EFFECT OF OPERATIONAL PARAMETERS ON LABORATORY DETERMINATION OF MALT FERMENTABILITY

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment
for the Degree of
MASTER OF SCIENCE

Major Program
Cereal Sciences

October 2013

Fargo, North Dakota

North Dakota State University
Graduate School

Title

Effect of Operational Parameters on Laboratory Determination of Malt

Fermentability

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MASTER OF SCIENCE

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ABSTRACT

Prediction and variability in malt fermentability is concern for breeders and brewers. Primary purpose of this study was to determine variation in fermentability using two different laboratory mash methods. Another objective was to develop relationship between malt/wort parameters and malt fermentability with addition of adjunct. The prediction of malt fermentability was achieved by using stepwise multiple linear regression analysis to evaluate which quality factors were able to explain the observed variation in fermentability. The hot water extract (HWE) mash protocol showed wider range of fermentability values than did the Congress mash. However, HWE mash method clearly showed better discriminative power. Diastatic Power (DP) explained about 50% of the variation in fermentability when using Congress mash with all malt (i.e. 100%) and also with adjunct. While in case of HWE extract protocol, limit dextrinase activity was able to explain 32% of variation in fermentability with all malt and also with adjunct.

ACKNOWLEDGMENTS

I would like to express my deepest appreciation to my advisor Dr. Paul Schwarz for his excellent guidance, caring, patience, and providing me with an excellent atmosphere for doing this research. The completion of this thesis would not have been possible without his guidance and support. I would also like to thank to all my committee members; Dr. Senay Simsek, Dr. Clifford Hall and Dr. Scott Pryor for their valuable support. Also I would like to thank every member of Barley and Malt Quality Project Team.

I am always grateful to my parents, Jai Bhagwan and Sunita and my sister Saraswati for their unconditional love and support. I owe them everything and wish I could show them just how much I love and appreciate them. They not only gave me the opportunity to be here, but also to be what I am right now.

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LIST OF ABBREVIATIONS

ASBC.........................American society of brewing chemist

HWE………………...Hot water extract

RDF………………....Real degree of fermentation

ADF………………....Apparent degree of fermentation

DP…………………...Diastatic power

KI……………………Kolbach Index

FAN………………....Free amino nitrogen

DU…………………..Dextrinizing unit

ºC……………………Degree Celsius

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CHAPTER 1. INTRODUCTION

The fundamental ingredients that are used in the production of beer are malt, water, adjunct (e.g. corn grits), hops and yeast. Malted barley or malt is the primary source for brewers to produce fermentable sugars, and provides flavor and color to the finished product (Freeman, 1999). Traditionally, a major factor in the determination of malt quality has been its total extract, which has been viewed as of extreme economic importance because it determines the amount of malt needed to produce a given amount of beer. However, it is becoming more apparent that the fermentability of the extract is equally important in determining the amount of malt required to produce a given amount of beer, or more specifically, a given amount of alcohol. Brewers need highly fermentable malts in order to produce beers with low residual carbohydrates. Information on the variability in malt fermentability would be of great value to brewers and would further help malt performance or quality.

The prediction of malt fermentability is a complex process, as there are several malting and brewing processing factors which are related to fermentability. In the malting process these factors include malt enzymes, fermentable sugars, free amino acids, minerals and vitamins, while in the brewing process, factors includes such as adjunct use and type, mash temperature, yeast strain, wort oxygenation and fermentation temperature.

The objectives of this research were to study the variation in the fermentability among barley cultivars and lines using two different laboratory mashes, and also to define the relationship between malt and wort quality parameters (e.g. enzymes, fermentable sugars, free amino acids etc.) and malt fermentability. This will aid the efforts of barley breeders, maltsters, and brewers to better understand or improve fermentability.

CHAPTER 2. LITERATURE REVIEW

2.1. Background

Maltsters and brewers always look for barley varieties with malt parameters that meet product quality and processing needs. Higher malt extract has been a target of selection since barley breeding began in the early 20th century. This is because extract determines the amount of malt needed in brewing formulations, and as such, is an important economic factor. Breeding efforts from the mid-20th century have improved extract levels in North American six-rowed malting varieties from around 75% (Schwarz and Horsley, 1995) to nearly 81%. The majority of this extract comes from starch that is solubilized and then converted to a mixture of fermentable sugars and non-fermentable dextrins during mashing. A small portion of the total extract is from soluble nitrogenous compounds referred to as soluble nitrogen or soluble protein.

However, it would seem that gains in extract will become progressively more difficult to achieve because a portion of the kernel (e.g. husk, embryo, etc) is not extractable. In fact, extract gains in some recent varieties have been made by increased soluble nitrogen and not carbohydrate extract. This extract does not directly translate into gains in beer production, and high soluble nitrogen levels actually can be problematic (Li et al. 2007). A close examination of calculations for brewing formulation, shows that a gain of 0.1 percentage points in malt extract actually only translates into reduction of malt usage of around 100 g/hectoliter beer (Schwarz and Li, 2010). This is about 1% of total malt requirement, and it would seem that greater gains in the economics of malt use in brewing are more likely to be achieved by increasing the proportion of the extract that is fermentable.

Fermentability of malt plays an important role for brewers to match their beer type. Some beer types are characterized by higher alcohol with less residual unfermentable dextrins, while others require more unfermentable dextrin for greater mouthfeel. Low calorie or light

beers, which account for the majority of the North American market, require malts with high fermentability. This is because the major objective is to reduce caloric content by lowering the percentage of carbohydrate that is not fermentable.

Breeders have paid little attention to fermentability in the past because the methodology is difficult, time consuming, and not amenable to high-throughput analysis. Fermentable sugars in wort can be measured by high performance liquid chromatography (HPLC), but it must be remembered that they are not the only malt-based factor that influences fermentability. A further factor complicating the determination of fermentability is that worts from laboratory or Congress mash actually need to be fermented. This is not viable for a large number of samples on a routine basis. In addition, Congress worts are not considered ideal for determination of fermentability or fermentable sugars. This is because the rest at 70°C results in extensive inactivation of β-amylase and lower values for fermentable sugars are obtained (Evans et al. 2005; Schwarz et al. 2007). However, the Hot Water Extract (HWE) laboratory mashing method, which is used in the United Kingdom, is carried out at 65°C and typically increases the yield of fermentable sugars. Ultimately, the screening of breeding lines and populations for fermentability will require either an additional laboratory test (s), and/or a better understanding of how currently measured traits contribute to fermentability.

2.2. Definition and Laboratory Determination of Malt Extract and Fermentability

Extract can be defined as the percentage of dry matter, which is solubilized from the grist during mashing process (Kunze, 2004). The malt extract (%) can be calculated using following equation:

$$\textit{Extract (as is)}\ \% = \frac{P(M\ x\ 800)}{100 - P} \quad \text{(ASBC, 2009)} \qquad \text{(Eq. 1)}$$

Where P (Plato) is the weight of extract (g)/100 g wort and M is the moisture content (%) of malt.

The fermentability of wort is generally defined by the terms apparent and real degree of fermentation (ADF and RDF). The terms real and apparent attenuation limits (RAL and AAL) are used as alternatives by some. The extract or gravity of the wort is determined before and after fermentation. The difference gives an indication of the amount of extract that has been fermented. Apparent degree of fermentation (ADF) does not take into account the formation of alcohol and mass of carbon dioxide produced during fermentation. Alcohol is less dense than water so the specific gravity is only an indication of the "apparent extract" left in the wort. The apparent extract is always lower than the real extract but is still a useful indicator of fermentation process and can be used as a beer specification American Society of Brewing Chemists (ASBC 2009),(Method of Analysis Wort-2).

Determination of RDF involves the removal of alcohol or correction for its presence, as well as a correction for the mass lost by CO_2 evolution (ASBC 2009) (Method of Analysis- Beer-6b). Apparent degree of fermentation values affects characteristics of beer like mouthfeel, body and alcohol content. Typical values for ADF in brewery worts fall between 75% and 85% (Carey and Grossman, 2006).

Different laboratories use different methods for measuring fermentability. According to the ASBC Method of Analysis Wort-5a (2009), the determination of fermentability or RDF can be made by measuring the change in extract content during fermentation with an excess amount of yeast under specific conditions. Busch Agriculture Resources Inc. (BARI, Fort Collins, CO.) developed a method for measuring the RDF value of breeder's lines (Dr. Jolanta Menert, personal communication). This was accomplished by making some modifications of the ASBC

method. The amount of yeast was changed from 5 to 2g and the amount of wort from 250mL to 70mL. The fermentation time was reduced from 48 to 15h. Wort was obtained by the standard Congress mash. Fermentation was carried out at room temperature (22 to 25ºC) with constant shaking at 150 rpm on a rotary shaker. Values for ADF were obtained by measuring the specific gravity of filtered wort before and after fermentation, and RDF was estimated using a correction factor (ADF * 0.88).

2.3. Review of the Malting and Brewing Processes

Both extract and fermentability are influenced by the composition of barley and by the malting and brewing processes. An understanding of these factors is important, and this portion of the literature review is intended to provide the reader with a basic understanding of the barley and malt extraction composition, as well as the fundamental processes of malting and brewing. Readers desiring more detailed information are advised to consult textbooks by Kunze (2004), Lewis and Young (1995), Briggs (1998), and Ullrich (2010).

2.3.1. Barley Composition

2.3.1.1. Starch: Starch is the major and largest component of barley grain, and is found exclusively in the endosperm. It composes 58-65% of dry weight of the kernel (De Clerck, 1957). Starch is a mixture of two polysaccharides, amylose (25%) and amylopectin (75%). In barley, starch exists in two forms; small (1-5 µm) and large (10-25 µm) spherical or egg-shaped granules (Briggs, 1998). Bathgate et al. (1978) stated that there was a higher content of amylose in small granules than in large. Proportionally, the number of small granules is greater, but a large granule constitutes 80-90% of total starch weight. There are variations among the barley cultivars for the granule ratio. During malting, only 10 to 12% of the starch is degraded, with the small granules being more readily degraded than the large (Lewis and Young, 1995). This is

beneficial as small granules have a higher gelatinization temperature than large granules, and would thus be more slowly degraded in mashing, which could lead to lower fermentability (Briggs 1998., Lewis and Young, 1995).

2.3.1.2. Protein: Protein is found throughout the barley kernel, but the largest amount is present in the endosperm. The protein content of barley is commonly measured as the total nitrogen content multiplied by a conversion factor (N x 6.25). Six-rowed and two-rowed malting barley generally range from 12-13.5% and 10-13% protein, respectively (Schwarz and Horsley, 1995). Barley with high protein levels will generally contain proportionally less starch in the endosperm, and therefore will be expected to have less fermentable extract than the lower protein barley. Proteins in barley grain can be separated into albumin, globulin, prolamin (hordein) and gluten fractions based on solubility (Shrewry, 1993). The major endosperm proteins are the alcohol-soluble hordeins, which comprise 30 to 50% of total grain. Albumins and globulins are categorized as water soluble proteins and comprise about 10-20% of the total grain (Shrewry, 1993).

2.3.1.3. Cell Wall Polysaccharides: Beta-glucans and arabinoxylans are the two major components of barley endosperm and aleurone cell walls. The barley endosperm cell wall contains about 70 to 75% β-glucans and 3 to 11% arabinoxylans (Han and Schwarz, 1996). The β-glucans are degraded by the action of endo-β-glucanases ((1, 3; 1,4)–β-glucan endohydrolases), which are developed during the germination process. The arabinoxylans are mainly degraded by three enzymes; endoxylanases, β-D- xylosidases and α-L-arabinofuranosidases (Delcour et al. 1996). Degradation of endosperm cell walls in malting is important as they limit the access of enzymes to the endosperm starch and storage protein.

2.3.1.4. Lipids: The total lipid content of barley kernel ranges from 2-3.5% of grain dry weight. According to Morrison (1978) the lipid in barley grain contains 65-78% neutral lipids (triacylglycerides), 7-13% glycolipids and 15-26% of phospholipids. Lipase and lipoxygenase (LOX) enzymes activity are responsible for lipid degradation and oxidation during malting and mashing processes (Schwarz et al. 2002), and it is believed that high lipid content can lead to stale or off-flavor in beer (Kunze, 2004).

2.3.1.5. Ash: Barley grain contains 2-3% of ash, and this is mainly composed of potassium, phosphate, silica, iron, magnesium, calcium and sodium (Kunze, 2004). Potassium and phosphate are not only the main component of inorganic material, but also help yeast growth during fermentation process (De Clerck, 1957).

2.3.2. Barley Endosperm Structure

The endosperm is the major part of barley kernel. It is separated from the germ (embryo) region by a thin tissue layer known as scutellum where growth hormone gibberellins (GA) are synthesized. Synthesis and release of enzymes occurs in aleurone layer, which separates the endosperm from the testa and pericarp. Barley endosperm cells contain mostly starch granules surrounded by a matrix of storage protein. As previously stated, the endosperm cell walls are composed of β-glucan and arabinoxylans layers. The thickness of cell wall is considered an important factor because it affects the malting properties of barley. Barley kernels with thicker cell walls (mainly β-glucan content) and higher protein level hinder the degradation of starch during malting. This would result in poor malt modification and ultimately lead to low extract level and fermentability.

2.3.3. Composition of Malt Extract (wort)

Maltsters and brewers know that the quality and brewing performance of malt extract is judge by the quantity of specific compounds present in the wort. The quantity of materials such as carbohydrates (fermentable and non-fermentable sugars), nitrogenous compounds (protein, peptide and amino acids) and other minor compounds influences the fermentation process in the brewery. In a laboratory wort or extract about 75-80% of the malt mass is extracted during the mashing process (Horsley and Schwarz, 1995). Kunze (2004) reported that extract contains 63-68% fermentable sugars (i.e. 65% maltose, 17.5% maltotriose, 5% saccharose, 12% glucose and fructose) and 20-25% non-fermenatble sugars (mostly dextrin molecules). However, fermentable sugars are the major energy source for yeast, and are consumed and converted into alcohol and carbon dioxide in fermentation process (Kunze, 2004). Soluble nitrogen normally comprises 0.55 to 0.75% of dry weight of the Congress wort extract. Soluble protein is actually composed of approximately 30% of amino acids, and 30% peptides, with the remainder being proteins in excess of 30 amino acid units (Meilgaard, 1988). A major function of amino acids and dipeptides is to provide nutrition for the yeast growth during fermentation.

