum for two to three years at 12-23°C gives a higher level of amylases (57-73%); approximately 25% higher compared to newly harvested grains (Owuama 1999; Ogbonna 1992; Novellie 1966).

Sorghum malting yields high proportions of hydrolytic enzymes such as α-glucosidase, and α- and β-amylases (Table 1.9) (Owuama 1999). Agu (2005) reported that when sorghum grain is malted, sufficient hydrolytic enzymes are produced to extract

the sugars and proteins needed for beer production. Initial studies on the malting of sorghum did not employ a definite malting temperature. This lack of consistency slowed the development of sorghum as a source of malt for brewing procedures (Agu 2005).

Enzyme development during germination of sorghum differs from that of barley. In the germination process of barley, hormonal signals cause the production of endosperm degrading enzymes in the aleurone layer. Ogbonna (1992) reported that in sorghum, production of α-amylase and carboxypeptidases are produced by the scutellum. Endo-β-glucanase, limit dextrinase, an endo-protease enzyme development occurs in the starchy endosperm. In sorghum malting, α-amylase is produced in embryos of sorghum while β-amylases are activated from latent form in starch endosperm (Owuama 1999). Another difference in the malting of barley versus sorghum is evident in the microscopic studies of the endosperm of the malted grain. Malted barley cell walls are degraded extensively whereas sorghum cell walls are left intact except for small portals through which amylolytic and proteolytic enzymes pass to degrade starch and protein reserves (Ogbonna 1992).

Enzymes in Sorghum Malt

Owuama (1999) reported that sorghum malt contained the highest levels of α-glucosidase activity whereas the sorghum wort produced had the lowest level of glucose – which may suggest that α-glucosidase is not the dominant glucose producing enzyme. Lipase converts free fatty acids to hydroperoxides and aldehydes that have detrimental effects on beer. Lipase activity varies among different sorghum cultivars and decreases after kilning (Owuama 1999). Peroxidases formed during germination are important in sorghum beer to prevent the formation of lipid oxidation products during mashing that

are detrimental to nutrients available to yeast along with beer flavor and colloidal stability (Owuama 1999). Endopeptidases including carboxypeptidases and proteinases are important in grain germination to hydrolyze proteins into free α-amino nitrogen (FAN), which is necessary for yeast metabolism (Owuama 1999).

Dewar et al. (1997) reported that steeping conditions applied to sorghum brewing indicates malt quality. Measured parameters of diastatic power, FAN content, and extract increased when steeping time was increased from 16 to 40 h and optimum temperature was between 25° and 30°C. The study showed that aeration during steeping was necessary to maximize malt quality. Quality of sorghum malt was found to be directly related to steep-out moisture of the grain. Additionally, steeping temperature is related to cultivar and is important to malt quality.

Agu and Palmer (1996) reported the relationship between germination temperature and β-amylase production. The studies found that more β-amylase is present in sorghum malts produced at 25°C and 30°C, producing 66% more maltose during mashing than malts produced at 20°C.

Other key factors to sorghum malting are the treatment of the grain prior to malting to reduce microbial growth (Okungbowa 2002). Steeping is a very important aspect of sorghum malting, and dilute alkaline steeping improves the overall quality of the malt (Obeta 1999). A study by Lefydei and Taylor (2006) indicated that steeping in 0.2% sodium hydroxide solution reduced fungal and bacterial contamination without causing cycotoxicity, along with increasing the diastatic power by increased water absorption in the grains (Figure 1.13).

Optimum germination temperature of sorghum is about 25°C (Palmer et al. 1989). In sorghum malt, this temperature is optimal for amylase and diastatic power development while encouraging vigorous respiration and high malting losses (Owuama 1999).

Malting quality of sorghum is determined by physical and biochemical factors such as temperature and time of steeping and kilning temperature (Owuama 1999).

Increase in diastatic power, FAN, extract, and malting loss with germination time is seen in sorghum malts from grains steeped with air rest period and steep out moisture of 33-35%, and with high moisture during germination, along with germination temperatures of 24-28°C.

Figure 1.11 Malted grain sorghum.

Source: Original photograph by Chris Martens, USDA-ARS Cereal Crops Research Unit (2008).

Table 1.10 Comparison between sorghum malt and barley malt.

Factor	Sorghum malt	Barley malt
Starch gelatinization Temperature range (°C)	64-68[a]	55-59[a]
Diastatic power	19[b]	53[c]
(°Lintner)	20[d]	80[d]
β-Amylase activity (°Lintner)	10[e]	56[e]
α-Amylase activity	53[d]	35[d]
(Dextrinizing units)	29[f]	24[f]

[a]From Taylor (1989).
[b]Determined by standard method for sorghum South African Bureau of Standards (1970).
[c]Determined by EBC method European Brewery Convention (1987).
[d]From Palmer et al. (1989).
[e]Determined by modified sorghum diastatic power method Taylor (1990).
[f]Determined by Phadebas method Axcell (1979).
Source: Taylor (1992).

Traditional and Modern Barley Brewing

Brewing begins with the milling of malt to reduce endosperm particle size for extraction shown in Figure 1.13. The goal of malt milling is to produce a particle size distribution that is best suited for the subsequent process in the brew house (Bamforth 2003). The brew house process can vary from brewery to brewery and require different particle size. The reduction of particle size allows for water to better penetrate the kernel. It is important to produce grist and not flour, as flour will cause problems in the brewing process. In barley milling there must be a compromise between endosperm particle size reductions and keeping the husk intact for good filtration (Bamforth 2006).

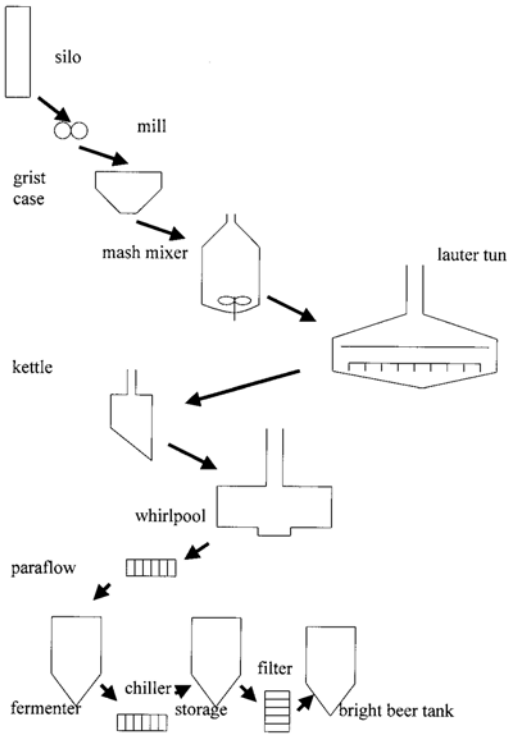


Figure 1.12 Overview of the brewing process.
Source: Bamforth (2003).

Following milling, the ground malt, now referred to as grist, is transferred to the mash tun, where grist is mixed with warm water to begin enzymatic hydrolysis. Mashing is the process where warm water is mixed with grist at various temperatures, allowing enzymes to convert starches to sugars, solubilize proteins and high molecular weight substances, and dissolve sugars creating wort (Table 1.10) (Bamforth 2006; Owuama 1999). Enzymes (amylases, proteases, peptidases, transglucosidases, and phosphorlyases) carefully controlled by temperature, pH, time, and concentration of the wort hydrolyze carbohydrates and proteins, shown in Figure 1.14 (Owuama 1999). There are several types of mashing procedures including infusion, decoction, double mash, and temperature programmed. Infusion mash is the simplest and is traditionally used for English ales (Briggs et al. 1981). Infusion mashing combines grist and warm water at a

single holding temperature where starch gelatinization occurs. At this temperature the enzymatic conversion of starch to sugars occurs. After a set amount of time, the mash is separated into spent grains and wort by filtration. The second type of mashing is decoction mashing, which was developed in ages past where malt quality was not as consistent as modern day and temperature could not be accurately measured (Cordey 2006; Palmer 2006). The decoction mash process begins at a lower temperature, which hydrolyzes the β-glucans. The temperature is then raised to gelatinize starch, followed by further enzymatic hydrolysis. A graph of the process illustrates a stepwise increase in temperature over certain periods of time (Figure 1.15). After mash-in (combining of grist and water), the mixture, referred to as mash, is allowed to rest. Then a portion, referred to as a decoction, of the mash is removed and heated. When the decoction is added back to the original mash, the entire mash temperature rises to the next step. This is repeated until mash out, where the temperature is raised to stop all enzymatic process. In temperature-programmed mashing, the stepwise increase of decoction mashing is followed. However, modern technology means this can occur in a single vessel using a steam-jacketed mash tun, shown in Figure 1.15 (Bamforth 2006).

Table 1.11 Basic mashing process.

Thermal process	Process time	Biochemical action	Importance of action
Mash in at 35°C	30 min rest	Hydrate malt	
Raise to 45°C	Hold for 20-30 min	Allows enzymes susceptible to heat to act	Proteolytic enzymes
Raise to 60°C	Hold for 20-30 min	Optimum temperature for β-amylase	
Raise to 70°C	Hold for 20 min	Maximum conversion of starch to maltose	Also, modification of barley protein

Source: Hoseney (1994).

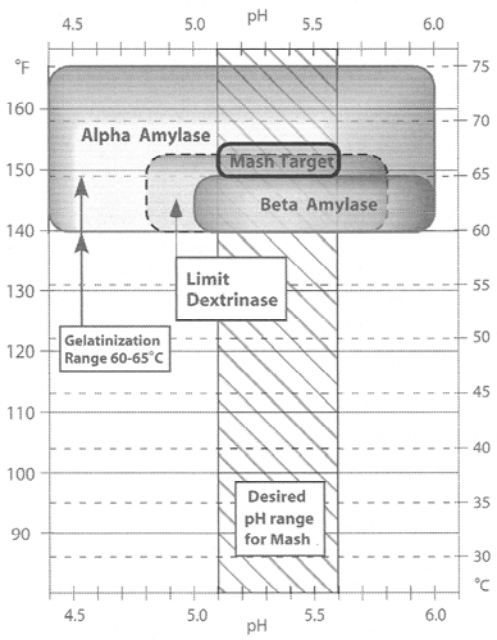


Figure 1.13 Chart of enzyme activity in relationship to pH and temperature.
Source: Palmer (2006).

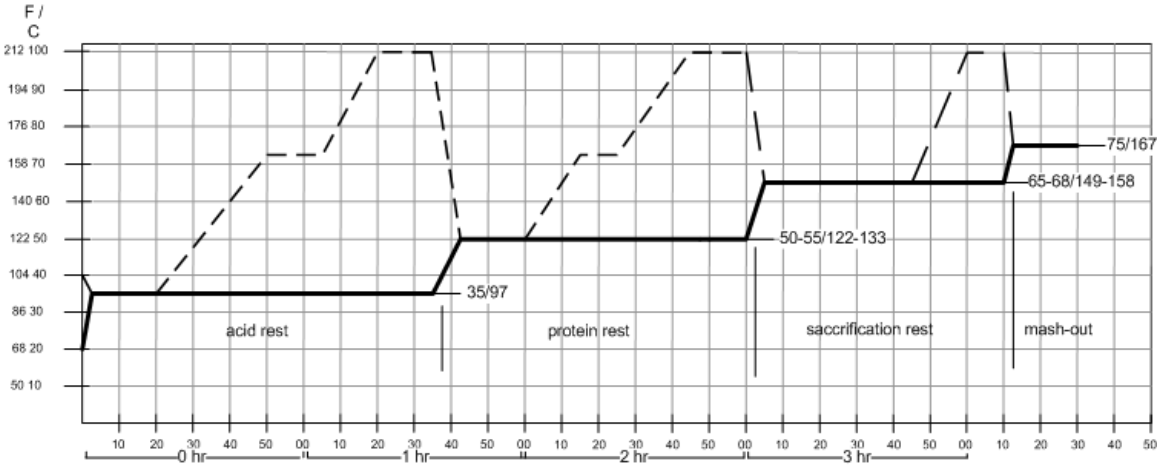


Figure 1.14 Diagram of a triple decoction mash.
Source: BrauKaiser (2008).

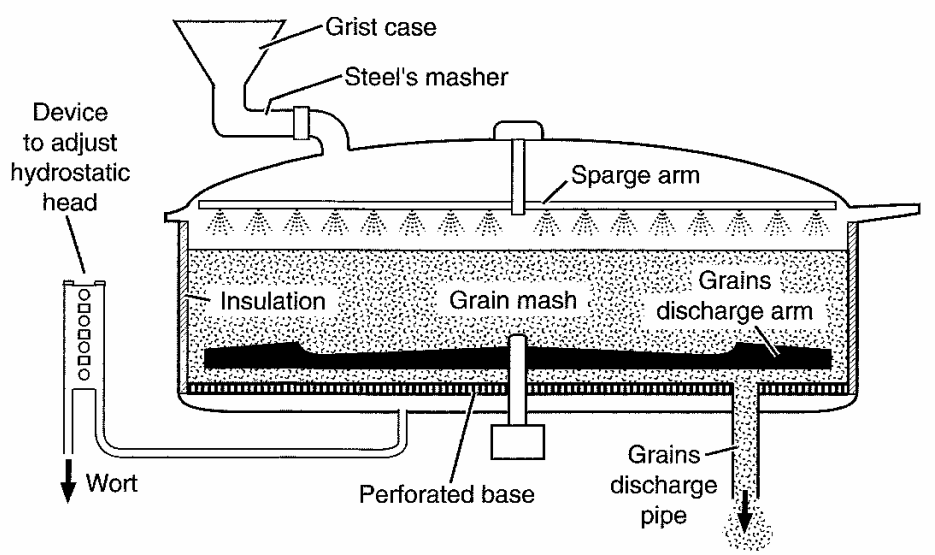


Figure 1.15 Diagram of a mash tun.
Source: Bamforth (2006).

After mashing, the spent grains and wort are separated. This process is referred to as lautering and occurs in a vessel called a lauter tun shown in Figure 1.16. The simplest explanation of a lauter tun would be a cylindrical vessel with a strainer at the bottom. In modern brewery systems this can be a straight-sided cylindrical vessel with a slotted base

and run-off pipes through which the wort is recovered. Lautering begins with a procedure called vorlauf, in which wort is recycled through grain to set the filter bed of husk and grist in the lauter tun, which will clarify the wort. The next step is to add water, called "sparge," to rinse the grist. Sparge water is hotter than the mash-out temperature and is sprayed onto the grains to dissolve remaining sugars into the wort. Wort is slowly drained from the lauter tun and is collected in the brew kettle.

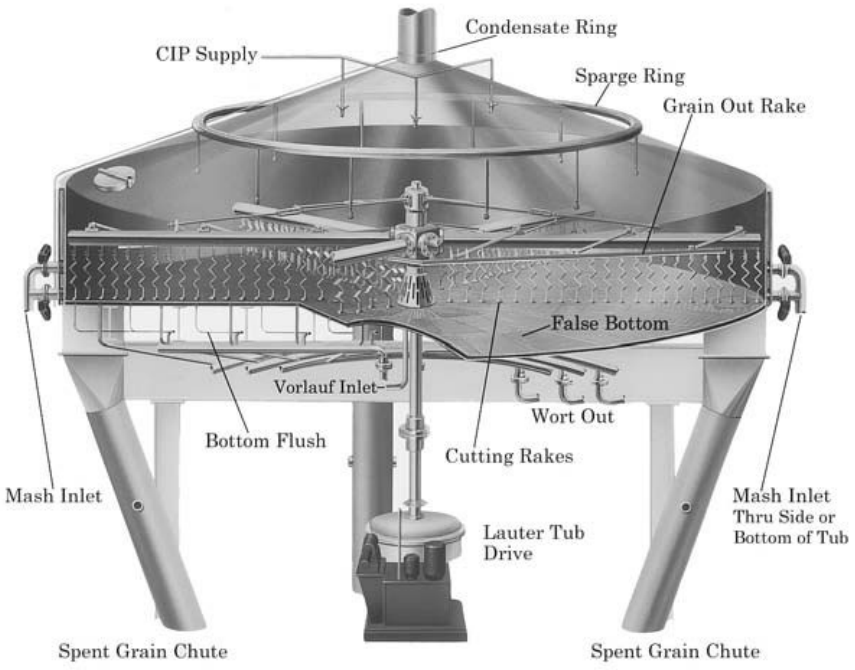


Figure 1.16 Diagram of a lauter tun.
Source: Bamforth (2003).

The separated wort is boiled. At this point several events are taking place, including: inactivation of remaining enzymes, isomerization of α-acids from hops, sterilization, precipitation of proteins called the "hot break," concentration of the wort, and color formation (Bamforth 2006). At this point in the brewing process, hops are

added to the wort. The heat of the boil isomerizes the α-acids, imparting a bitter flavor to the beer. Heat sterilizes the beer to ensure that there are no other microorganisms that will later compete with the yeast. During the boil, proteins present begin to coagulate. Due to high heat the protein from the malt cross links with tannins from malt and hops. Visible precipitation of the protein coagulation is referred to as the "hot break." Removal of these proteins reduces the visible haze in the final beer (Bamforth 2003). Once the wort has sufficiently boiled, spent hops are removed through the creation of a whirlpool or other filtration device.

After the boil, the wort must be cooled and aerated before yeast can be added. During cooling, the wort reaches a point called the "cold break" where protein precipitates out of the solution. After cooling, measurements are taken on the cooled wort as check-points for calculation of sugar content and potential alcohol content. At this point, it is very important to maintain the sterility of the wort by thoroughly cleaning fermentation vessels to minimize bacterial infection.

Pitching refers to the addition of yeast slurry to the cooled wort. Once yeast is added, the wort is referred to as beer. Primary fermentation is the first stage of fermentation, where yeast converts the sugars into alcohol and carbon dioxide, along with other by-products that contribute to flavor, shown in Equation 1.3. Rate of fermentation is dependent on strength of the wort, yeast pitching rate and viability, oxygen, and temperature (Bamforth 2006). At this point vigorous fermentation is visible, and a foam called the krausen forms on top of the wort. As fermentation continues, both specific gravity and pH decline and can be measured to indicate the rate at which yeast is growing.

Post Fermentation Processing:

Once fermentation is complete, beer is carefully siphoned off from the yeast that has settled to the bottom of the fermentation vessel. From here, the beer can proceed to secondary fermentation, if necessary. Secondary fermentation most often occurs in lager beer brewing. During secondary fermentation, yeast produces additional compounds that contribute to flavor and increase carbonation.

Following secondary fermentation, the beer is often filtered and possibly pasteurized, then bottled or kegged. Some processers pump carbon dioxide into the package, while others allow remaining yeast cells to further carbonate the beer in the container through a process called bottle conditioning.

Equation 1.3 Fermentation Equation.

$$C_6H_{12}O_6 \rightarrow 2\ CO_2 + 2\ CH_3CH_2OH + 22\ \text{calories}$$

Source: Hoseney (1994).

Traditional African Sorghum Brewing

Sorghum beer is brewed most predominantly in Africa, although Ogbonna (1992) references beer produced in Mexico, India, and Sri Lanka whose success has stimulated awareness of the brewing potential of sorghum.

Traditional sorghum beer production in Africa is commonly referred to as opaque beer and is identified as Bantu beer, kaffir beer, utshwala, joala, busaa, and dolo, depending on the region (Daiber and Taylor 1995). Novellie (1962; 1966) stresses that sorghum beer utilizes different ingredients and techniques compared to traditional barley

beer and thus bears little resemblance. Opaque sorghum beer is not hopped like conventional beer (Taylor 1992). This beverage is produced in homes and in local villages. Opaque beer is an important source of energy and nutrition for the African people because of the high level of complex carbohydrates and nutrient content (Daiber and Taylor 1995; Novellie 1966; Taylor 1992; Kayodé et al. 2007). The source of fermentable carbohydrate is most commonly malted sorghum or millet (Daiber and Taylor 1995). Traditionally, sorghum malting occurs outdoors by placing the steeped sorghum grain in thin layers on covered or uncovered floors. Following a 4-6 day germination period, the green malt is dried in thin layers in the sun (Daiber and Taylor 1995). The traditional brewing process utilizes malted grain sorghum and two different fermentations. The first fermentation is by lactic acid bacteria to produce lactic acid, which provides the characteristic flavor and lowers the pH, thus reducing microbial growth. The second fermentation is by yeast, *Saccharomyces cerevisiae*, to produce alcohol (Watson and Novellie 1975).

A study by Novellie (1966) utilized the following process for sorghum beer production. Processing begins with the souring step by combining sorghum malt and water; a cereal adjunct may be added to the mixture. The mixture undergoes lactic acid bacteria fermentation at 50°C until the pH decreases to 3. The soured mixture is then diluted and boiled. After cooling the mixture to 60°C, additional malt is added and the mixture is mashed for 2 h. During the mash, not all of the starch is hydrolyzed, which yields a high viscosity beverage, characteristic of opaque beer. The mash is then cooled to 30°C and pitched with *Saccharomyces cerevisiae* (Daiber and Taylor 1995). A

different opaque sorghum beer brewing process, the Reef-type sorghum process, is shown in Figure 1.19 (Taylor 1992).

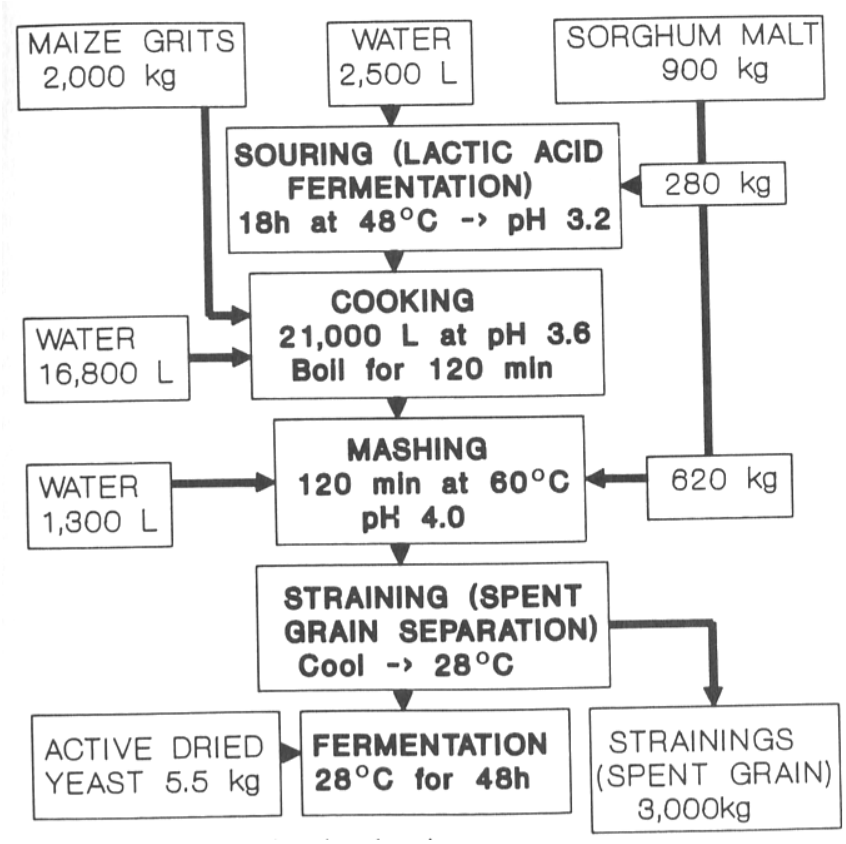


Figure 1.17 Reef-type sorghum beer brewing process.
Source: Daiber and Taylor (1995).

Brewing Conventional Beer with Sorghum

A number of the many varieties of sorghum work well as sorghum malt. These varieties possess beneficial qualities for beer brewing, such as good diastatic power, α- and β-amylase activities, and extract recovery (Owuama 1999).

Brewing beer with malted grain sorghum is best achieved with several modifications to the traditional brewing procedure due to higher starch gelatinization

temperature of starch versus barley (Agu 2005). Taylor (1992) reports that the starch gelatinization temperature for sorghum malt starch is 64-68°C, while barley is 55-59°C. Ogbonna (1992) reviews several studies that found that an increase in gelatinization and saccrification temperatures along with the development of decantation mashing procedures improved sorghum beer studies. Igyor (2001) found that increasing the mash temperature to 100°C during decoction produced a better beer with more alcohol and flavor components.

Sorghum Beer Brewing Process

The method for production of ale-style gluten-free beer from malted grain sorghum was developed on a laboratory scale after review of several sorghum brewing studies.

Taylor (1992) evaluated several mashing methods, including the traditional Reef-type sorghum beer brewing process, constant temperature infusion mashing, rising temperature mash, and a triple-decoction mash. Analyses of diastatic power and fermentable sugars indicated the triple decoction mashing procedure produced the highest extract and fermentable sugars. Taylor (1992) reasons that decoction type mashing is effective because the removal and boiling of portions of the mash causes the starch to gelatinize, enabling enzymes to convert starch to sugar. The 60°C mashing period enables β-amylase to act on the starch, while the gelatinization that occurs during the boiling of decoction enables α-amylase to saccrify the starch. The study also showed that higher temperatures and calcium ions increased extract and fermentable sugars. Taylor (1992) also found that removal of rootlets is important to sorghum beer production. Rootlets or vegetative parts cause a strong grassy flavor to be present in the beer.

A study by Igyor (2001) showed that the mashing procedures developed for barley are not suitable for sorghum malt. The study utilized barley malt and sorghum malt steeped at two temperatures, 20°C and 25°C, by evaluating three different mashing procedures: infusion at 65°C, decantation mashing at 80°C, and decantation mashing at 100°C. Evaluation of the wort and beer indicated the decantation mashing at 100°C produced better wort due to better starch gelatinization. Igyor (2001) also found the flavor compounds of the sorghum beer mashed at 100°C similar to those of malted barley beer.

Osorio-Morales et al. (2000) evaluated four different types of waxy, heterowaxy, normal, and brown sorghums as adjuncts for cost reduction in the Mexican brewing industry. The formulation included 63.3% sorghum brewing adjuncts and 36.7% commercial diastatic malt and employed a double-extraction mashing method. The study proved the mashing method to be effective in the evaluation of sorghum malts.

Daiber and Taylor (1995) reports and diagrams several mashing schedules to employ unmalted sorghum as an adjunct for barley brewing. Other considerations when brewing with sorghum include finding solutions to filtration problems during the sparge and breeding of sorghum varieties suitable for malting and brewing.

A study by Pozo-Insfran et al. (2004) evaluated enzyme addition on wort composition in sorghum lager beer. The study reports the positive attributes of waxy sorghum but also the negative attributes such as difficulty in hydrolysis, which causes problem with filtration and beer haziness. Results indicated that producing a sorghum lager is feasible when ingredients are carefully selected.

Research by Okafor and Aniche (1987) evaluated the brewing of lager beer from Nigerian sorghum. A triple decoction mashing method was used. Wort was analyzed on brewing day and throughout fermentation, a taste panel was conducted, and shelf life was evaluated. Results compared to barley wort indicated sorghum worts were similar, and sorghum was suitable for producing lager beer.

Demuyakor et al. (1994) utilized a triple decoction procedure to evaluate various ratios of malted sorghum to malted barley in beer production; see Figure 1.20 for the decoction graph. Conclusions of the study stated that increasing the amount of sorghum caused filtration problems, and the high level of FAN found in sorghum wort does not have adverse effects on the final product.

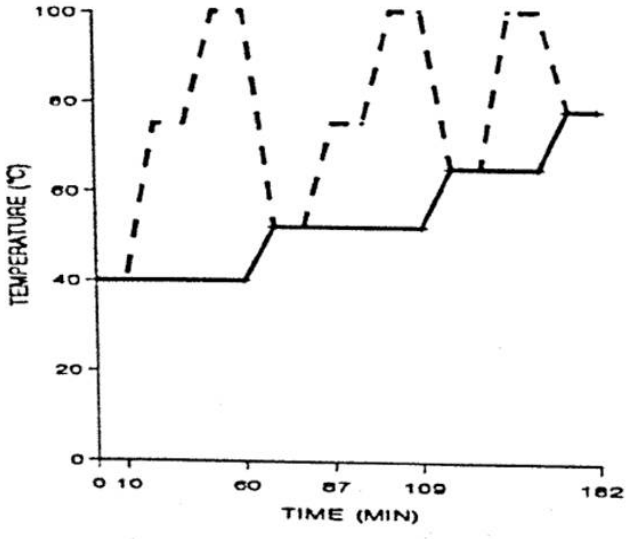


Figure 1.18 Mashing process used for a 66:34 sorghum-barley malt blend.
Solid line = temperature in mash tank
Dotted line = temperature in boiling kettle during decoction
Source: Demuyakor (1994).

Background on Analytical Tests and Methods Chosen

α-amylase

α-amylase, the liquefying amylase, is an endoenzyme hydrolyzing α 1→4 bonds within amylose and amylopectin that develops during the germination phase of malting (Bamforth 2006; Owuama 1999). α-amylase activity in sorghum malt is 25-183 Units/g depending on sorghum variety and increases with sorghum diastatic power. Diastatic power is measured in sorghum diastatic units (SDU) in cultivars with SDU values greater than 30 (Owuama 1999). Studies have shown that sorghum malt α-amylase levels are correlated with steeping and kiln conditions (Okungbowa et al. 2002). Aisien and Ghosh (1978) report that α-amylase rather than β-amylase is the primary amylase that degrades starch during malting. Pozo-Insfran et al. (2004) discuss conflicting research where one study found that barley malt has 3-4 times more diastatic and β-amylase activities than sorghum malt, and another study evaluated 16 different sorghums and found two genotypes that had amylase activities similar to those of barley malt.

β-amylase

Okungbowa et al. (2002) reported that β-amylase is the key saccrifying enzyme in brewers' malt. β-amylase, the saccharifying amylase, catalyzes the hydrolysis of α(1→4) glucosidic bond at a non-reducing end of polysaccharides, causing the release of maltose. Non-germinated sorghum grain exhibits virtually no β-amylase activity (Owuama 1999). β-amylase activity in sorghum malt is 11-41 SDU/g and constitutes 27-49% of total diastatic activity (Owuama 1999). Novellie performed studies on the production of beer from malted grain sorghum in the late 1950s and 1960s. In these studies, Novellie

investigated amylase activity in sorghum malt and found 18-30% of the saccrifying activity of sorghum malt was due to β-amylase (Ogbonna 1992). Taylor (1992) reports that the low ratio of β-amylase to α-amylase in sorghum malt along with mashing at high temperatures produces worts rich in complex carbohydrates and low in fermentable sugars, shown in Table 1.11. Pozo-Insfran et al. (2004) reports that this negatively affects wort properties.

Table 1.12 Wort composition after mashing with sorghum malt extracts.

Factor	Control (α- and β-Amylase)[a]	α-Amylase only[a]	β-amylase only[a]
Extract	9.91	9.59	7.77
Total fermentable sugars	6.92	1.74	2.46
Free glucose	0.17	0.18	0.15

[a]Grams per 100g of wort.
Source: Taken from Taylor (1992).

HPLC

High Performance Liquid Chromatography (HPLC) is an effective analytical technique that can be applied to the analysis of any compound with solubility in a liquid that can be used as the mobile phase. HPLC has often been used in the food industry to separate and identify sugars (Rounds and Gregory 2003). Taylor (1992) used HPLC analyses to measure water-soluble carbohydrates in beer wort produced from the Reef-type sorghum brewing process. Results showed that the fermentable sugars glucose, maltose, and maltotriose are present in approximately the same ratio as barley malt wort, 1:3:1. Taylor (1992) reports that maltose is the predominant fermentable sugar in sorghum beer wort. HPLC was utilized by Pozo-Insfran et al. to determine the glucose,

maltose, and maltotriose to be 35%, 48%, and 17% respectively. Comparison of wort sugars using HPLC was utilized for a study evaluating different mashing procedures (Igyor 2001). Fermentable sugar composition for sorghum wort was determined using HPLC (Dufour et al. 1992; Figueroa et al. 1995).

Free α-amino Nitrogen (FAN)

Evaluation of free-amino nitrogen (FAN) content in wort indicates how well yeast can grow and reproduce. Owuama (1999) reports that a high level of FAN in wort is necessary to support rapid and proper fermentation. Taylor and Boyd (1986) define FAN as the product of protein degradation. Dewar et al. (1997) defines FAN as the protelolytic break down of endosperm proteins, composed of amino acids and small peptides which serve as the nitrogen source for the yeast. FAN is necessary for yeast to synthesize structural and enzymatic proteins required for normal growth as well as the metabolic processes that affect the flavor and stability of beer (Taylor and Boyd 1986; Pickerell 1985). Dewar et al. (1997) writes that FAN is one of the primary terms to define sorghum malt quality for beer brewing. Pickerell (1986) studied the interactions of FAN and sugar in sorghum beer fermentations. Results showed the exhaustion of nitrogen is the limiting factor in yeast fermentations. The study also found correlations between initial FAN content of wort and the rate of ethanol production. Taylor and Boyd (1986) evaluated FAN in the traditional Reef-type production of sorghum beer. The findings indicated that FAN was produced in malting and mashing, and alterations in mashing procedures were reflected in the FAN levels. The study also found that wort FAN is correlated with malt FAN and can be used to evaluate sorghum malts.

Malting Loss

Owuama (1999) defines malting loss as the summation of leaching/steeping, metabolic/respiration, and vegetative/sprout losses. In short, malting is the loss in the weight of grains after malting. In sorghum malt, high malting loss is linked to good diastatic power.

Single Kernel Characterization System (SKCS)

Single Kernel Characterization System (SKCS) was developed for the objective classification of hard and soft classification in wheat grading (Osborne and Anderssen 2003). The instrument is composed of an indented wheel and vacuum which separates each individual kernel. The kernels are subsequently weighed then crushed between a toothed rotor and crescent, shown in Figure 1.20 (Osborne and Anderssen 2003). During the sorghum malting process, the enzymes produced begin to break down the starch and protein reserves, subsequently causing a decrease in the density of the caryopsis and reducing milling energy (Ogbonna 1992, Owuama 1999). Sorghum endosperm is composed of two regions: vitreous and floury. The vitreous portion of the endosperm correlates with grain hardness, which influences grain milling energy and malt milling energy since this portion is largely under-modified during malting. As a result, a positive correlation between grain milling energy and malt milling energy (Owuama 1999). A loss in malt milling energy caused by starch granule modification during malting may be responsible for the significant correlation between diastatic power and malt milling energy (Owuama 1999).

