Fermentability of Canadian Two-Row Barley Malt: Wort Turbidity, Density, and Sugar Content as Measures of Fermentation Potential

by

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DEDICATION PAGE

To my Mom, Dad, and Christa, who are in perpetual wonderment that I might pursue a degree in beer!

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ABSTRACT

The primary goal of this study was to investigate and compare the fermentation performance of malt produced from eleven Canadian two-row barley varieties grown during the 2007 and 2008 crop seasons. Common malting varieties tested included Harrington, AC Metcalfe, CDC Copeland, CDC Kendall, and feed varieties CDC Dolly, CDC Bold, CDC Helgason and McLeod. As well, three experimental varieties, TR251; TR306; and BM9752D-17, were included in this study due to their varied display of enzymatic activity; of chief interest was the β-amylase thermal stability. Fermentations were carried out using the standard miniaturized fermentation assay and SMA yeast. Apparent Extract (AE), absorbance, fermentable carbohydrates, and ethanol were measured throughout fermentation. Attenuation, carbohydrate and ethanol data were modeled using the logistic equation, and absorbance data was modeled using the newly developed Tilted Gaussian equation. Results indicate that all but the feed varieties fermented well, achieving low final attenuation, and exhibiting similar fermentation characteristics. Despite only minor performance differences among the top fermenters, it was found that between crop seasons both AC Metcalfe and CDC Copeland fermented as well or better than Harrington, as measured by their respective Apparent Degree of Fermentation (ADF). Harrington displayed substantial performance variation between seasons, while test variety BM9752D-17 fermented the most consistently between years, displaying enhanced fermentation to that of Harrington in 2008. Despite high β-amylase thermostability, BM9752D-17 did not display enhanced fermentation performance to that of CDC Copeland or AC Metcalfe.

LIST OF ABBREVIATIONS AND SYMBOLS USED

A	Amplitude (of the "Tilted Gaussian" function)
A.A.L.	Apparent Attenuation Limit
Abs	Absorbance
AC	Agriculture Canada
ADF	Apparent Degree of Fermentation
AE	Apparent Extract
ANOVA	Analysis of Variance
ASBC	American Society of Brewing Chemists
ASE	Asymptotic Standard Error
AVC	Average Cell Count
AU	Absorbance Units
B	A Function of the Slope at the Inflection Point of the Logistic Model
CDC	Crop Development Centre
CMBTC	Canadian Malting Barley Technical Centre
CGC	Canadian Grain Commission
DP	Diastatic Power
D.U.	Dextrinizing Units
EBC	European Brewery Convention
FAN	Free Amino Nitrogen
HPLC	High Performance Liquid Chromatography
KI	Kolbach Index
L.D.	Limit Dextrinase
M	Time at Inflection (of the Logistic function)
MBAA	Master Brewers Association of the Americas
μ	Midpoint (of the "Tilted Gaussian" function)
OE	Original Extract
P_e	Asymptotic Plato Value at Equilibrium (of the Logistic function)
P_i	Initial Asymptotic Plato Value (of the Logistic function)
$P_{(t)}$	Plato at Time=t

PYF	Premature Yeast Flocculation
R	Slope Term (of the "Tilted Gaussian" function)
$RA_{(t)}$	Relative Absorbance at Time=t
RDF	Real Degree of Fermentation
RE	Real Extract
RI	Refractive Index
RPM	Revolutions Per Minute
RSS	Residual Sum of Squares
r^2	Coefficient of Determination
SD	Standard Deviation
SG	Specific Gravity
SE	Standard Error
σ	Width Factor (of the "Tilted Gaussian" function)
YIS	Yeast in Suspension
VLB	Versuchs-und Lehranstalt für Brauerei
w/v	Weight/Volume
w/w	Weight/Weight
^oC	Degrees Celsius
^oL	Degrees Lintner
^oP	Degrees Plato

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1. Introduction

Canadian malting barley varieties account for a large majority of the total barley grown in western Canada, with a smaller but substantial proportion grown for use specifically as animal feed. Over the past twenty five years, Canada's portfolio of malting barley varieties has increased, offering greater yields, resistance to disease, and crop viability then previous varieties (28). Indeed, since the development and registration of the globally successful variety, Harrington, in the early 1980's, Canadian two- and six-row malting barley has shown increasing success on a global market. The terms "two-row" and "six-row" derive their names from the physical growth characteristics of the plant, where kernels develop in rows of either two or six along the axis of the plant. Malting barley varieties now account for nearly 60% of the barley-seeded acreage in Western Canada (Aaron McLeod, CGC, Winnipeg, MN, 2013 personal communication), and have been reported to be as high as nearly 80% in all of Canada (28).