2.3.4. The Malting Process

Malting is the controlled germination process of the grain (Briggs, 1998). The major objectives of the malting process are 1) to develop enzymes needed in the brewing process, 2) to change the structure of the barley endosperm into a form that is more readily utilized or extracted in the brewing process, and 3) to develop distinctive malt colors, aromas and flavors. If the cleaning and sizing of grain is to be ignored, the malting process can be divided into three major steps which include 1) steeping, 2) germination and 3) kilning.

2.3.4.1. Steeping: Steeping is the first step of the malting process. The purpose of steeping is to activate the enzymes which are already present in mature grain (i.e. β-amylase), and help promote the *de novo* synthesis of other enzymes (i.e. α-amylase) (Briggs, 1998). During steeping, barley is immersed into water, interrupted by air rests during which the accumulated carbon dioxide is removed. Barley is generally steeped to reach ~45% moisture. Typically, steeping process for brewer's pale malt takes 36-48 hour at 16ºC. Higher moisture levels help promote diffusion of the enzymes through the endosperm and in turn, modification of the endosperm

2.3.4.2. Germination: Following steeping, the barley is transferred to germination compartments, where moisture is maintained at ~45% and the barley is allowed to germinate at 16-18ºC and ~95% relative humidity. This stage is important for, synthesis/activation of enzymes (amylolytic and proteolytic) in the aleurone layer (Zhang and Jones, 1995). As these enzymes diffuse into the endosperm the breakdown of cell wall polysaccharides and protein matrix begins. Starch granules become exposed, and are thus more easily broken down in mashing. The process of endosperm breakdown is called malt modification. Modification typically requires 4 to 6 days.

2.3.4.3. Kilning: The primary objective of kilning is to reduce the moisture content of green malt from ~45% to 4%. The bulk of the moisture is removed at temperatures below 60ºC, in order to help prevent inactivation of enzymes. Enzymatic activity in the final malt is halted due to restricted moisture levels. During the later phases of kilning, high heat promotes the Maillard reaction that results in the development of malt colors and flavors (Kunze, 2004). Kilning of pale malts typically is completed in ~24 hrs with temperatures being increased in stepwise manner form 50ºC to 90ºC.

2.3.5. The Brewing Process

Brewing can be divided mainly in four stages that include 1) brewhouse operations, 2) fermentation, 3) cellar operations, and 4) packaging. As the brewhouse operations and fermentation have the greatest impact on extract and fermentability, they will be discussed in the greater detail.

2.3.5.1. Brewhouse operations:

2.3.5.1.1. Mashing: Mashing is basically an extraction process that is conducted in a vessel called a mash tun. In the mashing process the coarsely crushed malt is mixed with water in an approximately 1:3 ratio. The major biochemical events of mashing are conversion of some soluble protein to amino acid, and the solubilization, and then conversion of starch to fermentable sugars (Kunze, 2004). As mentioned earlier, hot water extract (HWE) and Congress mash are the main mashing methods that are used to produce laboratory extract. However, in commercial brewing, mashing protocols can vary widely. Commercial mashes are almost always much thicker, meaning a greater ratio of malt to water. Non-malt adjuncts are frequently used in commercial practice, and may constitute up to 50% of the grain bill (Li and Schwarz 2010). Corn grits and rice are generally first prepared in a separate vessel called a cereal cooker. Here the starch is gelatinized and partially degraded through the action of added amylase or malt. Once processed, the cereal mash is combined with the main mash. Pre-gelatinized grits or starch adjuncts are added directly to the mash tun along with the malt. Corn syrups, in which the starch has already been converted to fermentable sugars, are added to the brew kettle.

Mashing is carried out in a stepwise manner with several temperature rests and ramps, which are designed to optimize the activities of proteolytic and amylolytic enzymes. For a poorly modified malt or mash containing adjunct it is desirable to begin the mash at lower temperature

(45ºC). This allows the proteases and other heat labile enzymes to produce desirable compounds such as soluble protein, free amino nitrogen, which can be metabolized by yeast during fermentation (Briggs et al. 2004). As temperature increases to 60-65ºC, starch degradation becomes the major reaction. The degradation of starch and dextrins to into maltose by β-amylase is particularly important within this temperature range. In addition, starch gelatinization occurs at 60-64ºC. This further increases the susceptibility of the starch to enzymatic hydrolysis. Lewis and Young (1995) reported that a temperature rest of 60-65ºC will results in higher fermentability but lower extract yield. As the temperature increases above 65ºC, β-amylase activity is denatured, and only α-amylase remains. The presence of only α-amylase results in worts that have more unfermentable dextrins. Mashing at the higher temperature range leads to higher extract level and a reduction in wort viscosity. Lewis and Young (1995) referred to "brewer's window" as a range of mash temperatures, where both extract and fermentability are high.

2.3.5.1.2. Wort Separation (Lautering): Lautering is conducted in a vessel called a lauter tun, or alternately in a mash filter. Upon completion of mashing process the mash consist of a mixture of dissolved and undissolved substances. The aqueous solution is called "wort" and the solid portion is the "spent grain". The spent grain consists mainly of husks and coagulated protein. The husks play an important role in wort filtration, as they sediment to the bottom of the lauter tun and acts as a filtration bed. This separation process is called lautering. A portion of extract that is retained by the spent grain is washed out by hot water in a process known as sparging. Prolonged sparging results in higher extract yield but increases the proportion of unwanted material sourced from spent grain such as bitter substances from the husk (Briggs, 1998).

2.3.5.1.3. Wort Boiling: After lautering, the filtered wort is transferred to the brew kettle, and vigorously boiled for 50 to 60 minutes. During wort boiling hops are added at times and amounts, which are based on beer style. The essential purposes of wort boiling are; i) extraction and transformation of hop components, ii) formation and precipitation of protein-polyphenol complexes, iii) wort sterilization, iv) wort color and v) evaporation of water (Kunze, 2004). Boiled wort is cooled before it is transferred to fermentation tanks.

2.3.5.2. Fermenatation: Fermentation is the process where fermentable sugars are converted by the enzymes of the yeast to produce alcohol and carbon dioxide. During the fermentation other by-products (e.g. aldehydes, esters) are also produced which influence the flavor and aroma of finished beer (Kunze, 2004). In general fermentation is categorized into four yeast growth phases: i) lag, ii) exponential, iii) stationary and iv) death. During the first phase yeast cell absorbs oxygen and start growing. While the first phase is aerobic, all subsequent stages are anaerobic. Yeast cells reproduce exponentially in the second phase, and consume fermentable carbohydrates (glucose, fructose, maltose and maltotriose) as source of energy. This results in production of alcohol, carbon dioxide and other by-products. Yeast typically consumes monosaccharides (glucose, fructose) first, followed by maltose and maltotriose. As the fermentable sugar is depleted, the fermentation phase changes to stationary and subsequently to a so-called death phase (Kunze, 2004). At the end of fermentation yeast flocculates at the bottom of tank.

Wort fermentabilty largely depends on three basic factors which are wort composition (biochemical components, inorganic nutrients, and specific gravity), fermentation processing parameters (e.g. temperature, time, oxygen, size and shape of fermentation vessel) and yeast strain. As mentioned earlier, yeast needs adequate nutrients (e.g. sugars, free amino acids,

minerals and vitamins) to grow and achieve optimum fermentability. A wort deficient in nutrients and/or with high gravity will result in poor fermentability. In the case of high gravity, the high concentration of sugar results in increased osmotic pressure that restricts yeast growth. Hence, it is important to have good quality malt, which can be achieved following proper malting and mashing procedures.

Fermentation temperature also has a considerable impact on wort fermentability. As fermentation temperature increases the rate of fermentation becomes faster but the production of undesirable compounds also increases (Kunze, 2004). However, fermentation temperature and time are determined based on the specific style of beer to be produced and yeast strain.

2.3.6.1. Cellar Operations: At the end of fermentation process, the fermented beer is called green or immature beer. It is not suitable for consumption due to the presence of many undesirable flavor and aroma compounds. During aging or cellaring, the green beer is stored at cold temperature (-1 to 4ºC) for several weeks. The main purpose of cellar operations or aging is to reduce the level of undesirable flavor compounds (e.g. diacetyl) required to levels desired in a mature beer (Schwarz and Li, 2010). The other functions which are achieved during cellar operations are clarification, carbonation, stabilization and blending/standardization of the finished beer. The beer is packaged following cellar operations.

2.4. Barley and Malt Factors Affecting Malt Extract and Fermentability

2.4.1. Barley and Malt Physical Factors

Barley kernel size and weight have long thought to be related to the amount of extract in malt. In practice these observations have often been applied to fermentable extract even though fermentability was not actually measured. De Clerk (1957) reported that large-grained barley contains proportionally less husk and therefore, gives higher yields of extract. Large and plump

grains also suggest greater kernel fill and a higher proportion of starch. Shape was found to be dependent upon variety, and varieties with short, plump kernels generally gave a higher extract since they had less husk. Agu et al. (2007) mentioned that larger kernels yielded malts with higher levels of diastatic power (DP), and if the percentage of larger kernels was higher, more fermentable sugar was produced. Screening of barley samples prior to malting removes a large proportion of the very thin kernels present. Where there is a wide distribution of kernel sizes in malting, grain modification will not be uniform, as kernels of different sizes will modify at different rates during malting (Palmer, 1986). Fermentability, in turn, might be affected by the modification of the malt.

The 1000-kernel weight for malting barley generally varies between 35 to 45g. The kernel weight can be used to indicate the extract of the malt, and it is reflective of the nitrogen content. The lower the 1000-kernel weight, the higher the nitrogen content (Briggs et al. 1998). This is because protein is largely synthesized before starch during kernel development. It has been shown that for every 0.1% increase in total nitrogen, there is a corresponding increase of 0.6% of protein. For every 0.6% increase of protein there is a decrease in the yield of extract by about 1% (Bishop, 1957).

2.4.2. Barley and Malt Chemical Factors

Malt extract is largely carbohydrate in nature and factors such as increased starch content or factors that lead to greater solublization and degradation of starch will likely lead to greater extract. However, soluble protein also makes a contribution to extract percentage. The situation with fermentability becomes more complex because many factors which impact yeast growth and metabolism also impact the formation and yield of fermentable sugars. The profiles of

fermentable sugars present in the wort are a direct result of the starch degrading activities present in the malt.

2.4.2.1. Malt Enzymes:

2.4.2.1.1. Diastatic Power (DP): Maltsters and brewers have traditionally used DP as an indicator of the total starch degrading activity in the malt, and in a sense, the fermentability. Several hydrolytic enzymes contribute to DP including α-amylase, β-amylase, limit dextrinase, and α-glucosidase (Arends et al. 1995), but the term DP actually predates knowledge of all individual activities. As will be discussed in a following section, DP is generally assumed largely reflect the activity of β-amylase. Diastatic power activity is reported in °ASBC in North America and typical values fall within a range of 110 to 160 °ASBC. Activity is based upon the release of reducing sugars from a soluble starch substrate.

2.4.2.1.2. Alpha-Amylase (EC 3.2.1.1): catalyzes the cleavage of α-(1, 4)-D-glucosidic linkages (Figure 1) in amylose and amylopectin (Briggs, 1998). The initial reaction products are branched and linear dextrins, which in turn also are subject to α-amylase activity. As an endo-enzyme, it plays a great role in reduction of the molecular weight or size of starch and large dextrins. This reduces viscosity and provides an additional substrate (oligosaccharides and limit dextrins) for β-amylase.

To maltsers and brewers α-amylase is a measure of the malt's dextrinizing capacity, and its activity is expressed in dextrinizing units (DU). Typical values in malt range from 45 to 60 DU (Schwarz and Horsley, 1995). Alpha-amylase is more thermostable (Temp. optimum 50 >70°C) than β-amylase (Temp. optimum < 65°C), and a higher level of α-amylase theoretically can provide more substrate to β-amylase.

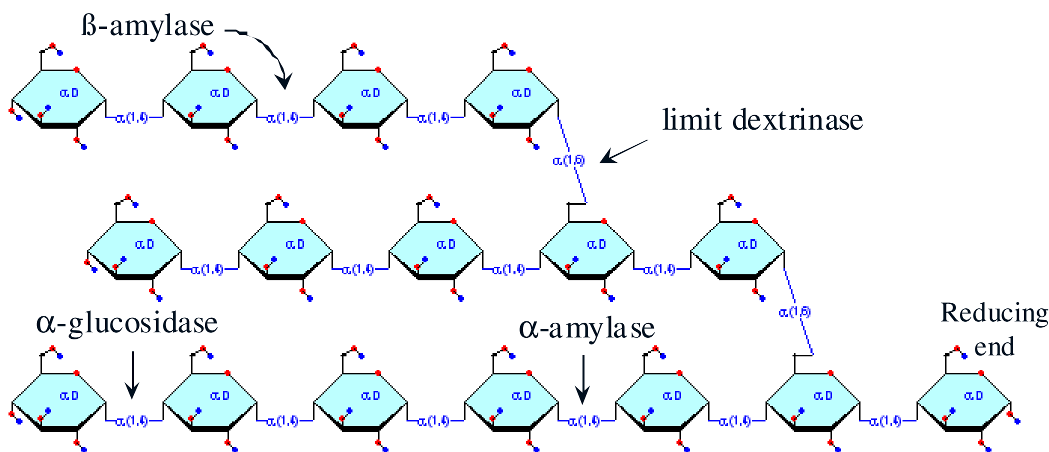

Figure 2.1. Schematic diagram of starch hydrolysis by α-amylase, β-amylase, α-glucosidase breaking (α-1, 4-D-glucosidic linkages) and limit-dextrinase breaking (α-1, 6 glycosidic linkages) (Source: Evans et. al., (2009).

2.4.2.1.3. β-Amylase (EC 3.2.1.2): is an exo-acting enzyme which hydrolyzes α-(1, 4)-D-glucosidic linkages in starch and oligosaccharides to yield maltose from the non-reducing end of starch and dextrins molecules (Figure 1). As such, β-amylase is a major contributor to the formation of fermentable sugars (maltose) in wort. Because it cannot by-pass the α-(1,6) linkages (branch-points) in amylopectin, branched β-limit dextrins remain as end-products. These are not fermentable, and are the major carbohydrate present in beer.