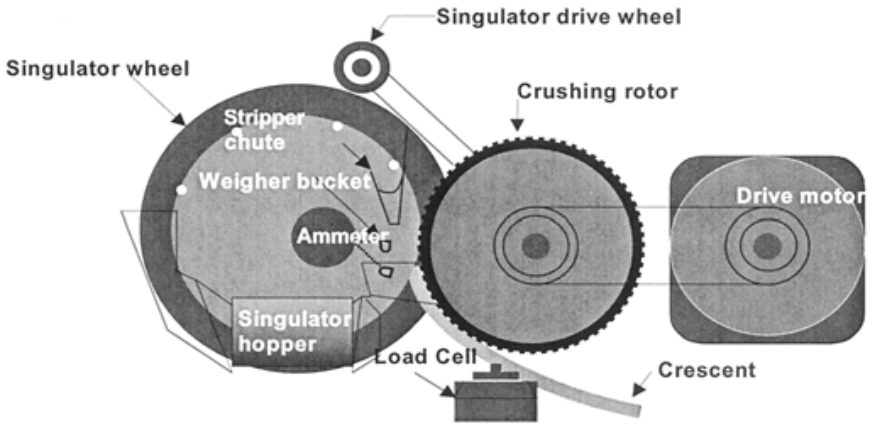


Figure 1.19 Diagram of crushing mechanism within the SKCS 4100 instrument. Source: Osborne and Anderssen (2003).

DSC

Differential Scanning Calorimetry (DSC) is one of the most commonly used methods of thermal analysis in food products (Schnez 2003). The analysis measures the differential temperature to and from a sample versus a reference material that is displayed as a function of temperature or time. Akingbala et al. (1988) reported the technique detects the heat flow associated with order-disorder transitions to quantify geleatinization. One application of DSC is starch analysis. A study by Mestres et al. (1996) evaluated a method developed to determine amylase content of starches using DSC.

References

Agu, R.C., Palmer, G.H. 1996. Enzymic breakdown of endosperm proteins of sorghum at different malting temperatures. Journal of the Institute of Brewing. 102:415-418.

Agu, R.C. 2005. Some relationships between amylolytic enzymes developed in malted barley, extract recovery, and sugar profile. Technical Quarterly of the Master Brewers Association of the Americas. 42(4):315-318.

Agu, R.C. 2005. Some relationships between enzyme development, extract recovery, and sugar profile in malted sorghum. Technical Quarterly of the Master Brewers Association of the Americas. 42(2):120-124.

Aisen, A.O., Ghosh, B.P. 1978. Preliminary studies of the germination behavior of guinea corn (Sorghum vulgare). Journal of Food Science and Agriculture. 29(10):850-852.

Akingbala, J.O., Gomez, M.H., Rooney, L.W., Sweat, V.E. 1988. Thermal properties of sorghum starch. Starch/Stärke. 40(10):375-378.

Anheuser-Busch, Inc. Brewers of Redbridge Beer. Redbridge Beer. www.redbridgebeer.com (accessed Feb 19, 2009).

Bamforth, C.W. 2003. Beer: tap into the art and science of brewing [Online]. Oxford University Press. New York, NY. http://www.KSU.eblib.com/EBLWeb/patron/?target=patron&extendedid=P_279517_0 (accessed Feb 4, 2009).

Bamforth, C.W. 2006. Scientific principles of malting and brewing. American Society of Brewing Chemists. St. Paul, MN.

Bard's The Original Sorghum Beer. www.bardsbeer.com (accessed Feb 19, 2009).

Beta, T., Rooney, L.W., Waniska, R.D. 1995. Malting characteristics of sorghum cultivars. Cereal Chemistry. 72(6):533-538.

Braidwood, R.J., Suaer, J.D, Helback, H., Mangelsdorf, P.C., Cuter, H.C., Coon, C.S., Linton, R., Steward, J., Oppenheim, A.L. 1953. Did man once live by beer alone? American Anthropologist. 55(4):515-526.

BrauKaiser. 2009. Decoction mashing (part 1), decoction mash (part 2), decoction mash (part 3). http://braukaiser.com/wiki/index.php?title=Decoction_Mashing (accessed Apr 28, 2009).

Brewers Association. Craft Brewing Statistics. http://www.beertown.org/craftbrewing/statistics.html (accessed Mar 26, 2009, Apr 16, 2009).

Briggs, D.E., Hough, J.S., Steven, R., Young, T.W. 1981. Malting and brewing science, Volume 1, 2nd Ed. Chapman and Hall, New York, NY.

BurnSilver, G. 2007. Gluten-free brewing. Brew Your Own. March-April: 2007:32-37.

Caicci, C., Maiuri, L., Caporaso, N., Bucci, C., Guidice, L.D., Massardo, D.R., Pontieri, P., Fonzo, N.D., Bean, S.R., Ioerger, B. Londei, M. 2007. Celiac disease: in vitro and in vivo safety and palatability of wheat-free sorghum food products. Clinical Nutrition. 26(6):799-805.

City of Manhattan. 2007. 2007 Water Quality Report. City of Manhattan, KS. June 2008.

Cordrey, D. 2006. Decoction mashing, part 1, demystification of the decoction mash. Strand Brewer's Club. http://www.strandbrewers.org/techinfo/decoct1.htm on (accessed Jul 23, 2008).

Daiber, K.H., Taylor, J.R.N. 1995. Opaque beer. As found in Dendy, D.A.V. 1995. *Sorghum and millets: chemistry and technology*. American Association of Cereal Chemists, Inc. St. Paul, MN.

De Jonge, M. 2005. Decoction mashing. Knights of the Mashing Fork. http://kotmf.com/articles/decoction.php?PHPSESSID=f9d3a402563a75e4ef37f39939667254 (accessed Jun 26, 2008).

Demuyakor, B., Ohta, Y., Nakatani, K., Fukui, N., Kanagawa, K. 1994. Brewing of beer with Sorghum vulgare malt with minimal barley malt blending. Journal of the American Society of Brewing Chemists. 52(3):111-115.

Dendy, D.A.V. 1995. Sorghum and millets: production and importance. In *Sorghum and millets: chemistry and technology*; Dendy, D.A.V. 1995. American Association of Cereal Chemists, Inc.: St. Paul, MN, 1995.

Dewar, J., Taylor, J.R.N., Berjak, P. 1997. Determination of improved steeping conditions for sorghum malting. Journal of Cereal Science. 26:129-136.

Dufour, J.P., Mélotte, L., Srebrink, S. 1992. Sorghum malts for the production of a lager beer. Journal of the American Society of Brewing Chemists. 50(3):110-119.

FAO. 2006. FAO statistical yearbook for 2004 [Online]. FAO. http://www.fao.org/statistics/yearbook/ (accessed Feb 20, 2009).

Fasano, A., Catassi, C. 2001. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. Gasterenterolgy. American Gastroenterological Association. 120:636–651.

Figueroa, J.D.C., Martínez, B.F., Ríos, E. 1995. Effect of sorghum endosperm type on quality of adjuncts for the brewing industry. Journal of the American Society of Brewing Chemists. 5391):5-9.

Food Standards Agency. 2009. New rules for 'gluten-free' foods [Online]. http://www.food.gov.uk/news/newsarchive/2009/jan/newrulesforglutenfree (accessed Feb 4, 2009).

Hoseney, R.C. 1994. Principles of cereal science and technology. 2nd ed. American Association of Cereal Chemists. St. Paul, MN.

Hughes, K. 2007. Gluten-free looking good. Prepared Foods. Sept. 2007.

Igyor, M.A., Ogbonna, A.C., Palmer, G.H. 2001. Effect of malting temperature and mashing methods on sorghum wort composition and beer flavour. Process Biochemistry. 26:1039-1044.

INSORTMIL. 2008. INSORTMIL Report No. 17, Jan. 15, 2008. INSORTMIL Management Entity: University of Nebraska, Lincoln, Nebraska.

Katz, S.H., and Maytag, F. 1991. Brewing an ancient beer. Archeology. 44(4):24-27.

Kayodé, A.P.P., Hounhouigan, J.D., Nout, M.J.R. 2007. Impact of brewing process operations on phytate, phenolic compounds, and in vitro solubility of iron and zinc in opaque sorghum beer. LWT. 40:834-841.

Kitsock, G. 2007. Try these for a better gut reaction. *The Washington Post*, Feb. 14, 2007. pF02.

Lake Front Brewery. http://lakefrontbrewery.com/sorghum.html (accessed Feb 19, 2009).

Leeds, J.S., Hopper, A.D. 2008. Coeliac disease. British Medical Bulletin. 88:157–170.

Lefydei, M.L., Taylor, J.R.N. 2006. Effect of dilute alkaline steeping on the microbial contamination, toxicity, and diastatic power of sorghum malt. Journal of the Institute of Brewing. 112(2):108–116.

Lewis, M.J., Bamforth, C.W. 2006. Essays in Brewing Science [Online]. Springer. http://www.myilibrary.com/Browse/open.asp?ID=111797&loc=116 (accessed Feb 16 2009).

Martens, C. 2008. Photograph of malted grain sorghum USDA-ARS Cereal Crops Research Unit.

Maytag, F. 1991. Sense and nonsense about beer. Chemtech. 22(3):138-141.

Mestres, C., Matencio, F., Pons, B., Yajid, M., Fliedel, G. 1996. A rapid method for determination of amylase content by using differential scanning calorimeter. Starch/Stärke 48(1):2-6.

NDDIC. 2009. Celiac disease awareness campaign from the National Institutes of Health. http://celiac.nih.gov/ (accessed Feb 6, 2009).

Nelson, M. 2005. The barbarian's beverage: a history of beer in ancient Europe. Routledge. New York, NY.

Novellie, L. 1966. Biological ennoblement and Kaffir beer. Food Technology. 20(12):101-102.

Novellie. L. Kaffircorn malting and brewing studies. 11. Effect of malting conditions on diastatic power of kaffircorn malt. Journal of Food Science and Agriculture. 13(2):115-120.

O'Brien, K. 2007. Taste good, no gluten. *The Boston Globe*, Jan. 17, 2007, pD10.

Obeta, J.A.N., Okungbowa, J., Ezeogu, L.I. 1999. Malting of sorghum: further studies on factors influencing α-amylase. Journal of the Institute of Brewing. 106(5):295-304.

Odibo, F.J.C., Nwankwo, L.N., Agu, R.C. 2002. Production of malt extract and beer from Nigerian sorghum varieties. Process Biochemistry. 37:851-855.

Ogbonna, A.C. 1992. Developments in the malting and brewing trials with sorghum. World Journal of Microbiology and Biotechnology. 8(2):87-91.

Ogu, E.O., Odibo, F.J.C., Agu, R.C., Palmer, G.H. 2004. Malting studies of some selected brewing sorghum varieties. Technical Quarterly of the Master Brewers Association of the Americas. 41(4):386-389.

Okafor, N., Aniche, G.N. 1980. Brewing a lager beer from Nigerian sorghum. Brewing and Distilling International. 10:32-35.

Okafor, N., Aniche, G.N. 1986. Studies on the brewing of lager beer from Nigerian sorghum. Journal of Food Science and Technology. 24(3):131-134.

Okungbowa, J., Obeta, J.A.N., Ezeogu, L.I. 2002. Sorghum β-amylase production: relationship with grain cultivar, steep regime, steep liquor composition, and kilning temperature. Journal of the Institute of Brewing. 108(3):362-370.

Osborne, B.G., Anderssen R.S. 2003. Single-kernel characterization principles and applications. Cereal Chem. 80(5):613-622.

Osorio-Morales, S., Serna Saldivar, S.O., Chaves Contreras, J.C. 2000. Production of brewing adjuncts and sweet worts from different types of sorghum. Journal of the American Society of Brewing Chemists. 58(1)21-25.

Oregon State University Barley Project. Barley Project Oregon State University Crop and Soil Science Department. http://barleyworld.org/whatisbarley.php (accessed Mar 20, 2009), Barleyworld.org. 2006. What is barley?.

Owuama, C.I. 1997. Review sorghum: a cereal with lager beer brewing potential. World Journal of Microbiology and Biotechnology. 13:253-260.

Owuama, C.I. 1999. Brewing beer with sorghum. Journal of the Institute of Brewing. 105(1):23-34.

Owuama, C.I., Okafor, N. 1987. Studies on mashing methods for beer brewing with sorghum. MIRCEN Journal. 3:243-253.

Palmer, G.H., Etokakpan, O.U., Igyor, M.A. 1989. Review: sorghum as a brewing material. MIRCEN Journal. 5:265-275.

Palmer, J.J. 2006. How to brew. Everything you need to know to brew beer right the first time. Brewers Publications. Boulder, CO.

Papazian, C. 2003. The complete joy of homebrewing. 3rd ed. Harper-Collins. New York, NY.

Pickerell, A.T.W. 1986. The influence of free α-amino nitrogen in sorghum beer fermentation. Journal of the Institute of Brewing. 92:568-571.

Pozo-Insfran, D.D., Urias-Lugo, D., Hernandez-Brenes, C., Saldivar, S.O.S. 2004. Effect of amyglucosidase on wort composition and fermentable carbohydrate depletion in sorghum lager beer. Journal of the Institute of Brewing. 110(2):124-132.

Presutti, R.J., Cangemi, J.R., Cassidy, H.D., Hill, D.A. 2007. Celiac disease. American Family Physician. 76(12):1795-1802.

Rounds, M.A., Gregory III, J.F. 2003. High performance liquid chromatography. In *Food Analysis*; 3rd ed.; Nielsesn, S.S. Ed.; Springer Science+Business Media, Inc.: New York, NY.

Schenz, T.W. 2003. Thermal analysis. In *Food Analysis*; 3rd ed.; Nielsesn, S.S. Ed.; Springer Science+Business Media, Inc.: New York, NY.

Sweeney, S. 2002. The gluten-free beer challenge; brewing without barley, wheat, or rye. American Homebrewers Association. Zymurgy. 25(6):40-44.

Taylor, J.R.N. 1992. Mashing with malted grain sorghum. Journal of the American Society of Brewing Chemists. 50(1):13-18.

Taylor, J.R.N., Boyd, H.K. 1986. Free α-amino nitrogen production in sorghum beer mashing. Journal of the Science of Food and Agriculture. 37:1109-1117.

Taylor, J.R.N., Dewar, J. 1994. Role of alpha-glucosidase in the fermentable sugar composition of sorghum malt mashes. Journal of the Institute of Brewing. 100:417-419.

Taylor J.R.N., Dewar J. 2001. Developments in sorghum food technologies. Advances in Food and Nutrition Research. 43:217–264.

Taylor, J.R.N., Schober, T.J., Bean, S.R. 2006. Novel food and non-food uses for sorghum and millets. Journal of Cereal Science. 44:252–271.

US Grains Council. Barley. http://www.grains.org/barley (accessed Mar 20, 2009).

USDA NASS 2008, Agricultural Statistics 2008 [Online]. http://www.nass.usda.gov/Publications/Ag_Statistics/2008/TOC2008.pdf (assessed Nov 6, 2009).

USDA NASS .2009. Barley national statistics [Online]. http://www.nass.usda.gov/QuickStats/index2.jsp (accessed Mar 20, 2009).

USDA, NASS, Kansas Field Office. Kansas farm facts 2008 [Online]. http://www.nass.usda.gov/Statistics_by_State/Kansas/Publications/Annual_Statistical_Bulletin/ff2008.pdf (accessed Feb 20, 2009).

Watson, T.G., Novellie, L. 1976. The development of amylase and maltase during the malting of Sorghum vulgare. Agrochemophysica. 7(4):61-74.

Weiser, H., Kiehler, P. 2008. The biochemical basis of celiac disease. Cereal Chem. 85(1):1-13.

CHAPTER 2 - Preliminary Work

Introduction

The brewing process has been described as not only a science but also an art (Bamforth 2003). The development of a process to brew gluten-free beer required an understanding of the traditional brewing process as well as an evaluation of research procedures used for brewing with sorghum.

Previous work on the development of sorghum beer focused on the substitution of barley malt with sorghum using established barley malt brewing techniques (Okafor and Aniche 1980). A study by Taylor (1992) utilized malted grain sorghum to produce sorghum beer because malted sorghum provides a better buffering effect than un-malted sorghum. Agu (2005) reported brewing beer with malted grain sorghum was best achieved using several modifications to the mashing procedure, to account for the higher starch gelatinization temperature of sorghum starch. In addition, studies by Okafor and Aniche (1980), Taylor (1992), Osorio-Morales et al. (2000), and Igyor (2001) evaluated traditional African, European, and modified methods for the production of barley beer with sorghum adjuncts and 100% sorghum beer. The overall findings indicated modification of the mashing portion of the brewing process was most critical to sorghum beer production due to the higher gelatinization temperature of sorghum starch.

The objective of this portion of the research was to evaluate brewing procedures and develop a procedure for the production of an ale style gluten-free beer.

Materials and Methods

Brewing With Barley Kits

Initial investigations into sorghum beer included brewing first with three barley extract kits, followed by three all-grain barley kits (Northern Brewer, St. Paul, MN) in order to better understand the traditional brewing procedure. Barley kits were evaluated on specific gravity, Brix, and pH.

Material and Methods for Barely Kits

The brewing procedure began once all surfaces, equipment, and utensils were cleaned and sanitized using One Step sanitizer (LD Carlson Company, Inc., Kent, OH). Appropriate sanitation is necessary to prevent bacterial infection of the yeast that can spoil the beer and cause fermentation problems and off flavors.

Barley Syrup Kits

The first step in extract kit brewing was to heat 9.5 L of water in a 11.4 L kettle and slowly heat to boiling, on a Corning International PC-620 (Lowell, MA) hotplate set to 5. Once boiling, the kettle was removed from the hotplate and fermentable sugars, mainly extract syrups, were stirred in until all sugars were dissolved. The mixture, now referred to as wort, was returned to heat and brought to a boil. The wort was boiled for 60 min. Hops were added during the boil at the times noted in each kit. The wort was cooled to 20–22°C using an immersion wort chiller (Northern Brewer. St. Paul, MN.). During the cooling period the yeast, included in the kit, was prepared according to package directions.

The remaining cooled wort was transferred to a clean and sanitized 5 gallon glass carboy (Northern Brewer. St. Paul, MN). Clean, cool tap water, approximately 11.4 L, was added to the carboy to yield a volume of 18.9 L (5 gallons). Yeast was pitched into the cooled wort. A sanitized drilled carboy bung and fermentation air lock filled with sanitizer was placed into the neck of the carboy. The carboy was covered with a dark cloth cover/jacket, and the beer was allowed to ferment. When the krausen had dissipated and the specific gravity measurement remained constant for two consecutive days, bottling commenced.

Barley All-Grain Kits

The first step in barley all-grain kit brewing was to heat 1.2 L of water per pound of grain of sparge water to 79°C and transfer to the mash tun. The barley grist was slowly added while stirring to prevent clumps. The temperature stabilized at approximately 68°C. The mash was allowed to rest for 60 min. During the rest, sparge water was prepared by heating 2.0 L of water per pound of grain to 79°C. Mash-out began by adding hot water, at approximately 93°C, to raise the mash temperature to 76°C. The mash was allowed to rest at this temperature for 15 min. The sparge began by recirculation of the liquid portion of the mash to allow the barley hulls to create a filter bed. One L of runoff was collected from the lauter tun valve and slowly added back to the top of the lauter tun without disturbing the grain bed. The recirculation was repeated with another 1 L of runoff. Next the lauter tun valve was opened allowing the wort to drain from the grain, the sparge water was also added, which rinses any remaining sugars from the grain. The collected wort was heated to boiling and boiled for 60. Hops were added during the boil at the times noted in each kit. The wort was cooled to 20–22°C

using an immersion wort chiller (Northern Brewer. St. Paul, MN.). During the cooling period the yeast, included in the kit, was prepared according to package directions.

The remaining cooled wort was transferred to a clean and sanitized 5 gallon glass carboy (Northern Brewer. St. Paul, MN). Yeast was pitched into the cooled wort. A sanitized drilled carboy bung and fermentation air lock filled with sanitizer was placed into the neck of the carboy. The carboy was covered with a dark cloth cover/jacket, and the beer was allowed to ferment. When the krausen had dissipated and the specific gravity measurement remained constant for two consecutive days, bottling commenced.

Bottling

To begin bottling, the work area, along with all surfaces and equipment, were cleaned and sanitized. The equipment was included: mash paddle, bottle tree drainer ART. 15231 (Ferrari Group, Italy), 48 12-ounce brown glass bottles (Northern Brewer, St. Paul, MN), bottling bucket (Northern Brewer, St. Paul, MN), Fermtech Auto Siphon (Fermtech Ltd., Kitchener (ON), Canada), tygon siphon tubing (Northern Brewer, St. Paul, MN), Fermtech bottle filler (Fermtech Ltd., Kitchener (ON), Canada) and Red Baron (Emily) Capper bottle capper (Northern Brewer, St. Paul, MN).

Approximately 473 mL of water was heated to boiling. When boiling, 141.7 g of priming sugar (corn sugar (dextrose)) were added to the water. The priming sugar was stirred to dissolve, and the solution was boiled for 5 min to sanitize. Approximately 710 mL (3 cups) of water and 48 bottle caps were placed on the hot plate and heated to boiling. The caps were boiled for 5 min to sanitize.

Beer from the carboy was siphoned to the sanitized bottling bucket with tygon siphon tubing and Fermtech bottle filler (Northern Brewer, St Paul, MN) attachment,

ensuring the trub on the bottom of the carboy was not disturbed. The cooled priming sugar solution was added and gently stirred. The Fermtech bottle filler was inserted into the bottom of the sanitized bottle, and beer was filled to the top rim of the bottle. When the bottle filler was removed, the bottle had the correct amount of beer to yield the appropriate headspace to allow for bottle conditioning. The bottle filler was removed and immediately capped. Caps were labeled with the beer abbreviation and date. Bottles were allowed to condition at room temperature for 14 days.

Wort and Beer Analysis

Specific gravity measures the density of the liquid wort and beer. Brewers utilize this measurement to indicate the amount of sugar in solution and to determine the rate of fermentation. Specific gravity was measured using a triple scale hydrometer (Northern Brewer, St. Paul, MN.). Approximately 237 mL of wort or beer was placed into a 1 in. plastic cylinder (included with the hydrometer). The hydrometer was placed in the liquid within the column and gently spun to prevent the hydrometer from sticking to the side. The liquid level was read at eye level for all three scales. The alcohol content by volume was calculated with the Equation 2.1.

Equation 2.1 Alcohol by volume

(Original specific gravity - final specific gravity) × 105 = % Alcohol by volume

Source: Papazain (2003).

Brix measures the amount of dissolved solids, indicating the amount of sugar in wort and beer. Also, indicates of the rate of fermentation. Brix was measured with a

Huake RHB-32ATC refractometer (Huake Instrument Co. Ltd., Shenzhen, China). Three drops of beer were placed onto the viewing window and the plastic cover was closed, ensuring no air bubbles were present between the window and the cover. The measurement was recorded based on the view from the view finder.

pH was measured to evaluate the brewing process and the rate of fermentation. The pH was measured using a Hanna HI98129 handheld pH meter (Hanna Instruments, Woonsocket, RI). Approximately 25 mL of wort or beer were placed into a 50 mL beaker. The probe was inserted into the liquid and gently stirred until a stable pH and temperature reading were displayed.

Results of Barely Kits

For the barley syrup kits (Table 2.1), the specific gravity ranged from 1.022 to 1.060; in the all-grain processing, specific gravity ranged from 1.015 to 1.061. Brix and pH ranged from 5.0 to 16.0 and 4.88 to 5.77 respectively.

Table 2.1 Northern Brewer initial barley kits used to evaluate protocol for beer production.

Kits	Original SG[a]	Initial Brix	Initial pH	Final SG[a]	Final Brix	Final pH	% ABV[b]
BARLEY SYRUP:							
Oktoberfest	1.06	14.4	5.70	1.06	14.2	6.12	n/a
Sweet Stout	1.02	9.2	4.88	1.21	9.0	4.95	n/a
St. Paul Porter	1.06	13.8	5.38	1.02	8.2	5.06	5.25
BARLEY ALL-GRAIN:							
Nut Brown Ale	1.06	16.0	5.77	1.01	8.6	4.70	6.69
Phat Tyre Amber Ale	1.03	8.0	5.76	1.02	6.2	4.55	1.18
Bavarian Hefe Weizen	1.02	5.0	4.93	1.01	5.0	4.96	0.66

[a]Specific gravity
[b]Alcohol by volume

Sorghum Beer Formula Development

Materials and Methods of Sorghum Syrup Based Beer

Sorghum syrup (Northern Brewer, St. Paul, MN) was initially used to evaluate hop additions and a gluten-free yeasts variety. Two different formulas obtained from Briess Malt & Ingredients Company for home-brewed sorghum syrup beer were used as a starting point (Briess Malt & Ingredients Company, 2008). The formulas were for two styles, ale and lager. Refrigeration facilities were unavailable; therefore, both formulas were fermented at 18-22°C with Danstar Nottingham dry ale yeast (Lallemand, Inc., Montreal, QC, Canada) chosen because propagation occurred on gluten-free mediums. A third formula was created to evaluate molasses as a source of fermentable sugar. Each formula was brewed once and yielded approximately 18.9 L (5 gallons) of beer per formula.

The brewing procedure began once all surfaces, equipment, and utensils were cleaned and sanitized using One Step sanitizer (LD Carlson Company, Inc., Kent, OH).

The next step was to heat 9.5 L of water in a 11.4 L kettle and slowly heat to boiling, on a Corning International PC-620 (Lowell, MA) hotplate set to 5. Once boiling the kettle was removed from the hotplate and fermentable sugars were stirred in following Formula 1, 2, and 3 (Table 2.2 and 2.3) until all sugars were dissolved. The third formula followed Formula 2 (Table 2.3) with 226.8 g of corn syrup replaced by 226.8 g of molasses (B&G Food, Inc., Parsippany, NJ). The mixture was returned to heat and brought to a boil. The wort was boiled for 60 min. Each formulation utilized different hop varieties (Table 2.2 and 2.3) added to the boil at different times, referred to as the hop schedule. The hop schedule directed that hops were added at the beginning of

the boil, 5 or 10 min before the end of the boil (depends on formula), and at the end of the boil. The wort was cooled to 20–22°C using an immersion wort chiller (Northern Brewer. St. Paul, MN).

During the cooling period, two packages (22 g) Danstar Nottingham dry ale yeast (Lallemand, Inc., Montreal, QC, Canada) were prepared according to package directions. Approximately 200 mL sterilized (boiled) water at 30–35°C was place into a sanitized 150 mL beaker. Yeast was gently sprinkled on top of the water. The yeast was allowed to hydrate for 15 min, then gently stirred to incorporate all yeast particles, and allowed to set for an additional 5 min.

The remaining cooled wort was transferred to a clean and sanitized 5 gallon glass carboy (Northern Brewer. St. Paul, MN). Clean cool water, approximately 11.4 L was added as necessary to the fill the carboy to yield a volume of 18.9 L (5 gallons). Yeast was pitched into the cooled wort. A sanitized drilled carboy bung and fermentation airlock filled with sanitizer was placed into the neck of the carboy. The carboy was covered with a dark cloth cover/jacket, and the beer was allowed to ferment. When the krausen had dissipated and the specific gravity measurement remained constant for two consecutive days, the beer was bottled using the previously mentioned procedure. Bottles were allowed to condition at room temperature for 14 days.

Specific gravity, Brix, and pH analysis of the wort was done just prior to pitching and again after fermentation just prior to bottling using the procedures and equipment outlined previously for barley extract kits.

Formula 1 and 2 were tasted by 14 untrained, non-celiac consumers to evaluate the different a hop schedules. Beer were judged blind against two gluten-free beer on the

market: RedBridge (Anheuser-Bush, St. Louis, MO) and New Grist (Lakefront Brewery, Milwaukee, WI). All beer were ranked on a scale of 1 being "Not Acceptable" to 9 being "Acceptable" for 4 parameters: flavor, color, mouthfeel, and overall. Panelists were also asked if they would purchase the sample and allowed to provide comments.

Table 2.2 Formula 1 for pale ale style sorghum syrup based gluten-free beer.

Amount (g)	Ingredient	Supplier City, State
3401.9	White Sorghum Syrup	Northern Brewer, St. Paul, MN
22.8	Water	City of Manhattan, Municipal Water, Manhattan, KS
28.4	Cascade pellet hops 6.3% AA[1] (beginning of boil)	LD Carlson Company, Inc., Kent, OH
14.2	Cascade pellet hops 6.3% AA[1] (5 min before end of boil)	LD Carlson Company, Inc., Kent, OH
28.4	Cascade pellet hops 6.3% AA[1] (end of boil)	LD Carlson Company, Inc., Kent, OH
28.4	Cascade pellet hops 6.3% AA[1] (dry hop)	LD Carlson Company, Inc., Kent, OH
22.0 (2 pkgs)	Nottingham dry ale yeast	Lallemand, Inc., Montreal, QC, Canada
226.8	Honey (bottling)	Northern Brewer, St. Paul, MN

[1]Alpha acids

Source: Adapted from Briess Malt and Ingredients, Co. (2008).

Table 2.3 Formula 2 for lager style sorghum syrup based gluten-free beer.

Amount (g)	Ingredient	Supplier, City, State
1696.4	White Sorghum Syrup	Northern Brewer, St. Paul, MN
22.8	Water	City of Manhattan, Municipal Water, Manhattan, KS
680.4	Honey, Light amber blend	Northern Brewer, St. Paul, MN
226.8	Corn Syrup	ACH Food Companies, Memphis, TN
14.2	Hallertau pellet hops 6% AA[1] (beginning of boil)	Northern Brewer, St. Paul, MN
21.3	Cascade pellet hops 6.3% AA[1] (10 min before end of boil)	LD Carlson Company, Inc., Kent, OH
21.3	Czech Saaz pellet hops 2.5% AA[1] (end of boil)	Northern Brewer, St. Paul, MN
22.0 (2 pkgs)	Nottingham dry ale yeast	Lallemand, Inc., Montreal, QC, Canada
226.8	Honey (bottling)	Northern Brewer, St. Paul, MN

[1]Alpha acids

Source: Adapted from Briess Malt and Ingredients, Co. (2008).

Table 2.4 Sorghum syrup trials

Formula	1	2	3
Carbohydrate source	3401.9 g sorghum syrup, 226.8 g honey (bottle conditioning)	1696.4 g sorghum syrup, 680.4 g honey, 226.6 g corn syrup, 226.8 g honey (bottle conditioning)	1696.4 g sorghum syrup, 680.4 g molasses, 226.6 g corn syrup, 226.8 g honey (bottle conditioning)
Hop varieties	Cascade, Cascade, Cascade	Hallertau, Cascade, Czech Saaz	Hallertau, Cascade, Czech Saaz
Yeast	Nottingham ale yeast	Nottingham ale yeast	Nottingham ale yeast

PLEASE WRITE IN THE SAMPLE NUMBER
AND CIRCLE YOUR CHOICE.

Do you suffer from celiac disease? Yes No

Sample No. _____

Flavor

Not Acceptable *Acceptable*

1 2 3 4 5 6 7 8 9

Color

Not Acceptable *Acceptable*

1 2 3 4 5 6 7 8 9

Mouthfeel

Not Acceptable *Acceptable*

1 2 3 4 5 6 7 8 9

Overall

Not Acceptable *Acceptable*

1 2 3 4 5 6 7 8 9

Would you purchase this product? Yes No

Comments:

Figure 2.1 Sample ballot for sensory test.

Results of Sorghum Syrup Formulation

The processing data collected for all three sorghum syrup formulations indicates the variations in carbohydrate source and the hop source (Table 2.5). Formulations 2 and 3 were expected to have higher Brix and specific gravity due to the addition of corn syrup, honey, and molasses respectively. On brewing day the specific gravity or original gravity ranged from 1.036 to 1.040 and at bottling the final gravity ranged from 1.006 to 1.011. From these numbers the alcohol content was calculated and ranged from 3.81 to 5.37. Based on final alcohol contents, the yeast produced more alcohol from the corn syrup, honey, and molasses with the highest alcohol content seen for Formula 2 which included sorghum syrup, corn syrup, and honey. Brix values ranged from 4.8 post-fermentation to 11.2 prior to fermentation and pH values ranged from 4.20 after fermentation to 6.36 prior to fermentation.

The variation in hop schedule created different and distinct flavors due to isomerization of alpha acids during the boil and addition of aroma imparting essential oils as noted by the taste panel.

The overall scores from the taste panel for sorghum syrup formulas 1 and 2 were 4.6 and 4.0, respectively. Comments by panelists indicated that sorghum flavor paired better with Formula 2, which utilized hops traditionally found in lager style beer versus Formula 1 which utilized hops tradition found in pale ale style beer. RedBridge received the highest score of the market beer with an overall ranking of 6.1, with New Grist ranked 4.3.

Table 2.5 Processing data collected during brewing and fermentation of sorghum syrup beer.

Sorghum Syrup Beer Formula	Processing point	Brix	SG[1]	pH	ABV[2]	Overall Sensory Score[3]
1	Post brewing	9.9	1.040	6.36		
	Fermentation check	6.2	1.011	4.65		
	Bottling	6.2	1.011	4.65	3.81	4.6±1.4
2	Post brewing	8.4	1.036	6.35		
	Fermentation check	5.0	1.008	4.22		
	Bottling	5.0	1.008	4.20	6.68	4.0±1.8
3	Post brewing	11.2	1.047	6.14		
	Fermentation check	4.8	1.006	4.39		
	Bottling	4.9	1.006	4.40	5.37	

[1]Specific gravity
[2]Alcohol by volume
[3]Overall score on a scale of 1 = not acceptable, 9 = acceptable

Sorghum Brewing Process Development

Brewing Equipment

The equipment used for all grain barley brewing is the same for sorghum beer with a few modifications. A mash and lauter tun was constructed by modifying a 5 gallon (18.93 L) Rubbermaid cooler with a spigot, ball valve, false bottom, and appropriate rubber tubing (Northern Brewer, St. Paul, MN). Sorghum is a huskless grain that often clogs the false bottom of the lauter tun during the sparge and lauter. For the preliminary research, an additional 14 mesh stainless-steel mill screen obtained from the milling lab was cut to size and sewn to the existing false bottom using unflavored, wax-coated dental floss (Figure 2.1).

Figure 2.2 Modified mash tun and lauter tun used in brewing sorghum beer.

Materials and Methods of All-Grain Sorghum Brewing

Preliminary research was conducted with a malted sorghum grain sample donated by the Bard's Tale Research Group (Lee's Summit, MO) to determine a suitable procedure. Malt was milled at the KSU Grain Science Department. The sorghum malt was milled to produce grist, using an experimental roller mill (Ross, Oklahoma City, OK) on 6x10 in. smooth rolls 1.5:1, set to a 0.060 in. gap. Grist was sifted for 3 min on a 150-micron sieve.

Preliminary procedure work began by following a combination of traditional and modern brewing methods. All of the methods followed the basic brewing process; however, the mashing step required greater modification due to the differences between barley and sorghum composition. Mashing method 1 utilized an infusion mash procedure by Robert Hinterding (2004). Mashing method 2 was a double decoction mash from

Taylor (1992). Mashing method 3 used a traditional triple decoction mash as described for barley beer with adjustments for the high starch gelatinization temperatures of sorghum (Palmer 2006; Taylor 1992). The triple decoction procedure was based on a series of BrauKaiser videos (Figure 2.3) (BrauKaiser 2008). Mashing method 4 was a double mash, double decoction procedure from Osorio-Morales et al. (2000) and Barredo Moguel and Rojas de Gante (2001) (Table 2.7). Each mashing method was brewed once and yielded approximately 9.5 L of beer per brew.

The brewing procedure began once all surfaces, equipment, and utensils were cleaned and sanitized. One Step sanitizer (LD Carlson Company, Inc., Kent, OH) was used to sanitize all surfaces and equipment. Approximately 2270 g malted sorghum grist was mashed with 6 L of water following four different mash schedules (Table 2.7).

The lautering process was modified by adding 15% by total weight of the rice hulls to the mash just before sparging to improve filtration because sorghum is a huskless grain. Approximately 9.5 L of sparge water was heated to 80°C and transferred to the lauter tun. Approximately 340.5 g rice hulls, incorporated as a filter aid, were stirred into the mash. The continuous sparge equipment was assembled. The grain bed was set by performing a Vorlof (or recirculation) where 0.5 L of the wort in the mash tun were removed and gently poured into the top of the mash tun without disturbing the grain bed. The Vorlof was repeated. Following Vorlof, the sparge removed the wort from the spent grains. The sprinkling of the sparge water on the grain rinses the grain of any remaining sugars. The water level was maintained 1 in. above the grain bed during sparging. The wort was collected into the 37.85 L brew pot with spigot and false bottom (PolarWare, Kiel, WI).

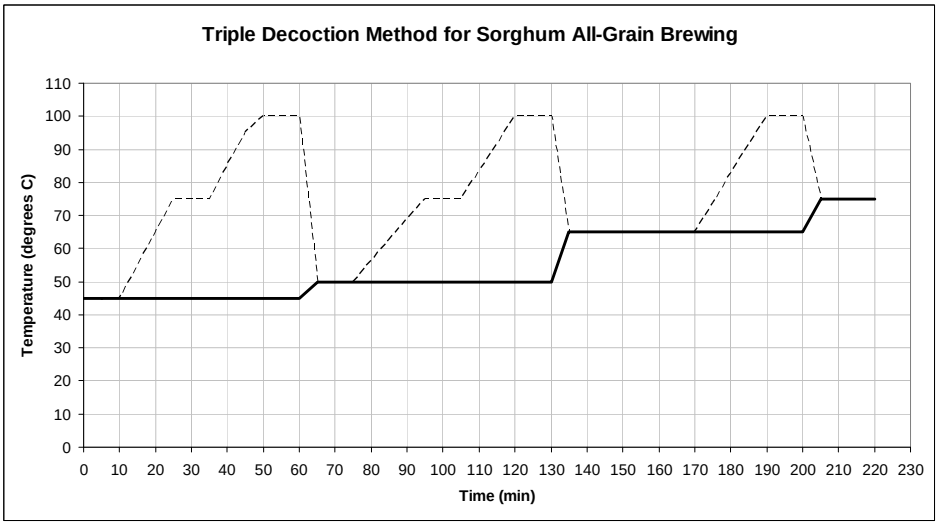
The 37.85 L brew pot was placed on a Barnstead International SP47230 (Dubuque, IA) and set to 8. Once the wort began to boil, the timer was set for 60 min, and 10.3 g of Hallertau hops were added to impart a bitter flavor. When 10 min remained in the boil, 10.6 g of Cascade hops were added to impart bitterness and aroma. At the end of the boil 10.6 g of Czech Saaz hops were added to impart aroma. The wort was cooled to 20–22°C using an immersion wort chiller (Northern Brewer. St. Paul, MN).

During the cooling period, one package (11 g) Danstar Nottingham dry ale yeast (Lallemand, Inc., Montreal, QC, Canada) was prepared according to package directions. Approximately 100 mL sterilized (boiled) water at 30–35°C was place into a sanitized 150 mL beaker. Yeast was gently sprinkled on top of the water. The yeast was allowed to hydrate for 15 min, then gently stirred to incorporate all yeast particles, and allowed to set for an additional 5 min.

The remaining cooled wort was transferred to a clean and sanitized 11.36 L glass carboy (Northern Brewer. St. Paul, MN). Yeast was pitched into the cooled wort. A sanitized drilled carboy bung and fermentation air lock filled with sanitizer was placed into the neck of the carboy. The carboy was covered with a dark cloth cover/jacket, and the beer was allowed to ferment. When the krausen had dissipated and the specific gravity measurement remained constant for two consecutive days, the beer was bottled using the previously mentioned procedure. Bottles were allowed to condition at room temperature for 14 days.

Specific gravity, Brix, and pH analysis of the wort was done just prior to pitching and again after fermentation just prior to bottling using the procedures and equipment outlined previously for barley extract kits.

Figure 2.3 Triple decoction method for sorghum all-grain brewing.



The dotted line is for the decoction, the solid line is for the mash.
Source: Adapted from BrauKaiser (2008).

Table 2.6 Mashing methods evaluated for the production of sorghum beer.

Method 1[a]	**Method 2**[b]	**Method 3**[c]	**Method 4**[d]
Mash-in • 40°C, 20 min	Mash-in • 45°C, 30 min	Mash-in • 45°C, 10 min	Mash-in **A** •50.5°C, 30 min •Boil 30 min •Cool to 65°C
Infusion • Raise entire mash to 50°C • 30 min rest	Decoction • Boil 1.4L of mash 10 min • Combine mash • Rest at 60°C, 60 min	Decoction • Heat calculated L of mash to 75°C, rest 10 min • Boil 15 min • Combine mash • Rest at 50°C, 10 min	Mash-in **B** •45.5°C, 30 min •Combine Mash **A** and Mash **B**
Decoction • Boil entire mash 20 min • Combine mash • Rest at 65°C, 1.5 h	Decoction • Boil 1.4L of mash 10 min • Combine mash • Rest at 70°C, 60 min	Decoction • Heat calculated L of mash to 75°C, rest 10 min • Boil 15 min • Combine mash • Rest at 65°C, 35 min	Decoction •Boil calculated L of mash 10 min •Combine mash •Rest at 67.5°C, 30 min
Infusion • Raise mash to 70°C • 20 min rest	Mash-out • Raise mash to 75°C • 15 min rest	Decoction • Boil calculated L of mash 10 min • Combine mash • Rest at 75°C, 15 min	Decoction •Boil calculated L of mash 10 min •Combine mash •Rest at 76.5°C, 30 min
Mash-out • Raise mash to 75°C		Mash-out • Raise mash to 76.5°C for 30 min	Mash-out • Raise mash to 76.5°C for 30 min

[a]Hinterding (2004)
[b]Taylor (1992)
[c]Palmer (2006), Taylor (1992)
[d]BrauKaiser (2008); Osorio-Morales et al. (2000); Barredo Moguel and Rojas de Gante (2001)

Results of Sorghum Process Development

Brew day measurements on Brix ranged from 7.6 to 11.0, original specific gravity from 1.031 to 1.045, and pH from 5.64 to 6.45. At bottling following fermentation, the final specific gravity ranged from 1.008 to 1.016, which produced alcohol contents ranging from 2.23% to 4.46%.

Method 4 was chosen because for the high specific gravity post brewing and for ease of procedure. Method 4 was easier to execute with existing equipment and provided different points for evaluation of starch gelatinization and sugar extraction throughout the process. Igyor et al. (2001) reported decoction mashing at 100°C produced better results in terms wort properties because boiling the mash adequately gelatinized sorghum starch and also produced flavor compounds similar to those of malted barley."

Table 2.7 Data collected from preliminary all malted sorghum grist brews.

Method No.	Processing point	Brix	SG[e]	pH	ABV[f]	Yield
1[a]	Post brewing	11.0	1.045	6.45		
	Fermentation check	5.8	1.011	4.88		
	Bottling	5.8	1.011	4.92	4.46	17 bottles
2[b]	Post brewing	7.6	1.031	6.34		
	Fermentation check	4.0	1.007	4.38		
	Bottling	4.0	1.008	4.35	3.02	22 bottles
3[c]	Post brewing	9.4	1.039	5.64		
	Fermentation check	5.1	1.014	4.35		
	Bottling	5.1	1.014	4.32	3.28	21 bottles
4[d]	Post brewing	8.0	1.033	5.67		
	Bottling	5.6	1.016	4.51	2.23	18 bottles

[a]Hinterding (2004)
[b]Taylor (1992)
[c]Palmer (2006), Taylor (1992)
[d]BrauKaiser (2008); Osorio-Morales et al. (2000); Barredo Moguel and Rojas de Gante (2001)
[e]Specific gravity
[f]Alcohol by volume

References

Agu, R.C. 2005. Some relationships between amylolytic enzymes developed in malted barley, extract recovery, and sugar profile. Technical Quarterly of the Master Brewers Association of the Americas. 42(4):315-318.

Bamforth, C.W. 2003. *Beer: tap into the art and science of brewing* [Online]; Oxford University Press. New York, NY, 2003. http://www.KSU.eblib.com/EBLWeb/patron/?target=patron&extendedid=P_279517_0 (accessed Feb 4, 2009).

Barredo Moguel, L.H., Rojas de Gante, C. 2001. Comparisons between a commercial wort and a waxy sorghum wort fermented into lager beer with emphasis on yeast growth and ethanol production. Journal of the American Society of Brewing Chemists. 59(1)24-27.

Bean, S.R., Chung, O.K., Tuinstra, M.R., Pedersen, J.F., Erpelding, J. 2006. Evaluation of single kernel characterization system (SKCS) for measurement of sorghum grain attributes. Cereal Chem 83(1):108-113.

BrauKaiser. 2009. Decoction mashing (part 1) decoction mash (part 2) decoction mash (part 3). http://www.youtube.com/user/BrauKaiser (accessed Jun 26, 2008). Transcript of video available at http://braukaiser.com/wiki/index.php?title=Decoction_Mashing_Video_-_Transcript.

Briess Malt & Ingredients Company. 2008. Gluten-free beer recipes. http://www.briess.com (accessed Jan 18, 2008).

City of Manhattan, KS. 2008. Consumer confidence report 2008 Water Quality Report.

Dewar, J., Taylor, J.R.N., Berjak, P. 1997. Determination of improved steeping conditions for sorghum malting. Journal of Cereal Science. 26:129-136.

Hinterding, R. 2004. Brewing gluten-free beer. http://www.sillyyak.com.au/beer/index.html on (accessed Jan 18, 2008). Silly Yak Beverages Pty Ltd.

Igyor, M.A., Ogbonna, A.C., Palmer, G.H. 2001. Effect of malting temperature and mashing methods on sorghum wort composition and beer flavour. Process Biochemistry. 26:1039-1044.

Lallemand Danstar. 2004. Danstar Nottingham *Saccharomyces cerevisiae* British ale yeast. http://www.danstaryeast.com/tds/nottingham.pdf (accessed Jan 20, 2008).

Lefyedi, M.L., Taylor, J.R.N. 2006. Effect of dilute alkaline steeping on the microbial contamination, toxicity, and diastatic power of sorghum malt. Journal of the Institute of Brewing. 112(2):108-116.

Okafor, N., Aniche, G.N. 1980. Brewing a lager beer from Nigerian sorghum. Brewing and Distilling International. 10:32-35.

Okungbowa, J., Obeta, J.A.N., Ezeogu, L.I. 2002. Sorghum β-amylase production: relationship with grain cultivar, steep regime, steep liquor composition and kilning temperature. Journal of the Institute of Brewing. 108(3):362-370.

Osorio-Morales, S., Serna Saldivar, S.O., Chaves Contreras, J.C. 2000. Production of brewing adjuncts and sweet worts from different types of sorghum. Journal of the American Society of Brewing Chemists. 58(1):21-25.

Palmer, J.J. 2006. How to brew: Everything you need to know to brew beer right the first time. Brewers Publications. Boulder, CO. Images from http://www.howtobrew.com/images/f83.pdf (accessed May 30, 2008).

Papazian, C. 2003. The complete joy of homebrewing, 3rd ed. Harper Collins. New York, NY.

Park S.H., Bean S.R., Wilson J.D., Schober T.J. 2006. Rapid isolation of sorghum and other cereal starches using sonication. Cereal Chem. 83(6):611-616.

Taylor, J.R.N. 1992. Mashing with malted grain sorghum. Journal of the American Society of Brewing Chemists. 50(1):13-18.

CHAPTER 3 - Experimental Work

Introduction

Beer is the most consumed alcoholic beverage in the world (Nelson 2005). Beer is most commonly brewed using traditional processes developed centuries ago for malted barley grain (Papazain 2003). Throughout history other grain sources have been investigated when environmental and economic conditions increased the cost of malted barley. In modern times, other grains have been investigated to create gluten-free beer for persons suffering from celiac disease. Celiac disease is an autoimmune disorder in which the consumption of wheat, barley, and/or rye proteins causes damage to the small intestine (Fasano and Catassi 2001). Beer has traditionally utilized one or all of these grains as a source of fermentable sugars. Sorghum, a grain grown around the equator in semi-arid climates, is safe for celiac patients to consume (Owuama 1997). Incorporation of sorghum into brewing practices developed for barley has been unsuccessful due to structural and chemical differences.

Previous work investigated modern and traditional small scale barley brewing including using extract kits and all-grain brewing techniques. The results of this work were to better understand the processes and the application to sorghum brewing. Work was also done to investigate extract brewing with sorghum syrups for sensory analysis of sorghum beer. The last step was to develop an all-grain brewing procedure for use with malted sorghum as grain sources for gluten-free beer.

Development of an all-grain brewing procedure for malted grain sorghum found the critical modifications to the brewing procedure are to alter the mash temperatures to account for the higher gelatinization temperature of sorghum starch and to add a filter medium, most commonly rice hulls, to the lauter steps because sorghum is a husk less grain. The procedure developed was used to evaluate four different sorghum hybrids to determine which hybrids may have better malting and brewing properties and the impact of the properties on gluten-free beer. The objective of this study was to determine differences of sorghum hybrids through the malting and brewing process of a gluten-free beer.

Grain

Four hybrids of food grade sorghum grain were selected from a collection of the Grain Marketing and Production Research Center (GMPRC) at the United States Department of Agriculture – Agricultural Research Service (USDA-ARS) in Manhattan, KS. Three hybrids of were chosen from a study conducted by the USDA-ARS in Mead, NE in 2006 (Unpublished data). 82G63 and 83G66 were commercial red (non-tannin) hybrids from Pioneer Hi-Bred International. RN315 was the third sorghum from the study; it was a white sorghum from USDA-ARS sorghum breeding program in Lincoln, NE. The fourth sorghum hybrid used was X303, a white hybrid, produced in Healy, KS.

Malting

Sorghum grain samples were packaged and shipped in 9 kg increments to the USDA-ARS Cereal Crops Research facility in Madison, WI for malting. Preliminary fractions were malted at the USDA-ARS Cereal Crops Research facility in Madison, WI

with sodium hypochlorite and sodium hydroxide disinfection. Once 0.2% sodium hydroxide solutions was chosen, the pre-steep times were evaluated at 5 and 6 h to assess the effectiveness of the pre-steep to minimizing fungal contamination based on the work of Lefyedi and Taylor (2006) (Figure 3.2).

The sorghum was cleaned on a 51/2/64 in. (approximately 2.3 mm) slotted, pan sieve screen to remove broken kernels and foreign material. 800 grams (dry basis) of each sorghum sample were placed into Joe White malting boxes (1000-gram capacity, stainless steel with mesh screen bottoms).

Each container/malting box was washed for 30 seconds under running tap water to remove dust. The contents of each container were transferred to 2 L beakers and immersed in a 1% available chlorine solution from commercial bleach (sodium hyperchlorite) for 20 min as described in Okungbowa et al. (2002). The samples were returned to the large Joe White malting boxes, and rinsed for 30 seconds under running tap water. The boxes were placed in 70 L (112cm x 50cm x 16cm) containers (Stearlite Corporation, Townsend, MA) containing enough 0.2% sodium hydroxide (21°C) to fully immerse the sorghum. Samples were tempered in this solution, without agitation, with one change of solution at 3 h, for 6 h in total based on Lefyedi and Taylor (2006). After 6 h, the sorghum samples were rinsed for 15 seconds under running tap water, and transferred to the Joe White Micromalter (Adelaide, SA, Australia).

Sorghum grain was steeped at 24°C for 6 h wet, 3 h air, 8 h wet, 2 h air, 8 h wet. The air rests allowed for adequate respiration in embryo while removing carbon dioxide and ethanol, which restrain respiration (Bamforth 2006). During air rests, total flow was

30% with 0% air recirculation. Temperature and steep time were based on work by Dewar et al. (1997).

Germination occurred under 30% total flow and 0% air recirculation, with 4 full turns every 2 h (turning the grain boxes helps keep the rootlets from matting together and helps aerate the grain). Germination was performed at 26°C for 60 h. Each grain box was spritzed with 30 mL tap water twice daily, and "suspect" grains starting to show fungal growth were removed (Figure 3.1). Sorghum is different from barley in that it must be watered during germination (Dewar et al. 1997).

Sorghum grain was kilned in the Joe White Micromalter for 24 h at 50°C with 75% total flow and 0% recirculation. After kilning, samples were cleaned by knocking the rootlets off by rubbing the grain over a 41/2/64 in. slotted sieve screen. The resultant malted sorghum was sealed into plastic bags and shipped to Manhattan, KS.

Figure 3.1 Malted grain sorghum sample 83G66 shown after germination.
Source: Photograph by Chris Martens, USDA-ARS Cereal Crops Research Unit (2008).

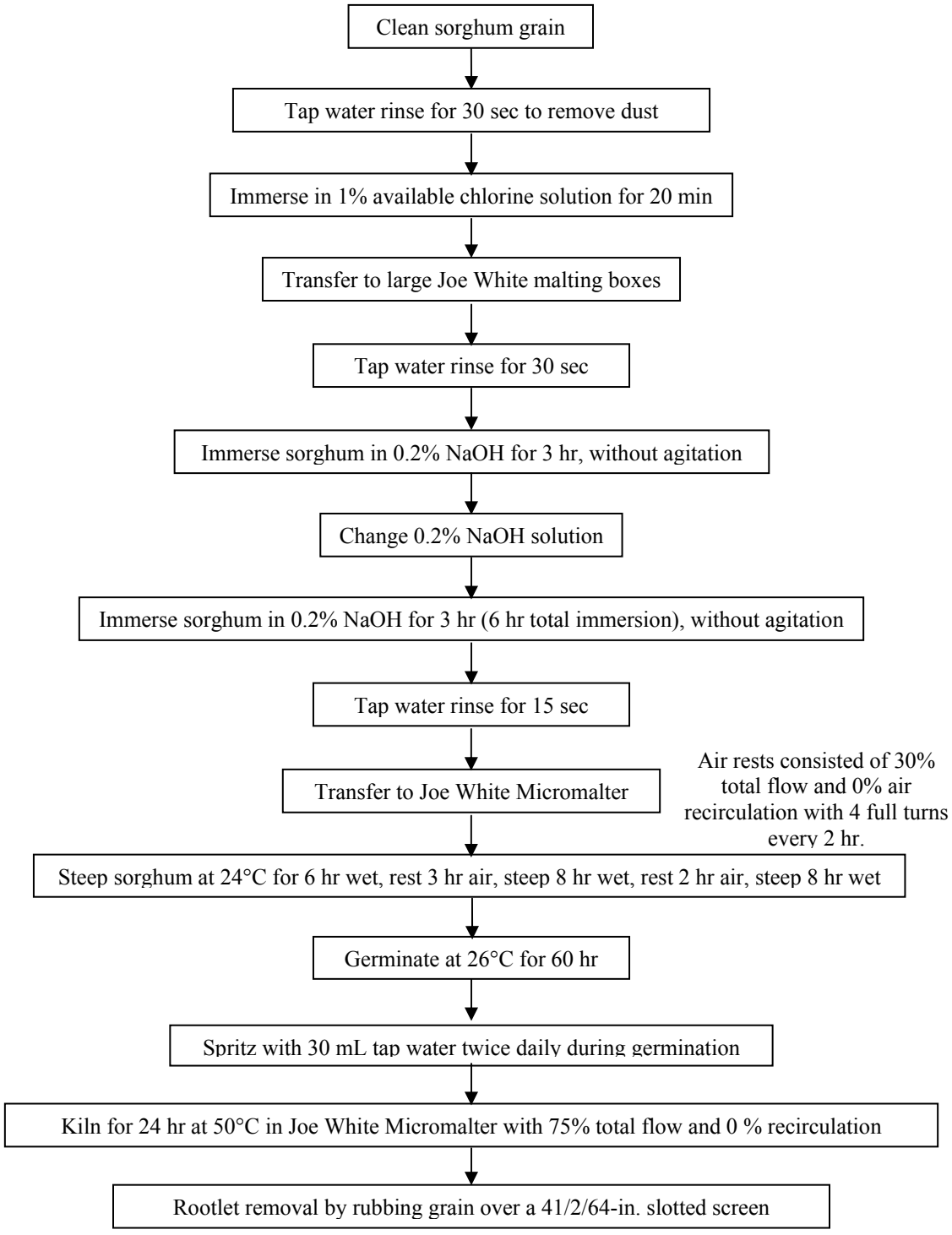


Figure 3.2 Flow chart of sorghum malting process.

Milling

The malted sorghum grain was milled at the Kansas State University Grain Science and Industry department milling lab. Milling began when the grain was placed in a Forster Lab Scourer (Forster, Wichita, KS) set on the slowest speed (by manual belt adjustment) to remove remaining rootlets (Figure 3.3). The lights were measured by weight and discarded because flour particles cause filtration problems during the lauter and sparge (Hallgren 1995). The fines portion was set aside and transferred to the aspirator, and the through portion was transferred to the Carter-Day Dockage tester (Figure 3.3) (Carter-Day, Minneapolis, MN.). The grist was then aspirated to remove remaining rootlets, which can cause a strong grassy flavor to be present in the beer (Taylor 1992).

The Carter-Day feed rate was set to 6 and the air was set to 7.5. The screens used were #6 round, #3 round, and #8 0.089 in. triangle screens were used in the Carter-Day for rootlet removal. Coarse portions and through fines were transferred to the Kice Aspirator (Figure 3.4) (Kice, Wichita, KS) to remove remaining contaminants and vegetative cells.

The air setting of the aspirator was 454. After both the coarse and fines were aspirated, the two fractions were blended together and milled on an experimental roller mill (Figure 3.4) (Ross, Oklahoma City, OK) on 6x10 smooth rolls 1.5:1, set to a 0.075 in. gap (Figure 3.5). Following milling, grist was sealed in 3.79 L (2 gallon) zip-top bags and placed in -10°C frozen storage until brewing or analysis was conducted.

Figure 3.3 Left, Forster Lab Scourer. Right, Carter-Day Dockage tester.

Figure 3.4 Left, Kice aspirator. Right, Ross experimental roller mill.

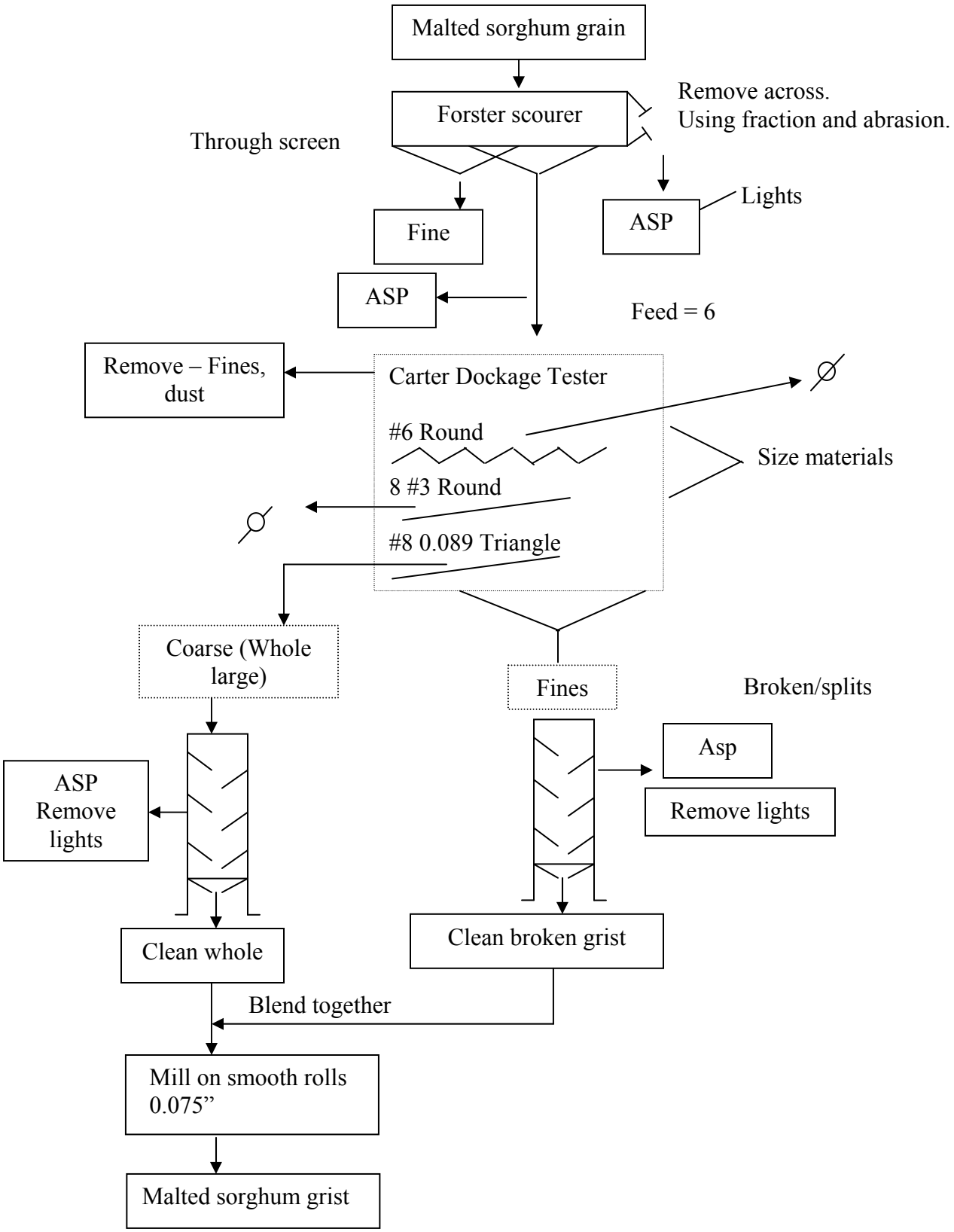


Figure 3.5 Flow diagram of sorghum grist milling process.

Grain and Malt Analysis

Prior to analysis, sorghum grain and malt were removed from frozen storage and allowed to equilibrate to 23°C overnight.

Malt Analysis at Malting Facility

All four sorghum hybrids were individually analyzed for the following parameters at the malting facility to evaluate how the malting process would proceed: percent moisture, percent nitrogen, and germinative energy. After malting percent steep out, and germination end were calculated. Percent moisture was measured to determine how much water the kernel may absorb and percent nitrogen was measured to evaluate the kernels ability to germinate. Briggs et al (1981) defines germinative energy as the proportion of grains (%) that will germinate under the conditions of a specified test. The test is performed by placing 100 grains in a Petri dish that contains paper or graded sand and counting the number of germinated kernels at 1, 2, and 3 days.

Proximate Analysis

Proximate analysis was performed on 30 g of whole kernel unmalted and malted sorghum samples to measure dry matter using AOAC Method 390.15, crude protein using AOAC Method 990.03, crude fat using AOAC method 920.39, and ash using AOAC Method 942.05 (AOAC International, 2000).

Crude fiber was measured using the ANKOM Technology equipment and procedure (Macedon, NY). The reagents used were sulfuric acid solution 0.255$\pm$0.005N 1.25g sulfuric acid/100 mL distilled water, 0.313$\pm$0.005N 1.25g sodium hydroxide/100 mL distilled water, and acetone. An ANKOM$^{200/220}$ Fiber Analyzer (Macedon, NY) was

used with ANKOM Technology F57 filter bags, an impulse bag sealer, and ANKOM Technology MoistureStop weigh pouch – F39. The F57 filter bag was weighed and tarred. Samples were ground to pass through a 1mm screen and 1.0±0.05g weighed directly into a filter bag. The bag was sealed closed within 0.5cm from the open edge using a heat sealer. The sample was then spread uniformly inside the filter bag by lightly shaking to eliminate clumping. A blank bag was weighed and included in digestion to determine the blank bag correction. The fat was extracted when sample bags were placed into a 500 mL bottle and completely covered with acetone and securely capped. The bottle was shaken 10 times, and the bags were allowed to soak for 10 min. The procedure was repeated once with fresh acetone. Acetone was then poured out and the bags were placed on a wire screen to air dry for approximately 5 min. Bagged samples were then placed onto a bag suspender tray. Approximately 1900-2000 mL of ambient temperature 0.255N H_2SO_4 solution was added to an ANKOM Fiber Analyzer vessel. The bag suspender tray was submerged, and the timer was set for 45 min and set to Agitation and Heat. Once the bag suspender was agitating, the lid was tightly sealed. After 45 min, the exhaust valve was released, the solution exhausted, and the exhaust valve closed. Approximately 1900-2000 mL of hot rinse water was added, Agitation was turned on and Heat was turned off, and samples were agitated for 3-5 min. The hot water rinse was repeated twice (a total of three times). Bagged samples were removed and excess water was gently pressed out. Bags were placed into a 250 mL beaker, acetone was added to cover, and allowed to soak for 2-3 min. Bags were removed, excess acetone was lightly pressed out, and the bags were then spread out and allowed to air dry. Samples were completely dried in an oven at 105°C for 2-4 h. After removal from the

oven, samples were placed in a desiccant pouch until cooled to ambient temperature and weighed. The entire sample and filter bag were placed in a pre-weighed crucible for 2 h at 550°C, cooled in a desiccator, and weighed for organic matter calculation (see equation 3.1).

Equation 3.1 Calculation for percent crude fiber (dry matter basis) using ANKOM technology

Calculate percent CFOM (Crude Fiber Organic Matter) (Dry matter basis) = $[(W_4-(W_1\times C_2)) \times 100]/(W_2 \times DM)$

W_1 = Bag tare weight
W_2 = Sample weight
W_3 = Weight after extraction process
W_4 = Weight of organic matter (OM) (Loss of weight on ignition of bag and fiber residue)
C_2 = Ash corrected blank bag (Loss of weight on ignition of bag/original blank bag)

Single Kernel Characterization System

Prior to analysis, grains were sorted manually to remove debris, and broken kernels. The single kernel characterization system (SKCS) 4100 (Perten Instruments, Inc., Springfield, IL) was used to analyze 300 kernels of each unmalted and malted variety as described in Bean et al. (2006).

Sample Preparation

Unmalted grain and malted grist were milled with a Cyclone sample mill with a 0.5-mm screen (Udy Corporation, Fort Collins, CO).

α-amylase

The quantity of α-amylase present in both the unmalted and malted sorghum flours was found using the Megazyme Alpha-Amylase Assay Procedure (Ceralpha Method) Assay Kit, K-CERA 08/05 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). The enzyme was extracted from the unmalted sorghum grain flour following the wheat and barley procedure, and from the malted sorghum following the malt procedure outlined by Megazyme (2004). The method uses non-reducing-end blocked *p*-nitrophenyl maltoheptaoside (BPNPG7) in the presence of excess levels of a thermostable α-glucosidase. The oligosaccharide was hydrolyzed by endoacting α-amylase, while the excess α-glucosidase gives quantitative hydrolysis of the *p*-nitrophenyl maltosaccharide fragment to glucose and free *p*-nitrophenol. To summarize this method — the cereal flour extract was incubated with the substrate mixture under defined conditions, and the reaction was terminated and color developed by the addition of a weak alkaline solution. Absorbance was measured at 400nm and the amount of α-amylase is calculated against a blank and reported in Ceralpha units/gram. Megazyme reports the definition of one Ceralpha Unit of activity, as the amount of enzyme, in the presence of excess thermostable α-glucosidase, required to release one micromole of *p*-nitrophenol from BPNPG7 in 1 min under the defined assay conditions (Megazyme 2004).

β-amylase

The amount of beta-amylase present in both the unmalted and malted sorghum flours was found using the Megazyme Beta-Amylase Assay Procedure (Betamyl-3 Method) Assay Kit, K-BETA2 12/04 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). The method uses Megazyme Betamyl-3, β-amylase test reagent

composed of high purity β-glucosidase and *p*-nitrophenyl-β-D-maltotrioside (PNPβ-G3). The hydrolysis of PNPβ-G3 to maltose and *p*-nitrophenyl-β-D-glucose by β-amylase, causes the *p*-nitrophenyl-β-D-glucose to be immediately cleaved to D-glucose and free *p*-nitrophenol by the β-glucosidase present in the substrate mixture. The rate of release of *p*-nitrophenol relates directly to the rate of release of maltose by β-amylase. When the reaction was stopped, the phenolate color was developed upon addition of a high pH Trizma base solution. Absorbance was measured at 400 nm, and the amount of β-amylase was calculated against a blank and reported in Betamyl-3 units/gram. Megazyme reports the definition of one Betamyl-3 Unit of activity as the amount of enzyme, in the presence of excess thermostable β -glucosidase, required to release one micromole of *p*-nitrophenol from PNPb-G3 in 1 min under the defined assay conditions (Megazyme 2004).