Several studies have found that β-amylase is the major component of DP, because its turnover number (K_{cat}) is considerably higher than the other starch degrading enzymes (Englard et al. 1998). It has been suggested that there are at least four allelic forms of β-amylase in cultivated barley that exhibit subtle but significant differences in thermostability and electrophoretic mobility (Eglinton et al. 1998; Kihara et al. 1998). Increasing thermostability has been linked to increased levels of fermentability of wort produced during mashing by these authors. Beta-amylases are optimally active at 60°C to 65°C in mashing and are sensitive to higher temperatures. Fermentability can be affected by several factors that make screening of

breeding lines for fermentability and performing quantitative trait locus (QTL) analysis more difficult. Gunkel et al. (2002) investigated the effect of malting barley genotype on fermentability and attributed the variation in fermentability to the genotype-dependent difference in beta-amylase thermostability. Isoelectric focusing (IEF) and molecular mapping (markers, i.e SNP) techniques were used to identify four β-amylase alleles B*myl*-Sd1, -Sd2L, -Sd2H and B*myl-Sd3*. The first three alleles were identified in cultivated barley, while B*myl-Sd3* was identified in a sample of wild barley (*Hoerdeum vulgare ssp. Spontaneum).* It has been reported that malt containing the *Sd1* and *Sd2H* alleles of β-amylase showed higher ADF % than malt containing (*Bmyl*-Sd3) (Eglinton et al. 1998). However, *Bmyl*-Sd3 is more thermostable and expected to increase the fermentable sugars during the brewing process (Eglinton et al. 1998). Evans et al. (2005) estimated that the impact of malt containing the *Sd2H* allele resulted in a commercially important 2% point increase in ADF. However, malt that contains very high levels of β-amylase activity can also compensate for the absence of the more thermostable beta-amylase types (Edney et al. 2007). Hence, β-amylase thermostability could play an important role in predicting fermentability.

2.4.2.1.4. Limit Dextrinase (LD): Limit dextrinase also known as pullulanase or *α*-dextrin 6-glucanohydrolase (EC 3.2.1.41), is the key barley enzyme in the debranching of starch. Limit dextrinase hydrolyses α-(1, 6) glucosidic linkages in amylopectin molecules and branched dextrin molecules yielding linear dextrins (Bamforth et al. 2009). These in turn can be further degraded by the α- and β-amylases. Limit-dextinase activity is not routinely measured as part of malt analysis. Its activity in malt has been reported to range from 50 to 160 (U/Kg) (Duke and Henson, 2009).

Limit dextrinase and α-glucosidase were found to be relatively heat labile in kilning and mashing (Sissons et al. 1995). Limit dextrinase has an optimum temperature of 50°C to 60°C in mashing. Sissons and coworkers showed that 25-85% of limit dextrinase activity is lost during the kilning process. Studies performed by Evans et al. (2005) indicated that an elevated level of limit dextrinase can result in a 2-4% increase in wort fermentability due to the availability of substrate to α-amylase.

2.4.2.1.6. Alpha-glucosidase (EC 3.2.1.20): or maltase, primarily catalyzes the hydrolysis of α-(1, 4)-D-glucosidic linkages in oligosaccharides (Sun and Henson, 1991). It is an exo-enzyme which acts from the non-reducing end to liberate glucose. It is unstable in solution at temperatures of 45ºC and above, but MacGregor et al. (1987) reported that it largely survives the kilning process. In later studies MacGregor et al. (1999) reported that α-glucosidase may help to increase the effectiveness of α-amylase and β-amylase activity during mashing by removing maltose which is considered a competitive inhibitor. Sun and Henson (1990) found that the combined activity of α-glucosidase and α-amylase were synergistic in starch hydrolysis, which resulted in higher extract levels.

Alpha-glucosidase is less thermostable than α-amylase, β-amylase and limit-dextrinase. A study conducted by Muslin et al. (2003) showed that only 5% of *α*- glucosidase activity remains after exposure to high mash temperatures (65-75°C) when compared to a 30°C control. They also found that malt containing more thermostable *α*-glucosidase showed significantly higher fermentable values. But others have stated that the importance of *α*-glucosidase in the brewery is still not clearly understood (Bamforth, 2003).

2.4.3. Protein/Soluble Protein

As previously discussed there is generally considered that there is an inverse relationship between the level of protein in barley (malt) and malt extract, with higher protein implying a lower proportion of starch. On the other hand, if the levels of barley protein drop below 8% then the amount of hydrolytic enzymes, particularly α-amylase, become too low for the standard malting and brewing process and ultimately, would lower the extract level (Edney et al. 2007). Likewise worts that are high in soluble protein would be expected to contain proportionally lower levels of fermentable sugars. However, yeast does require specific amino acids for growth and as such, free amino nitrogen (FAN) can have an impact upon the fermentability of the wort. As an example, Paik et al. (1990) analyzed forty commercial malt samples for fermentable values and reported that the samples with higher levels of FAN showed higher fermentable values than samples with lower levels of FAN. Beer that utilizes high levels of adjunct require greater levels of malt soluble proteins, as adjunct provides virtually no soluble nitrogen. During the fermentation process a minimal of (150 mg/L) amount of FAN is needed for the optimum yeast growth (Pierce, 1966).

2.4.4. Cell Wall Polysaccharides

The major polysaccharides present in the barley cell wall are arabinoxylans and (1-3), (1-4)-β-D-glucan or β-glucan. The β-glucans are extensively degraded during the malting process, while the arabinoxylans are to a lesser degree (Schwarz and Li, 2010). Beta-glucans appeared to affect fermentability and extract in a number of ways including restriction of enzyme movement due to high viscosity during mashing, and also by causing the late release of starch from poorly modified endosperm (Edney et al. 2007). Moreover, since high molecular weight β-glucans are mainly responsible for possible gel formation, they consequently cause filtration problems in the brewing process. Lautering becomes more difficult, which could result in lower extract level.

(Schwarz et al. 2007) reported that average β-glucans values were approximately 40% lower in Congress mashes when compared to hot water extracts (65ºC isothermal mashes). The maximum degradation of β-glucan can also play a great role in improving malt fermentability by producing more glucose. Bamforth and coworkers (2001) reported that accessibility to β-glucan is hindered by arabinoxylans. Endo-xylanase is the primary enzyme associated with the degradation of arabinoxylans during germination. Xylose is not fermentable by brewer's yeast (Briggs, 1998).

2.4.5. Modification

Malt modification is a complex process and is highly important for the quality of malt. The term modification is used to encompass the physical and biochemical changes which occur within the barley endosperm during the malting process (Lewis and Young, 1995). In general, well-modified malts produce higher levels of extract and fermentability, than poorly or over modified malts. Well-modified malt is friable and easily crushable, while the poorly modified malt is steely and hard.

The physical or biochemical changes in the endosperm principally include degradation of the cell walls and protein matrix that surround the starch. This degradation makes the starch more accessible to the amylases, and easier to extract and degrade in the brewing process (Bamforth et al. 2001). Hydrolysis of starch requires adequate degradation of the endosperm cell wall that surrounds the protein matrix and starch (Voragen et al. 1987). High levels of β-glucan in the endosperm can cause cell wall degradation problems. If there is not adequate cell wall degradation, proteolytic and amylolytic enzymes will have limited interaction with barley endosperm cell contents (Gill et al. 1982). Edney and coworkers (2007) have found that malt modification can have a significant impact on malt extract and fermentability. Poorly modified malt limits the extract availability and vice-versa. An over modified malt would also result in

reduced malt yield and lower extract because of more extensive starch degradation with the resultant glucose consumed through respiration (Kunze, 2004).

It has been reported that barley endosperm modification is affected by barley genetics, growing environment and malthouse processing conditions (time, temperature and moisture). The effect of time on malt modification is direct, as the levels of hydrolytic enzymes continue to increase during the germination process. Increase in germination time would result in a greater amount of enzymatic activity and more enzyme-substrate interaction (Kunze, 2004). This could result in over modification of malt and subsequently would lower the extract level. (Smart et al. 1995) have reported that increasing the moisture level of the germinating grain from 42-45 to 48% favors over-modification and reduces extract. They have also found that an increase in temperature would result a faster germination process, which affects the overall malt modification. In another study Pollock et al. (1962) reported that high values of extract are reached earlier when germination occurs at higher temperature, but the maximum level of malt extract is generally produced when grain is malted at lower germination temperatures for a longer time.

Assessment of malt modification is very important and can be tested in many ways. Tests include malt friability, fine and coarse-grind extract difference, β-glucan content, wort viscosity, and Kolbach Index (KI) (Schwarz and Li 2010). Kolbach Index is the ratio of wort soluble protein to total protein or (S/T). Li et al. (2008) demonstrated that modification of protein had a significant contribution to the extract level among different barley cultivars. Bathgate et al. (1978) reported that over modified malt could result in reduction of fermentability due to the presence of higher levels of soluble protein, and proportionally reduced fermentable

carbohydrates. On the other hand, use of well modified malts also ensures that there is a sufficient level of amino-acid available to the yeast growth.

2.5. Research Studies on Factors that influence the Malt Extract and Fermentability

2.5.1. Extract

Maltsters and brewers consider malt extract as an important malt quality parameter for purchase or selection decisions. Malt extract has traditionally been held to be of extreme economic importance, because it determines the amount of malt needed to produce for a given amount of beer (Schwarz et al. 2007). As a consequence numerous research studies have investigated factors which influence extract.

2.5.1.1. Determination of Malt Extract: As stated earlier, laboratory extract value for brewer's pale malt generally falls between 79-82%, which is always slightly higher than the actual brewhouse extract (Schwarz and Li, 2010). There are two methods that are used for the determination of laboratory extract, and both were developed only with this goal in mind. However, in the years following the introduction of these methods, the resultant worts have been used for an increasing battery of quality tests (Schwarz et al. 2007).

The Institute of Brewing (IoB) developed a laboratory mashing method referred as hot water extract (HWE). This method has only been widely used in the UK and former colonies, but was later also adopted by the European Brewery Convention (EBC). The HWE method is carried out isothermally at 65ºC for 1 hour with a mixture of 50 g finely or coarsely ground malt in 360 ml distilled (DI) water. Then mash is cooled and then adjusted to 450 g or (515 ml) by adding distilled water.

The method in more commonly used method, by both the EBC and ASBC, is the Congress mash. Here, 50 g of finely (or coarsely) ground malt is mixed with 200 mL of DI water

and mash begins at 45°C with continuous stirring. After 30 minutes of mashing, temperature is increased by 1°C/min until 70°C. As temperature reaches 70°C, there is addition of 100 mL (70°C) distilled water. After 1 hour, the mash is cooled to 30°C and adjusted to a volume of 515 mL or a weight of 450g.

Schwarz et al. (2007) conducted a study to evaluate the influence of mash temperature, grind (coarse and fine) and original gravity (water:grits ratio) on laboratory extract. In this study, two malting barley cultivars with different modification levels, three level of grind, three water:grits ratios and two different mash temperature profiles were used. The mash temperature profiles were modified Congress and HWE methods. The results of this study indicated that mash temperature affected the extract levels, but to a more limited extent when compared to other wort quality parameters (i.e. β-glucan, wort color, FAN, soluble protein and fermentable sugars). When extract was averaged across all treatments, the Congress mash showed that level of averaged extract was 1.3 percentage points higher than for the HWE mash. The authors suggested that this was due the proteolytic rest (45ºC) in Congress mash which promoted the degradation of protein and β-glucans, and perhaps made the starch more susceptible to the amylolytic enzymes. The HWE mashes had higher levels of fermentable sugars (maltose) than Congress mashes, which is due to more production of more maltose by β-amylase at the 65ºC rest, as opposed to the 70°C rest. This would result in higher fermentable values.

2.5.1.2. Prediction of Malt Extract: Several authors have described predictive equations for the estimation of malt extract ((Bishop et al. 1948; Harris and Banasik, 1952) Bishop and coworkers developed the following equation based upon analysis of 851 malt samples:

$$T.E. = A - 11N + 0.22G \qquad \text{(Eq. 2)}$$

Where T.E. is abbreviated as True Extract N as Nitrogen (%), G as 1000 kernel weight and A is used as constant dependent on barley variety.

Recently, a study conducted by Li and coworkers (2008) have also proposed an equation model to predict malt extract. In this model, they have included the contribution of malt modification, barley and malt proximate analyses within a single barley cultivar (Tradition). The equation (3) is as follows and model was developed using stepwise linear regression;

Malt Extract = 106.4 –1.34(Barley protein, % dry basis) + 0.14(1000-Kernel weight, g) + 0.0089 (Diastatic power, °ASBC) + 0.018(Wort β-glucan, mg/L) – 8.17(Ash, % dry 31.13(Kolbach Index, %) – 0.21(Starch, % dry basis) (Eq. 3)

Both studies found that the protein content and 1000 kernel weight are the common factors in predicting the malt extract but the contribution was not high. They also found that 81% of the variation in the extract was explained by malt modification (KI), and that the DP also showed a significant impact in predicting extract. Traditionally, maltsters use DP as indicator of total starch breakdown activity in malted barley. Their study also suggested that increasing the amount of soluble protein would increase the malt extract.

2.5.2. Fermentability

There is growing interest in fermentability in the malting and brewing industry due to the fact that fermentability is also a determinate in the amount of malt required to produce a given amount of beer at specific alcohol content. It is well known that there are many factors which impact fermentability, and most studied include; malt enzymes, mash temperature, fermentable sugars and free amino nitrogen. Several studies have evaluated the correlation between the malt enzymes (i.e. α-amylase, β-amylase and limit-dextrinase) , their thermostability, starch

gelatinisation temperatures ,the yield of fermentable sugars, and consequently malt fermentability during mashing process (Evans et al. 2003, 2005; Evans and Eglinton, 2009).

Brewers generally have considered DP as a predicator of malt fermentability, but in recent years some have lost confidence in this predictor, as there are cases of adequate DP, but lower fermentability. To clarify brewer's concerns Evans et al. (2003) performed a study on more than forty commercial malts samples and developed a equation to predict malt fermentability. These malt samples were infusion mashed with a slight modification of the Congress mash method and the ADF was measured. There was significant variation in the thermostability of beta-amylase and limit dextrinase in the malt samples, and these factors were found to significantly contribute to wort fermentability. Among the malt quality parameters, DP alone was only able to explain about 50% of the variation observed for ADF. Results of this study indicated that mashing at 65ºC showed maximum wort fermentability when compared to in comparison the congress mash (at 70ºC). This at least partially because, the production of maltose by beta-amylase is greater when mashing at 65ºC than at 70ºC. The starch gelatinization temperature showed negligible contribution to wort fermentability in this study. On the other hand, Stenholm et al. (1996) reported that starch gelatinization temperatures in excess of 65ºC could result in low level of fermentable values.