Starch Isolation

Starch was isolated from unmalted and malt flours using a modified sonication method from Park et al. (2006). Samples were prepared with the grain and malted grist from the Ceralpha and Betamyl tests. The whole unmalted grain and malted grist was milled with a Cyclone sample mill (Uday Corporation, Fort Collins, CO) and collected in 50 mL plastic centrifuge tubes and sealed.

The process began with the preparation of 500 mL of pH 10 buffer. The buffer was prepared by combining 50 mL of 125 mM sodium borate adjusted to pH 10 with 0.21g boric acid and 1.1 g borax in 50 mL of distilled water. This solution was diluted to 500 mL with distilled water and 2.5 g sodium dodecyl sulfate (SDS) and 2.5 g sodium

metabisulfite were added to the solution. The solution was placed on a hot plate on low heat and stirred slowly to dissolve particulates.

Five grams of sorghum flour, used for starch isolation, were mixed with 100 mL of buffer, creating at a 1:20 ratio. The solution and flour were gently stirred to ensure all flour particles were moistened. The beaker containing the solution and flour was then sonicated for 100 seconds in a Sonics VibraCell VCF-1500 ultrasonic processor (Sonics & Materials, Inc., Newton, CT). The glass beaker was set in ice water to reduce the temperature generated by sonication. Following sonication, the solution was transferred to a 50 mL plastic centrifuge bottle, and centrifuged for 10 min at 4000 rpm, and the supernatant was decanted. The precipitate was rinsed with 40 mL of distilled water and passed through a 62 μm screen. The suspension was centrifuged for 5 min at 4000 rpm, and the remaining liquid was decanted. The precipitate was resuspended in 40 mL of distilled water and centrifuged for 5 min at 4000 rpm and decanted. Following centrifuging, the liquid was again decanted and the dry pellet containing sorghum starch was freeze-dried (Labconco Freezone 6 Freeze Dryer (Labconco Corporation, Kansas City, MO). Amylose content and Differential Scanning Calorimetry (DSC) analysis was subsequently performed.

Amylose

The amylose content of each starch for both grain and malt was determined using Megazyme Amylose/Amylopectin Assay Kit, K-AMYL 04/06 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). Dimethyl sulphoxide (DMSO) and heat help disperse the starch at the start of this enzymatic test. Ethanol is added to remove lipids, and the amylopectin is precipitated with concanavalin A (Con A). Amylose is hydrolyzed with a

glucose oxidase/peroxidase reagent, and the samples are compared to the total starch sample (Megazyme 2006).

Differential Scanning Calorimetery

Differential Scanning Calorimetery (DSC) was used to evaluate the properties of the starch from both the grain and the malt using a PerkinElmer Diamond DSC (Waltham, MA) with an autosampler. Approximately 7 g of starch were weighed in a large-volume stainless steel pan. Water was added at a ratio of 1:3 and the pan was sealed. The sample was allowed to hydrate for 24 h in a refrigerator. The scanning range was 5-130°C at 10°C per min. Thermograms were analyzed for gelatinization attributes using Pyris software (PerkinElmer, Waltham, MA).

Statistical Design

Four treatments of sorghum grain were evaluated for grain and malt characterizations. Two replications were performed for DSC, amylose content, α-amylase, β-amylase and proximate analysis; and one replication was used for SKCS.

All data were analyzed using SAS, Software Release 9.1 (SAS Institute, Inc., 2003). When treatment effects were found to be significantly different ($\alpha = 0.05$), the least square means with Tukey-Kramer groupings were used to differentiate treatment means.

Formula

The formula for ale-style sorghum beer is shown in Table 3.1. Ingredients used were sorghum grist, rice hulls (Northern Brewer, St. Paul, MN.), Vanguard pellet hops with 4.9% alpha acids (AA) (Northern Brewer, St. Paul, MN.), Cascade pellet hops with

6.3% AA (LD Carlson Company, Inc., Kent, OH), and Czech Saaz pellet hops with 2.5% AA (Northern Brewer, St. Paul, MN), Danstar Nottingham dry ale yeast (Lallemand, Inc., Montreal, QC, Canada), and 15.7 L of water (City of Manhattan municipal water, Manhattan, KS).

Table 3.1 Formula for all grain ale style sorghum beer.

Amount (g)	Ingredient	Supplier, City, State
2270.00	Malted sorghum grist	USDA-ARS GMPRC[b], Manhattan, KS.
340.50	Rice hulls	Northern Brewer, St. Paul, MN
10.30	Vanguard pellet hops (4.9%AA[a]) (60 min)	Northern Brewer, St. Paul, MN
10.60	Cascade pellet hops (6.3%AA[a]) (10 min)	LD Carlson Company, Inc., Kent, OH
10.60	Czech Saaz pellet hops (2.5%AA[a]) (end)	Northern Brewer, St. Paul, MN
11.00	Danstar Nottingham dry ale yeast (1 pkg)	Lallemand, Inc., Montréal, QC, Canada
70.87	Priming sugar (Corn sugar (dextrose))	LD Carlson Company, Inc., Kent, OH
15.94 L	Water	City of Manhattan Municipal Water Source, Manhattan, KS

[a]Alpha acids
[b]United States Department of Agriculture-Agricultural Research Service Grain Marketing and Production Research Center (GMPRC)

Hop calculation

All hops were used in pellet form because of ease of use and availability. The formula is dependent on the variety of hop pellet, the alpha acid percentage and the time of addition to the 60 min boil. The formula chosen by the taste panel during preliminary research was modified due to hop shortages. Hallertau hop pellets were replaced with Vanguard hop pellets, which are similar to Hallertau (Papazain 2003). The International Bitter Units (IBUs) provided in the initial formula (Equation 3.2) for Hallertauer hops,

percent alpha acids, and the usage amount was used to recalculate the correct amount of Vanguard hops required to achieve the appropriate bitterness level.

Equation 3.2 International Bittering Units (IBU) Predicted. From Mosher (2004).

Hop quantity (oz) × AA% × Utilization% × Correction factor = IBU predicted

Water

Water was from the City of Manhattan municipal water source. Using a water quality report, Consumer Confidence Report (CCR), supplied by the City of Manhattan (Table 3.2) and the water guidelines provided by Palmer and Papazain (2006; 2003), the city water source was determined to contain the appropriate elements for quality beer production. A nomograph can be used to evaluate what color of beer can be brewed from a particular water source (Figure 3.4) (Palmer 2006). After denoting calcium and magnesium, a line is drawn between the two points indicating effective hardness. Then a line is drawn between effective hardness through the corresponding residual alkalinity and estimated mash pH (Palmer 2006).

Table 3.2 City of Manhattan 2007 Water Quality Report.

Mineral (ion)	Level Detected
Ca (ppm)	42
SO_4 (ppm)	100
Mg (ppm)	5.4
Na (ppm)	61
Cl (ppm)	89
Alkalinity as $CaCO_3$ (ppm)	26.7
pH (pH units)	9.3

Source: Consumer Confidence Report (CCR) City of Manhattan, Kan. June 2008.

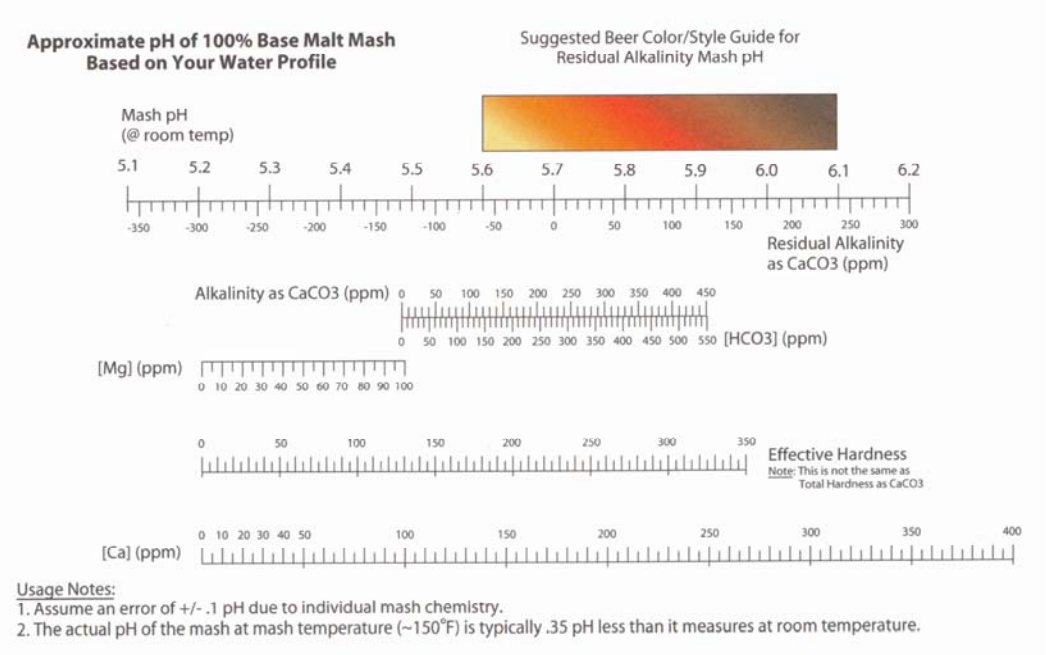


Figure 3.6 Nomograph used to calculate residual alkalinity of brewing water.
Source: Palmer (2006).

Usage Notes:

1. Assume a correlation error of +/-.1 pH and a range of at least 5 SRM due to individual mash chemistry.
2. The actual pH of the mash at mash temperature (-150°F) is typically .35 pH less than it measures at room temperature.
3. For best results, the mash pH should always be between 5.2 and 5.6, regardless of beer style, when measured at mash temperature.

Brewing

The brewing procedure began once all surfaces, equipment, and utensils were cleaned and sanitized. One Step sanitizer (LD Carlson Company, Inc., Kent, OH), an oxygen based sanitizer was used to sanitize all surfaces and equipment. Appropriate sanitation is necessary to prevent bacterial infection of the yeast that can spoil the beer and cause fermentation issues and off flavors. This procedure used a double decoction mash (Figure 3.13).

For the experimental research, an additional screen was added to the false bottom of the lauter tun. A 14 mesh stainless-steel mill screen obtained from the Kansas State University milling lab was cut to size and sewn to the existing false bottom using unflavored, wax coated dental floss.

Mash 1

For the initial mash in, approximately 1135 g malted sorghum grist was gently stirred into the mash tun without a false bottom, containing 3.1 L of water at 60°C (Figure 3.7). The temperature was allowed to stabilize for 5 min; the mash was gently stirred, and the temperature recorded. After the temperature stabilized at 50.5°C, the mash was allowed to rest 25 min (30 min total). Following the rest, the mash was transferred to a 11.4 L kettle and slowly heated to boiling, on a Corning International PC-620 (Lowell, MA) hotplate set to 5, over 10 min (96°C). The mash was stirred frequently over the 30-min boiling period. When the mash reached 75°C, a visual observation of the change in viscosity due to the amylase enzyme reaction, served as a control point. Following boiling, the mash was cooled to 65°C using an immersion wort chiller (Northern Brewer. St. Paul, MN). Mash 2 began when the boil timer was set.

Figure 3.7 Mash 1 during the boil.

Mash 2

Approximately 1135 g malted sorghum grist was gently stirred into 3.1 L water at 55°C in the mash tun with false bottom for the second mash-in (Figure 3.13). The temperature was allowed to stabilize for 5 min; the mash was gently stirred, and the temperature recorded. The grist and water continued to rest at 45.5°C for an additional 25 min. Following the 30-min total rest, mash 1 was combined with mash 2 in the mash tun with the modified false bottom.

Combined Mash 1 and 2

The combined mash was allowed to rest for 5 min. The mash was gently stirred, and the temperature measured. The decoction equation (Equation 3.3) and the measured mash temperature were used to determine the number of liters to remove to achieve a desired mash temperature of 67.5°C. The decoction, or removed portion, was heated to boiling over to 10 min, then allowed to boil for 10 min while stirring frequently, and

added back to the mash tun (Figure 3.8). The combined mash temperature stabilized at 67.5°C and rested for 30 min.

Following the 30-min rest, the mash was gently stirred, and the temperature measured. The decoction equation was again used to determine the number of liters to be removed to achieve the desired mash temperature of 76.5°C. The decoction was removed and boiled for 10 min with frequent stirring, then added back to the mash tun. The temperature of the mash stabilized at approximately 76.5°C (Figure 3.9).

Equation 3.3 Mash equations used to calculate amount of decoction to remove.

$$V_{mash} = M_{grain}\,(kg) \times (R + 0.68\ L/kg);$$

where R = water to grain ration in L/kg (1.57 L/kg usually)

$$V_{decoction} = V_{mash} \times \frac{(T_{target} - T_{start})}{(T_{boil} - T_{start})}$$

Note: Add 20% buffer to $V_{decoction}$ calculation. (Total $V_{decoction} = V_{decoction} \times 1.2$)

M = weight of mash in kg
V = volume of decoction to remove
Source: BrauKaiser (2008).

Figure 3.8 Decoction 1.
Left to right, removal of sorghum mash, addition of liquid, and decoction boil.

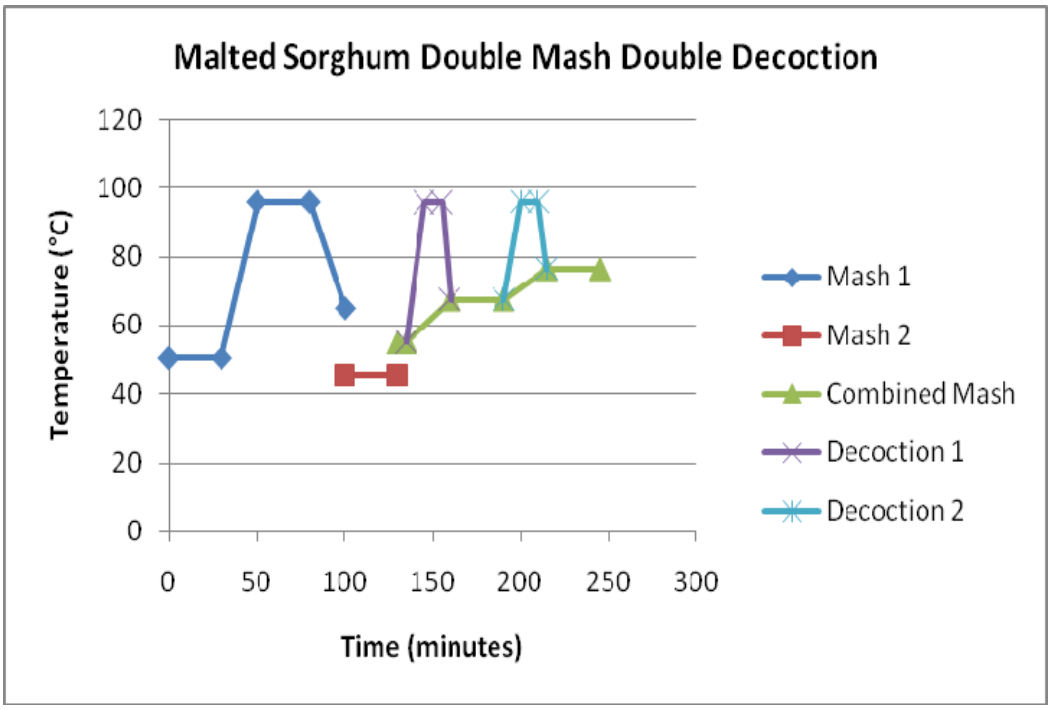


Figure 3.9 Representative image of a double mash double decoction profile.

Lauter

Mashout was conducted by allowing the grain to rest at 76.5°C for 30 min. Approximately 9.5 L of sparge water (water used to rinse the grain) was heated to 80°C and transferred to the lauter tun (Figure 3.9). Approximately 340.5 g rice hulls, incorporated as a filter aid, were stirred into the mash. The continuous sparge equipment was assembled. The grain bed was set by performing a Vorlof (or recirculation) where 0.5 L of the wort in the mash tun were removed and gently poured into the top of the mash tun without disturbing the grain bed. The Vorlof was repeated. Following Vorlof, the sparge removed the wort from the spent grains. The sprinkling of the sparge water on the grain rinses the grain of any remaining sugars. The water level was maintained 1 in. above the grain bed during sparging. The wort was collected into the 37.85 L brew pot with spigot and false bottom (PolarWare, Kiel, WI).

Figure 3.10 Lauter and sparge of sorghum beer.
Left picture: top vessel (cooler) is sparge tank, middle vessel (cooler) is lauter tun containing grist, and bottom is brew pot for wort collection. Middle: Lauter tun and brew pot. Right: View of sparge apparatus delivering sparge water to lauter tun.

Boil and cool

The 37.85 L brew pot was placed on a Barnstead International SP47230 (Dubuque, IA) and set to 8. Once the wort began to boil, the timer was set for 60 min, and 10.3 g of Vanguard hops were added to impart a bitter flavor. When 10 min remained in the boil, 10.6 g of Cascade hops were added to impart bitterness and aroma. At the end of the boil 10.6 g of Czech Saaz hops were added to impart aroma. The wort was cooled to 20–22°C using an immersion wort chiller (Northern Brewer. St. Paul, MN) (Figure 3.11).

During the cooling period, one package (11 g) Danstar Nottingham dry ale yeast (Lallemand, Inc., Montreal, QC, Canada) was prepared according to package directions. Approximately 100 mL sterilized (boiled) water at 30–35°C was place into a sanitized 150 mL beaker. Yeast was gently sprinkled on top of the water. The yeast was allowed to hydrate for 15 min, then gently stirred to incorporate all yeast particles, and allowed to set for an additional 5 min.

A pint of cooled wort was placed into a freezer-safe plastic container and frozen (-10°C) for later analysis. The remaining cooled wort was transferred to a clean and sanitized 11.36 L glass carboy (Northern Brewer, St. Paul, MN).

Yeast was pitched into the cooled wort. A sanitized drilled carboy bung and fermentation air lock (Northern Brewer, St. Paul, MN) filled with sanitizer were placed into the neck of the carboy. The carboy was covered with a dark cloth cover/jacket, and the beer was allowed to ferment (Figure 3.12).

Figure 3.11 Cool and filter.
Left: Cooling of wort using immersion wort chiller.
Right: filtering of wort while transferring to glass carboy.

Figure 3.12 Fermentation vessels and equipment.
Left, 11.36 L (3 gallon) glass carboy. Center, carboy bung (bottom white rubber) and fermentation lock (top). Right, fermenting beer in cover carboy.

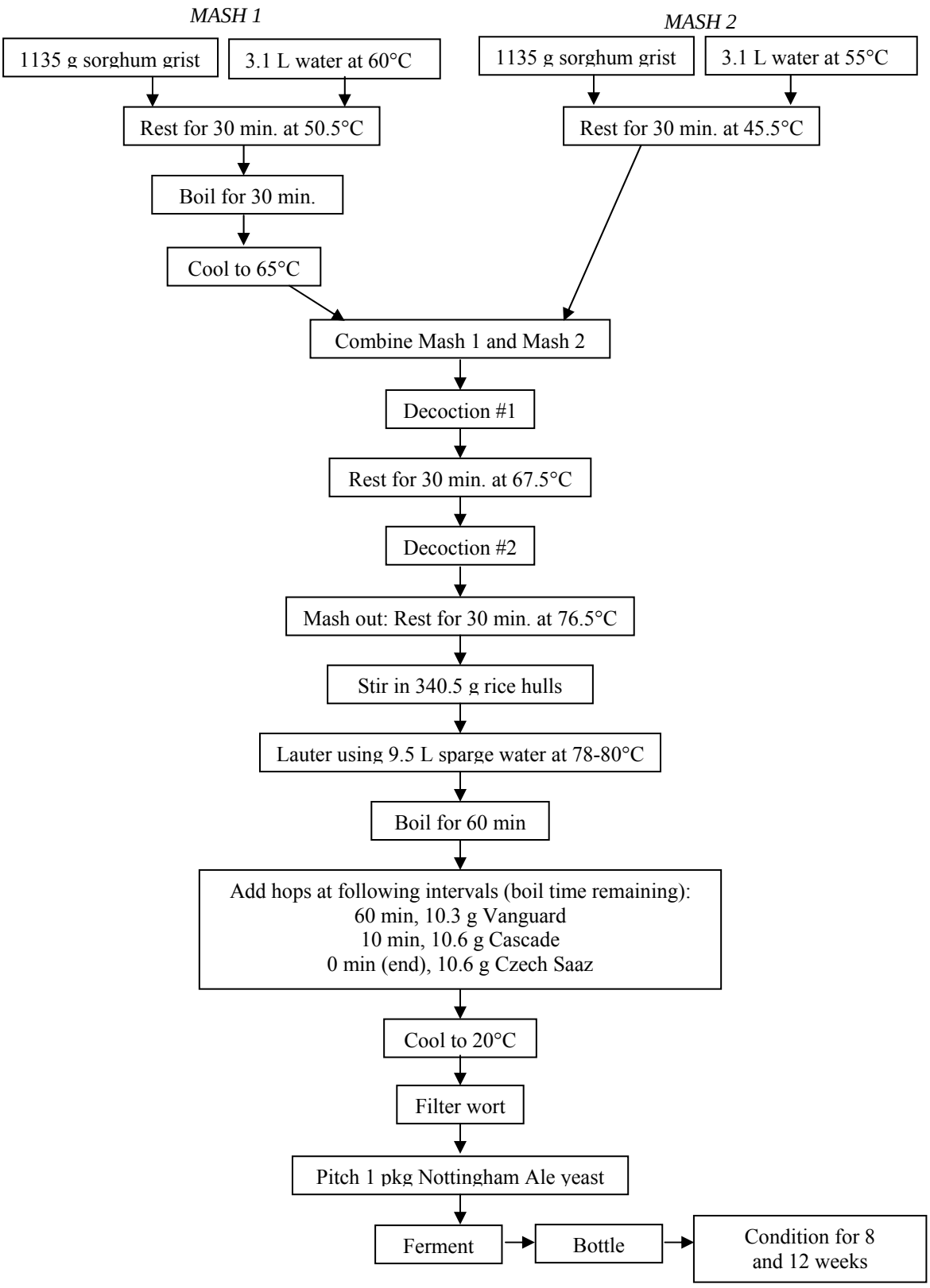


Figure 3.13 Flow diagram for sorghum beer brewing procedure.

Fermentation

Active fermentation was visible by bubbling in the air lock after approximately 6 h from the time the yeast was pitched. At approximately 12 h, a krausen developed along the top of the liquid beer (Figure 3.14). Bubbling in the air lock was continuous at this point. When bubbling had slowed to less than one bubble per min and the krausen had dissipated, a sample was aseptically removed to evaluate the rate of fermentation.

Figure 3.14 Sorghum beer fermenting in a glass carboy.
Foam on top of beer is referred to as the krausen.

Specific Gravity of Wort

One Step sanitizer (LD Carlson Company, Inc., Kent, OH) solution was prepared according to package instructions. The plastic thief and top of carboy around the opening were sanitized. The fermentation airlock and drilled bung were removed. The plastic thief was used to remove enough beer for sample requirements, being careful not to let the thief contact any surfaces that could contaminate the beer. Once the beer sample was

removed, the air lock, drilled bung, and top of carboy were re-sanitized and the airlock and bung were replaced.

Bottling

When the krausen had dissipated and the specific gravity measurement remained constant for two consecutive days, bottling commenced. The work area, along with all surfaces and equipment, were cleaned and sanitized. The following equipment was sanitized for bottling: mash paddle, bottle tree drainer ART. 15231 (Ferrari Group, Italy), 24 twelve-ounce brown glass bottles (Northern Brewer, St. Paul, MN), bottling bucket (Northern Brewer, St. Paul, MN), Fermtech Auto Siphon (Fermtech Ltd., Kitchener, ON, Canada), tygon siphon tubing (Northern Brewer, St. Paul, MN), Fermtech bottle filler (Fermtech Ltd., Kitchener, ON, Canada) and Red Baron (Emily) Capper bottle capper (Northern Brewer, St. Paul, MN).

Approximately 237 mL (1 cup) of water was heated to boiling. When boiling, 70.87 g of priming sugar (corn sugar (dextrose)) were added to the water. The priming sugar was stirred to dissolve, and the solution was boiled for 5 min to sanitize. Approximately 355 mL (1.5 cups) of water and 24 bottle caps were placed on the hot plate and heated to boiling. The caps were boiled for 5 min to sanitize.

Beer from the carboy was siphoned to the sanitized bottling bucket, ensuring the trub on the bottom of the carboy was not disturbed. The cooled priming sugar solution was added to the beer in the bottling bucket and gently stirred. The tygon siphon tubing and Fermtech bottle filler (Northern Brewer, St Paul, MN) were attached to the bottling bucket. The Fermtech bottle filler was inserted into the bottom of the sanitized bottle, and beer was filled to the top rim of the bottle. When the bottle filler was removed, the

bottle had the correct amount of beer to yield the appropriate headspace to allow for bottle conditioning. The bottle filler was removed and immediately capped using a Red Barron capper (Figure 3.15) (Northern Brewer, St. Paul, MN). Caps were labeled with the beer abbreviation and date. Bottles were allowed to condition at room temperature for 8 and 12 weeks.

Figure 3.15 Red Barron (Emily) bottle capper.

Analysis of Wort

Specific gravity, Brix, and pH

Specific gravity was measured using a triple scale hydrometer (Northern Brewer, St. Paul, MN). Approximately 237 mL of wort or beer was placed into a 1 in. plastic cylinder (included with the hydrometer). The hydrometer was placed in the liquid within the column and gently spun to prevent the hydrometer from sticking to the side. The liquid level was read at eye level for all three scales. The alcohol content by volume was calculated with the Equation 3.4.

Equation 3.4 Alcohol by volume

(Original specific gravity - final specific gravity) × 105 = % Alcohol by volume

Brix was measured with a Huake RHB-32ATC refractometer (Huake Instrument Co. Ltd., Shenzhen, China). Three drops of beer were placed onto the viewing window and the plastic cover was closed, ensuring no air bubbles were present between the window and the cover. The measurement was recorded based on the view from the view finder.

The pH was measured using a Hanna HI98129 handheld pH meter (Hanna Instruments, Woonsocket, RI). Approximately 25 mL of wort or beer were placed into a 50 mL beaker. The probe was inserted into the liquid and gently stirred until a stable pH and temperature reading were displayed.

Wort High Pressure Liquid Chromatography

High pressure liquid chromatography (HPLC) was used to evaluate the wort for glucose and maltose. The instrument used was a HP/Agilent 1100 Series; (Santa Clara, CA) the column used was a Rezex ROA Organic Acid 300 × 7.80 mm (Phenomenex, Torrance, CA) with a 4 mM H_2SO_4 mobile phase at 0.6 mL/min flow rate. The detection occurred with a diode array detector (DAD) monitored at 192 nm; peaks were integrated with ChemStation Software (Agilent Technologies, Santa Clara, CA). Frozen wort was removed the day prior to HPLC, FAN, and color analysis and placed in a refrigerator and allowed to thaw overnight. The thawed wort was gently stirred to mix thoroughly. The wort was transferred to a 50 mL plastic centrifuge bottle, centrifuged for 2 min at 4000 RPM. Samples were placed into a 3 mL syringe and filtered using a Millipore 0.8 μm

filter (Millipore Corporation, Billerica, MA.). Filtered samples were placed into HPLC vials and ran.

Wort Free alpha-Amino Nitrogen (FAN)

The AOAC 945.30 Characteristics of Wort method was used for the FAN and color measurements. Wort samples were placed in 50 mL vials prepared by centrifuge tubes for 2 min at 4000 RPM to remove remaining hop residues. The AOAC 945.30 procedure was followed from this point forward. A Beckman DU®530 LifeScience UV/Vis spectrophotometer (Beckman Coulter, Fullerton, CA) was used.

Wort Color

For color analysis, AOAC Method 972.13 was used. The wort was filtered using a 3 mL syringe and a Milipore 0.8 µm filter. The filtered wort was placed into one-half in. cuvettes and absorbance was determined at 430 nm using a Beckman DU®530 LifeScience UV/Vis spectrophotometer (Beckman Coulter, Fullerton, CA).

Analysis of Beer at 8 and 12 Weeks of Age

Specific gravity and alcohol were measured using a triple scale hydrometer (Northern Brewer, St. Paul, MN) following the previously stated method. Brix and pH were measured using the above methods.

HPLC of Beer Samples

High pressure liquid chromatography (HPLC) was used evaluate the amount of glucose and ethanol present in the beer using previously mentioned equipment and parameters. The instrument used was a HP/Agilent 1100 Series; (Agilent Technologies, Santa Clara, CA) the column used was a Rezex ROA Organic Acid 300 × 7.80 mm

(Phenomenex, Torrance, CA) with a 4 mM H_2SO_4 mobile phase at 0.6 mL/min flow rate. The detection occurred with a diode array detector (DAD) monitored at 192 nm; peaks were integrated with ChemStation Software (Agilent Technologies, Santa Clara, CA). Samples were 8 and 12 weeks old and at 23°C at sampling time. Approximately 100 mL of beer were poured into a beaker. The samples were drawn into a 3 mL syringe and then filtered using a 0.8 μm Millipore filter (Millipore Corporation, Billerica, MA) attached to the syringe. Filtered samples were placed into HPLC vials and run.

Beer Color

Color was analyzed using AOAC method 976.08 and Beckman DU®530 LifeScience UV/Vis spectrophotometer. Unfiltered samples were placed directly into one-half in. cuvettes and read at 430 and 700 nm. Other samples were filtered using a syringe and 0.8 μm filter attached to the end of the syringe then read at 430 and 700 nm.

Statistical Design

Four treatments of sorghum grain were evaluated for grain and malt characterizations. For grain and malt characterization, two replications were performed for DSC, amylose content, α-amylase, and β-amylase; one replication was used for SKCS and proximate analysis.

Data was compiled in Microsoft Office Excel 2007. All data were analyzed using SAS, Software Release 9.1 (SAS Institute Inc., 2003). When treatment effects were found to be significantly different, the least square means with Tukey-Kramer groupings were used to differentiate treatment means. A level of significance was observed at $\alpha = 0.05$.

For each sorghum hybrid, beer was brewed three times. Analysis of wort and beer was performed for all three samples produced per sorghum hybrid.

Four treatments of sorghum ale-style beer were evaluated for all tests. For wort characterization, two replications were performed for HPLC, color, and FAN, one replication was used for specific gravity, Brix, and pH.

Results and Discussion

Introduction

After the brewing and analysis of the data collected for the experimental study analysis of collected data was evaluated in two sections grain and malt followed by wort and beer.

Grain Analysis

Single Kernel Characterization System (SKCS)

The data from SKCS is a useful tool for measurement of the physical parameters of sorghum grain and sorghum malt, as well as the subsequent evaluation of these parameters on processing (Pedersen et al. 1996). The SKCS 4100 (Perten Instruments, Inc., Springfield, IL) instrument analyzed 300 kernels of each unmalted and malted hybrid as described in Bean et al. (2006). Table 3.3 depicts the physical parameters reported for 300 kernels of each sorghum hybrid for comparison of the SKCS data for hardness, weight, and diameter for unmalted samples.

Table 3.3 Comparison of mean population and standard deviation hardness, weight, and diameter among four grain sorghum hybrids (n=300 kernels).

Sorghum hybrid	Hardness (scale)	Weight (mg)	Diameter (mm)
82G63	78.0±22.3	24.4±7.4	2.0±0.4
83G66	78.7±17.6	27.0±6.2	2.2±0.3
RN315	87.8±18.4	19.3±4.4	1.8±0.2
X303	96.3±22.7	20.9±6.1	1.9±0.3

The results from the SKCS data indicate the grain hardness for the 300 kernels ranged from 77.95 to 96.34 for 82G63 and X303 respectively. The two red hybrids, 82G63 and 83G66, were the softest whereas the white hybrids, RN315 and X303, were harder.

A study by Pederson et al. (1996) utilized 16 sorghum lines and found hardness values ranged from 67±19 to 116±18 on a hardness scale. Bean et al. (2006) found the mean hardness on a range of tannin, waxy, and heterowaxy sorghum grain samples to be 77.5±17.6 using the SCKS hardness index. Beta et al. (1995) evaluated 16 different sorghum cultivars grown at the Texas Agricultural Experiment Station in Lubbock, TX in 1992. The study found that hardness correlated with test weight and density of raw and malted grains and that sorghums containing pigmented testa, normally have softer endosperm textures. The standard deviation of hardness is high possibly due to two reasons. First, is that the SKCS instrument is designed for the classification and grading of wheat (Pedersen et al. 1996; Osborne and Anderssen 2003). The second reason may be the pattern of sorghum kernel growth varies among genotypes as well as positions in the panicle. This means that for one stalk of sorghum the individual kernels can vary in age from 8 or 9 days (Gambín and Borrás 2005).

The SKCS data evaluated weight of sorghum kernel samples with values ranging from 19.34 to 27.02 mg for RN315 and 83G66 respectively. A study by Pederson et al. (1996) found weight values ranged from 15.5±3.0 to 38±8.1 mg. Bean et al. (2006) found the mean weight of sorghum grain samples to be 26.3±5.5 mg. The diameter of sorghum grain ranged from 1.78 to 2.23 mm. A study by Pederson et al. (1996) found diameter values ranged from 1.83±0.20 to 3.06±0.54 mm. Bean et al. (2006) found the

mean weight of sorghum grain samples to be 2.2±0.3 mm. In direct comparison to sorghum grain in this study was in the range of typical sorghum grains.