The relationship between the malt quality parameters and malt fermentability was determined using stepwise multiple linear regression analysis. The predicted equation for malt fermentability includes α-amylase (A), limit-dextrinase (B), Kolbach Index (C), β-amylase (D), and β-amylase thermostability was able to explain about 95% of the observed variation.
The equation is as follows:

$$Malt\ Fermentability = 69.9 + 0.0174\ (A) + 9.602\ (B) + 0.1950\ (C) +$$

$$0.0070\,(D) + 0.5375\,(E) - 0.0008(D)*(E) \quad \text{(Eq. 4)}$$

Further analysis conducted by Evans et al. (2007) on a large scale in pilot brewing trials on series of malt supported their previous research on α-amylase, β-amylase and limit dextrinase activity. The results suggested that the conventional DP enzymes assessment could be replaced with the measurement of its component enzymes activities and malt modification level which played an important role in determining final wort fermentability.

CHAPTER 3. MATERIALS AND METHODS

The primary objective of this research was to determine the variation in the malt fermentability or RDF using two different laboratory mashing methods in different barley cultivars and lines. Second objective was to identify which malt/wort quality parameters have the greatest influence upon fermentability within the confines of the tests when a definite portion of malt is replaced with adjunct. Barley samples known to show differences in malting and brewing behavior and quality were be malted under standard laboratory conditions. The malt samples will be analyzed for quality and mashed under several different conditions, which are intended to limit or enhance differences in fermentability.

3.1. Materials

3.1.1. Barley Samples

Barley samples were selected for this study with the aim of (NDSU) barley breeding, 2-rowed cultivars and lines from several European breeding programs and a two-rowed feed maximizing variability in malt quality. The samples selected represented a mixture of Midwestern six- and two-rowed barley cultivars, lines from the North Dakota State University barley cultivar from the Montana Agricultural Experiment Station (Table 1). All samples were grown at Fargo, ND location in 2010.

Table 3.1. Barley cultivars and lines used for the evaluation of malt quality and fermentability.

Sample Number	Cultivar/Line	Row Type	Origin
1	Lacey	6	University of Minnesota
2	Tradition	6	Busch Ag. Resources, Inc. Fort Collins, CO.
3	Stellar-ND	6	NDSU Agricultural Experiment Station Fargo, ND
4	Jennifer	2	Saatzucht Dr. J. Ackermann and Co. Irlbach, Germany
5	Sunshine	2	Saatzucht Josef Breun GmbH & Co. KG Herzogenaurach, Germany
6	Lilly	2	Saaten-Union GmbH, Isernhagen, German
7	ND07/551/23	2	NDSU Agricultural Experiment Station Fargo, ND
8	ND07/550/1	2	NDSU Agricultural Experiment Station Fargo, ND
9	ND07/604/24	2	NDSU Agricultural Experiment Station Fargo, ND
10	ND07/604/29	2	NDSU Agricultural Experiment Station Fargo, ND
11	NORD 2505	2	Saaten-Union GmbH, Isernhagen, Germany
12	NORD 2509	2	Saaten-Union GmbH, Isernhagen, Germany
13	Robust	6	Minnesota Agric. Experiment Station St. Paul, Minnesota
14	Legacy	6	Busch Ag. Resources, Inc. Fort Collins, CO.
15	Conlon	2	NDSU Agricultural Experiment Station Fargo, ND
16	Haxby	2	Montana Agricultural Experiment Station Bozeman, Montana

3.2. Methods

3.2.1. Barley Quality Analysis

3.2.1.1. Kernel Plumpness: Kernel assortment was determined according to American Society of Brewing Chemists Barley Method 2-B (ASBC, 2009) using a Eureka-Niagara sample barley grader (S. Howes Co., Inc., Silver Creek, NY, USA).

3.2.1.2. Protein and Moisture: Barley protein and moisture contents were determined by Near Infrared Reflectance (NIR), on a FOSS (model # Infratec 1241) whole grain analyzer (FOSS NIR Systems, Inc., Laurel , MD), using the calibrations supplies by the manufacturer

3.2.2. Pilot-Malting

Micro-malting was conducted according to the standard laboratory procedure described by Karababa et al. (1993). The time required for each sample to reach 45% steep-out moisture was determined by pilot-steeping a 10 g sample. Malting was performed on 300 g (dry basis) and samples were malted in multiple batches. Steeping was performed at 16°C with a 1 hr air rest included with each 12 hr of steeping. Germination was for 96 hr at 16°C and ~95% relative humidity. During germination, samples were turned daily by hand to prevent matting, and the sample weight was adjusted to 45% moisture (575 g) with distilled water. Kilning was conducted in a Joe White laboratory malting unit (Joe White Malting. Melbourne, Australia). Total kiln time was 24 hr, during which temperature was ramped from 49 to 85°C (Karababa et. al., 1993). Rootlets were removed by hand from kilned malt prior to analysis.

3.2.3. Malt Quality Analysis

3.2.3.1. Friability: Friability and percent of unmodified malt were determined according to ASBC Malt Method-12 (ASBC, 2009) using a Pfeuffer friabilimeter (Pfeuffer GmbH, Kitzingen, Germany).

3.2.3.2. Malt Milling: Coarse-grind malt used in mashing was prepared using a Buhler-Miag disc mill (model# DLFUW-11060, Uzwil, Switzerland). The setting for coarse-grind was calibrated according to the procedure in ASBC Malt Method-4 (ASBC, 2009). Malt used for the determination of enzymes activities was ground with a Udy Cyclone mill (Udy Corporation, Fort Collins, CO) to pass through 0.5 mm screen.

3.2.3.3. Moisture Content: Malt moisture was determined by heating a 5.5 g ground sample in a moisture tin for 3-4 hrs at 104°C as described in ASBC-Malt Method-3 (ASBC, 2009).

3.2.4. Enzymes Analyses

3.2.4.1. Alpha-amylase Activity: The level of α-amylase activity on all malt samples were analyzed by using a Technicon Instrument Corporation (Tarrytown, NY USA) flow auto-analyzer according to a modification of ASBC Method Malt-6 (ASBC, 2009) as previously described (Karababa, 1993). Malt samples (5 g) were extracted with 100 mL of buffer solution (0.5% NaCl solution) for 2.5 hr at 20°C, with agitation every 20 min. The extracts were filtered through 32-cm fluted filter paper in a conical funnel with diameter of 20 cm. A 5 ml aliquot was assayed according to the ASBC Malt Method-6 (ASBC, 2009). Alpha-amylase activity is expressed as dextrinizing unit (DU).

3.2.4.2. Diastatic Power: Diastatic Power (DP) was determined using a Technicon Instrument Corporation, Tarrytown, NY flow auto-analyzer according to a modification of ASBC Method Malt-6A (ASBC, 2009) as previously described (Karababa, 1993). Extract preparation for measurement of DP was the same as for the α-amylase. Diastatic Power is expressed as °ASBC (unit).

3.2.4.3. β- Amylase: The level of β-amylase activity in the malt samples was determined using Betamyl kits (Megazyme Ltd., Wicklow, Ireland) according to instructions of manufacturer. This method uses a *p*-nitrophenyl-*α*-D-maltopentaoside (PNPG5) substrate in the presence of excess α-glucosidase. β-amylase activity is based on the release of p-nitrophenol from the PNPG5 substrate and is expressed as Units/gram (U/g).

β-amylase thermostability was determined by heating the enzyme extract (0.2ml) for 10 min at 60°C as described by Evans et al. (2005). After exactly 10 min, the samples were cooled to 4°C by keeping them on ice. After cooling, the samples were centrifuged for 10 min at 13,000 × g, and then assayed according to Evans et al. (2005). Values reported for both β-amylase activity and thermostability were the average of three concordant determinations.

3.2.4.4. Limit Dextrinase: Limit Dextrinase (LD) activity of the malt samples were determined using Limit Dextrinase kits (Megazyme Ltd., Wicklow, Ireland) according to the instructions of manufacturer. This assay uses an insoluble Azurine-crosslinked-pullulan as a substrate. The substrate is hydrolyzed by limit dextrinase to yield soluble-dyed fragments. Enzyme activity can be directly related with the rate of release of water soluble-dyed fragments, which were measured at 590 nm. Malts samples (0.25 g) were suspended in 4ml of extraction buffer (sodium maleate 100mM plus sodium azide 0.02% and dithiothreitol 25 mM) and extracted at 40°C over a period of 5 to 6 hrs. A 0.5 ml aliquot of the enzyme extract was assayed. Each of the samples were assayed in triplicate, and the level of activity was calculated from a standardard curve based on manufacturer's method.

3.2.4.5. α-glucosidase: Alpha-glucosidase activity in the malt samples were determined according to a modification of the method described by Evans et al. (2005), which in turn was based upon the method developed by Sissons and MacGregor (1994). The assay method uses *p*-

nitrophenyl-α-D-glucopyranoside (Sigma, St. Louis, MO) as a substrate, and α-glucosidase activity is amount of enzyme required to release 1µmole *p*-nitrophenol in one minute. Activity unit is expressed as units/g. Malt flour (0.4 g) was extracted with 4 mL of extraction buffer (0.1*M* acetate buffer, 0.1% BSA, 0.02% sodium azide) for 1 hr with constant mixing at room temperature. After centrifugation at 13,000 × *g* for 5 min., a 0.2 mL aliquot was assayed according to the Evans et al. (2005). The absorbance was measured at 400 nm and the level of α-glucosidase activity was calculated as $\Delta E400 \times 9.39$ units/g. Each sample was assayed in triplicate.

3.2.4. Mashing Methods (Malt Extract)

Laboratory mash procedures utilized in this study were the ASBC Malt Method-4, commonly known as the Congress Mash (ASBC, 2009), and the hot water extract (HWE), method (4.6) of the European Brewery Convention (EBC, 1998). Both methods were conducted in a Weber-Ehrenfeld mash apparatus (Weber Brothers Metal Works. Chicago, IL). The temperature and time profiles of the two methods are shown in figure 2.

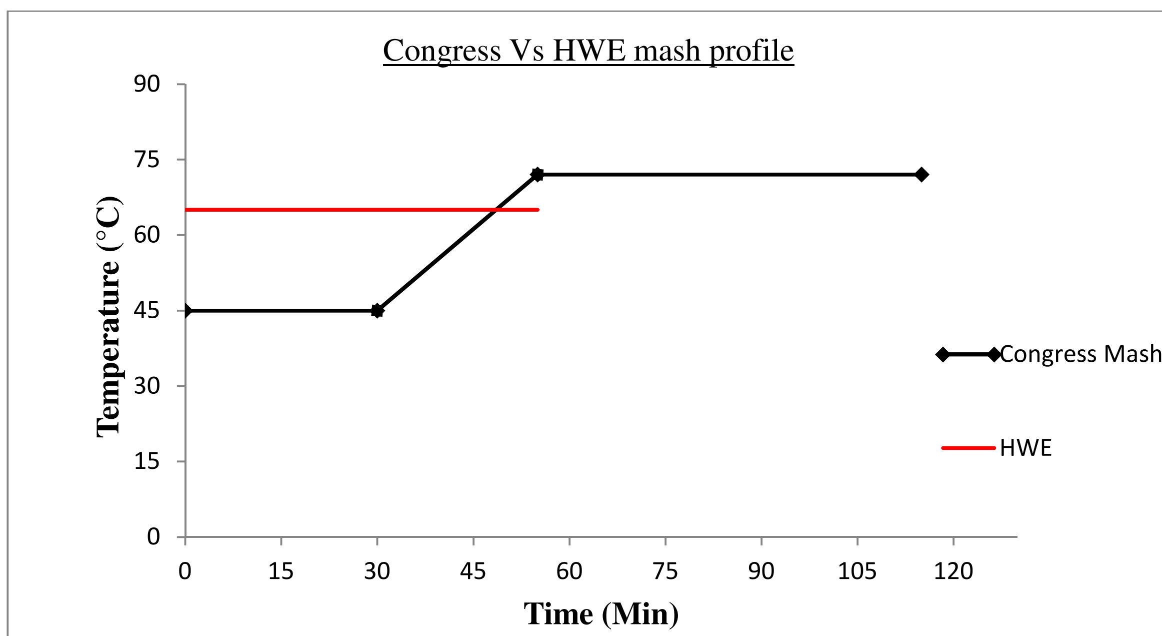

Figure 3.1. Time and temperature profiles of laboratory mash methods.

Both methods used 50 g of coarsely ground malt. The Congress mash begins at 45°C with the addition of 200 mL distilled water to the ground malt. The mash was continuously stirred. Following 30 min then temperature is increased at a rate of 1°C/ min. until 70°C. When the temperature reaches 70°C, there was addition of 100 mL (70°C) water to the mash. Then mash was maintained at 70°C for 1 hr followed by cooling to around 30°C. The final mash was adjusted to a weight of 450 g. The extract was filter through a fluted 32 cm filter paper (catalog# 509, Ahlstrom Filtration, Inc., Mount Holly Spring, PA). Extract was determined using an Anton-Parr DMA 5000 density meter (Anton Parr GmbH. Graz, Austria).

The HWE mash begins with the addition of 350 mL of 65°C water to the ground malt. The mash was held at 65°C for 60 min with continuous stirring. The mash was cooled to 20°C by circulating cool water in the mash apparatus, the weight is adjusted to 450 g with the addition of distilled water. Filtration and extract determination were as described for the Congress mash.

The inclusion of non-malt adjunct was a second treatment, and was considered due to the fact that α-amylase, DP, and FAN, are all likely to be in excess in the standard all malt laboratory mashes. As such detection of differences in fermentability may be more difficult. Replacement of a portion of the malt with adjunct thus may impact the measurement of fermentability. Adjunct:malt (malt:corn grits) ratios, of 0, 20 and 40% were based upon contributions to total extract (Table 3.2.)