The results from the SKCS data indicate the malted sorghum grain hardness ranged from 30.81 to 77.95 for 83G66 and 82G63 on the hardness scale respectively (Table 3.4). A reduction in hardness was observed for hybrid 83G66, RN315, and X303 after malting. Owuama (1999) also observed that malting caused a decrease in sorghum grain caryopsis. The research suggested that vitreous portion of the kernel contributed to the hardness of the kernel and was largely unmodified during malting. Bamforth (2006) reported that a decrease in hardness is commonly found in barley grain after malting due to the breakdown of starch and proteins as the kernel begins the germination process. When brewing with barley, malted grain is preferred because the softer kernel is easier to mill and enzymes are available for starch degradation after germination.

Table 3.4 Comparison of mean population and standard deviation hardness, weight, and diameter among four malted sorghum hybrids using Single Kernel Characterization System (n=300 kernels).

Sorghum hybrid	Hardness (scale)	Weight (mg)	Diameter (mm)
82G63	78.0±31.3	22.3±6.1	1.9±0.3
83G66	30.8±42.3	22.7±5.4	2.0±0.4
RN315	67.1±37.9	16.1±4.0	1.6±0.2
X303	60.2±35.6	17.5±5.1	1.7±0.3

At this time there was no literature reporting SKCS data for malted grain sorghum, however, Nielsen (2003) found SKCS to be a useful tool for screening purposes in malting barley breeding programs and that the SKCS relative hardness index is the most important tool for predicting malting performance. Nielsen also reported SCKS for

relative hardness on four malted barley samples grown in Denmark range from 49.7 to 67.3.

Proximate analysis

Proximate analysis measured the percents of dry matter, crude protein, crude fat, and ash. The data from proximate analysis was used as a tool to evaluate the changes in the sorghum hybrids before and after malting (Table 3.5). Proximate analysis was performed on 30 g of whole kernel unmalted and malted sorghum samples to measure dry matter using AOAC Method 390.15, crude protein using AOAC Method 990.03, crude fat using AOAC method 920.39, and ash using AOAC Method 942.05 (AOAC International, 2000). Crude fiber was measured using the ANKOM Technology equipment and procedure (Macedon, NY).

Table 3.5 Comparison of proximate analysis among unmalted and malted sorghum hybrids.

Sorghum Hybrid		Dry Matter [a] (%)	Crude Protein [b] (%)	Crude Fat (%)	Crude Fiber (%)	Ash (%)
82G63	Grain	90.62	9.85	2.96	3.29	1.60
	Malt	93.36	9.47	2.35	3.16	1.02
83G66	Grain	87.16	10.60	3.59	3.38	1.60
	Malt	93.53	9.93	2.56	3.53	1.40
RN315	Grain	90.64	11.42	3.19	3.34	1.62
	Malt	93.70	10.52	2.34	3.52	1.22
X303	Grain	91.47	10.77	3.34	3.52	1.87
	Malt	93.00	9.74	2.66	3.62	1.44

[a]Results are reported on a 100% Dry Matter Basis.
[b]Calculated using a 6.25 conversion.

Proximate analysis for sorghum grain yielded values for dry matter from 87.16 to 91.47%, crude protein from 9.85 to 11.42%, crude fat from 2.96 to 3.59%, crude fiber

from 3.29 to 3.52% and ash from 1.6 to 1.87%. Sorghum malt dry matter values ranged from 93.00 to 93.70%, crude protein from 9.47 to 10.52%, crude fat from 2.34 to 2.66%, crude fiber from 3.16 to 3.62% and ash from 1.02 to 1.44%.

Ortega Villicaña and Serno Saldivar (2004) reported proximate composition of sorghum malt to be 5.83% moisture, 12.34% protein, 1.38% ash, 1.03% fat, and 2.23% fiber. Urias-Lugo and Serno Saldivar (2005) reported proximate composition of sorghum malt to be 8.6% moisture, 11.9% protein, 1.1% ash, 2.7% fat, and 2.0% fiber. Aisen and Ghosh (1978) evaluated a red sorghum from South Africa and white sorghums from Nigeria and Australia. Results for unmalted sorghum found the percent protein ranged from 10.3-12.7% on a dry matter basis. The study also found percent fat ranges from 3.0-3.7%, with the red variety containing more fat. Beta et al. (1995) reported protein percent ranged from 11.9-14.6%. Osorio-Morales et al. (2000) evaluated 4 normal and waxy sorghums and reported moisture ranged from 9.6-13.5%, protein from 11.0-11.7%, and ash 1.2-1.7%. A study by Agu (2005) evaluated four white, red, and yellow sorghum varieties from The Crop Research Institute in Nigeria, found a lower percentage of protein which ranged from 9.4 to 10.6%. This is most similar to the sorghum hybrids used in this study.

Aisen and Ghosh (1978) evaluated a red sorghum from South Africa and white sorghums from Nigeria and Australia. Results for malted sorghum found the percent protein ranged from 11.7-14.6% on a dry matter basis and fat ranged from 2.4-3.5%. Sorghum hybrids with lower protein contents may contain more starch which is beneficial to the malting and brewing processes. Lower fat contents are desirable to reduce oxidation off flavors in the final beer.

Differential Scanning Calorimeter (DSC)

Differential Scanning Calorimetery (DSC) was used to evaluate the properties of the starch from both the grain and the malt using a PerkinElmer Diamond DSC (Waltham, MA) with an autosampler. The thermograms of the DSC measured the thermal energy required to gelatinize starch, which is critical in the mashing step of brewing. Table 3.6 depicts as comparison of the DSC data across the four different unmalted sorghum grains.

Table 3.6 Comparison of DSC data among four unmalted sorghum hybrids.

Sorghum	Onset temperature (°C)
82G63	64.38 ± 1.62^{b}
83G66	65.51 ± 0.14^{a}
RN315	61.75 ± 0.13^{b}
X303	63.97 ± 0.39^{b}

[ab]Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Sorghum hybrid 83G666 exhibited a significantly higher onset temperature compared to the other hybrids. There were no significant differences among the other hybrids with respect to onset temperatures. The DSC onset temperature values for sorghum grain starch were significant and ranged from 61.75 to 65.51°C for 83G63 and RN315 respectively. The significant difference in onset temperature indicates the samples began to deviate from the baseline at different points. No significant difference was found among hybrids as function of peak temperature, which indicates the maximum difference between the baseline and DSC curve (Brown 2001). The peak temperature values ranged from 69.33 to 71.09°C for 82G63 and RN315 respectively. No significant difference was found for enthalpy (ΔH) with values ranging from 8.25 to 10.66 J/g for

X303 and 82G63 respectively. Hoseney (2004) reported similar results as the sorghum hybrid starch gelatinization temperature in his study ranged from 68 to 78°C. Akingbala et al (1988) reported the thermal properties using DSC of twenty-five sorghum varieties grown in India. The study reported onset temperature, peak temperature, and end temperature was 71.0±1.0°C, 75.6±0.9°C, 81.0±1.1°C respectively with gelatinization energies ranged from 2.51 to 3.95 cal/g. Akingbala et al (1984) reported that sorghum starch onset temperature, peak temperature, and gelatinization temperatures averaged at 70°C, 73°C, and 76°C, respectively.

A comparison of the DSC data obtained from the four malted sorghum hybrids found no significant differences for onset temperature, peak temperature, and enthalpy (ΔH). Onset temperature values for sorghum grain starch ranged from 65.25 to 66.77°C. All four hybrids exhibited a higher onset temperature after the grain was malted, most likely due to changes in starch composition during germination and kilning. This value is within the range for sorghum malt starch gelatinization. Overall, peak temperature tended to decline after malting and values ranged from 64.49 to 69.99°C. Enthalpy (ΔH) values ranged from 13.59 to 14.85 J/g respectively. Taylor (1992) reported the starch gelatinization of sorghum malt ranged from 64-68°C, whereas barley malt ranged from 55-59°C. Comparison of the DSC data for the grain and malt may indicate that during the malting process, the starch within the kernel may have been modified by enzymatic reactions resulting in an increased amount of energy required for gelatinization but at a lower temperature and a decrease the temperature required for starch gelatinization.

Malting process data

All four sorghum hybrids were individually analyzed for the following parameters at the malting facility to evaluate how the malting process would proceed: percent moisture, percent nitrogen, and germinative energy. After malting percents steep out moisture and germination end were calculated. Percent moisture was measured to determine how much water the kernel may absorb. Briggs et al (1981) defines germinative energy as the proportion of grains (%) that will germinate under the conditions of a specified test. The test is performed by placing 100 grains in a Petri dish that contains paper or graded sand and counting the number of germinated kernels at 1, 2, and 3 days. Table 3.7 indicates the parameters measured immediately after malting grain for all four sorghum samples. Dewar et al. (1997) defined steep-out moisture as the mass of spin-dried steeped grain calculated as a percentage used.

Table 3.7 Malting process analysis of four different sorghum hybrids.

Sorghum	Nitrogen (%)	Moisture (%)	Germinative Energy (%)[a]	Steep-out moisture (%)[b]	Germination end (%)
82G63	1.45	12.77	84	39.5	38.6
83G66	1.48	12.48	98	39.1	38.7
RN315	1.55	12.57	95	41.0	41.3
X303	1.49	13.80	94	41.4	41.2

[a]Percent of grain germinated in 3 days.
[b]Mass of spin-dried steeped grain, calculated as a percentage.

Data collected to evaluate the malting process showed the nitrogen values ranged from 1.45 to 1.55% and moisture from 12.48 to 13.80%. The germinative energy ranged

from 84 to 98%, the steep out ranged from 39.1 to 41.4%. The overall malting loss was 33%.

The current study was in agreement with a study by Agu (2005), the researcher evaluated four white, red, and yellow sorghum varieties from The Crop Research Institute in Nigeria, found total nitrogen ranged from 1.5 to 1.7% as is, moisture from 9.4 to 12.3%, and germinative energy ranged from 95.5 to 99.0 %. A study by Beta et al. (1995) found the germinative energy of 16 sorghum cultivars grown in Lubbock, TX ranged from 43-99%. Demuyakor and Ohta (1992) found the germinative energy of sorghum varieties grown in Ghana ranged from 72-90%. The germinative energy of sorghum hybrids used in this study was equal to or higher than the literature indicating a higher percentage of the kernels successfully germinated. Dewar et al. (1997) reported steep-out moisture in malted grain sorghum range from 30 to 36%, which is slightly lower than the amounts found in this study. In barley malting the common steep out moisture is 45% (Briggs et al. 1981).

Amylose

Amylose and amylopectin are the two components of starch. Amylose is a linear polymer of glucose units while amylopectin is a branched polymer of glucose units. Amylose content was evaluated to quantify the amount of starch present due to the relationship between ethanol content and starch. During the malting and brewing processes starch is converted to sugars by enzymatic reactions. Conversion of starch to sugars is necessary for yeast to metabolize the sugars and produce alcohol. The amylose content of each starch for both grain and malt was determined using Megazyme

Amylose/Amylopectin Assay Kit, K-AMYL 04/06 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland).

No significant difference was found for amylose content in the isolated sorghum starch. The amylose content in the sorghum hybrids ranged from 26.46 (82G63) to 33.68% (82G66) of the total starch of the sorghum hybrids. Sorghum hybrid X303, a white food grade sorghum, exhibited the higher amount of amylose prior to malting. Beta et al. (1995) reported that the percentage starch content in different sorghum grain hybrids ranged from 67.4-73.1%.

No significant difference was found for amylose content in the isolated starch of malted sorghum grain. The amylose values ranged from 27.10 to 29.34% of starch for sorghum hybrids X303 and RN315 respectively. After malting, RN315 contained the higher amount of amylose. Overall, the values of malting did not have large impact on the amylose content.

α-amylase and β-amylase

The quantity of α-amylase and β-amylase was measured in both the unmalted and malted sorghum hybrid flours to evaluate the amount of enzymes produced during malting. One of the goals of malting is to produce high enzyme activity (Hoseney 1994). When the kernel is moistened, the embryo and endosperm become hydrated switching on embryo metabolism. Subsequently, a hormonal signal triggers the synthesis of enzymes responsible for digestion of starch endosperm, as a source of energy for the developing embryo. As the growth process proceeds, enzymes break down cell walls and some of the protein in the starchy endosperm, the grain's food reserve, causing the grain to become more friable. The enzymes produced, especially amylases, are important for

breaking down the starch during the mashing process in the brewery (Bamforth 2006). Owuama (1999) reported that important starch degrading enzymes, including α- and β-amylase, are present in germinating grains and causes the hydrolysis of terminal, non-reducing α-(1→4) glucosidic linkages in both oligosaccharides and α-glucans yielding glucose. Owuama (1999) also reported that β-amylase activity produced maltose during mashing. Measurement of enzymatic activity is important in sorghum malt because β-amylase is often low (Dewar et al. 1997).

The quantity of α-amylase present in both the unmalted and malted sorghum flours was found using the Megazyme Alpha-Amylase Assay Procedure (Ceralpha Method) Assay Kit, K-CERA 08/05 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). The amount of beta-amylase present in both the unmalted and malted sorghum flours was found using the Megazyme Beta-Amylase Assay Procedure (Betamyl-3 Method) Assay Kit, K-BETA2 12/04 (Megazyme International Ireland Ltd., Co. Wicklow, Ireland).

The α-amylase content for sorghum grain was significantly different (Table 3.8) and ranged from 0.16 to 0.53 Ceralpha units/g. No significant difference was found for β-amylase content among the sorghum grain samples. The values ranged from 6.46 to 18.20 Betamyl units/g.

Table 3.8 Comparison of α- and β-amylase contents of selected sorghum grain hybrids.

Sorghum	α-amylase (Ceralpha units/g)	β-amylase (Betamyl units/g)	Ratio α-amylase to β-amylase
82G63	0.58±0.01[a]	6.46±2.49	0.09:1
83G66	0.16±0.02[c]	12.33±0.83	0.01:1
RN315	0.45±0.01[b]	13.50±0.83	0.03:1
X303	0.19±0.02[c]	18.20±2.49	0.01:1

[abc]Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

The mean α-amylase content measured for each malt are significantly different indicating the amount of α-amylase was different among the sorghum hybrids (Table 3.9). The values for malted sorghum grain α-amylase content ranged from 71.63 to 96.44 Ceralpha units/g. No significant difference was found for β-amylase content among the malted sorghum grain samples. The values ranged from 18.78 to 39.33 Betamyl units/g.

Table 3.9 α-amylase and β-amylase content of malted sorghum.

Sorghum	α-amylase (Ceralpha units/g)	β-amylase (Betamyl units/g)	Ratio α-amylase to β-amylase
82G63	92.68±0.27[a]	18.78±0.00	4.89:1
83G66	81.78±3.46[c]	38.74±1.66	2.13:1
RN315	96.44±24.19[b]	18.20±17.43	5.33:1
X303	71.63±10.37[c]	39.33±4.15	1.82:1

[abc]Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Beta et al. (1995) evaluated 16 different sorghum cultivars grown at the Texas Agricultural Experiment Station in Lubbock, TX in 1992. Malted cultivars RTx345 and Black Tx430, a yellow and a black, had the highest α-amylase activities of 167 and 169 U/g. β-amylase for the same cultivars was 26 and 22 U/g. The study also found β-amylase was low for all cultivars after malting and that the ratio α-amylase to β-amylase ranged from 0.12 to 0.25. A study by Agu (2005) evaluated sorghum varieties from

Nigeria, found α-amylase values ranged from 63 U/g for a white variety and 135 U/g for the yellow. β-amylase values ranged from 99 to 168 U/g. Demuyakor and Ohta (1994) reported 100% sorghum malt extract had a α-amylase content of 95 α-amylase units compared to 100% barley malt which contained 365 α-amylase units. The study also evaluated β-amylase and found 100% sorghum malt contained 48 β-amylase units while 100% barley malt contained 1017 β-amylase units. Taylor and Robbins (1993) report β-amylase activity is significantly correlated with malt diastatic power therefore diastatic power can be used to select sorghums for malting and brewing. Both studies found higher α-amylase activities than the sorghum hybrids used in this study. However, the β-amylase was similar or higher than the sorghum grown in Texas. The higher β-amylase activity in sorghum malt did produce higher amounts of maltose in subsequent wort evaluation shown by a correlation coefficient of 0.76.

The enzyme activity of sorghum malt has been compared to barley malt in several studies. A study by Dufour et al. (1992) evaluated 49 different sorghum cultivars grown in 10 different Asian and Africa nations. The results showed that eighty percent of the sorghum malts exhibited α-amylase activities similar to or higher than industrial lager barley malts. The study also reported that the sorghum malts showed low β-amylase activity and almost 60% of sorghum malts contained very low enzyme activity. It was also reported that the white sorghums in the study performed better in malting and brewing than red sorghum. Taylor (1992) reported the total diastatic power of sorghum malt was half that of barley. β-amylase activity of sorghum malt was very low, while α-amylase activity appeared to be slightly higher, 53 DU versus 35 DU for barely. Taylor (1994) found that sorghum has a lower ratio of β- to α- amylase (0.2), which limits the

conversion of starch to simple sugars. Pozo-Insfran et al. (2004) compared barley malt to sorghum malt in a double mash, double decoction process and found barley malt had six times the diastatic activity of sorghum malt. One theory to explain the differences in diastatic activity is that in barley the enzymes are synthesized in both the aluerone and scutellar tissues whereas in sorghum α-amylase is synthesized in the endosperm layer (Aisen et al. 1983).

Wort and Beer Analysis

After grain analysis the each sorghum hybrid was brewed into three gluten-free beer. Each beer was analyzed after brewing at the wort stage, at bottling, then at eight weeks and twelve weeks after bottling.

Wort Specific gravity, Brix and pH

Specific gravity, Brix and pH were measured after the brewing process for an indication of the success of the brewing process and to serve as a starting point for evaluation of fermentation. Specific gravity measures the density of the liquid wort and beer. Brewers utilize this measurement to indicate the amount of sugar in solution and to determine the rate of fermentation. Specific gravity was measured using a triple scale hydrometer (Northern Brewer, St. Paul, MN.) Brix measures the amount of dissolved solids, indicated the amount of sugar in wort and beer. Also, indicates of the rate of fermentation. Brix was measured with a Huake RHB-32ATC refractometer (Huake Instrument Co. Ltd., Shenzhen, China). The pH was measured using a Hanna HI98129

handheld pH meter (Hanna Instruments, Woonsocket, RI) and was measured to evaluate the brewing process and the rate of fermentation.

There was no significant difference among the hybrids with respect to specific gravity, Brix, and pH when measured at the end of fermentation and bottling which indicates that the fermentation of each beer occurred consistently. Specific gravity ranged from 1.020 to 1.023, Brix from 5.9 to 6.7 for sorghum hybrid X303 and 83G66 respectively, and pH from 2.24 to 4.64. For all three parameters measured sample 83G66 exhibited the highest values. Sample X303 had the lowest value for Brix and pH.

Ortega Villicaña and Serno Saldivar (2004) reported 100% sorghum malt wort had a pH of 5.20 and 1.064 specific gravity. Urias-Lugo and Serno Saldivar (2005) reported 100% sorghum malt wort had a pH of 5.20 and 1.055 specific gravity. Demuyakor and Ohta (1994) reported 100% sorghum malt extract had a pH of 5.73 compared to 100% barley malt which had a pH of 5.91. A study by Pozo-Insfran et al. (2004) reported that 100% sorghum malt wort had an initial pH of 5.20, whereas 100% barley malt wort had a pH of 5.40. Osorio-Morales et al. (2000) reported the wort pH ranged from 5.7-5.9 for sorghum malt worts from normal and waxy sorghums which was within the expected range of 5.2-5.8.

HPLC, Color, and Free alpha-amino nitrogen in wort

High pressure liquid chromatography (HPLC) was used evaluate the amount of maltose and glucose present in the beer using previously mentioned equipment and parameters.

HPLC analysis showed a significant difference among all hybrids for percent maltose in wort (Table 3.10). The difference between the highest and the lowest was

1.65%. Sorghum hybrid 83G66 wort contained the greatest percentage of maltose, followed by X303. No significant difference was found among the hybrids for percent glucose in wort. The values ranged from 1.05 to1.34%.

Dufour et al. (1992) used HPLC to analyze the sugar content of sorghum and barley malt worts. The study found sorghum malt wort contained 29.9+5.34% glucose and 52.5+_6.22% maltose, while barley malt wort contained 11.9+0.72 and 70.5+1.23%. A study by Pozo-Insfran et al. (2004) reported that 100% sorghum malt wort had an initial glucose and maltose contents of 35 and 48% respectively. The study also reported that the maltose amount was 40% lower than barley malt due to the lower β-amylase activity. A study by Pozo-Insfran et al. (2004) reported that 100% sorghum malt wort had an initial glucose content of 20.4 g/L while 100% barley malt wort contained 13.5 g/L. The study also reported initial maltose levels of sorghum malt wort to contain 27.9 g/L and barley malt wort to contain 46.4 g/L. Urias-Lugo and Serno Saldivar (2005) reported 100% sorghum malt wort contained 20.4 g/L of glucose and 27.9 g/L of maltose. A study by Igyor et al. (1997) used HPLC to quantify reducing sugars in sorghum malt wort and found a range of 186-422 μg/L. Ortega Villicaña and Serno Saldivar (2004) also quantified reducing sugars in sorghum malt wort and reported 108.8 mg maltose/mL.

For color analysis, AOAC Method 972.13, which utilized a spectrophotometer was used. Wort color was evaluated to determine if a color change occurred during fermentation and differences in wort color among the different color sorghum hybrids. Table 4.14 provides a comparison. No significant difference was found among the hybrids for wort color. The color values ranged from 2.53 to 5.56 °SRM. Orsorio-Morales et al. (2000) reported color values ranged from 3.91 to 5.64 °SRM and that these

were below optimum of 6.5 to 8.0. Ortega Villicaña and Serna Saldivar (2004) reported 100% sorghum malt wort color of 5.74 °SRM. Demuyakor and Ohta (1994) reported wort color of 1.89 °SRM for 100% sorghum malt wort.

The AOAC 945.30 procedure which utilized a UV/Vis spectrophotometer was used to quantify the FAN content. Dewar et al. (1997) defined FAN free-amino nitrogen (FAN) as the protelolytic break down of endosperm proteins and reported FAN is one of the primary terms to define sorghum malt quality for beer brewing. FAN is necessary for yeast to synthesize structural and enzymatic proteins required for normal growth as well as the metabolic processes which affect the flavor and stability of beer (Taylor and Boyd 1986; Pickerell 1985). Owuama (1999) reports that high levels of FAN (180 mg FAN/100g of malt) in wort are necessary to support rapid and proper fermentation.

A significant difference was found for FAN content of the wort indicating different malts released different amounts of FAN into the worts. The highest FAN content was for sorghum hybrid wort RN315, followed by 83G66, and X303 respectively (Table 3.10). Osorio-Morales et al. (2000) reported FAN levels of sorghum malt worts ranged from 104-165 mg/L. Demuyakor and Ohta (1994) reported 100% sorghum malt extract had a FAN content of 22.0 mg/100mL compared to 100% barley malt which contained 22.2 mg/100mL. Urias-Lugo and Serno Saldivar (2005) reported 100% sorghum malt wort had a FAN level of 75.28 mg/L. The FAN levels found in this study were below and within the range reported by Igyor et al. (1997) of 91-177 mg/L.

Table 3.10 Comparison of wort maltose content, and wort FAN.

Sorghum	Maltose (%)	FAN (mg/L)[1]
82G63	1.27±0.32[b]	65.15±20.15[c]
83G66	2.81±0.17[a]	151.37±41.11[a]
RN315	1.73±0.27[b]	191.34±2.34[a]
X303	1.93±0.32[b]	97.18±44.73[b]

[abc]Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)
[1]Free alpha-amino nitrogen

Beer Specific gravity, Brix and pH

Specific gravity, Brix and pH were measured at bottling and after the bottling process for an evaluation of secondary fermentation progress. The alcohol content by volume was calculated with the Equation 3.5. No significant difference was found for the specific gravity and pH parameters. A significant difference as found for the Brix parameter when compared against the 8 and 12 week samples.

Equation 3.5 Alcohol by volume

(Original specific gravity - final specific gravity) × 105 = % Alcohol by volume

Source: Papazain (2003).

Table 3.11 Brix content of sorghum beer.

Sorghum	Brix
82G63	6.6±0.2[ab]
83G66	5.4±0.2[ab]
RN315	6.6±0.1[b]
X303	5.9±0.4[a]

[ab]Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Ogbonna (1992) reported the results of a study that evaluated commercial beer. The evaluation found that sorghum beer had an original gravity of 10.97 and a pH value of 4.18. Ortega Villicaña and Serna Saldivar (2004) reported 100% sorghum malt beer pH of 4.11. Urias-Lugo and Serno Saldivar (2005) reported 100% sorghum malt beer pH of 4.12 and a specific gravity of 1.033. Barredo Moguel et al. (2001) found pH values of beer produced with 100% waxy sorghum grits and 22.8% regular sorghum grits adjunct followed typical brewery fermentation by becoming more acidic as fermentation progressed. The final pH values sorghum beer was slightly higher than commercial beer. Overall, the change in specific gravity, Brix, and pH from the wort to the beer indicates fermentation occurred and sugars were consumed.

HPLC of Beer

High pressure liquid chromatography (HPLC) was used evaluate the beer at 8 and 12 wk using previously mentioned equipment and parameters. The amounts of maltose, glucose, and ethanol were quantified to indicate the how the sugars produced through the malting and brewing processes were metabolized by the yeast into alcohol.

Evaluation of maltose curves indicated maltose was nearly entirely utilized during fermentation. A significant interaction was found between time and grain for percent ethanol indicating a malt effect for each level of time. A significant malt effect was found for percent glucose. Sorghum hybrid beer 83G66 was significantly different from the other hybrids. It had the highest percent ethanol and glucose percentages for eight and twelve weeks (Table 3.11).

Table 3.12 Comparison of beer ethanol content and beer glucose content among sorghum beer at 8 and 12 weeks.

Sorghum	Week	Ethanol (%)	Glucose (%)	Week	Ethanol (%)	Glucose (%)
82G63	8	3.62±0.84[b]	0.16±0.14[c]	12	2.85±0.65	0.18±0.12
83G66	8	4.17±0.56[a]	0.31±0.04[a]	12	3.51±0.49	0.31±0.03
RN315	8	3.31±0.60[b]	0.21±0.13[bc]	12	3.17±0.22	0.27±0.06
X303	8	3.28±0.38[b]	0.27±0.06[b]	12	3.02±0.32	0.30±0.07

[abc]Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Dufour et al. (1992) reported that the major difference between sorghum and barley malt worts was the residual glucose content. The study found sorghum malt wort glucose content to be 29.9% and maltose to be 52.5% whereas barley malt wort glucose content was 11.9% and maltose content was 70.5%. A study by Agu (2005) found glucose amounts ranged from 7.2 g/L to36.6 g/L and maltose levels from 27.6 g/L to 40.2 g/L. Ortega Villicaña and Serno Saldivar (2004) also quantified reducing sugars in sorghum beer and reported 42.8 mg maltose/mL. Urias-Lugo and Serno Saldivar (2005) reported 100% sorghum malt beer contained 3.6 g/L glucose and 13.0 g/L maltose by HPLC.

Evaluation of the HPLC data from the wort samples shows the higher ethanol contents seen in sorghum hybrids 83G66 and X303 also had higher contents of β-amylase in the malt and maltose in the wort. In addition, the HPLC for the beer indicates which sugars were utilized by the yeast indicated by a drop in specific gravity, Brix, and pH. A study by Igyor et al. (1997) used gas liquid chromatography (GC) to quantify ethanol in sorghum malt wort and found a range of 0.9-3.9%. Ortega Villicaña and Serno Saldivar (2004) measured ethanol in sorghum beer and reported 3.55% by gas chromatography (GC). Urias-Lugo and Serno Saldivar (2005) reported 100% sorghum malt beer

contained 3.95% ethanol measured by GC. Ogbonna (1992) reported the results of a study that evaluated commercial beer. The evaluation found that sorghum beer had an alcohol content of 3.49 % w/w. Barredo Moguel et al. (2001) compared two worts and two yeast inncoula preparations. One wort was produced from waxy sorghum grits and the second from a combination of adjuncts including 22.8% regular sorghum grits. The study found the ethanol composition was similar for both worts and range from 5 to 5.5%.

Beer Color

Color was analyzed using AOAC method 976.08 and Beckman DU®530 LifeScience UV/Vis spectrophotometer. Beer color was evaluated to determine if a color change occurred during fermentation and differences in final beer color among the different color sorghum hybrids. Table 4.14 provides a comparison of beer color measured at 8 and 12 weeks in degrees SRM among four selected sorghum hybrids. A significant difference was found among the malt indicating the initial malt color had an impact on final beer color. Ortega Villicaña and Serna Saldivar (2004) reported 100% sorghum malt wort beer of 4.24 Lovibond.

Table 3.13 Comparison of beer color measured at 8 and 12 weeks (°SRM).

Sorghum	Week	Color	Week	Color
82G63	8	4.6 ± 0.36^{a}	12	4.4 ± 0.4
83G66	8	2.9 ± 0.8^{b}	12	2.6 ± 0.6
RN315	8	3.9 ± 1.1^{a}	12	4.5 ± 2.2
X303	8	1.8 ± 0.4^{c}	12	3.2 ± 0.4

[abc]Means with different superscripts in columns indicate significant differences among treatments ($p<0.05$)

Overall Conclusions

Overall evaluation of the data collected from the initial grain to the final beer illustrates individual grain characteristics can provide information about the malting and brewing characteristics of a sorghum hybrid for use in all-grain gluten-free beer. Proximate analysis of the grain provides information for the malting process and can predict the success of malting. The characteristics of the sorghum hybrids after malting are indicative of the final beer by starch and enzyme contents. Amylose content indicates the amount of starch present and enzymatic components α-amylase and β-amylase play an important role in sugar extraction during brewing and subsequent fermentation. The amount of β-amylase present in the malt and the percent of maltose measured in the wort showed a correlation coefficient of 0.76 indicating greater amounts of β-amylase yielded a higher maltose content in the wort. Free α-amino nitrogen (FAN) was an important aspect of the wort for yeast fermentation. A correlation coefficient of 0.79 was found between FAN and alcohol content indicating higher FAN levels in the wort yielded better fermentation. Further investigation of the data indicated worts containing greater FAN contents showed a greater reduction in glucose during fermentation. Greater starch content and a high enzyme activity lead to higher ethanol production in the final beer.

Overall, no single hybrid showed superior performance. Sorghum hybrids should be chosen on the parameters of starch content, enzyme activity, and FAN which are critical for successful processing and fermentation. After these parameters, sorghum hybrids should be selected for the desired characteristics of the final beer. For example, pericarp color should be considered for darker color beer. Higher starch contents may be desirable for investigation of killing or roasting techniques.

Sorghum hybrid evaluation for the production of gluten-free beer should evaluate the amount of starch present in the unmalted and malted hybrids. Following malting the enzymatic activity of the malted grain is a critical aspect for the success of the mashing process during brewing. Measurement of FAN in both the malt and the wort is important to future evaluation to indicate if successful fermentation may occur.

The brewing process can be evaluated by analysis of starch gelatinization using DSC to provide parameters for establishing mash procedure in which successful gelatinization can occur allowing the enzymes to break down the starches. During the brewing and fermentation processes HPLC, FAN, specific gravity, Brix, and pH provide checkpoints for brewing process sugar extraction and fermentation progression. The process developed in this study worked for the sorghum hybrids used but may need alteration depending on crop year and grain storage prior to malting.

Future Research

Further research into gluten-free ale style beer using malted grain sorghum should investigate several items. Procedure refinement and controls should be applied to the malting and brewing processes to ensure that the equipment and procedures are optimized for the use of sorghum. The mashing process may be altered by enzyme addition to the mash for more complete starch degradation during mashing to increase alcohol content. Addition of a filtration procedure after the boil and before bottling may improve the appearance of the beer. Also, yeast cell count should be measured from pitching of the yeast throughout bottle conditioning.

Optimization of grain characterization can be used to improve the flavor profile to more closely resemble barley-based ales. One item is the effect of roasting processes

following malting. In traditional barley based brewing, ale style beer is distinguished by using roasted malts to impart a variety of flavors such as caramel, chocolate, and coffee. This process may improve the flavor profile as well as provide a final beer color that is more characteristic of traditional barley ales. Evaluation of other ingredients such as hops or yeast may help to improve the overall flavor profile. The propagation of yeasts that are better suited to ferment the sugar profile of sorghum wort can increase the alcohol content and improve the flavor profile of the final beer.

References

Agu, R.C. 2005. Some relationships between enzyme development, extract recovery, and sugar profile in malted sorghum. Technical Quarterly of the Master Brewers Association of the Americas. 42(2):120-124.

Akingbala, J.O., Gomez, M.H., Rooney, L.W., Sweat, V.E. 1988. Thermal properties of sorghum starch. Starch/Stärke. 40(10):375-378.

Aisen, A.O., Ghosh, B.P. 1978. Preliminary studies of the germination behavior of guinea corn (Sorghum vulgare). Journal of Food Science and Agriculture. 29(10):850-852.

Aisen, A.O., Palmer, G.H., Stark, J.R. 1983. The development of enzymes during germination and seedling growth in Nigerian sorghum. Starch/Stärke 35(9):316-320.

AOAC International. 2000. Standard Methods (CD-ROM).

Bamforth, C.W. 2006. Scientific principles of malting and brewing. American Society of Brewing Chemists. St. Paul, MN.

Barredo Moguel, L.H., Rojas de Gante, C. 2001. Comparisons between a commercial wort and a waxy sorghum wort fermented into lager beer with emphasis on yeast growth and ethanol production. Journal of the American Society of Brewing Chemists. 59(1)24-27.

BrauKaiser. 2009. Decoction mashing (part 1), decoction mash (part 2), decoction mash (part 3). http://braukaiser.com/wiki/index.php?title=Decoction_Mashing (accessed at Apr 28, 2009).

Bean, S.R., Chung, O.K., Tuinstra, M.R., Pedersen, J.F., Erpelding, J. 2006. Evaluation of the single kernel characterization system (SKCS) for measurement of sorghum grain attributes. Cereal Chem. 81(1):108-113.

Beta, T., Rooney, L.W., Waniska, R.D. 1995. Malting characteristics of sorghum cultivars. Cereal Chem. 72(6):533-538.

Briggs, D.E., Hough, J.S., Steven, R., Young, T.W. 1981. Malting and brewing science, Volume 1, 2nd Ed. Chapman and Hall, New York, NY.

Brown, M.E. 2001. Introduction to thermal analysis: techniques and applications. Kluwer Academic Publishers, Norwell, MA. http://books.google.com/books?id=1rw2eVYlI4oC&pg=PA65&lpg=PA65&dq=dsc+%22peak+temperature%22&source=bl&ots=X1ku-9XkcB&sig=zek9FIPzyJt_DE7ofRdtRaV0D9Q&hl=en&ei=adWmSof1FIS8NtK85acI&s

a=X&oi=book_result&ct=result&resnum=5#v=onepage&q=dsc%20%22peak%20temperature%22&f=false accessed on Sep 8, 2009.

Demuyakor, B. Ohta, Y. 1992. Malt characteristics of Sorghum vulgare varieties from Ghana. Journal of Food Science and Agriculture. 59:457-492.

Dewar, J., Taylor, J.R.N., Berjak, P. 1997. Determination of improved steeping conditions for sorghum malting. Journal of Cereal Science. 26:129-136.

Dewar, J. and Taylor, J.R.N. 1997. Role of α-glucosiodase in the fermentable sugar composition of sorghum malt mashes. Journal of the Institute of Brewing. 100(Nov-Dec):417-419.

Dufour, J.P., and Mélotte, L., Srebrink, S. 1992. Sorghum malts for the production of lager beer. Journal of the American Society of Brewing Chemists. 50(3):110-119.

Fasano, A., Catassi, C. 2001. Current approaches to diagnosis and treatment of celiac disease: an evolving spectrum. Gastroenterology. American Gastroenterological Association. 120:636–651.

Hallgren, L. 1995. Lager beer from sorghum. In *Sorghum and millets: chemistry and technology*; Dendy, D.A.V. 1995. American Association of Cereal Chemists, Inc.: St. Paul, MN, 1995.

Hoseney, R.C. 1994. Principles of cereal science and technology. 2nd ed. American Association of Cereal Chemists. St. Paul, MN.

Gambín, B.L, Borrás, L. 2005. Sorghum kernel weight: growth patterns from different positions within the panicle. Crop Science 45:553-561.

Lefyedi, M.L., Taylor, J.R.N. 2006. Effect of dilute alkaline steeping on the microbial contamination, toxicity, and diastatic power of sorghum malt. Journal of the Institute of Brewing. 112(2):108-116.

Megazyme. 2004. Megazyme alpha-amylase assay procedure (ceralpha method) assay kit, K-CERA 08/05. Megazyme International Ireland Ltd., Co. Wicklow, Ireland.

Megazyme. 2004. Beta-amylase assay procedure (betamyl method) K-BETA 12/04. Megazyme International Ireland Ltd., Co. Wicklow, Ireland.

Megazyme. 2006. Megazyme amylose/amylopectin assay kit, K-AMYL 04/06. Megazyme International Ireland Ltd., Co. Wicklow, Ireland.

Mosher, R. 2004. Radical brewing: recipes, tales, and world-altering meditations in a glass. Brewers Publications, Boulder, CO. 324 p.

Nelson, M. 2005. The barbarian's beverage: a history of beer in ancient Europe. Routledge. New York, NY.

Nielsen, J.P. 2003. Evaluation of malting barley quality using exploratory data analysis. II. The use of kernel hardness and image analysis as screening methods. Journal of Cereal Science. 38:247-255.

Ogbonna, A.C. 1992. Developments in the malting and brewing trials with sorghum. World Journal of Microbiology and Biotechnology. 8(2):87-91.

Okungbowa, J., Obeta, J.A.N., Ezeogu, L.I. 2002. Sorghum β-amylase production: relationship with grain cultivar, steep regime, steep liquor composition and kilning temperature. Journal of the Institute of Brewing. 108(3):362-370.

Ortega Villicaña, M.T.O. and Serna Saldivar, S.O. 2004. Production of lager beer from sorghum malt and waxy grits. Journal of American Society of Brewing Chemists. 62(4):140-146.

Osorio-Morales, S., Serna Saldivar, S.O., Chaves Contreras, J.C. 2000. Production of brewing adjuncts and sweet worts from different types of sorghum. Journal of the American Society of Brewing Chemists. 58(1)21-25.

Osborne, B.G., Anderssen R.S. 2003. Single-kernel characterization principles and applications. Cereal Chem. 80(5):613-622.

Owuama, C.I. 1997. Review sorghum: a cereal with lager beer brewing potential. World Journal of Microbiology and Biotechnology. 13:253-260.

Owuama, C.I. 1999. Brewing beer with sorghum. Journal of the Institute of Brewing. 105(1):23-34.

Park S.H., Bean S.R., Wilson J.D., Schober T.J. 2006. Rapid isolation of sorghum and other cereal starches using sonication. Cereal Chem. 83(6):611-616.

Palmer, J.J. 2006. How to brew everything you need to know to brew beer right the first time. Brewers Publications. Boulder, Colorado. Images http://www.howtobrew.com/images/f83.pdf (accessed on 5-30-08).

Papazain, C. 2003. The complete joy of homebrewing. 3rd ed. Harper Collins. New York, New York.

Pedersen, J.F., Martin, C.R., Felker, F.C., Steele, J.L. 1996. Application of the single kernel wheat characterization technology to sorghum grain. Cereal Chem. 73(4):421-423.

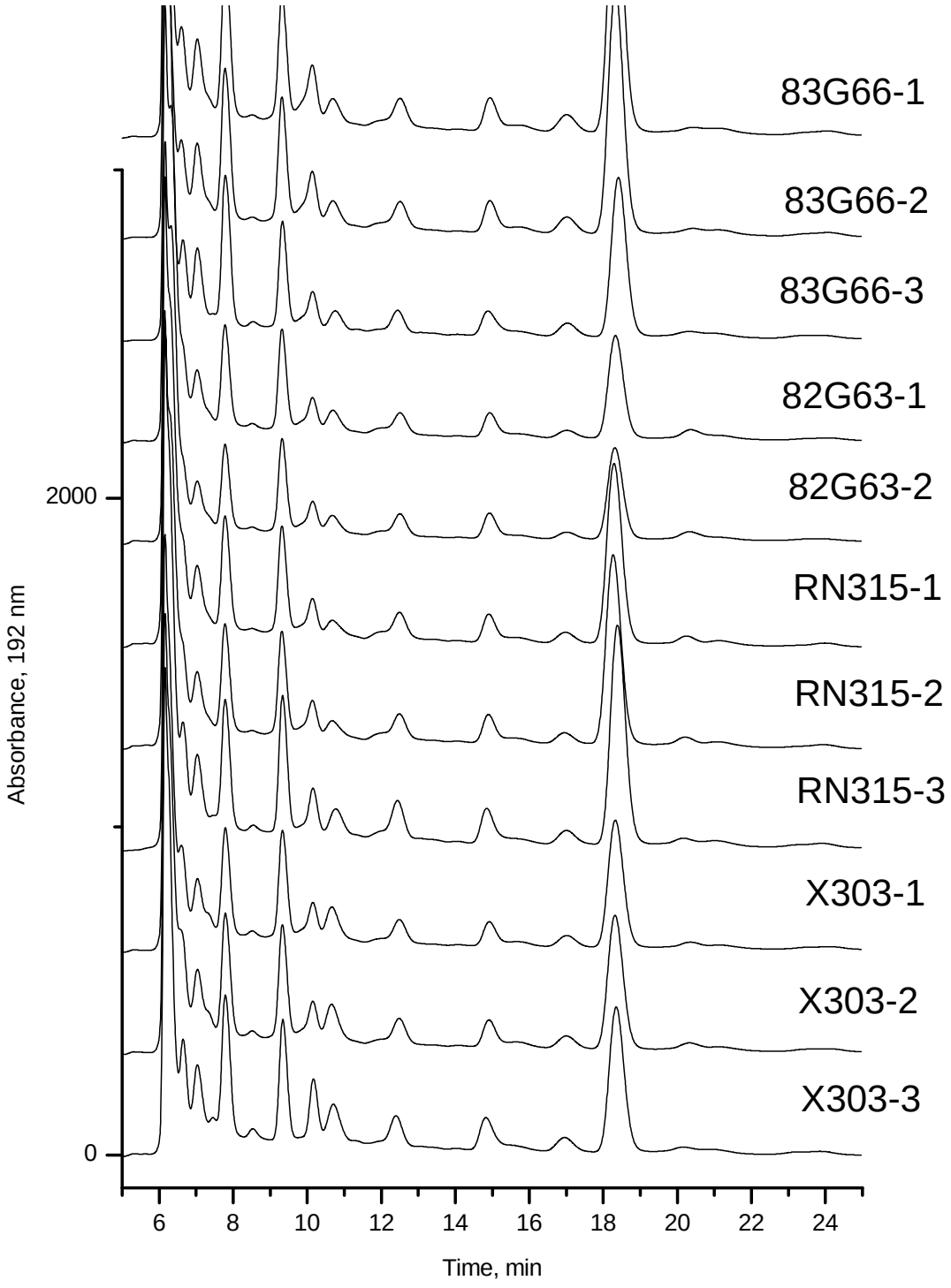
Pozo-Insfran, D.D., Urias-Lugo, D., Hernandez-Brenes, C., Serna Saldivar, S.O. 2004. Effect of amyglucosidase on wort composition and fermentable carbohydrate depletion in sorghum lager beer. Journal of the Institute of Brewing. 110(2):124-132.

Taylor, J.R.N. 1992. Mashing with malted grain sorghum. Journal of the American Society of Brewing Chemists. 50(1):13-18.

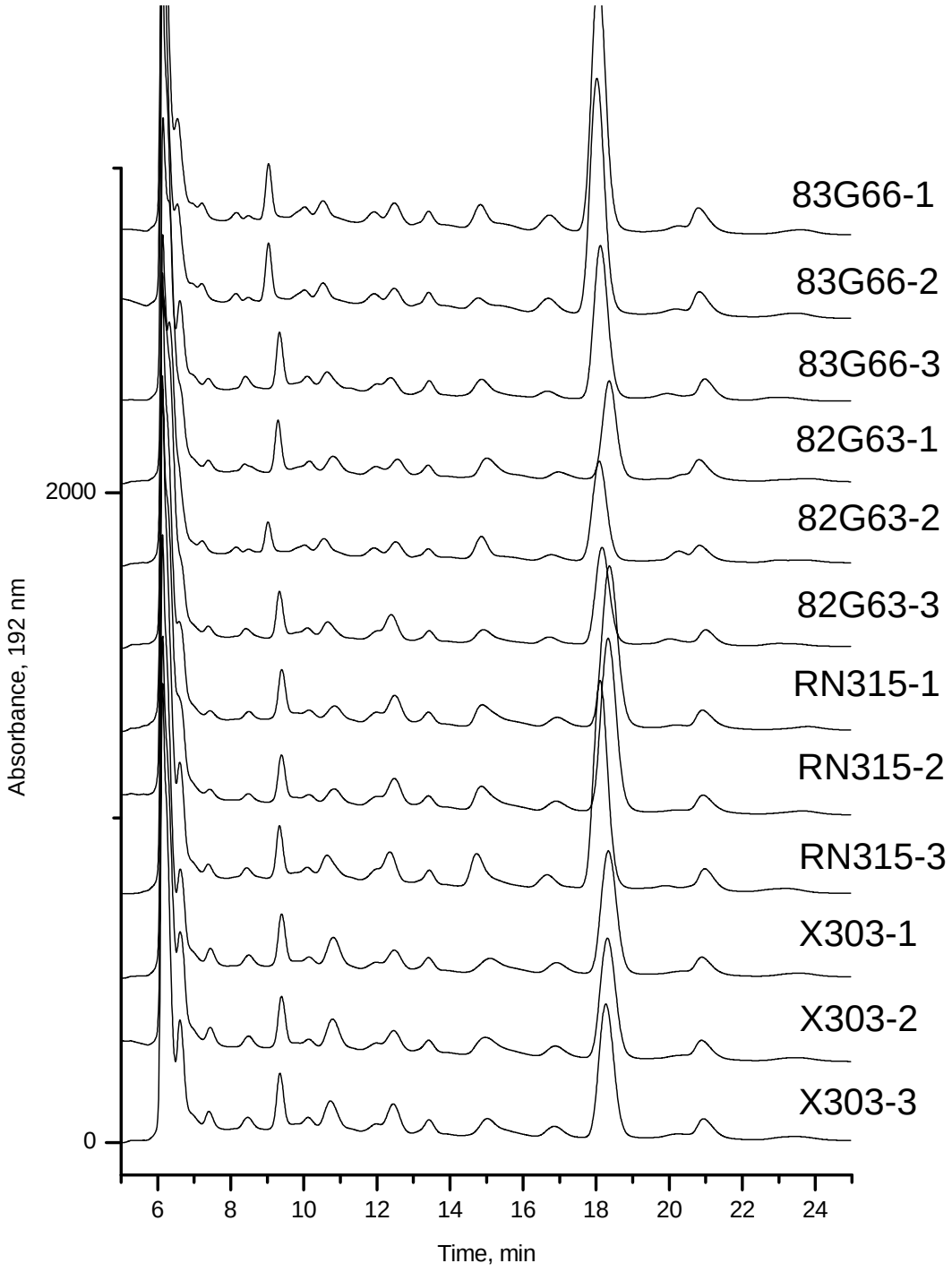
Taylor, J.R.N., Robbins, D.J. 1993. Factors influencing beta-amylase activity in sorghum malt. Journal of the Institute of Brewing 99:413-416.

Urias-Lugo, D., Serna Saldivar, S.O. 2005. Effect of amyglucosidase on properties of lager beers produced from sorghum malt and waxy grits. Journal of the American Society of Brewing Chemists. 110(2):124-132.

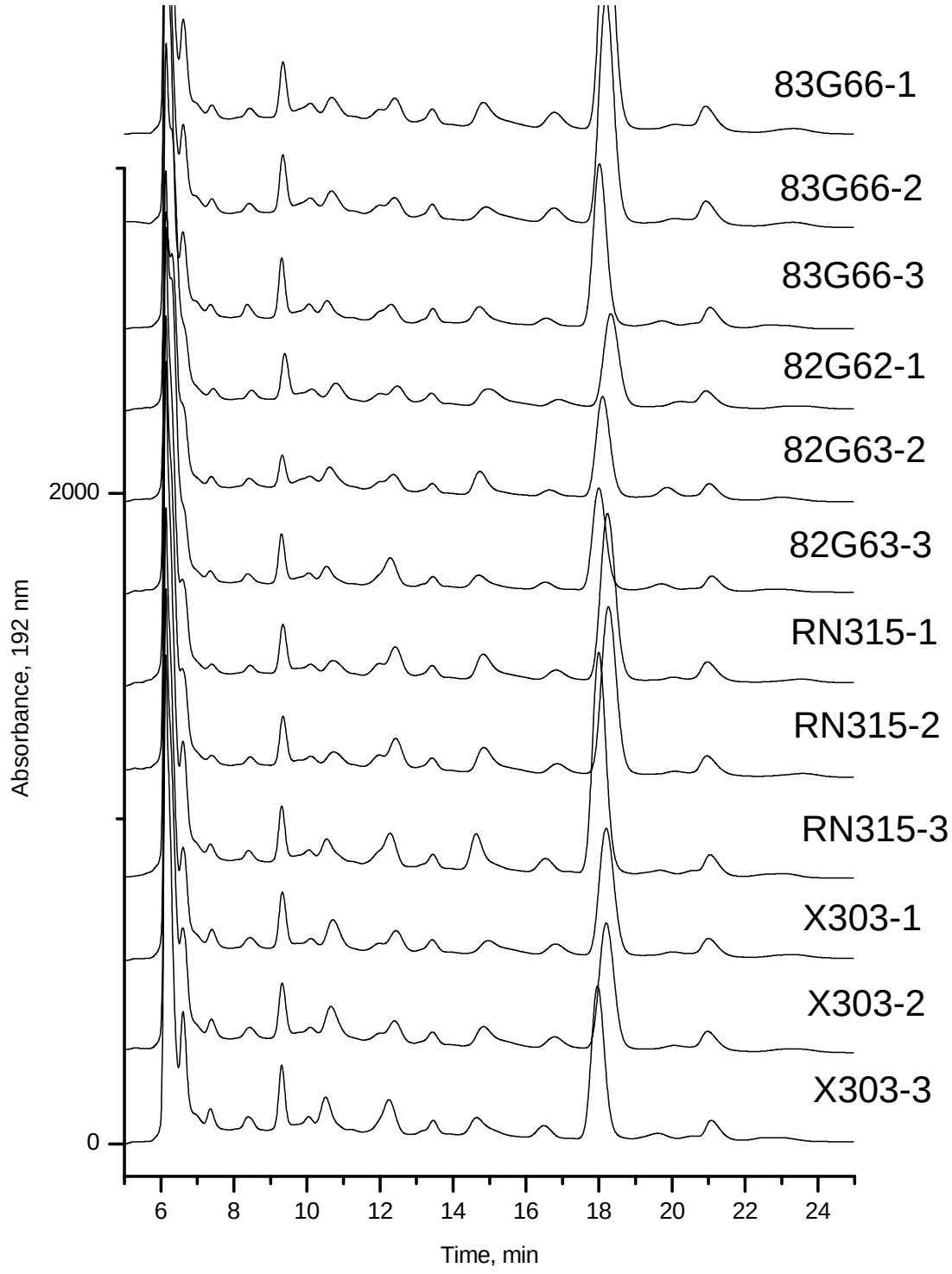
Appendix A - HPLC Curves



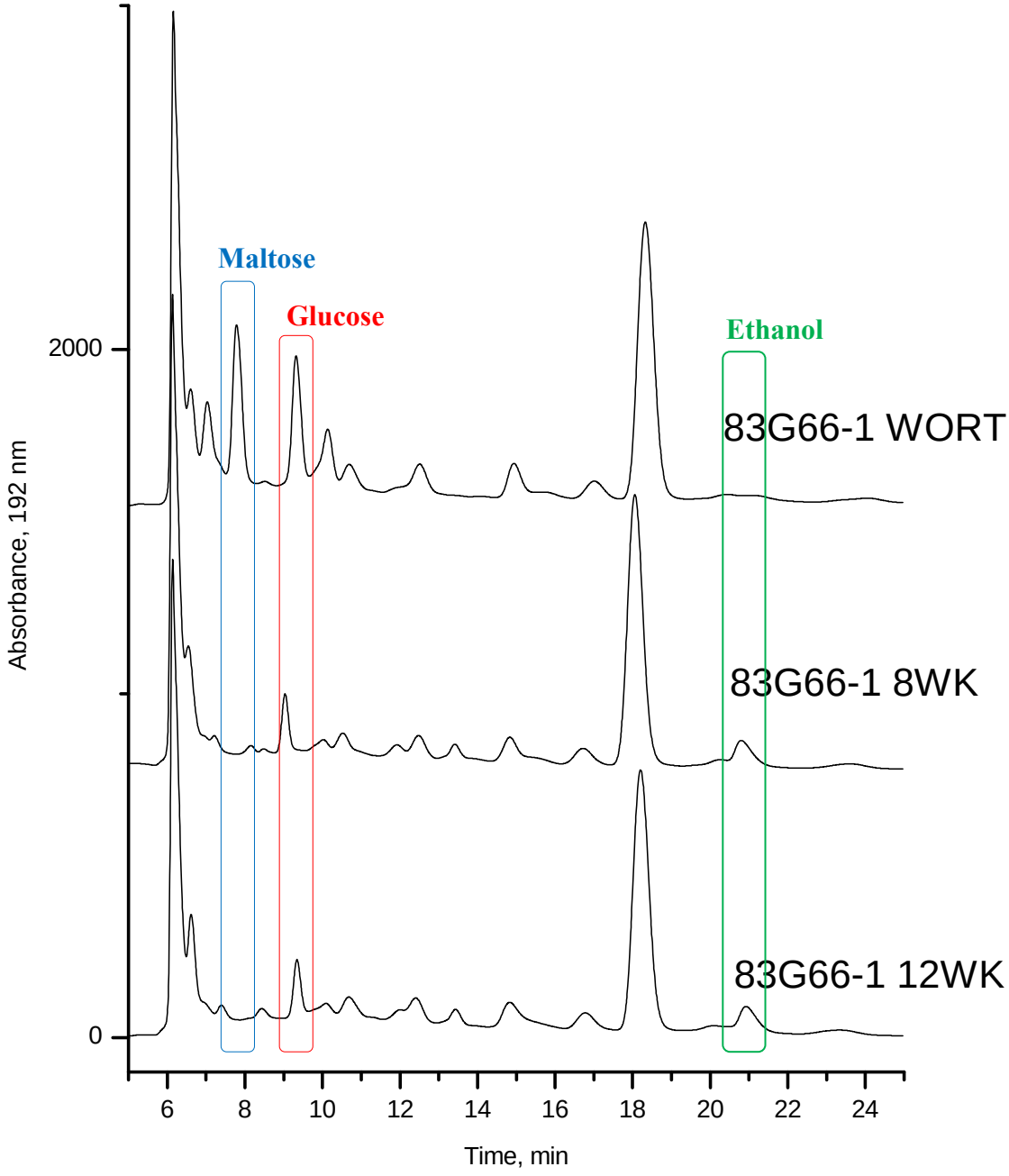
Appendix A 1 HPLC chromatogram for sorghum hybrid worts.



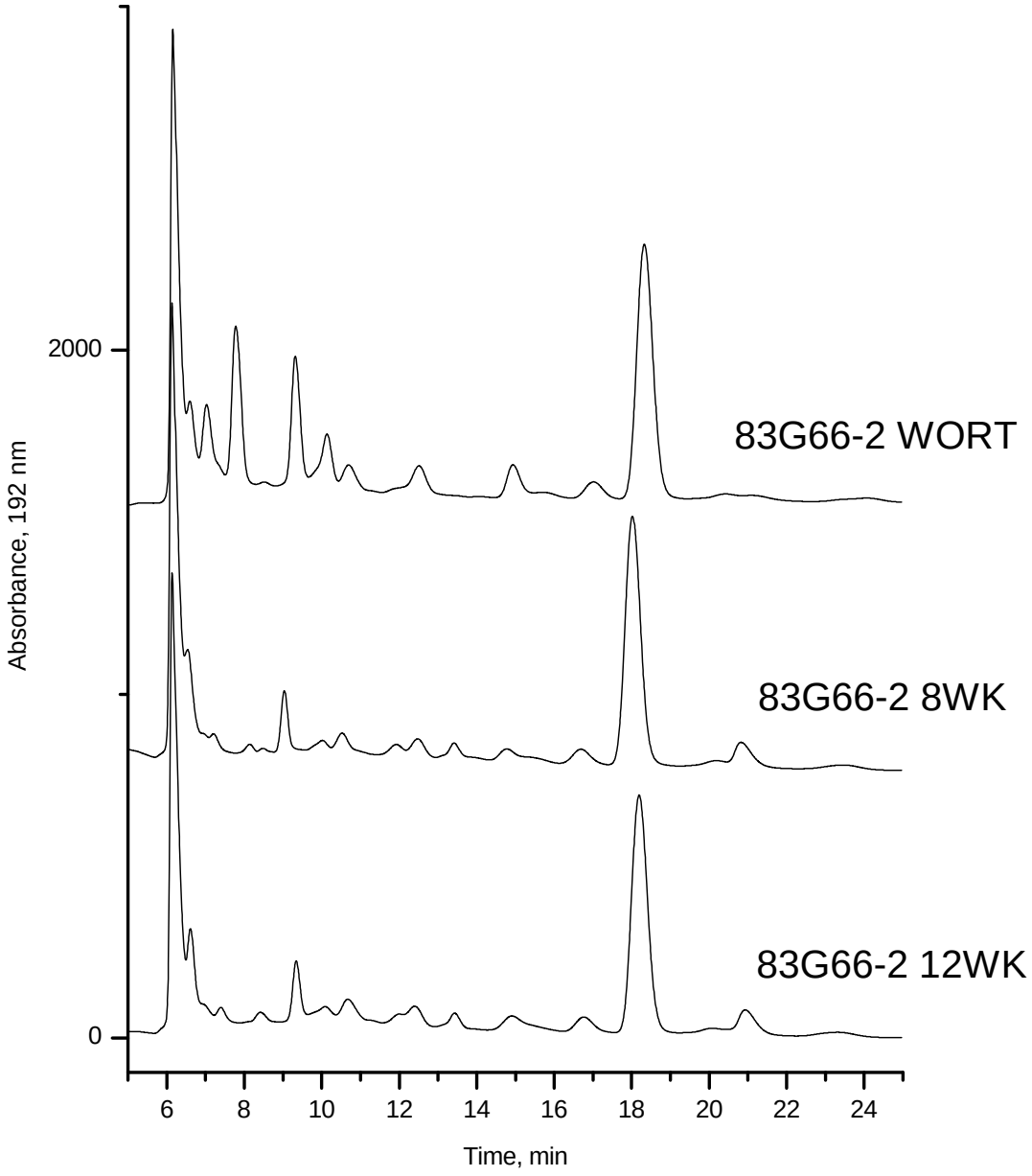
Appendix A 2 HPLC chromatogram for sorghum beer at 8 weeks.



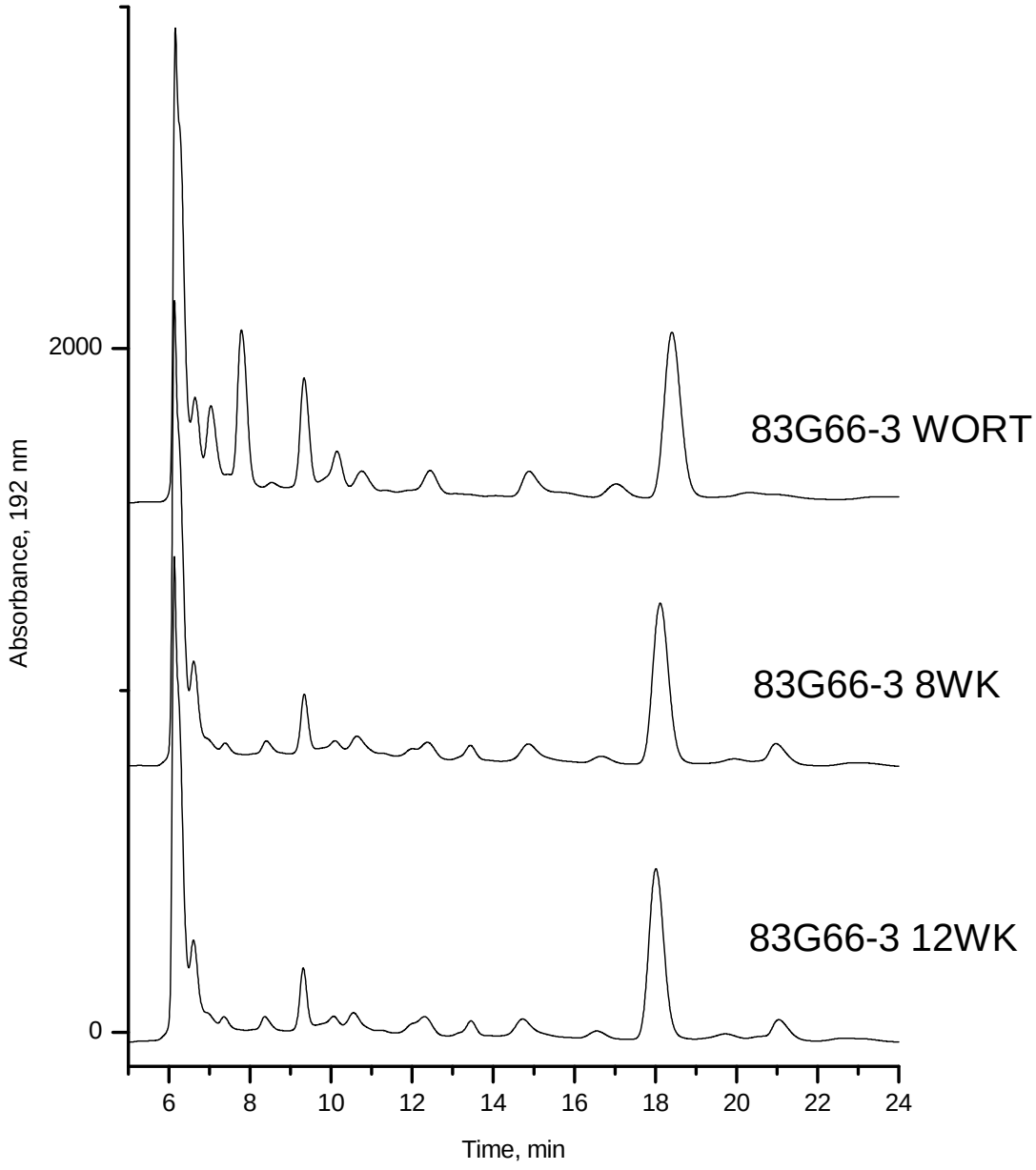
Appendix A 3 HPLC chromatogram for sorghum beer at 12 weeks.



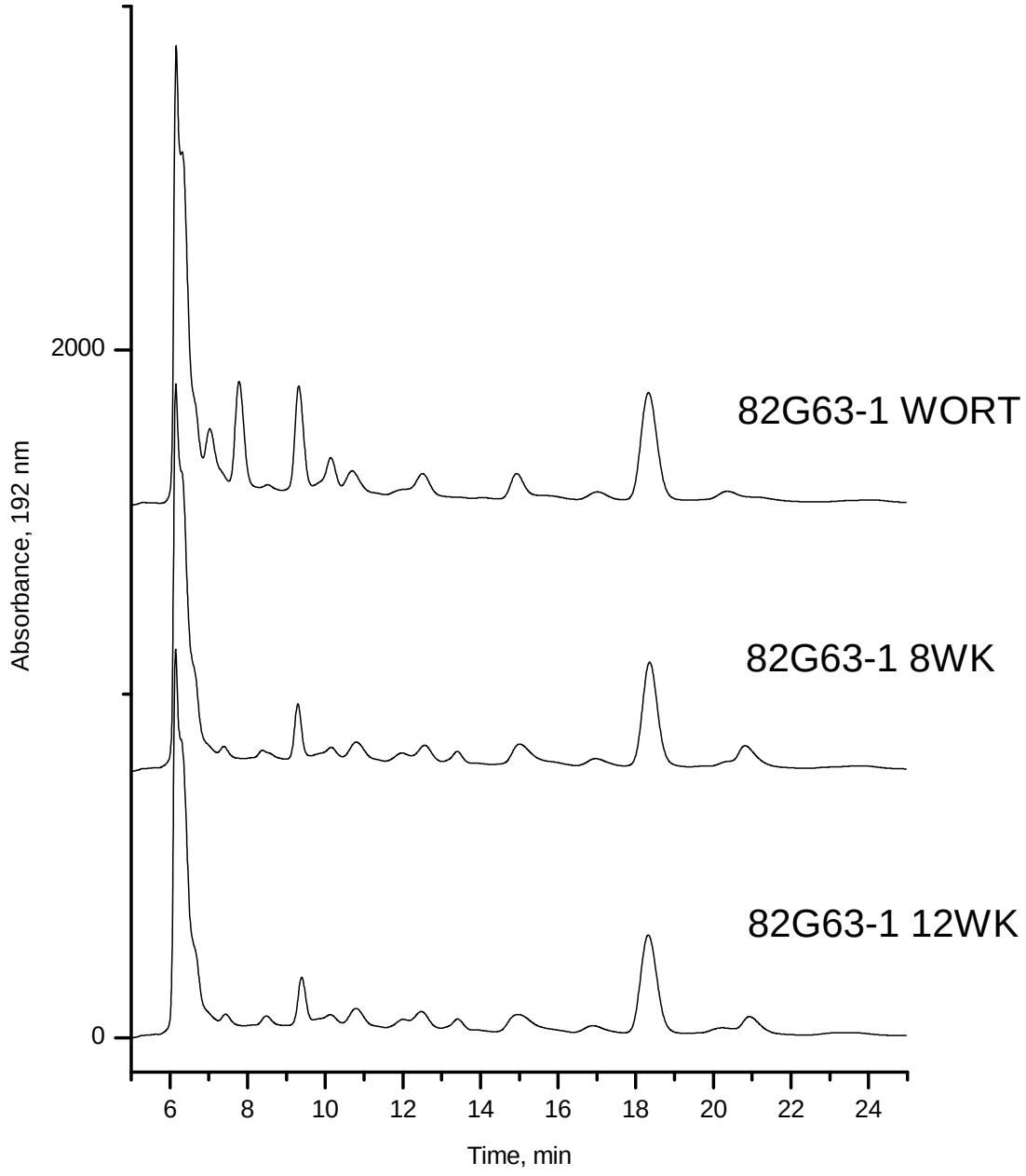
Appendix A 4 HPLC chromatogram for sorghum hybrid 83G66-1.