Table 3.2. Adjunct to malt ratios used in laboratory lashing

Level	Calculated Extract % from Malt	Calculated Extract % from Corn	Malt Used (g)	Corn Used (g)
1	100	0	50	0
2	80	20	40	10.6
3	60	40	30	21.3

Adjunct to malt ratios were calculated based on the individual extract values of malt and pre-gelatinized corn. The mean malt extract value for all barley cultivars and lines in this study was approximately 80%. The extract value for the pre-gelatinized corn grits was 75% percent (product # 5075, Briess Malt & Ingredients Co. Chilton, WI). The total amount extract requires was calculated based on the Lincoln equation as described below (Hardwick, 1995).

Lincoln equation:

$$\textit{Extract (kg/hl)} = 0.9974 \left[\frac{1}{\frac{1}{P} - 0.00382} \right] \qquad \text{(Eq. 5)}$$

P = gram of extract in 100 g wort (i.e. 8% Plato)

Pre-gelatinized corn grits were coarsely milled on Buhler-Miag disc mill (model# DLFUW-11060, Uzwil, Switzerland). Grits were mixed with ground malt prior to mashing process.

3.2.5. Wort Quality Analyses

3.2.5.1. Standard Analyses: Worts were analyzed for soluble protein, wort color, free amino nitrogen (FAN), wort viscosity, and wort β-glucan according to ASBC methods Wort-17, Wort-9, Wort-12, Wort-13 and Wort-18, respectively (ASBC, 2009). Kolbach Index or soluble protein to total protein ratio (S/T) was determined by using the barley protein values.

3.2.5.2. Wort Carbohydrate Analysis: Maltotriose, maltose and glucose concentrations were measured in the resultant wort samples. Fermentable sugar analysis was by high performance liquid chromatography (HPLC) (model# 1050, Agilent Technology, Santa Clara, CA) with an Aminex HPX-87 strong cation exchange column (Catalog# 125-0095, Bio Rad Laboratories Hercules, CA), as described in ASBC method Wort-14B (ASBC, 2009). The column temperature was maintained at 85°C with a flow rate of 0.5 µL/min.

3.2.6. Fermentability Assay

Fermentability of samples was measured by a slight modification of a method developed at Busch Agriculture Resources, LLC (Fort Collins, CO) (Dr Jolanta Menert, personnel communication). Dry yeast (1 g) *(Saccharomyces cerevisiae)* (Safale s-04, Fermentis LeSaffre Groups, Marcq-en Baroeul, France) was added to 70 ml of wort in a 250 ml Erlenmeyer flask. The flask was placed on a shaker and agitated at 150 rpm at room temperature (23 to 25°C) for 15 hr. After fermentation, the wort was vacuum filtered through 110 mm glass microfiber filters (catalog# 1820, Whatman International ltd. Maidstone, England). Specific gravity was determined using a DMA 5000 densitometer (Anton PAAR GmbH, Graz, Austria), and the RDF value was calculated as follows:

$$\text{Apparent Attenuation limit (AAL, \%)} = 100*(\text{original gravity} - \text{final gravity}) / (\text{original gravity} - 1) \quad \text{(Eq. 6)}$$

$$RDF\% = AAL\%*0.88 \quad \text{(Kunze, 2009)} \quad \text{(Eq. 7)}$$

3.3. Experimental and Statistical Analysis

A randomized complete block design was used throughout the study. Replication was accomplished repeating the experiment, on duplicate malt samples. Statistical analyses were performed by using procedures of the Statistical Analysis System (SAS® 9.3 System Options 2012 Second Edition; Cary, NC). Data was be analyzed by an analysis of variance (ANOVA) procedure using the general linear model. Means were separated using least significant differences (LSD) to determine the significant differences ($P \leq 0.05$).

The general linear model (GLM) was used to perform correlation and forward stepwise regression with α-to-enter = 0.15 and α-to-remove = 0.15. Stepwise regression was used to

determine how much variability could be explained by each independent variable (malt quality parameters) for the dependent variable malt fermentability or RDF.

CHAPTER 4. RESULTS AND DISCUSSION

The first objective of this research was to evaluate the impact of mashing methods on determination of the malt fermentability. In this study, sixteen malting barley cultivars were malted. The average barley and malt data for these samples is shown in Table 4.1.

Table 4.1. Barley and malt quality data averaged across the sixteen barley cultivars utilized in the study of malt fermentability*

Variable	Mean	Range	Std Dev.
Barley Protein (%)	12.8	11.6–13.6	0.6
Friability (%)	75	64.9–92.4	7.3
α-Amylase (U/g)	63	52.2–81.3	8.4
β-Amylase (U/g)	17.5	9.3–28.8	5.2
β-Amylase Thermostability(%)†	3.4	2.3–5.5	0.9
Limit-Dextrinase. (U/kg)	132.4	86.4–163.2	25
α-Glucosidase (U/g)	1.3	0.6–1.9	0.4
DP (°ASBC)	131.5	66.9–184.4	36.3

*N = 32, 16 cultivars with 2 replications of each.

†Thermostability is measured as the percentage of activity remaining after 10 min at 60ºC.

Table 4.1. shows that the selected cultivars generally yielded a wide range of values for the malt quality parameters. The exceptions were for β-amylase thermostability and α-glucosidase activity. Greater variation among the malt quality parameters levels was important as it contributed to the robustness to this study. The data for the individual cultivars is shown in appendix A1.

4.1. Impact of Different Mashing Methods on Wort Quality Parameters

4.1.1. 100% Malt Mash

The impact of mashing protocols on wort quality parameters was investigated using the ASBC Congress and HWE mash methods. The ASBC Congress mash starts at 45ºC and is then

ramped to 70ºC over 25 min. The temperature is held at 70°C for 1 hr. The HWE mash is conducted isothermally at 65ºC for 1 hr. Data for the wort quality parameters is shown in Table 4.2.

Table 4.2. Wort quality data averaged across 16 malt samples† mashed according to the congress and hot water extract (HWE) protocols.

Variable	ASBC Mash			HWE Mash		
	Mean*	**Range**	**StdDev.**	**Mean***	**Range**	**StdDev.**
Extract (%)	77[a]	73.8–79.2	1.5	75[b]	70.9–77.7	2
Wort β-glucan (mg/L)	129[a]	14.7–289	75.3	166[b]	16–319	98.7
Color (SRM)	2.4[a]	1.9–2.7	0.3	2.1[b]	1.8–2.5	0.2
Soluble Protein (%)	5.4[a]	4–6.7	0.8	4.9[b]	3.6–6.2	0.7
KI (%)	43.5[a]	33–53.6	5.7	39.6[b]	29.8–50	5.5
FAN (mg/L)	227[a]	161–287	31.8	194[b]	147–278	30.5
Glucose (g/100ml)	0.4[a]	0.15–0.5	0.1	0.2[a]	0.2–0.3	0.02
Maltose (g/100ml)	4.1[a]	3.3–4.9	0.5	4.7[b]	3.5–5.1	0.5
Maltotriose (g/100ml)	1.5[a]	1–1.4	0.1	1.3[a]	1–1.5	0.1

†N=32, 16 cultivars with 2 replications of each

*Means within a row followed by different letters differ significantly at ($P \leq 0.05$)

Results in (Table 4.2.) indicated that the mash temperature profile had a significant impact on extract ($P \leq 0.05$). When results for extract were averaged across all cultivars, the Congress mash protocol yielded extracts that were 2 percent points higher than the HWE mash protocol. Lewis and Young (1995) have suggested that the level of extract increases with an increase in the mash temperature. The Congress mash protocol with a rest at 70ºC rest will result in higher dissolution of solids than the HWE mash with a rest at 65ºC. In addition, a larger portion of the extract in the Congress mash is due to solubilized proteins, because proteases are active at the initial rest temperature of at 45ºC (Schwarz et al. 2007).

Mash method was also found to have a significant impact on wort β-glucan (*$P \leq 0.05$*). When values for β-glucan were averaged across all the cultivars, the HWE mash showed much higher β-glucan levels than Congress Mash. This was likely because HWE mash does not include a rest at 45ºC where the breakdown of β-glucan would occur. Malt modification is another factor which could play an important role in high/low levels of β-glucan in wort. Well modified malt (> 85%) tends to produce lower β-glucan levels than the poorly modified malt (Kunze, 2004). Wort color was also found to differ significantly ($P \leq 0.05$) between the two methods, although the overall difference was small. Wort color averaged across all cultivars was 0.3 SRM units higher in the Congress mash. The explanation of this finding might be due to greater formation of color precursors at the 45ºC rest (Kunze, 2004).

Soluble protein levels were significantly affected ($P \leq 0.05$) by the mash protocols. The values averaged across all cultivars (Table 4.2.) showed that Congress mash yielded higher levels of soluble protein percent than the HWE mash. This was not surprising because Congress mash includes a protease rest at 45ºC. Also, the soluble protein levels are greatly affected by the malt modification. More proteins are accessible to proteolytic activity in well modified malt during mashing, when compared to poorly modified malt. Li and Schwarz (2008) reported that mashing protocol becomes less important in differentiating the soluble protein levels with poorly modified malt. The Kolbach Index (KI) is an indication of malt modification, and more specifically, an indication of protein solubilization or modification. It is determining by dividing the wort soluble protein by total malt protein. Results in Table 4.2. shows that KI was significantly affected ($P \leq 0.05$) by mashing protocol. The explanation would be similar to that discussed for soluble proteins. Free amino nitrogen (FAN) was also significantly affected ($P \leq 0.05$) by the mash protocol. The values averaged across all the cultivars indicated that Congress

mash yielded a higher level of FAN (mg/L) than HWE mash. Again, this was because Congress mash has a rest at 45ºC, which favored peptidase activity. Owades et al. (1962) reported that higher values for FAN are obtained at a mash temperature between 30 to 45ºC, and as the temperature increases there is decreased proteolytic activity. Hence, the FAN level decreases in the resultant wort.

The mash profile was found to significantly affect ($P \leq 0.05$) the levels of maltose, but not the levels or either glucose or maltrotriose. The content of maltose in the HWE mash was 0.6 g/100 ml higher than that found in the Congress mash. This was probably because of greater stability and activity of β-amylase activity at the 65ºC rest in the HWE mash. The 70ºC rest in the Congress mash likely resulted in partial inactivation of β-amylase. A study by Evans et al. (2005) study showed the highest content of maltose in worts was obtained at mash temperatures between 62ºC to 67ºC. There was a trend towards higher maltotriose and glucose in the Congress mash, which might be explained by the prolonged action of α-amylase on dextrins and oligosaccharides.The statistical significance of mashing method and cultivar on RDF was determined using analysis of variance (ANOVA) (Table 4.3.).

Table 4.3. Pertinent Sources of Variation, Degrees of Freedom (DF), Mean Squares and results of F-tests for malt fermentability (RDF) across 16 barley cultivars mashed according to the Congress and hot water extract (HWE) protocols.

Source of Variation*	**DF**	**Type III SS**	**Mean Square**	**F-value**
Method	1	1799.3	1799.3	18053.9
Cultivar	15	71.9	4.8	48.06
Method x Cultivar	15	42.6	2.9	28.5

*Significant at $P \leq 0.05$ level of confidence.

The results (Table 4.3.) indicate that the malt fermentability (RDF) was significantly ($P \leq 0.05$) affected by both cultivar and mash method. However, the interaction between cultivar and

mashing method for RDF was also significant (*P≤ 0.05*). This interaction is graphically represented in figure 4.1. Since the HWE method yielded consistently higher values for RDF when compared to the Congress mash (Table 4.4.), the HWE data was transformed by subtracting ten from the original HWE RDF values. Figure 4.1. clearly shows that this is a true interaction, and is not due to differences in magnitude. Cultivars are clearly not responding in a uniform manner across the two mash methods.

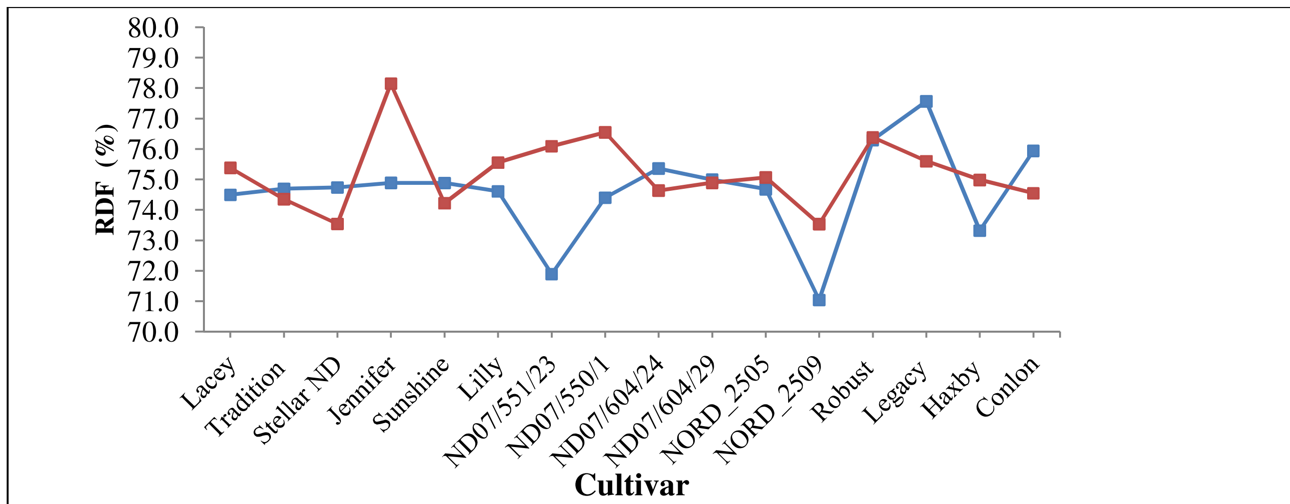

Figure 4.1. Illustration of the interaction between mash method and cultivar for real degree of fermentation ASBC Congress mash, Hot Water Extract mash.

Because of the significant interaction between cultivar and mash method for RDF, results for the Congress and HWE mashes are discussed separately. Data in (Table 4.4.) shows the RDF values obtained using the HWE and ASBC Congress mash methods for each cultivar. The HWE method consistently yielded higher RDF values than the Congress mash. This is likely because the 70°C rest of the Congress method results in greater inactivation of β-amylase and other amylolytic enzymes. This could explain the production of more fermentable sugars at 65ºC.

Table 4.4. Real degree of fermentation (%) or malt fermentability† values among sixteen barley cultivars using the ASBC Congress and hot water extract (HWE) mash protocols.

ASBC Mash		HWE Mash	
Variety	t-grouping*	Variety	t-grouping*
Legacy	77.6[a]	Jennifer	88.2[a]
Robust	76.3[ab]	ND07/604/24	86.5[b]
Conlon	75.9[ac]	Robust	86.4[b]
ND07/604/24	75.4[cd]	ND07/551/23	86.1[bc]
ND07/604/29	75.0[de]	Legacy	85.6[cd]
Sunshine	74.9[de]	Lilly	85.6[cde]
Jennifer	74.9[de]	Lacey	85.4[def]
Stellar ND	74.7[de]	NORD 2509	85.1[defg]
Tradition	74.7[de]	Haxby	85.0[efg]
NORD 2509	74.7[de]	ND/07/604/29	84.9[fgh]
Lilly	74.6[de]	ND07/550/1	84.6[ghi]
Lacey	74.5[e]	Conlon	84.5[ghi]
ND07/550/1	74.4[e]	Tradition	84.4[hi]
Haxby	73.3[f]	Sunshine	84.2[i]
ND07/551/23	71.9[g]	Stellar ND	83.5[j]
NORD 2505	71.0[h]	NORD 2505	83.5[j]

†N=32, 16 cultivars with 2 replications of each

*Treatment means in each column with different letters are significantly different at ($P \leq 0.05$) level of confidence

Also from Table 4.4. it appeared that the HWE mash protocol was better able to distinguish differences between cultivars for RDF, than the Congress mash. Perhaps the most interesting observation, and one implied by the significant interaction, was that the rank of the cultivars was not the same between the two mashing methods. This likely could be because different cultivars have different malt quality parameters (especially enzyme levels), and in turn behave differently at different mash temperatures.

For an example, the rank of the cultivar Jennifer rank was highest in the HWE method, but was more toward the middle with the Congress mash. This finding can likely be explained by the observation that Jennifer had high levels of β-amylase and limit-dextrinase (appendix A1.), which might have resulted in production of more fermentable sugar at the 65ºC rest. While in case of the Congress mash as the temperature was raised to 70ºC, the enzymes (β-amylase and limit-dextrinase) rapidly inactivated and which resulted in lower fermentable values. Conversely, the cultivar Conlon ranked higher in the Congress mash but gets lowered in the HWE mash. This could be explained because the cultivar Conlon had high level of α-amylase but low levels of β-amylase and limit-dextrinase (appendix A1.). The α-amylase has greater thermostability and survives longer at higher temperature which might have resulted in more fermentable sugars.

4.2. Prediction of Wort Fermentability Using Malt and Wort Characteristics

In the previous section we have seen that the wort obtained using HWE mash was higher in RDF, and this method showed greater discriminative power between cultivars. Apart from the impact of mash temperature profiles on enzyme activity, there are other malt and wort quality parameters which also can contribute to the malt fermentability. As stated earlier the second objective of this study was to evaluate the influence of malt and wort quality parameters upon fermentability or RDF.

Stepwise multiple linear regression (MLR) analysis was performed to determine the contribution of the independent variables representing malt and wort quality parameters to the dependent variable (i.e. RDF). The formation of MLR model includes the sequential addition and removal of predictive variables based on their individual contribution and statistical significance ($P \leq 0.15$ and $P \leq 0.05$) to the overall model. The analysis was performed across all malt samples (n=32). Data from the Congress and HWE mashes were analyzed separately.

Partial and model results from stepwise regression of the Congress mash data are shown in Table 4.5. These results indicate that diastatic power (DP) made the largest and most significant contribution to the RDF observed in the Congress mash. Diastatic power alone explained about 50% of the observed variation. Next in importance were α-amylase and KI which in turn explained an additional 18% and 8% of the variation, respectively. In total these three parameters, along with wort color, explained over 80% of the observed variation in RDF. Significant, but very small contributions to the model were made by the addition of wort β-glucan, glucose and β-amylase activity/thermostability.

Table 4.5. Partial and model (R^2) values for malt fermentability (RDF) from stepwise multiple linear regression (MLR) analysis across malt and wort quality parameters using Congress mash protocol.

Variable	RDF for all samples (n= 32)	
	Partial (R^2)*	**Model (R^2)**
DP (°ASBC)	0.51†	0.51
α-Amylase (U/g)	0.18†	0.69
Color (SRM)	0.07†	0.76
KI (%)	0.08†	0.84
β-Amylase Thermostability (%)¶	0.01	0.85
β-glucan (mg/L)	0.01	0.86
Glucose (g/100ml)	0.01	0.87
β-Amylase (U/g)	0.02†	0.89

*All variables in the model are significant at $P \leq 0.15$ level. No other variable met the 0.15 significance level for entry into the model

†Significantly different at $P \leq 0.05$ level of confidence.

¶Thermostability was measured as the percentage of activity remaining after 10 min at 60ºC.

Evans et al. (2003) reported that DP activity alone was able to explain 50% of the variation in RDF, when more than forty samples were mashed according to a modified Congress

method. Parameters of α-amylase, β-amylase, limit-dextrinase, enzyme thermostabilities and KI all made significant contributions to their predictive model for RDF, and in total, explain about 90% of the variation observed for RDF. One major difference between the results of Evans and coworkers (2003) and those of the current work reported that both β-amylase activity and its thermostability to make larger contributions to the model. The explanation as to why thermostability was not import in the current study is relatively straightforward. There was little variation in the thermostability of β-amylase between cultivatrs in the current study (see Table 4.1.) when compared to that of Evans et al. (2003). It is possible that none of the cultivars in the current study had the *Sd1* and *Sd2H* alleles, which in code for higher beta-amyalse thermostability stability (Eglinton et al. 1998).

The relatively small contribution of β-amylase to the model was particularly, surprising. DP was the largest component of the model. Diastatic power is considered as the combined activity of α-amylase, β-amylase and limit dextrinase (Arends et al. 1995). Appendix 3 shows that the DP when averaged across cultivars, was significantly ($P \leq 0.05$) correlated with β-amylase (r = 0.80), α-amylase (r = 0.65) and limit dextrinase (r = 0. 38) activities. This correlation between DP, and enzymes could possibly explain the contribution of individual DP enzymes to malt fermentability. These hydrolytic enzymes are responsible of producing fermentable sugars in wort during the mashing process, which would result in improved fermentable values.

The contribution of wort color to the RDF model is not clearly understood, and possibly just represents a correlated factor. Color is associated with a higher level of amino acids or FAN that serve as color precursors in the Maillard reaction, which occurs during malting and mashing. However, FAN did not make a significant contribution towards the model for RDF as shown in

Table 8. However, the simple correlation between FAN and RDF for the Congress mash was 0.46 (appendix A2.). In fact both KI and FAN were found to be significantly correlated with RDF, at almost identical (*r*) values. Kolbach Index (KI) was found to make a significant and relatively large contribution to the RDF model in the current study. However, the contribution in the current study was less, than observed by others. Several other studies have found that KI has a significant correlation (r = 0.42) with the malt fermentability (Evans et al. 2003 and Eglinton et al. 2007). Henson and Duke (2008) suggested that malts with high values of KI values might result in improved DP enzyme thermosatbility during mashing process, as a well modified malts will yield higher levels of solutes (e.g. soluble proteins, sugars etc.), which can have a protective effect.

Stepwise MLR was used to develop the following algorithmic equation (9) that used malt and wort quality parameters to predict the malt fermentability.

$$\textit{Malt Fermentability or RDF (\%)} = 70.91 + 0.084A + 0.092B - 0.412C + 0.021D - 0.0032E - 2.933F + 8.53G - 3.38H \quad \text{(Eq. 8)}$$

Where A = α-amylase (U/g), B = β-amylase (U/g), C = β-amylase thermostability (% Activity), D= Diastatic Power (ºASBC), E = beta-glucan (mg/L), F = Color (°SRM), G = Kolbach Index (%) and H = glucose (g/100ml).

This model was able to explain almost 90% of the variability observed in the RDF of worts prepared by Congress Mash. The regression coefficient observed here (R^2=0.89) was similar to that observed by others, but more variables were included in the current model. Also it is important to note that this RDF model was largely explained only by four variables, which included DP, α-amylase activity, wort color and KI. Addition of the other variables made negligible improvements in the RDF model.

Data in Table 4.6. represents the partial and cumulative (R^2) contribution of malt and wort quality parameters to the model for RDF model with the HWE mash protocol. Results in (Table 4.6.) depict that the variables are contributing in sequential manner to the RDF model and are statistically significant ($P \leq 0.15$ and $P \leq 0.05$) level.

Table 4.6. Partial and model $(R)^2$ values for malt fermentability (RDF) from stepwise multiple linear regression (MLR) analysis across malt and wort quality parameters using HWE mash protocol.

Variable	RDF for all samples (n= 32)	
	Partial (R^2)*	**Model (R^2)**
Limit-Dextrinase (U/kg)	0.30†	0.30
Color (SRM)	0.20†	0.50
Extract (%)	0.05	0.55
α-Glucosidase (U/g)	0.06	0.61
DP (°ASBC)	0.15†	0.76
β-Amylase Thermostability (%)¶	0.03	0.79
Maltose (g/100ml)	0.07†	0.86
α-Amylase (U/g)	0.03†	0.89
Glucose (g/100ml)	0.01	0.90
Wort β-glucan (mg/L)	0.01	0.91
Maltotriose (g/100ml)	0.01	0.93
β-Amylase (U/g)	0.01	0.94

*All variables in the model are significant at ($P \leq 0.15$) level. No other variable met the 0.15 significance level for entry into the model

†Significantly different at ($P \leq 0.05$) level of confidence.

¶Thermostability was measured as the percentage of activity remaining after 10 min at 60ºC.

Limit dextrinase activity showed the largest contribution (30%) to the RDF model followed by wort color (20%) and DP (15%) respectively. In total, these three parameters, along

with extract, maltose concentration and α-glucosidase activity explained over 80% of the observed variation in RDF model.

A significant but negligible amount of further contributions were made from wort β-glucan, glucose and maltotriose concentration, α-amylase activity and β-amylase activity and its thermostability. The contribution of limit dextrinase activity to this RDF model was not surprising. This could be explained by the greater stability of limit dextrinase activity in the 65ºC mash protocol as opposed to a 70°C rest. Limit dextrinase hydrolyzes α-(1, 6) glucosidic linkages in the starch molecule, which provides more substrate (linear dextrin molecules) to β-amylase. This additional substrate, in turn, can be converted to fermentable sugars (especially maltose). Evans et al. (2005) also reported that elevated levels of limit dextrinase activity could help enhance fermentability. The contribution of DP and wort color could be explained in a similar manner as discussed in with RDF model using the Congress mash.

Limit dextrinase activity, DP and color were able to explain about 65% of the observed variation in RDF. Further, a significant contribution was made by extract level (5%), α-glucosidase (6%) and concentration of maltose (7%) to the RDF model. Appendix 2. shows the level of maltose sugar averaged across cultivars was significantly ($P \leq 0.05$) correlated (r = 0.69) with RDF. β-amylase, which catalyzes the hydrolysis of α-(1, 4) glucosidic linkages in the starch molecule, likely and produced more maltose at 65°C. Also, the combined activity of limit dextrinase and α-amylase could result in maltose production but at a slower rate. The contribution of α-glucosidase is not clearly understood here. As it is thermolabile (≤ 50ºC) it is rapidly inactivated during the 65°C isothermal rest. Further, the contributions from extract and maltose concentration could be interrelated.

Stepwise MLR was used to develop the following algorithmic equation (10) that used malt and wort quality parameters to predict the malt fermentability.

$$Malt\ Fermenatbility\ or\ RDF\ (\%) = 46.81 + 3.62A - 0.062B - 0.056C - 0.920D + 0.039E + 2.70F - 0.32DP - 0.005G + 16.27H + 1.29I - 2.76J \quad \text{(Eq. 9)}$$

Where A= Extract (%), B = α-amylase activity (U/g), C = β-amylase activity (U/g), D = β-amylase thermostability (%), E = Limit-dextrinase (U/Kg), F = α-glucosidase (U/g), G = beta-glucan (mg/L), H = glucose (g/100ml), I = Maltose (g/100ml) and J = Maltotriose (g/100ml).

This model was able to explain almost 94% of the variability observed in the RDF of worts prepared by HWE Mash. The regression coefficient observed here (R^2=0.93) is slightly higher than the previous RDF model (equation 9). Also, more significant variables were observed in RDF model for HWE than in the Congress mash model.

4.3. Impact of Adjuncts on Fermentability and other Malt/Wort Quality Parameters

The final objective of this research work was to determine which malt and wort quality parameters are important in determining the RDF when a portion of the malt was replaced by adjunct. In this study pregelatinized corn flakes were used as an adjunct to replace 20% and 40% of the extract normally provided by malt. It is important to note that values reported for malt and adjunct percentages are the contributions to total extract and are not based on sample weight.

Table 4.7. shows the mean values of wort quality parameters mashed using the Congress and HWE mash protocols with three different levels of malt.

Table 4.7. Wort quality values† averaged across malt samples mashed according to the Congress and hot water extract (HWE) protocols with adjunct.

Variable	Congress Mash			HWE Mash		
	Malt Concentration (%)*			Malt Concentration (%)*		
	100	80	60	100	80	60
Extract (%)	76.7[a]	78.9[b]	80.9[c]	74.9[a]	76.5[b]	77.2[c]
Wort β-glucan (mg/L)	130.7[a]	106.0[b]	76.3[c]	166.2[a]	133.7[b]	98.1[c]
Color (SRM)	2.3[a]	1.9[b]	1.6[c]	2.1[a]	1.8[b]	1.5[c]
Soluble Protein (%)	5.4[a]	4.1[b]	2.9[c]	4.9[a]	3.8[b]	2.6[c]
FAN (mg/L)	227.6[a]	184.8[b]	136.7[c]	197.2[a]	145.8[b]	111.2[c]
Glucose (g/100ml)	0.33[a]	0.38[b]	0.43[c]	0.23[a]	0.22[a]	0.21[a]
Maltose (g/100ml)	4.1[a]	4.3[a]	4.2[a]	4.2[a]	4.5[b]	4.8[c]
Maltotriose (g/100ml)	1.3[a]	1.3[a]	1.2[a]	1.3[a]	1.3[a]	1.3[a]
RDF (%)	74.6[a]	73.4[b]	72.3[c]	85.2[a]	83.2[b]	79.8[c]

†N=32, 16 cultivars with 2 replications of each

*Means within a row followed by different letters differ significantly at $P \leq 0.05$ among each mashing protocol.