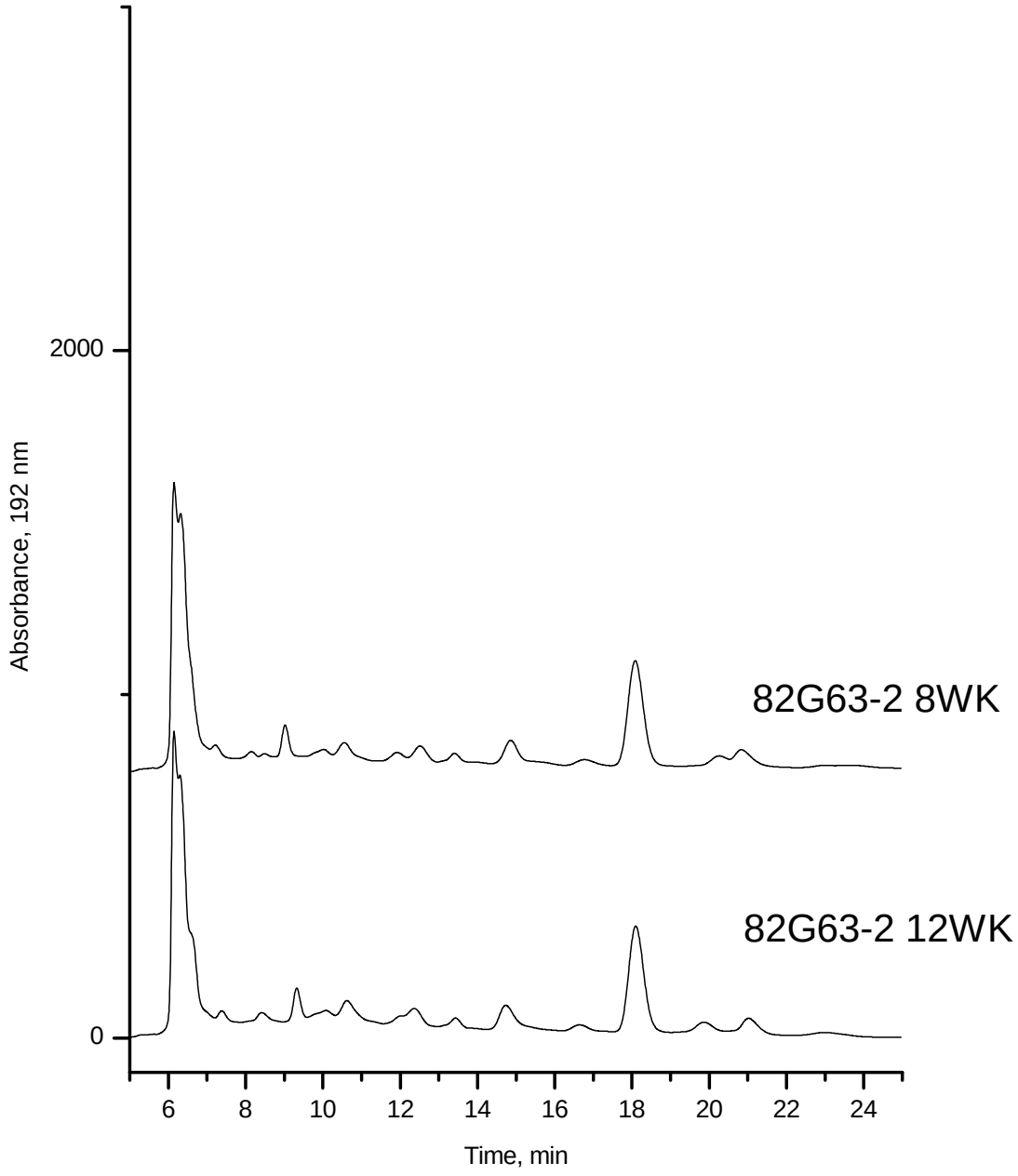
Appendix A 5 HPLC chromatogram for sorghum hybrid 83G66-2.



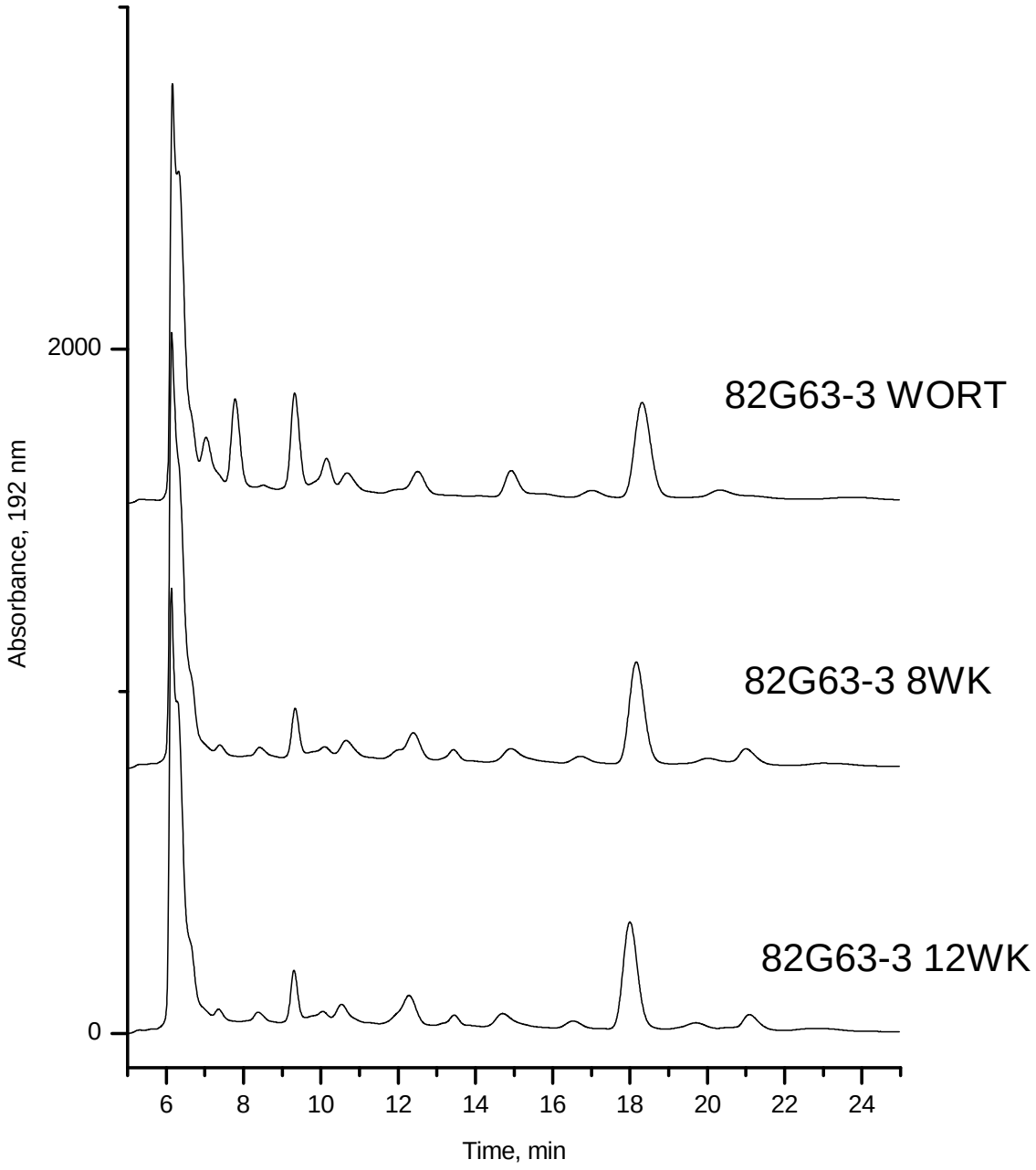
Appendix A 6 HPLC chromatogram for sorghum hybrid 83G66-3.



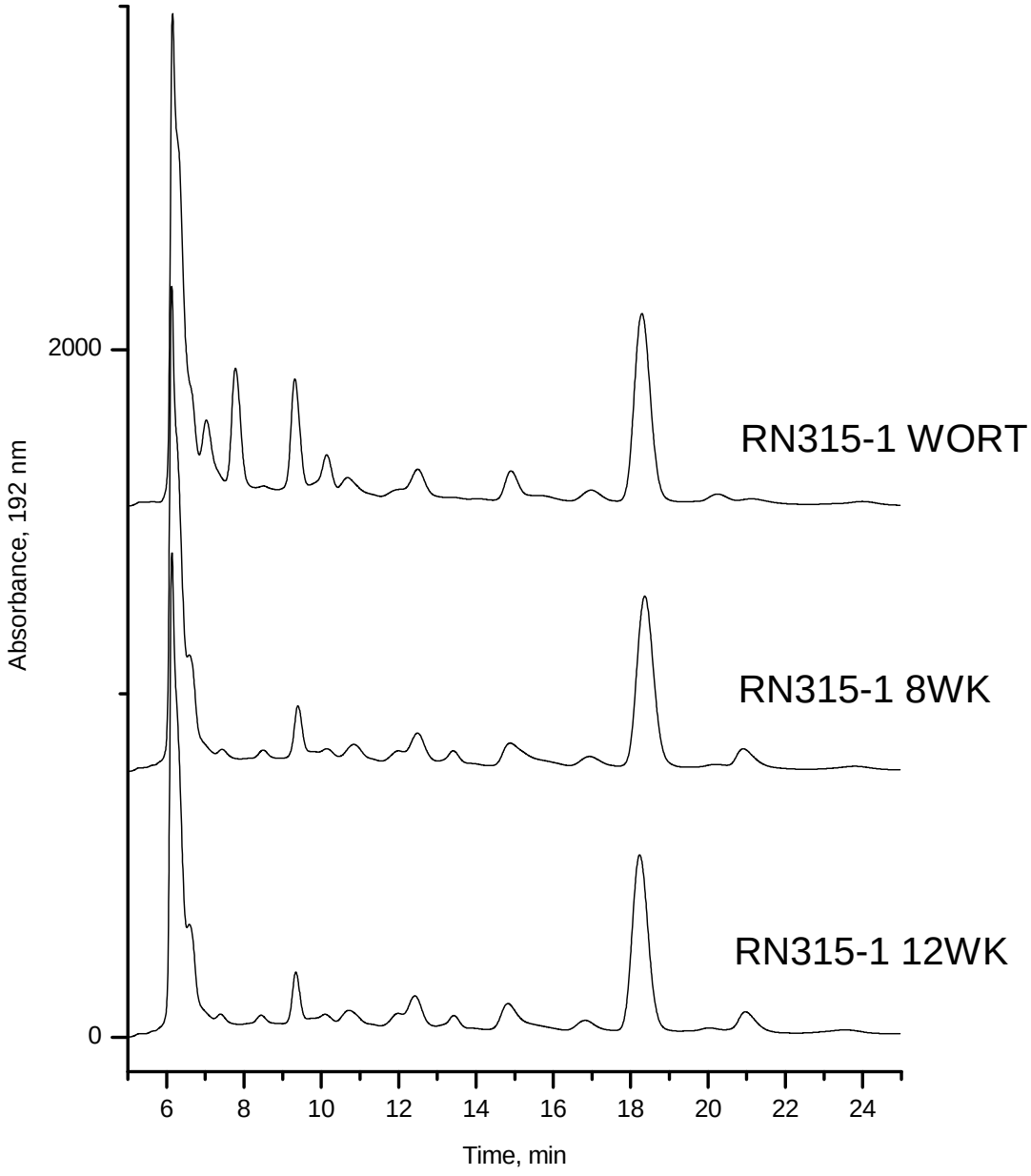
Appendix A 7 HPLC chromatogram for sorghum hybrid 82G63-1.



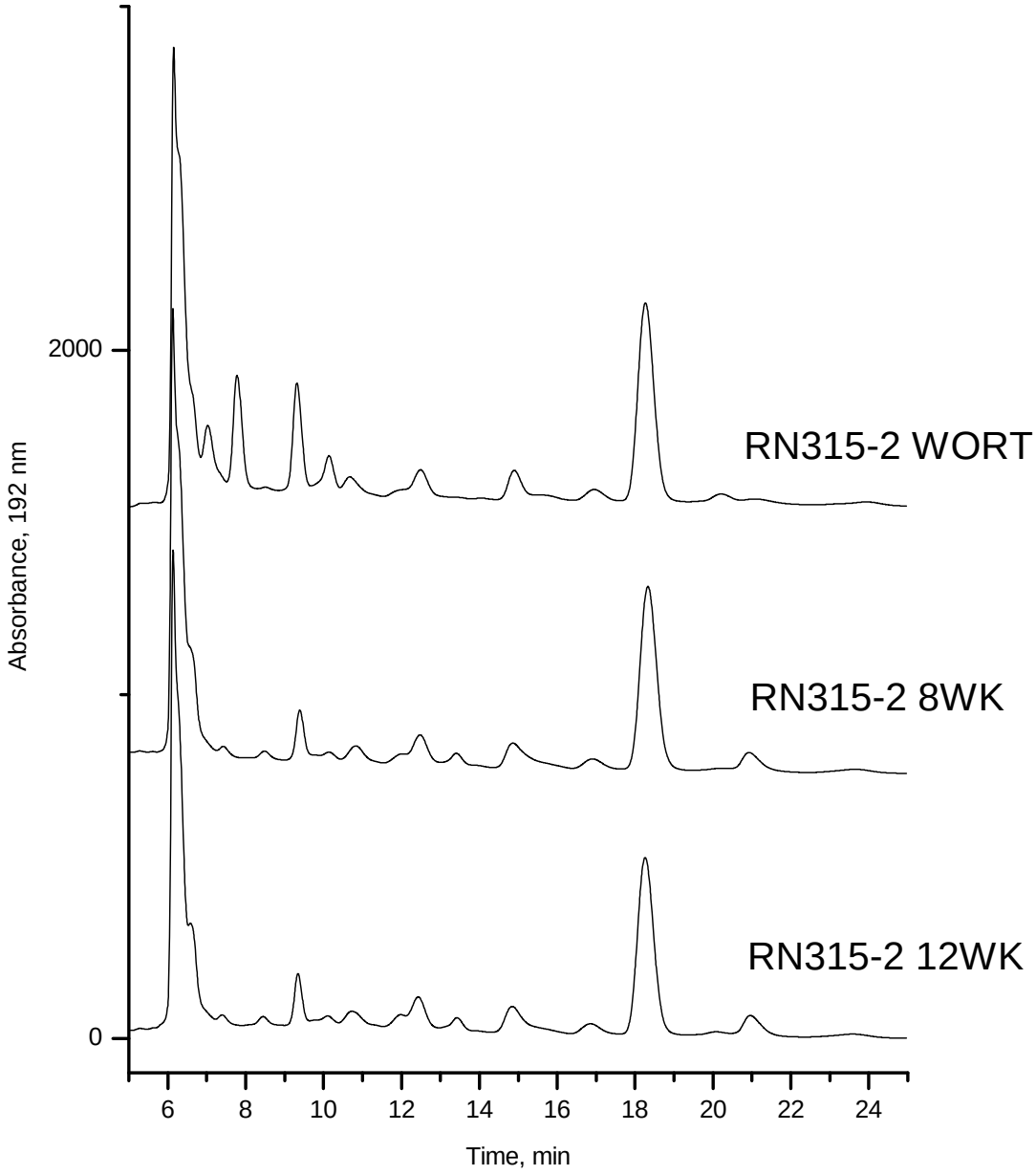
Appendix A 8 HPLC chromatogram for sorghum hybrid 82G63-2.



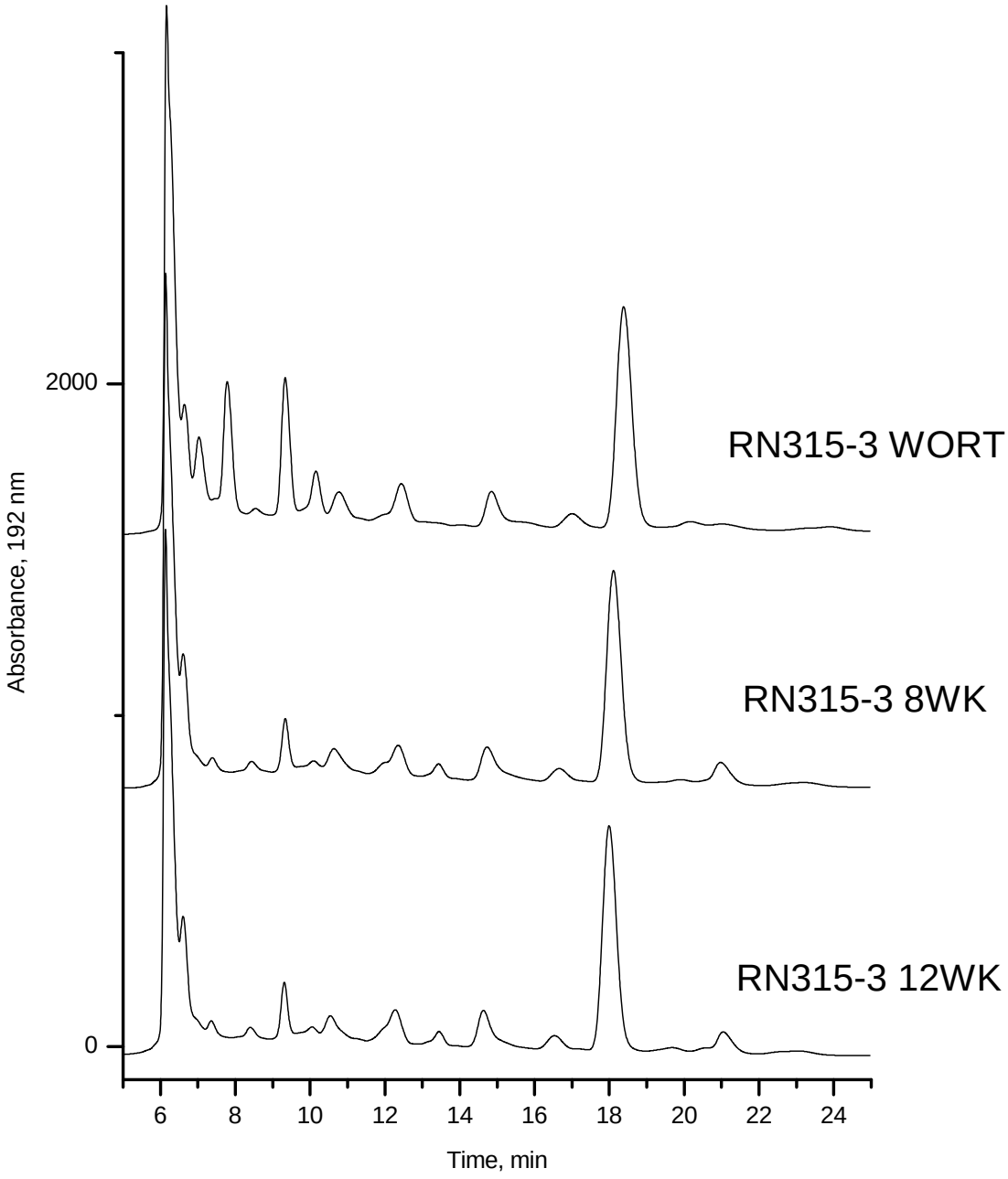
Appendix A 9 HPLC chromatogram for sorghum hybrid 82G63-3.



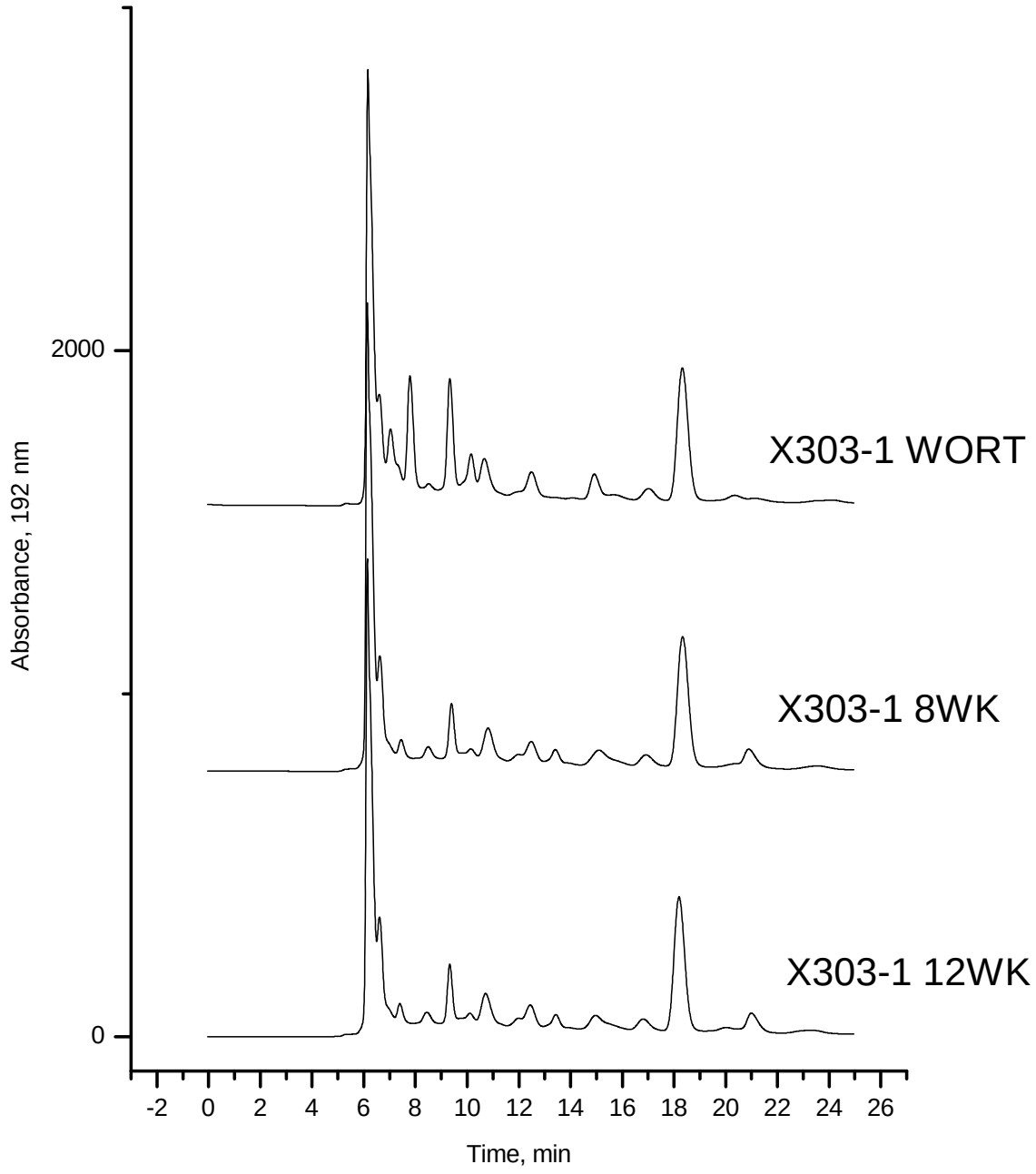
Appendix A 10 HPLC chromatogram for sorghum hybrid RN315-1.



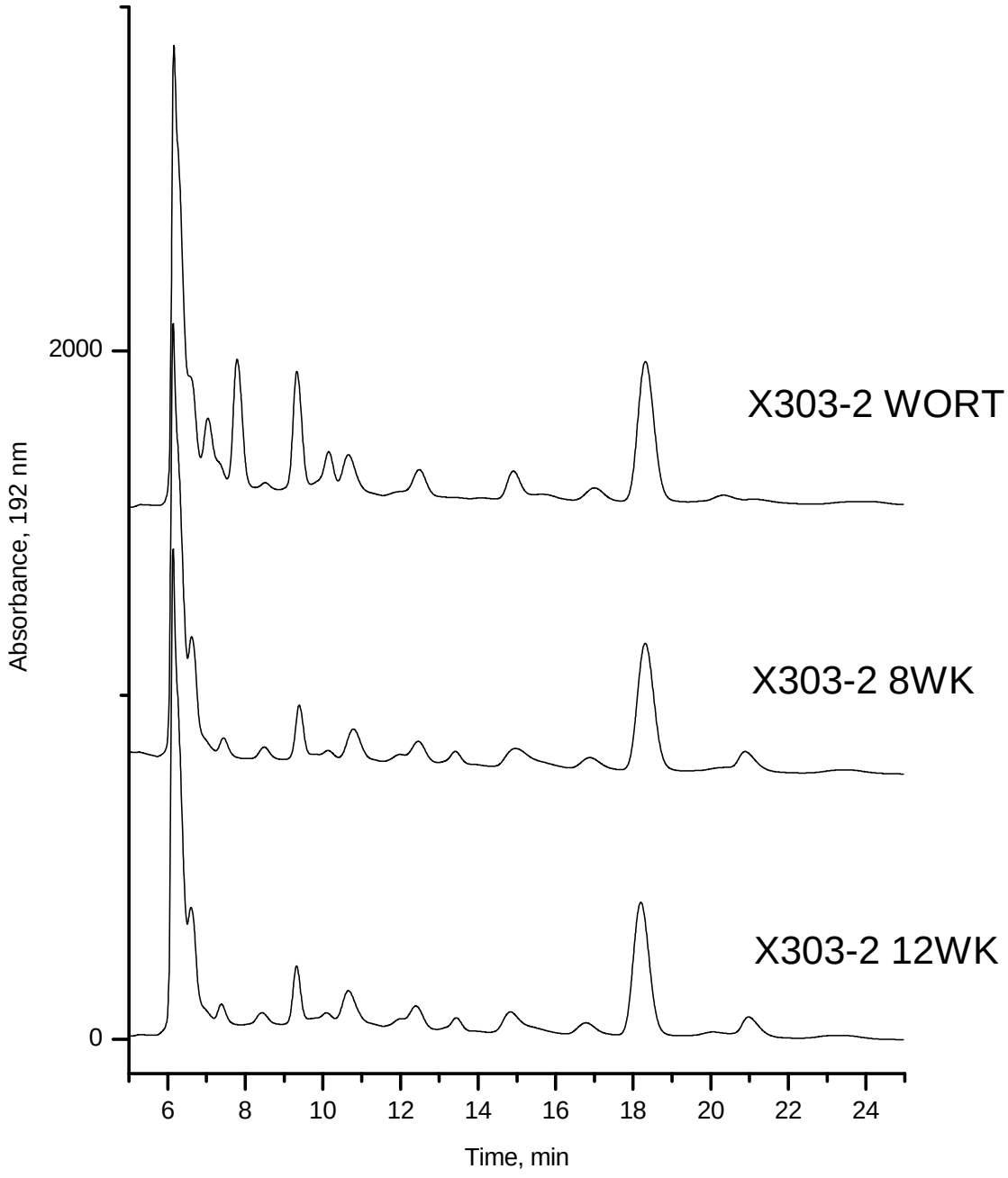
Appendix A 11 HPLC chromatogram for sorghum hybrid RN315-2.



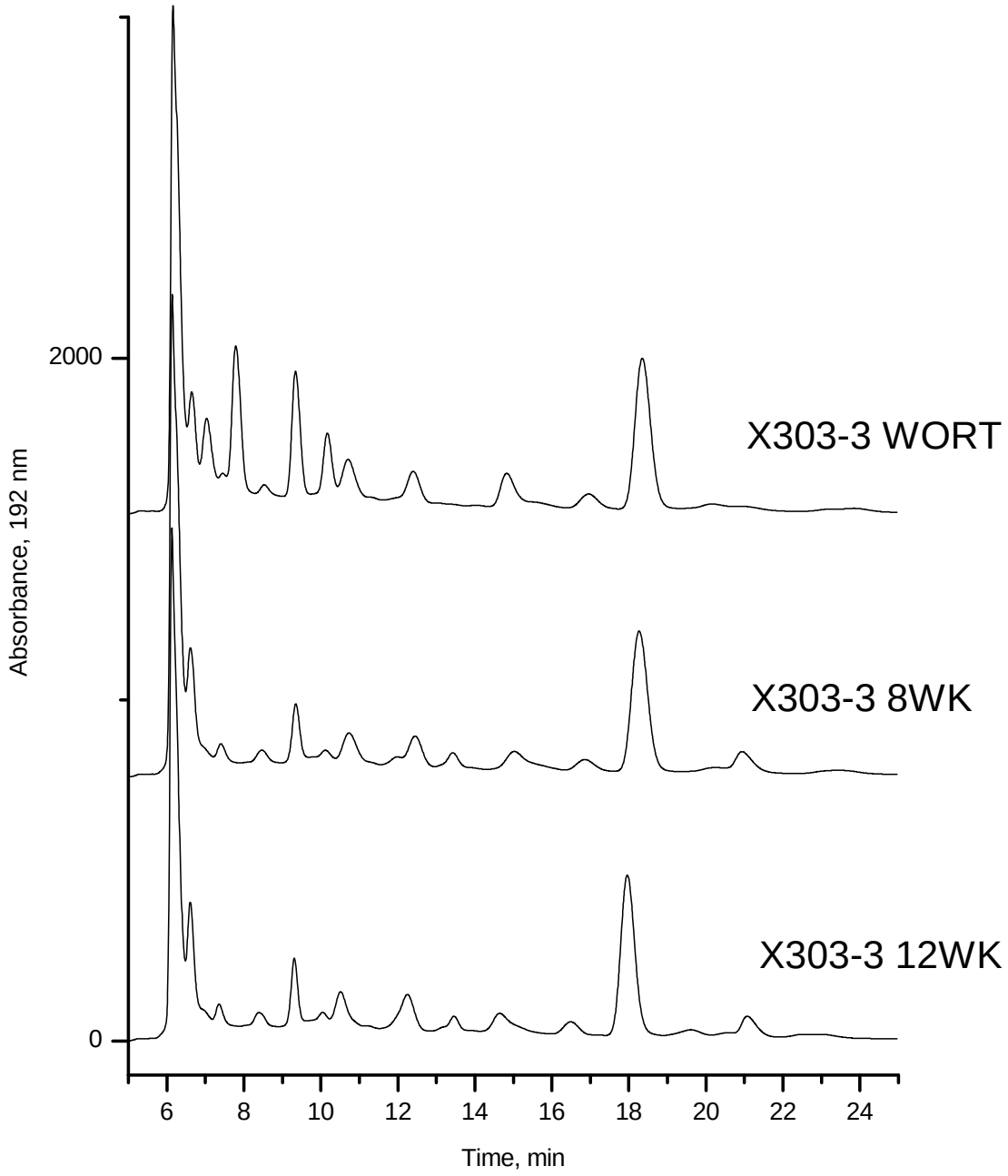
Appendix A 12 HPLC chromatogram for sorghum hybrid RN315-3.



Appendix A 13 HPLC chromatogram for sorghum hybrid X303-1.

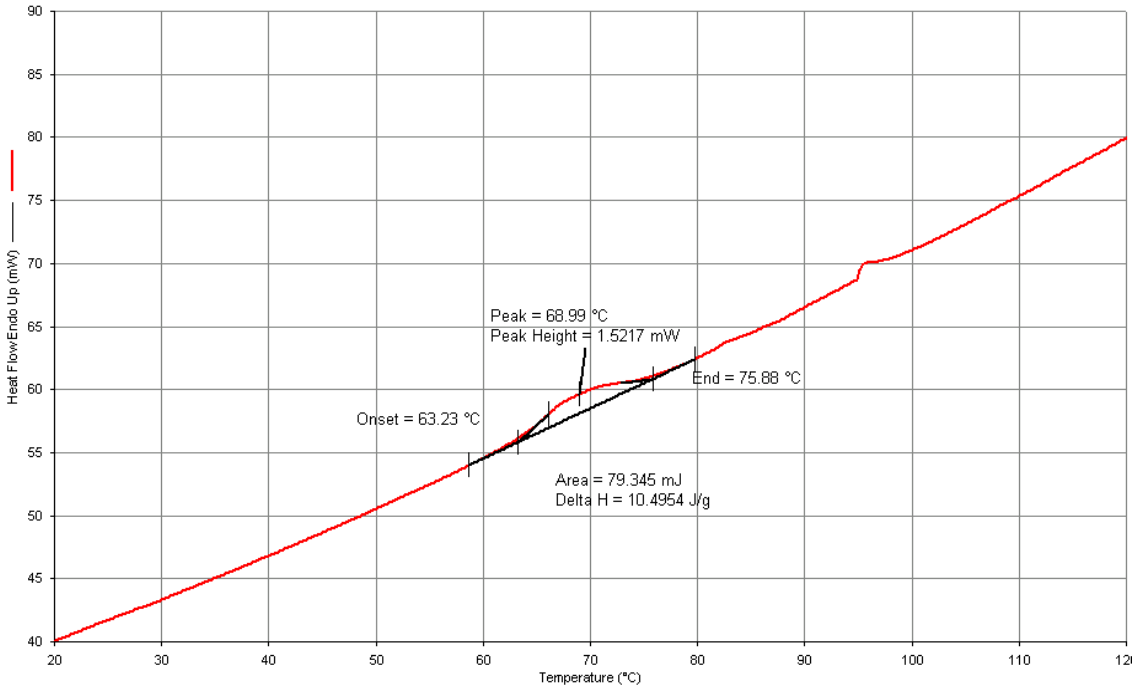


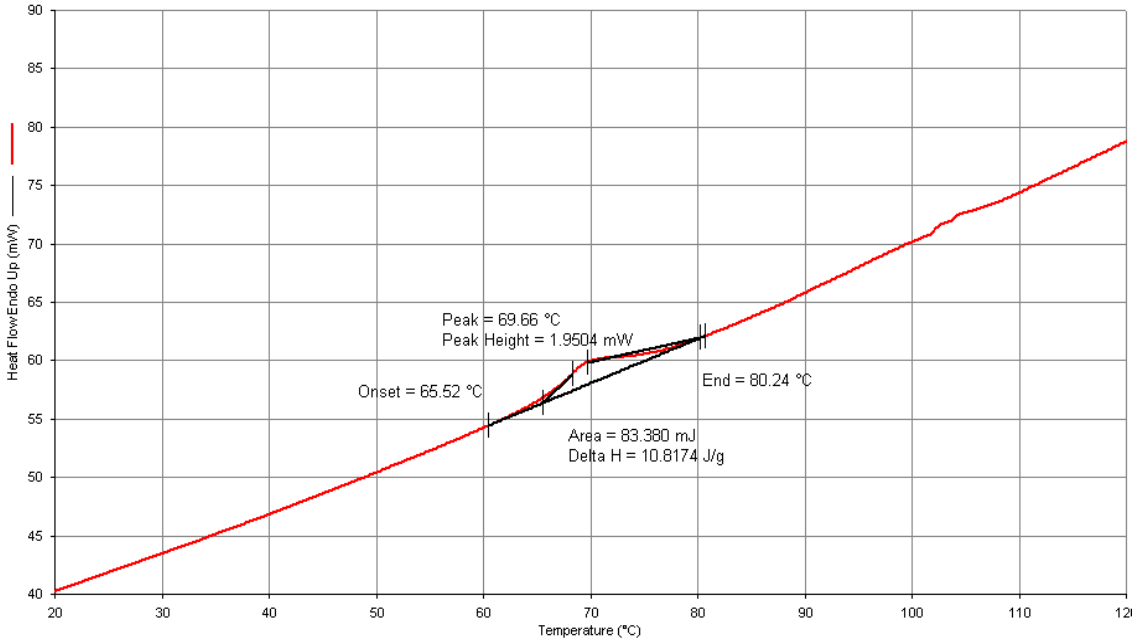
Appendix A 14 HPLC chromatogram for sorghum hybrid X303-2.