Results in table 4.7. indicated that different malt to adjunct ratio had a significant ($P \leq 0.05$) impact on extract values in both mashing protocols. When extract values were averaged across all the cultivars in each malt: adjunct ratio, the Congress mash protocol was found to yield higher extract levels than the HWE mash protocol. The explanation of this finding was described in previous section of the study, and relates to greater extraction at higher temperatures. Table 4.8. also shows that among both the mashing protocols; the 60:40 (malt to adjunct) ratio had the highest extract level followed by 80:20 ratio and then the all malt mashes, respectively. This was likely due to higher starch content in 60:40 ratio mashes. During mashing, this starch would ultimately be converted into fermentable sugars by the action of malt enzymes.

Malt to adjunct ratio was also found to have a significant impact on wort β-glucan ($P \leq 0.05$). When values for β-glucan were averaged across all the cultivars in each malt:adjunct ratio, the all malt mashes showed much higher β-glucan levels than 80:20 and 60:40 malt to adjunct mashes respectively. This was because the malt beta-glucans were diluted by the addition of adjunct. The adjunct likely contained little to no beta-glucan.

Wort color was also found to differ significantly ($P \leq 0.05$) between the all three malt; adjunct ratios in both mashing protocols. Table 4.7. shows that all malt mashes had higher wort color values than 80:20 and 60:40 ratios. This was due to the fact that adjunct contributes very little color, as a very low level of soluble nitrogen makes little contribution to color development during the mashing process. In industry adjuncts are used to provide starch, and can contribute lighter color and flavor to, which is generally desirable in light beers.

Soluble protein levels were significantly affected ($P \leq 0.05$) by the addition of adjunct among both the mashing protocols. The values averaged across all cultivars within each combination of malt:adjunct mashes. Table 4.7. shows that all malt mashes yielded the highest levels of soluble protein and FAN. As mentioned earlier, the adjunct used contains low protein levels, and makes little to no contribution to the wort soluble proteins and FAN. So the explanation for the lower level of soluble protein and FAN in the adjunct mashes is dilution with addition of adjunct. Eglinton and coworkers (1998) also showed the similar FAN results, when eighteen malt samples were mashed using modified infusion mash protocol and the 30% of malt portion was replaced with adjunct (i.e. rice). They also suggested that presence of lower FAN level would result in poor fermentable values of the resultant wort, as it provides the nutrition for yeast growth.

Table 4.7. shows that glucose concentration was significantly different ($P \leq 0.05$) between the three different malt:adjunct ratios (all malt, 80:20 and 60:40) in the Congress mash. The highest percentage of glucose was observed in 60:40 mashes followed by 80:20 mashes and all malt mashes, respectively. This was probably because of prolonged activity of α-amylase activity during the 1 hr 70ºC rest in the Congress mash. Additionally, α-glucosidase would be stable at the 45ºC rest. The α-glucosidase hydrolyzes (1,4) glucosidic linkages to form glucose. This may account for the observed differences in glucose concentration.

However, in the case of the HWE mash protocol, no significant differences in glucose were observed between the different malt:adjunct ratios. On the other hand, maltose concentration was also significantly affected ($P \leq 0.05$) by malt:adjunct ratio only in HWE mash. The explanation of this finding was described in previous section of current study, and relates to stability of β-amylase. In terms of maltotriose, no significant differences were observed between the three different malt:adjunct ratios for either mash method.

All three malt: adjunct ratio mashes were found to have a significant ($P \leq 0.05$) impact on RDF values across both mashing protocols. However, the HWE mash protocol shows higher RDF values when compared to the Congress mash. The explanation for this finding involves enzyme thermostability, and was discussed in a previous section. In terms of malt to adjunct ratio, the all malt mashes had the highest RDF values. This is clearly shown in Figure 4.2. This was likely because of greater levels of amylolytic enzymes in all malt mashes, and in turn these enzymes can produce a higher level of fermentable sugar.

A box-and-whisker plot (Figure 4.2.) was used to represent the range of RDF values across sixteen cultivars for each combination of mash method and malt:adjunct ratio. In a box-and-whisker plot, the bar represents the maximum and minimum values for RDF, and thus

provides the range. The box in the graph represents greater distribution of RDF values in this region. Furthermore, the mean values of RDF are indicated by the cross mark (X) in Figure 4.2.

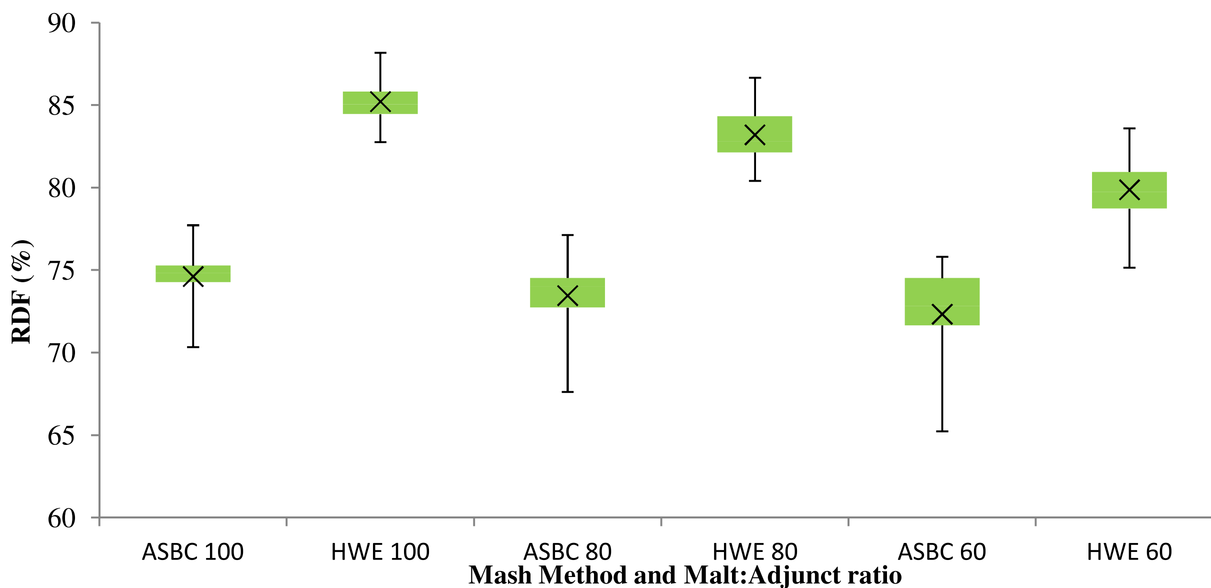

Figure 4.2. Box-and-Whisker represents the range and means of RDF values across both the mashing method (ASBC Congress and hot water extract mash) and all three malt to adjunct mashes (100:0, 80:20 and 60:40 ratio).

Results shown in Figure 4 indicate that 60:40 ratio mashes yielded the widest range of RDF values in the case of both mashing methods. The use of adjunct likely dilutes important malt quality parameters including (e.g. amylolytic enzymes, soluble protein, FAN etc). Some of these likely become limiting as the level of adjunct increases, whereas they are in excess in the all malt mashes.

As previously mentioned in the all-malt mash section of the discussion, the HWE mash protocol was better able to differentiate cultivars in terms of RDF when compared to the Congress mash. However, when the 60:40 ratio was used, the discriminative power among the cultivars increased with both mashing protocols.

Data in Table 4.8. shows the RDF values for each cultivar using the 60:40 ratio in the both mash methods.

Table 4.8. Real degree of fermentation (%)† values among sixteen. barley cultivars mashed according to the ASBC Congress and hot water extract (HWE) mash protocols with 60:40 malt:adjunct ratio.

ASBC (60:40 Ratio)		**HWE (60:40 Ratio)**	
Variety	**t-grouping***	**Variety**	**t-grouping***
Legacy	75.8[a]	Tradition	83.4[a]
ND/07/550/1	74.7[ab]	Jennifer	82.9[a]
Nord-2509	74.7[b]	Robust	81.9[b]
Tradition	74.6[b]	Lilly	81.3[bc]
Sunshine	74.1[b]	Legacy	80.8[cd]
Conlon	73.8[b]	Nord-2505	80.2[de]
Robust	73.8[b]	ND/07/604/24	80.0[de]
Stellar-ND	72.7[c]	Sunshine	79.9[ef]
Lacey	72.6[c]	ND/07/551/23	79.7[ef]
ND/07/604/29	72.6[c]	Lacey	79.6[efg]
ND/07/604/24	72.5[c]	Stellar-ND	79.2[fgh]
Jennifer	72.1[cd]	ND/07/604/29	78.9[gh]
Lilly	71.1[d]	ND/07/550/1	78.7[hi]
Haxby	68.0[e]	Conlon	77.9[ij]
ND/07/551/23	67.9[e]	Haxby	77.4[j]
Nord-2505	66.6[f]	Nord-2509	76.4[k]

†N=32, 16 cultivars with 2 replications of each

*Treatment means in each column with different letters are significantly different at $P \leq 0.05$ level of confidence

As was observed for the all-malt mashes, the rank of the cultivars in terms of RDF was not the same between the two mashing methods. This again is due to the fact that the cultivars have differences in malt quality parameters (e.g. especially enzyme levels), and in turn behave differently at different mash temperatures.

For an example, the rank of the cultivars Jennifer and Lilly rank was on higher end in the HWE method, but was more toward the middle or lower end in the Congress mash. As mentioned in the previous section of the study, this finding can likely be explained by the observation that Jennifer and Lilly had high levels of β-amylase and limit-dextrinase (appendix A1.), which might have resulted in production of more fermentable sugar at the 65ºC rest in HWE mash protocol. On the other hand, cultivar ND/07/550/1 showed higher rank in the Congress mash as compared with in the HWE mash. This was likely because the cultivar ND/07/550/1 had high level α-amylase and α-glucosidase activity which could have resulted in higher production of glucose in the wort. This can hydrolyze more fermentable sugar (e.g. glucose) in the wort.

The 80:20 ratio did show some increase discriminative power for RDF values when compared to all malt. However, since the increase for the 60:40 ratio was much more pronounced, only this data was shown.

Another interesting observation was that the rank of the cultivars observed in all malt mashes (see Table 4.4.) was not the same as observed with 60:40 ratio mashes. In terms of the Congress mash, the NDSU line ND/07/604/24 was high in relative fermentability with 100% malt, but was in the low group when 40% of the extract was replaced with adjunct. This finding can be explained based on the observation of level of enzymes during mashing; which becomes limiting with addition of adjunct. Also, the cultivar ND/07/604/24 had low level of protein values which could have resulted in low FAN levels and ultimately lower fermentable values in 40% adjunct diluted mash.

4.4. Prediction of Wort Fermentability in Worts Prepared with Adjunct

4.4.1. Congress Mash (60:40 Ratio)

In the previous section we have seen that the wort obtained using HWE mash with 60:40 ratio was lower in RDF, and showed greater discriminative power between cultivars, when compared to the all malt and 80:20 ratio mashes. Apart from the mashing temperature profiles there are other factors such as; malt/wort quality parameters also impact the malt fermentability or RDF values.

Stepwise multiple linear regression (MLR) analysis was performed to determine the contribution of the independent variables representing malt/wort quality parameters to the dependent variable (RDF). Data from Congress and HWE mash were analyzed separately.

Table 4.9. Partial and model (R^2) values for malt fermentability (RDF) from stepwise multiple regression (MLR) analysis across malt and wort quality parameters using the Congress mash protocol with a 60:40 malt to adjunct ratio.

Variable	RDF for all samples (n= 32)	
	Partial (R^2)*	**Model (R^2)**
DP (°ASBC)	0.49†	0.49
β-Amylase thermostability (%)¶	0.10†	0.59
α-Amylase (U/g)	0.06†	0.64
Glucose (g/100ml)	0.04	0.68

*All variables in the model are significant at $P \leq 0.15$ level. No other variables met the 0.15 significance level for entry into the model.

†Significantly different at $P \leq 0.05$ level of confidence.

¶Thermostability was measured as the percentage of activity remaining after 10 min at 60º.

Table 4.9. represents the partial and model results from stepwise regression of the Congress mash for the 60:40 ratio. These results indicate that DP made the largest and most significant contribution to the RDF. Diastatic power alone explained about 50 % of the observed variation, which is similar to the results observed for all malt data (Table 4.5.). This signifies that DP plays an important role in determining the RDF even with the diluted mash. As previously stated DP is the combined activity of α-amylase, β-amylase and limit dextrinase. The next important variables which contributed 10%, 6% and 4% to the RDF model are β-amylase thermostability, alpha-amylase activity and glucose concentration, respectively. In total these three variables along with DP explained about 70% variation observed for RDF. The contributions of α-amylase and glucose concentration are interrelated in this RDF model. This can be explained on the basis of α-amylase activity and its thermostability, which was already have discussed in the previous section.

A study conducted by Evans et al. (2005) using 30% rice adjunct showed that DP alone explained about 50% of the variation in ADF values. They have also observed that about 82% of variation could be explained when alpha-amylase, β-amylase and its thermostability, FAN and KI were added to the model. One of the major differences between their study and current results was the total number of significant variables in the model.

Again, stepwise MLR was used to develop the following algorithmic equation (10) that used malt and wort quality parameters to predict the RDF.

$$\textit{Malt Fermentability or RDF (\%)} = 58.46 + 0.082A - 0.58B + 0.051C + 11.22D \qquad \text{(Eq. 10)}$$

Where A = α-amylase (U/g), B = β-amylase thermostability (% Activity), C = Diastatic Power (°ASBC), D= glucose (g/100ml).

The regression coefficient observed here (R^2=0.68) was lower than that observed by (Evans et al. 2005) (R^2= 0.82) and was lower than the all malt mash

4.4.2. HWE Mash (60:40 Ratio)

Data in Table 4.10. represents the partial and cumulative (R^2) contribution of malt and wort quality parameters to RDF using the HWE mash protocol with a 60:40 ratio.