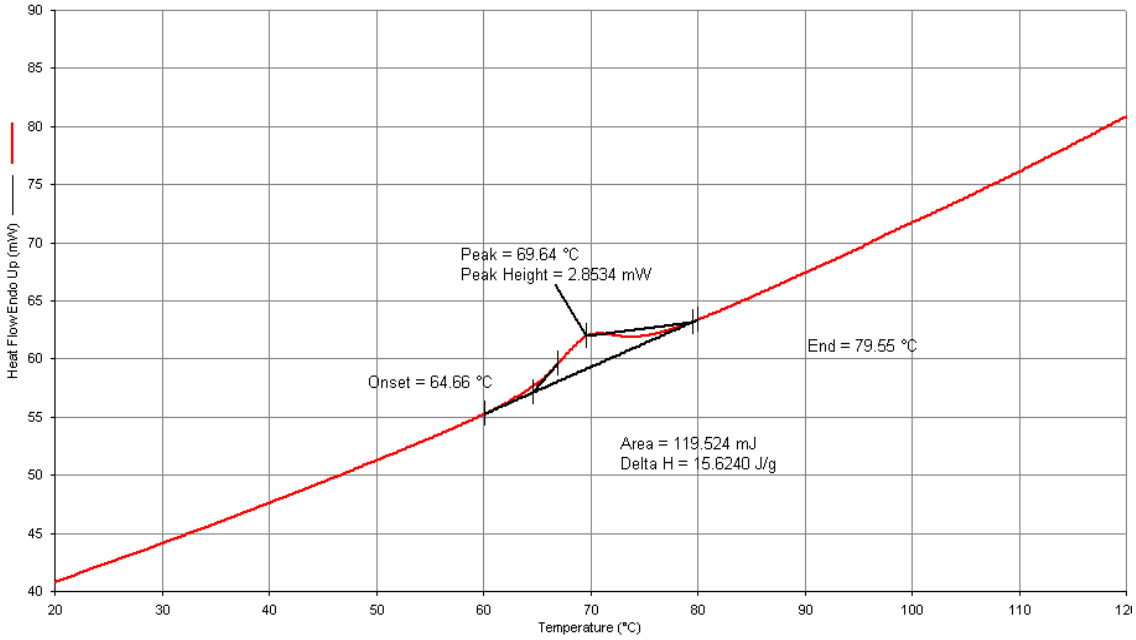
Appendix A 15 HPLC chromatogram for sorghum hybrid X303-3.

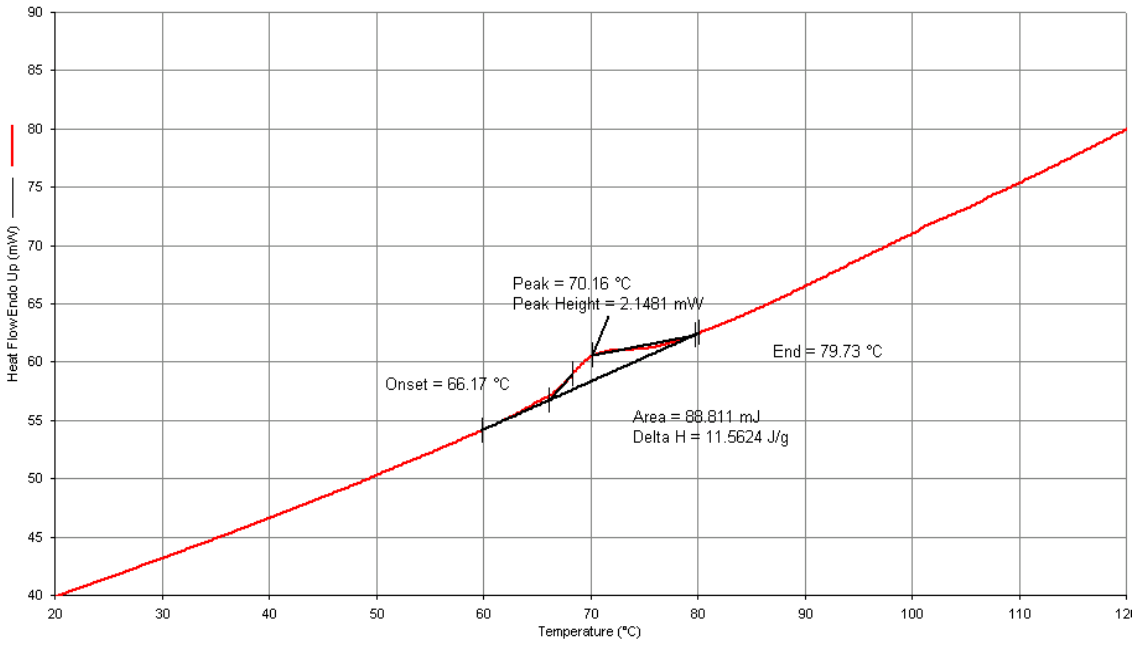
Appendix B - DSC Curves



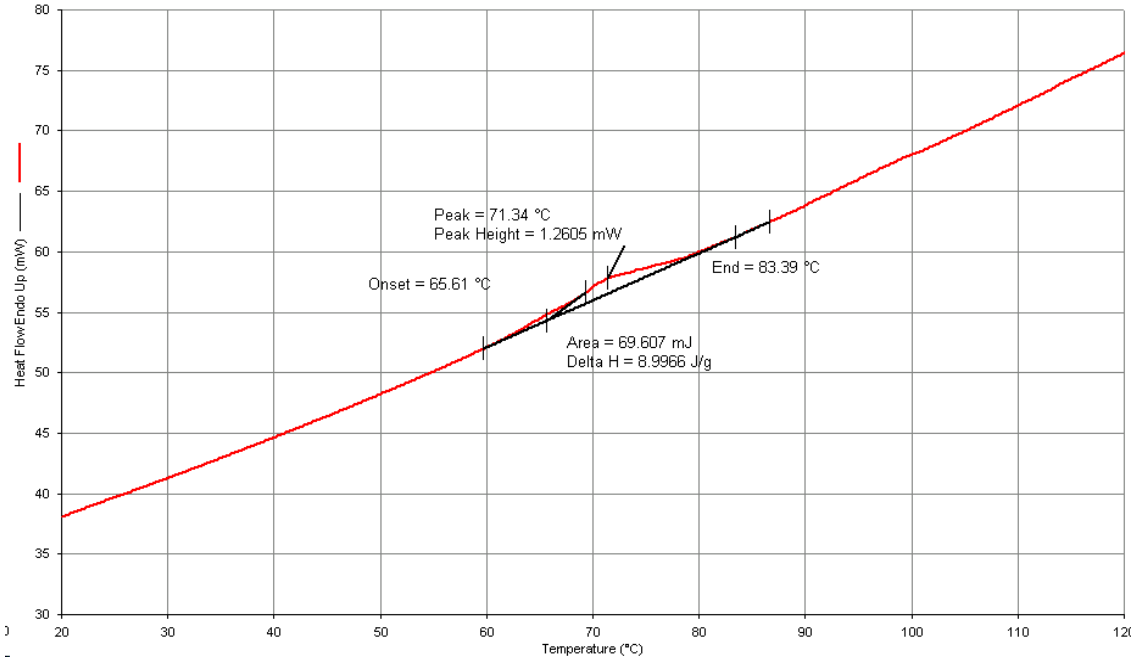


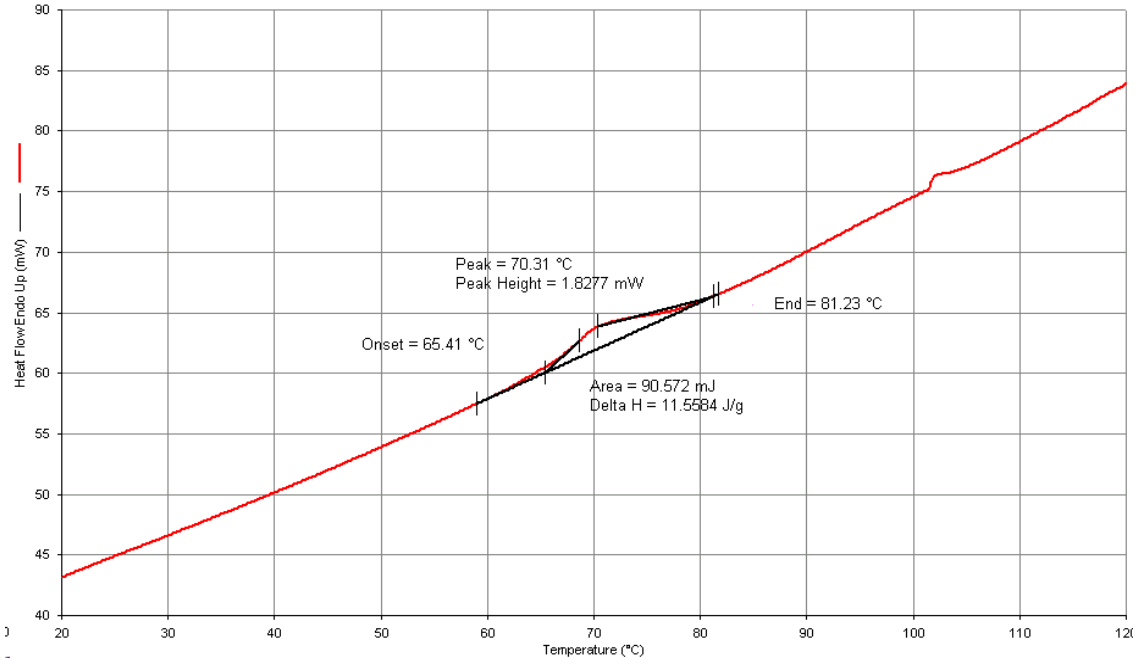
Appendix B 1 DSC curves for isolated starch of sorghum grain hybrid 82G63.



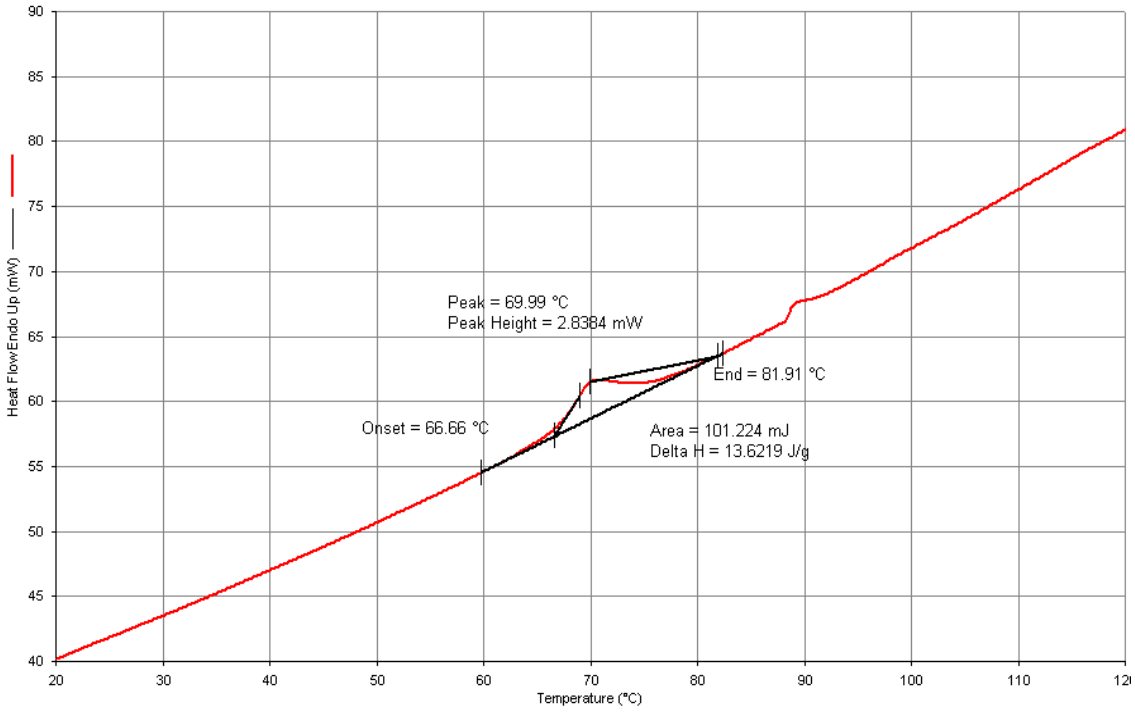


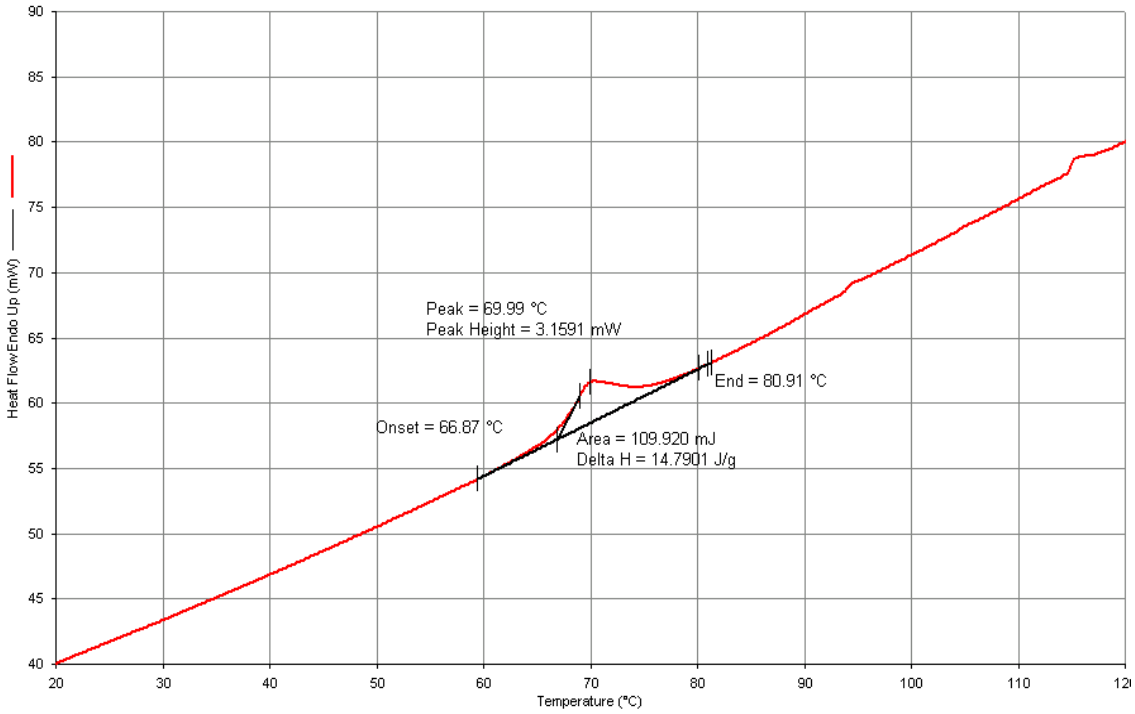
Appendix B 2 DSC curves for isolated starch of sorghum malt hybrid 82G63.



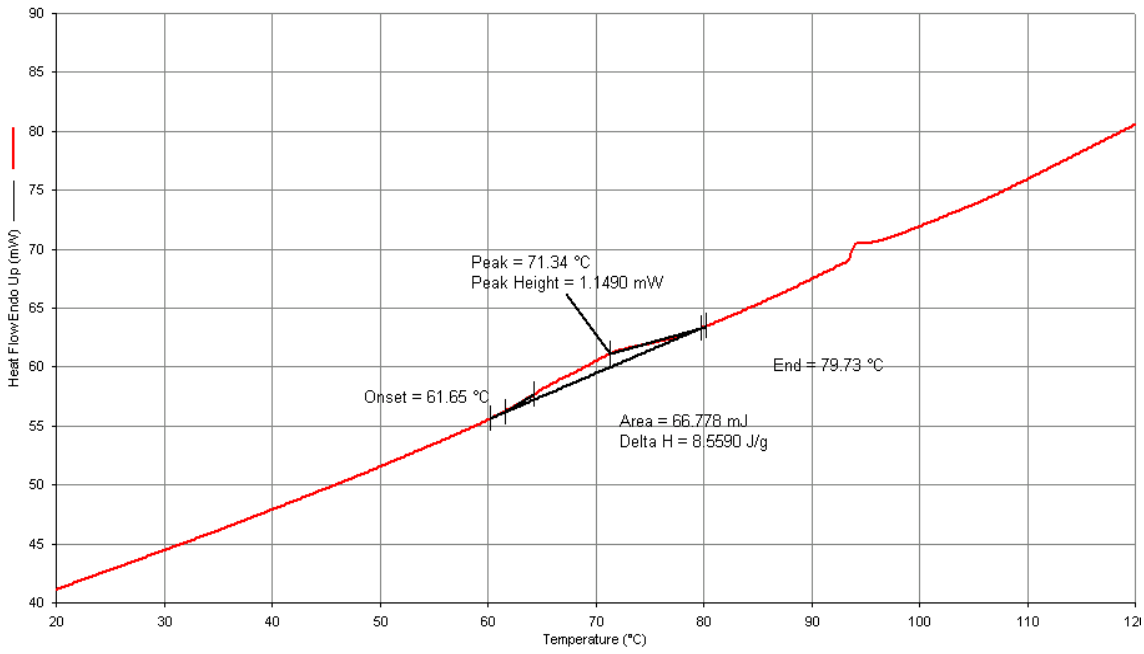


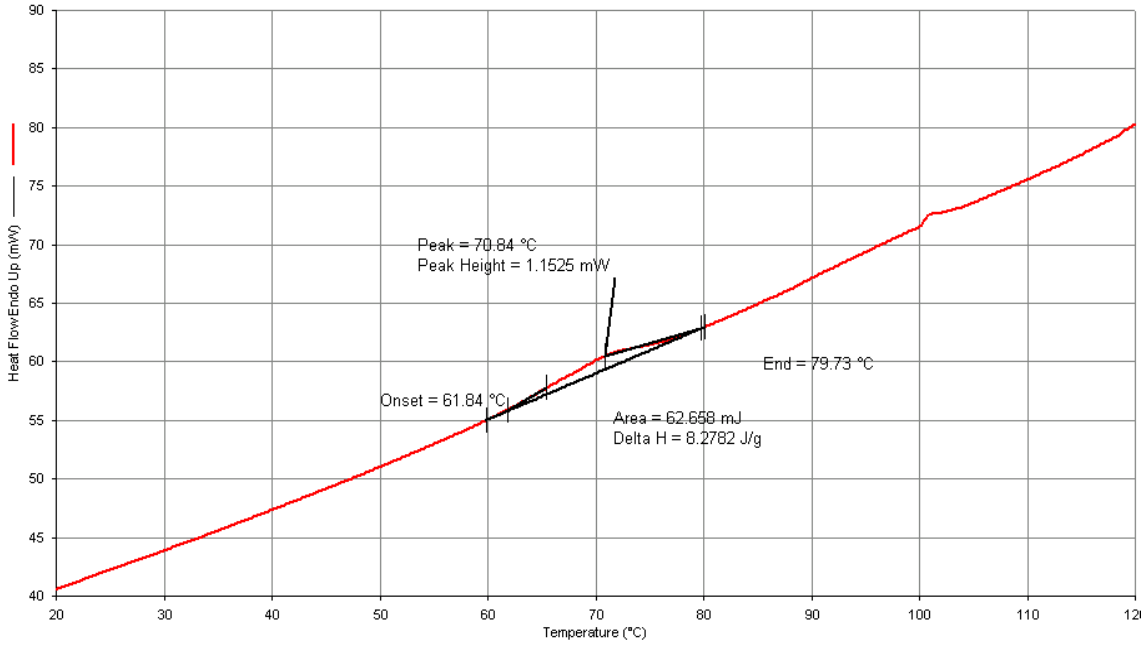
Appendix B 3 DSC curves for isolated starch of sorghum grain hybrid 83G66.



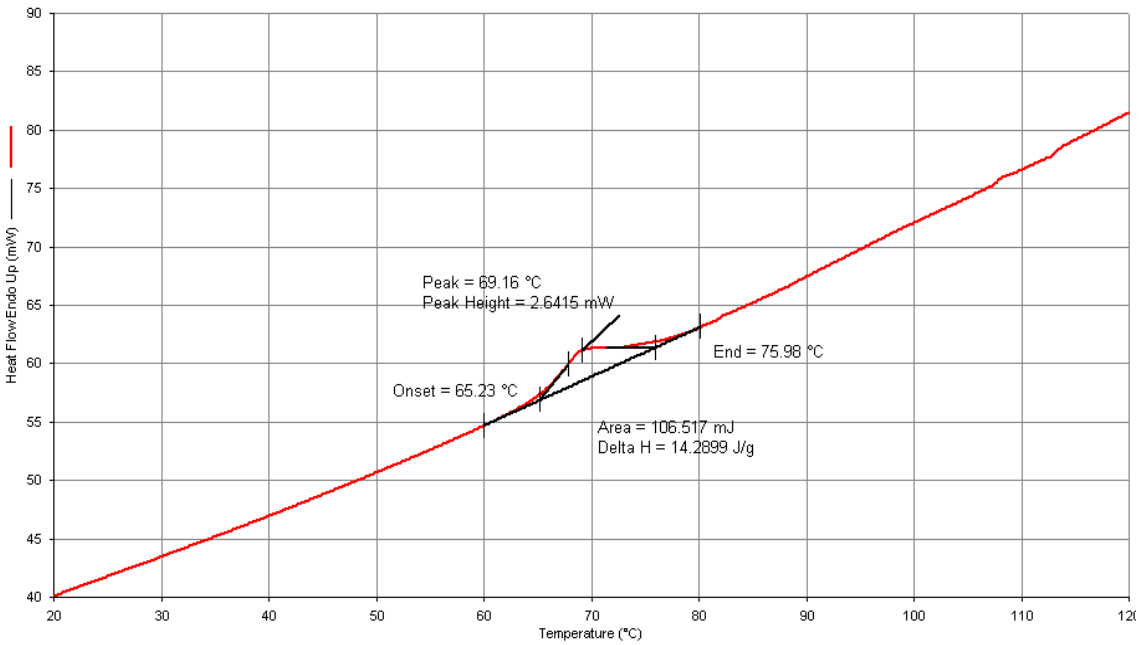


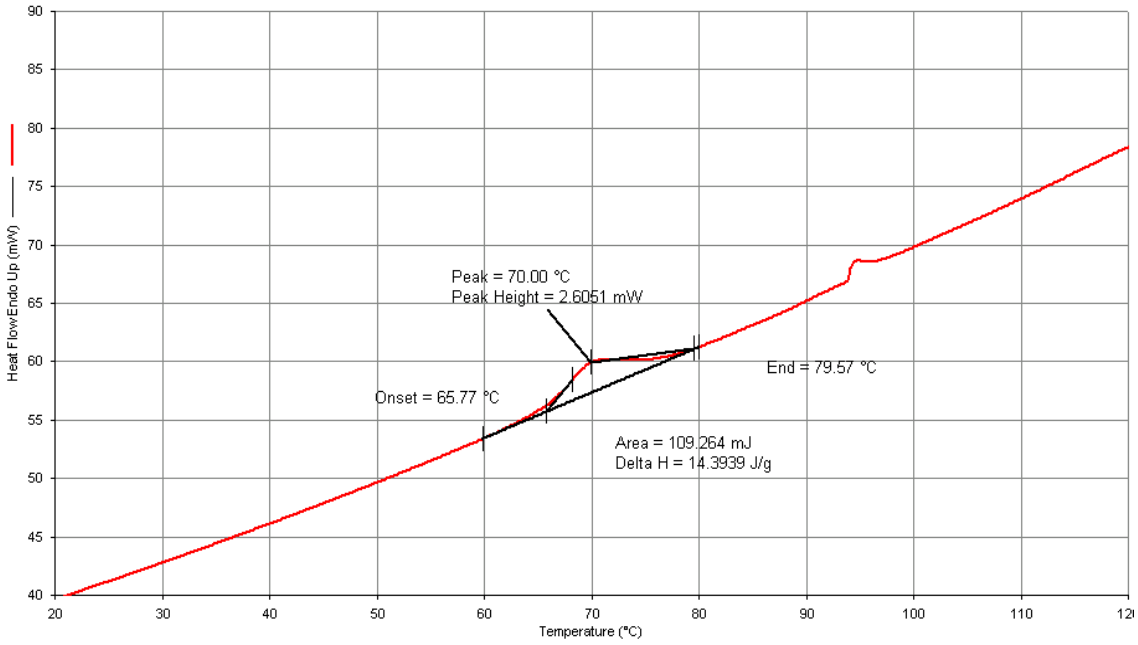
Appendix B 4 DSC curves for isolated starch of sorghum malt hybrid 83G66.



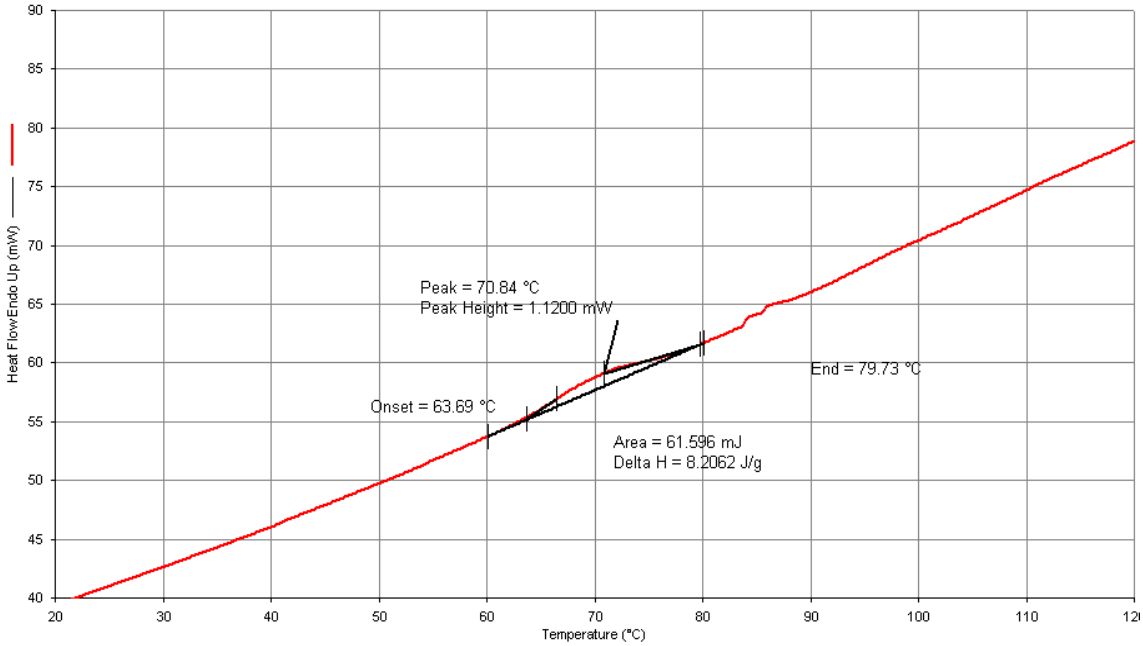


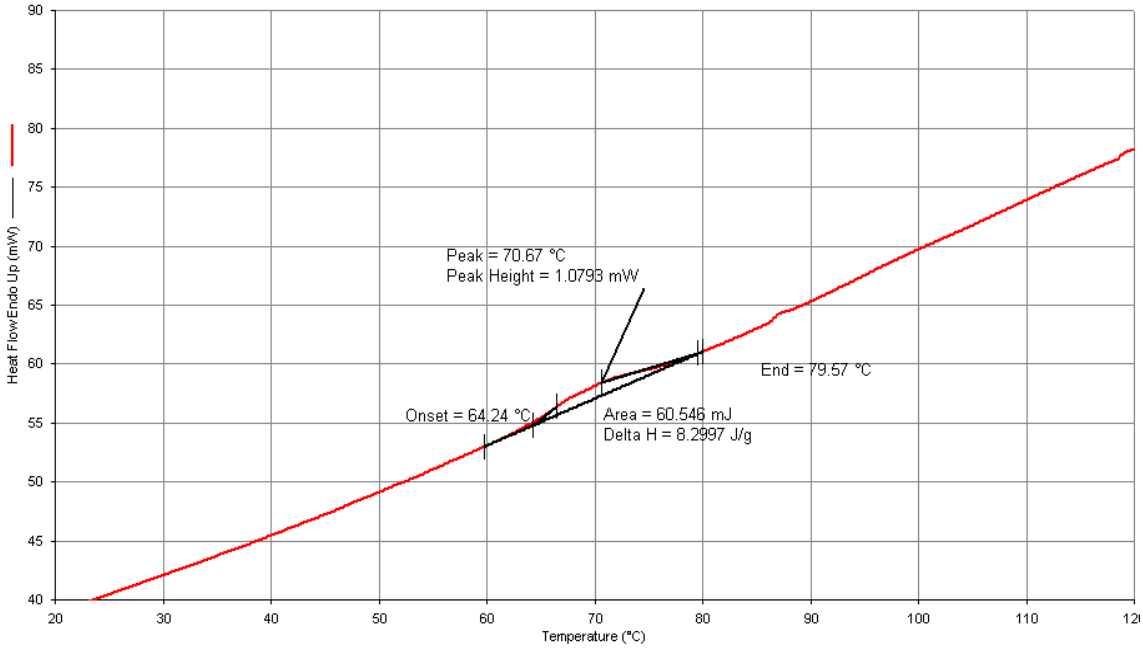
Appendix B 5 DSC curves for isolated starch of sorghum grain hybrid RN315.



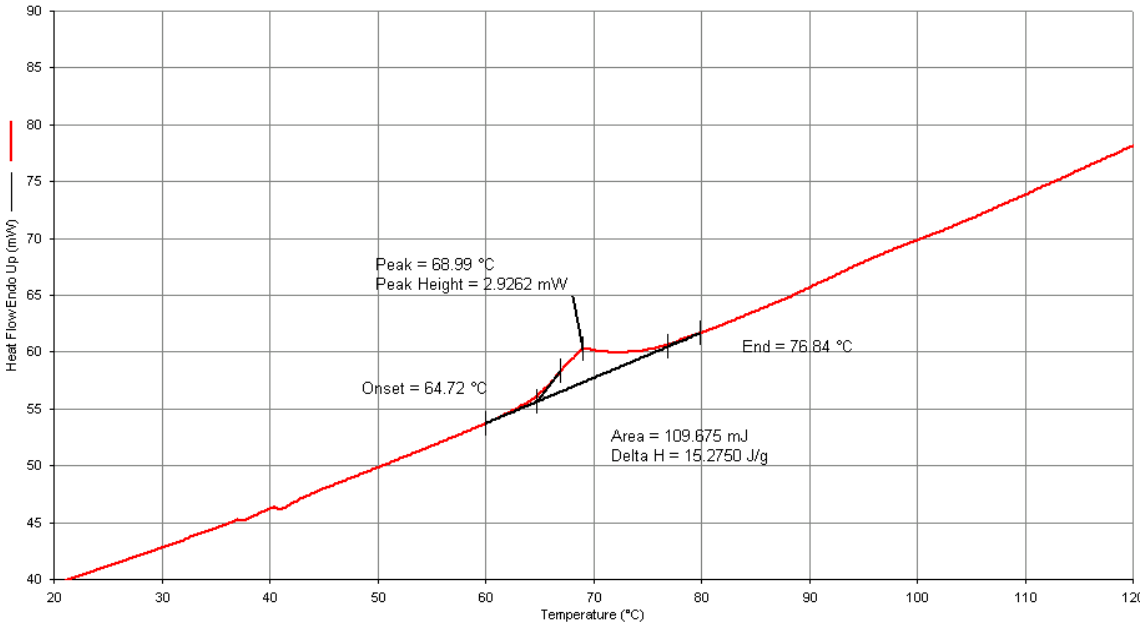


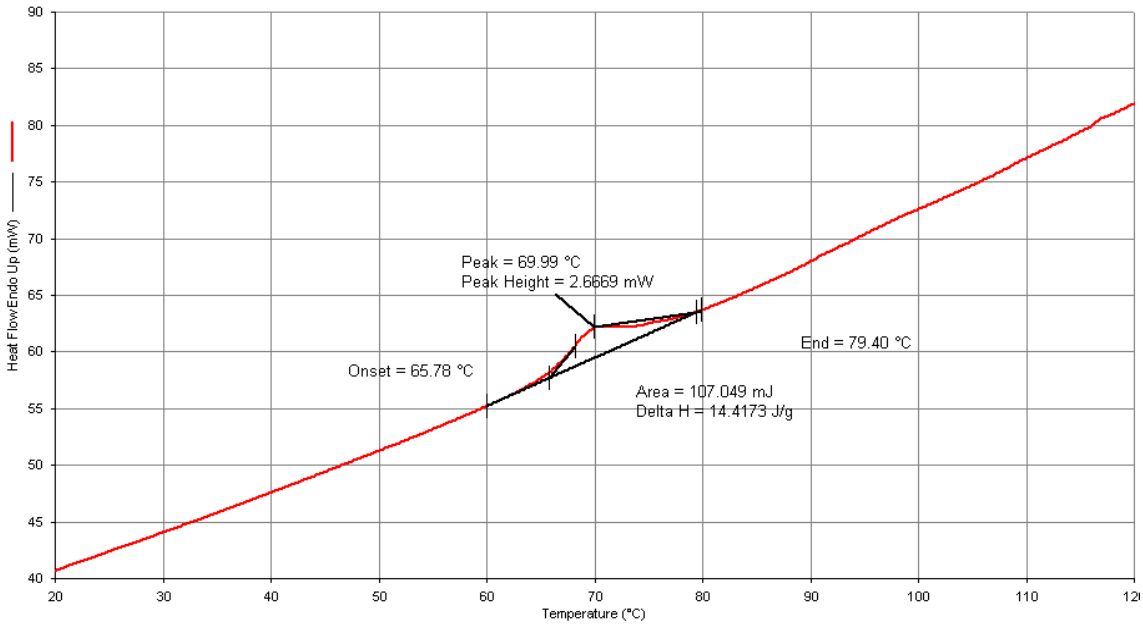
Appendix B 6 DSC curves for isolated starch of sorghum malt hybrid RN315.





Appendix B 7 DSC curves for isolated starch of sorghum grain hybrid X303.





Appendix B 8 DSC curves for isolated starch of sorghum malt hybrid X303.