Table 4.10. Partial and model (R^2) values for malt fermentability (RDF) from stepwise multiple regression (MLR) analysis across malt and wort quality parameters using the HWE mash protocol with a 60:40 malt to adjunct ratio.

Variable	RDF for all samples (n= 32)	
	Partial (R^2)*	**Model (R^2)**
Limit Dextrinase (U/kg)	0.32†	0.32
DPº(ASBC)	0.13†	0.45
KI (%)	0.16†	0.61
Maltose (g/100ml)	0.11†	0.72
β-Amylase (U/g)	0.06†	0.78
β-Amylase Thermostabilty (%)¶	0.04†	0.82
Glucose (g/100ml)	0.04†	0.87

*All variables in the model are significant at $P \leq 0.15$ level. No other variable met the 0.15 significance level for entry into the model

†Significantly different at P ≤ 0.05 level of confidence.

¶Thermostability was measured as the percentage of activity remaining after 10 min at 60º.

Limit dextrinase activity showed the largest statistically significant contribution (32%) to the RDF model followed by KI (16%) and DP (13%). In total, these three parameters, along with maltose, glucose concentration and β-Amylase activity and its thermostability explained 87% of the observed variation in RDF model. The variables observed in this RDF model are similar to those observed for the all malt HWE mash model, with the exception of KI. However, the

contribution of KI could be explained on the basis of malt modification. As described earlier well modified malt, as evidenced by higher KI values, provides higher availability of amylolytic enzymes to the starch matrix. The only major difference in this model, and that for the all malt HWE mash, is it includes less variables. The explanation for contribution of variables like limit-dextrinase, DP, maltose β-Amylase and its thermostability to RDF is the same as discussed for the all malt HWE mash.

Stepwise MLR was used to develop the following algorithmic equation (11) that used malt and wort quality parameters to predict the RDF model for 60:40 ratio using HWE mash protocol.

$$\textit{Malt Fermentability or RDF (\%)} = 30.76 + 0.65A - 0.065B - 0.13C - 0.67D + 4.18E + 0.41F - 14.78G \qquad \text{(Eq. 11)}$$

Where A = Kolbach Index (%), B = Maltose (g/100ml), C = β-amylase (U/g), D= β-amylase thermostability (% Activity), E = DP (°ASBC), F = Limit Dextrinase (U/kg), G = Glucose (g/100ml).

The regression coefficient observed here (R^2=0.87) was lower than that for the Congress model using a 60:40 ratio, but more variables were found to make a significant contribution to the current model. However, it is important to note that most of the variation in the current model was explained only by four variables; DP, β-amylase activity, KI and maltose concentration.

4.4.3. Congress Mash (80:20 Ratio)

Data shown in Table 4.11. represents the partial and cumulative (R^2) contribution of malt and wort quality parameters to the model the RDF model using Congress mash protocol for 80:20 ratio. Diastatic power (DP) made the largest (54%) and most significant contribution to the RDF. Other significant variables, which contributed to the model, included α-amylase (17%),

FAN (10%), color (4%) and KI (4%) respectively. In total about 90% of variation in RDF was explained by these five variables.

Table 4.11. Partial and model (R^2) values for malt fermentability (RDF) from stepwise multiple regression (MLR) analysis across malt and wort quality parameters using the Congress mash protocol with 80:20 malt to adjunct ratio.

Variable	RDF for all samples (n= 32)	
	Partial (R^2)*	**Model (R^2)**
DP (°ASBC)	0.54†	0.54
α-Amylase (U/g)	0.17†	0.71
FAN (mg/L)	0.10†	0.81
Color (SRM)	0.04†	0.85
KI (%)	0.04†	0.89
α-Glucosidase (U/g)	0.01	0.90
β-Amylase thermostability (%)¶	0.02†	0.92
Glucose (g/100ml)	0.02†	0.94
Extract (%)	0.01	0.95

*All variables in the model are significant at $P \leq 0.15$ level. No other variable met the 0.15 significance level for entry into the model

†Significantly different at $P \leq 0.05$ level of confidence.

¶Thermostability was measured as the percentage of activity remaining after 10 min at 60ºC.

With the exception of FAN, these five variables also showed the largest contribution to the all malt model. The contribution of variable FAN to this model seems reasonable as some cultivars with lower of FAN may not have provided enough amino nitrogen to support optimal

yeast during the fermentation of this dilute mash. Small contributions were made by α-Glucosidase, β-Amylase thermostability, glucose and extract.

Stepwise MLR was used to develop the following algorithmic equation (12) that used malt and wort quality parameters to predict the malt RDF for 80:20 ratio using the Congress mash.

$$Malt\ Fermentability\ or\ RDF\ (\%) = 57.46 + 0.19A + 0.09B - 0.77C + 1.77D + 0.03E - 0.007F - 3.99G + 0.10H - 8.92I \quad \text{(Eq. 12)}$$

Where A= Extract (%), B = α-amylase activity (U/g), C = β-Amylase thermostability (%), D = α-glucosidase (U/g) E= Diastatic Power (°ABSC), F = FAN (mg/L), G = Color (SRM) H = Kolbach Index (%), I = Glucose (g/100ml)

The regression coefficient observed here (R^2= 0.95) was similar to that observed for the all malt mash model (R^2= 0.90). The variables DP, color, α-amylase explained most of the variation in both models.

4.4.4. HWE mash Protocol (80:20 Ratio)

Results shown in Table 4.12. represents the partial and cumulative (R^2) contribution of malt and wort quality parameters to the model the RDF model using HWE protocol using 80:20 ratio. Table 4.12. depicts that the variables are contributing in sequential manner to the RDF model and are statistically significant a ($P \leq 0.15$ and $P \leq 0.05$).

As previously observed with the HWE mash protocol, limit dextrinase activity showed the largest statistically significant contribution (32%) to the current RDF model, followed by DP (15%), wort color (12%) and α-amyalse activity (7%) respectively. The explanation for contribution of the variables in this model would be similar to that discussed for the HWE all malt mash. Overall about 70% of variation was explained by these four variables. Beta-amylase

thermostability, maltose concentration, FAN and KI each made a significant but relatively low contribution (5%) to the current RDF model.

Table 4.12. Partial and model (R^2) values for malt fermentability (RDF) from stepwise multiple regression (MLR) analysis across malt and wort quality parameters using the HWE mash protocol with a 80:20 malt to adjunct ratio.

Variable	RDF for all samples (n= 32)	
	Partial (R^2)*	**Model(R^2)**
Limit-Dextrinase (U/kg)	0.32†	0.32
Color (ºSRM)	0.12†	0.44
Maltose (g/100ml)	0.06†	0.51
β-amylase Thermostability (%)¶	0.05†	0.56
DP (°ASBC)	0.15†	0.71
FAN (g/100ml)	0.05	0.76
α-Amylase (U/g)	0.07	0.83
KI (%)	0.05	0.88
Glucose (g/100ml)	0.01	0.89
β-amylase (U/g)	0.01	0.91

*All variables in the model are significant at $P \leq 0.15$ level. No other variable met the 0.15 significance level for entry into the model

†Significantly different at $P \leq 0.05$ level of confidence.

¶Thermostability was measured as the percentage of activity remaining after 10 min at 60ºC.

In total, all these variables along with glucose concentration and β-amylase explained about 90% of the observed variation.

Stepwise MLR was used to develop the following algorithmic equation (14) that used malt and wort quality parameters to predict the malt RDF for 80:20 ratio using the HWE mash.

$$\textit{Malt fermentability or RDF} = 84.58 + 0.53A + 0.069B + 3.705C - 0.013D - 0.023E - 0.056F - 0.008G + 2.454H - 0.178I - 34.44J \quad \text{(Eq.14)}$$

Where A= Wort Color (ºSRM), B = α-amylase activity (U/g), C = β-amylase (U/g), D = Limit dextrinase (U/kg), E= Diastatic Power (ºABSC), F = Maltose (g/100ml), G = FAN (mg/L), H = β-amylase thermostability I = KI (%), J = Glucose (g/100ml)

The regression coefficient observed here (R^2=0.91) that is slightly higher than the all malt RDF model using HWE mash protocol. Also, the number of variables was same observed in current RDF model and previous model (all malt HWE mash).

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

The overall objective of this study was to investigate the impact of laboratory mashing procedures on the fermentability of wort obtained from barley cultivars showing a wide range in quality parameters. The experiments conducted were divided into two primary sections. The variation in fermentability using two different laboratory mash methods (Congress and HWE mash) and 100% malt (all malt) was evaluated in the first section. In the second the relationship between malt/wort parameters and malt fermentability were determined under conditions where portions of the malt were replaced with adjunct (pregelatinzed corn flakes). In both cases differences in fermentability that could be attributed to the procedures were determined. In addition stepwise multiple linear regression analysis was performed to determine which quality factors were able to best explain the observed variation in fermentability.

Previous work in our laboratory has shown that worts obtained from ASBC congress mash method displayed a limited variation in fermentability. Although this method is in near universal use in North America for the evolution of malt quality, it might not be ideally suited for determination of fermentability. In the comparison of the two mash methods, the HWE mash protocol showed a wider range of fermentability values than did the Congress mash. If laboratory mashing were to be used for the determination of varietal differences in the fermentability, the HWE method clearly had better discriminative power. This was true in the case for both all malt and adjunct mashes. An interesting observation was the rank of the cultivars was not the same between the two mashing methods. The change in rank between methods was likely due to the difference in malt quality parameters (especially enzyme levels), which resulted in differential behavior in between the two different mash temperature profiles.

Diastatic Power was able to explain the largest portion of the variation in fermentability when using the Congress mash with all malt, and also with adjunct. In the case of HWE extract protocol the activity of limit dextrinase was able to explain a substantial portion of variation in fermentability. Factors such as alpha-glucosidase, color, FAN, KI, soluble proteins and Beta-amylase thermostability made significant but lesser contribution to RDF.

5.1. Recommendations

Quality testing in barley breeding is an expensive and often limiting in terms of sample throughput. While the laboratory mash procedures were evaluated as part of the current study, the intent was not to advocate that breeding programs switch mash protocol or adopt fermentation of laboratory worts as a routine test. This would be far too time consuming, and neither of mash methods are an ideal reflection of commercial brewing practice. However, the results of this study suggest that the definition of malt fermentability is not fixed, but can vary slightly with the intended use of the malt (e.g all malt beer as compared to adjunct beer), and the brewer's process.

Barley breeder's wishing to improve fermentability of their lines should continue to focus on parameters that are already measured, such as DP, KI and α-amylase, as these are all related to fermentability. Addition of tests for limit-dextrinase, beta-amylase and beta-amylase thermostability could improve the selection of lines for higher fermentability. Several of these tests can be adopted to a micro-plate format (Evans et al. 2008), which could speed throughput. Increasing limit dextrinase appears to be very important in the improvement of fermentability, especially when the malt is to be used in more traditional processes, such as infusion mashing with all malt. Both DP and α-amylase seem to be more important with adjunct mashes, and beta-amylase themostability becomes important as mash temperatures rises.

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APPENDIX

Table A1. Malt quality data* (enzymes) of all sixteen barley cultivars utilized in the study to determine the malt fermentability.

Cultivars	DP (°ASBC)	α- amylase (DU)	β-amylse(U/g)	β-amylse thermosatbility¶ (%)	Limit-dextrinase (U/Kg)	α-glucosidase U/g	Protein (%)
Tradition	163.0	52.2	20.4	2.6	115.2	1.7	12.4
Lacey	150.0	54.3	19.5	3.0	147.2	1.9	12.7
Stellar ND	179.0	59.0	21.9	3.1	92.8	1.3	12.0
Jennifer	127.0	66.5	16.6	3.9	156.8	1.6	12.1
Lilly	91.0	74.1	11.0	2.7	150.4	0.9	13.3
Sunshine	147.0	58.6	15.3	2.8	118.4	0.7	12.3
ND07/550/01	108.0	76.3	14.8	2.4	147.2	2.4	12.2
ND07/551/23	67.0	54.7	9.7	5.5	140.8	1.3	12.1
ND07/604/24	135.0	73.7	18.0	2.9	150.4	1.3	11.7
ND07/604/29	153.0	62.4	21.3	4.7	121.6	1.4	12.8
NORD2505	116.0	67.6	18.0	2.5	134.4	1.6	12.4
NORD2509	98.0	61.5	16.3	3.8	147.2	1.1	12.9
Robust	184.0	56.3	28.8	2.1	163.2	1.8	12.7
Legacy	180.0	81.3	24.7	2.7	150.8	1.7	12.5
Haxby	75.0	55.8	14.2	3.0	89.6	0.6	11.5
Conlon	134.0	75.9	9.3	3.2	86.4	1.9	11.0

* N = 32, 16 cultivars with 2 replications of each.

¶Thermostability is measured as the percentage of activity remaining after 10 min at 60ºC.

Table A2. Correlation coefficient (*r*) for relationship between malt fermentability (RDF) and malt and wort quality analyses in full malt using ASBC and HWE mash protocol

Variable	ASBC Mash	HWE Mash
	RDF (*r*)	**RDF (*r*)**
Friability (%)	Ns	0.37*
α-Amylase (U/g)	0.42*	ns
β-Amylase (U/g)	0.49*	62*
β-Amylase remaining activity (%)[¶]	Ns	ns
Limit-Dextrinase (U/kg)	Ns	0.47*
α-Glucosidase (U/g)	0.30*	ns
DP (°ASBC)	0.72*	0.17*
Extract (%)	0.46*	ns
Wort β-glucan (mg/L)	-0.24*	-0.41*
Color (SRM)	Ns	ns
Soluble Protein	0.43*	ns
KI (%)	0.47*	0.69*
FAN (mg/L)	0.46*	ns
Glucose (g/100ml)	Ns	ns
Maltose (g/100ml)	0.14*	0.42*
Maltotriose (g/100ml)	Ns	ns

*Significant at ($P \leq 0.05$) level of confidence and ns = Not Significant

¶Thermostability is measured as the percentage of activity remaining after 10 min at 60ºC.

Table A3. Correlation coefficient (*r*) value between the DP and the malt enzymes β-amylase, α-amylase and limit-dextrinase.

Variable	DP (r)*
β-Amylase	80.0
α-amylase	65.3
Limit-Dextrinase	38.6

*Significant at ($P \leq 0.05$) level of confidence.