However, after thirty years of successful cultivation and production, Harrington barley shows declining agronomic quality and disease resistance relative to varieties in other countries, which is typical of an aging genotype (71). This has led to an more urgent need to develop new barley varieties which offer increased malting and brewing quality, greater disease resistance and higher yields compared to Harrington, the previous Canadian 'gold-standard' (60). Newer varieties of Canadian malting barley include CDC Stratus, CDC Kendall, AC Metcalfe, Merit, and CDC Copeland (60). However, it is no surprise that with the emergence of new varieties comes also a dearth of adequate research to substantiate their putative high malting and brewing potential.

Preliminary reports and anecdotal evidence has suggested that Canadian barley contains high levels of modifying and starch degrading enzymes which ensures satisfactory brewing quality (60, 71). With the exception of Harrington there is little, if any, published literature to

confirm or refute the brewing efficiency or fermentability of novel Canadian malting barley varieties. Such inadequacies render Canadian varieties susceptible to the reservations of brewers and maltsters not willing to convert for fear of variable malting or brewing performance with untested barley. Therefore, it is the purpose of this investigation to assess the fermentability of prevalent Canadian two-row barley varieties in an effort to quantify their fermentation potential and brewing performance based on standard methods of analyses used in the brewing industry.

Fermentability is arguably the most important measure of barley quality to the brewer. It describes the proportion of fermentable wort carbohydrates, extracted from the barley grain after malting which are readily converted into carbon dioxide (CO_2) and ethanol by the fermenting yeast. The stoichiometry of the basic alcoholic fermentation is described in equation 1 (55):

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

One index of fermentability is the ratio of total fermentable sugars to total solids content of the wort (16). Quantifying such a trait allows the brewer to assess the extent to which fermentation will proceed under ideal conditions, which can help optimize the choice of an appropriate barley malt to be used in order to produce specific styles of beer. However, there are myriad underlying factors that can affect the fermentability of malt, and although some are still poorly understood, they are of considerable interest to breeders, malt researchers and brewing scientists and constitute a growing area of barley research. Indeed, one of the primary limiting factors of malt fermentability is the level, amount, and quality of starch degrading enzymes provided by the grain, substances which are necessary for the breakdown of barley starch and subsequent production of fermentable carbohydrates. However, it has also been noted that fermentation

performance can be influenced by the amino acid complement of the barley (32) as these provide the fermenting yeast with necessary nutrients for growth and metabolism (see section 2.2.2). Additionally, many varieties of yeast are utilized which can greatly influence the character of the final beverage, given different metabolic rates and biochemical strategies each strain employs to achieve fermentation. Though highly complex when scrutinized from its individual components, the concept of fermentability is fundamentally quite simple; it is a quantification of quality through the measurement of fermented extract produced by the malt. It is used as an indication of the extent to which a particular barley malt extract is expected to ferment.

The extent of fermentation is measured as the Apparent Attenuation Limit (AAL) or Apparent Degree of Fermentation (ADF). Such terms can bring about confusion given the similar nature of the phenomenon in which they describe. For the purposes of this report, AAL describes the maximum achievable attenuation possible for a given extract composition, assuming idealized conditions under which all fermentable material has been utilized. Meanwhile, ADF describes the maximum attenuation achieved in a specific fermentation regardless of extract potential. Attenuation describes the decline in specific gravity or density of the wort during fermentation as a result of sugar consumption and ethanol production by the yeast (36, 69). This value is often determined using a hydrometer, which measures specific gravity or density of a liquid in relation to pure water. Since attenuation levels change with carbohydrate consumption (which lowers specific gravity) and production of ethanol (which also lowers specific gravity), the hydrometer measurement results in a lower reading than would be observed from measurement of the solids in the absence of ethanol. This uncorrected value is termed the AE or “Apparent Extract” (44). As noted by McCabe (69) and Gales (44), the true (percent remaining) solids determined in the absence of alcohol is termed the Real Extract (RE).

The AAL is calculated as a percentage based on the difference between starting or Original Extract (OE) content of the wort and final, uncorrected, AE of the completely fermented beer (equation 2). The AAL is, therefore, defined as the maximum Apparent Degree of Attenuation that can be achieved during fermentation with a given extract composition, assuming that all fermentable material has been utilized (22). Conversely, the ADF, although calculated exactly the same way (equation 2), describes the extent to which a specific fermentation has attenuated regardless of whether all fermentable material has been utilized. Often the AAL for a given wort is determined in a laboratory setting by the excessive addition of yeast and subsequent gravity measure after two days of fermentation. The wort is said to have reached its final Apparent Extract when no further decline in gravity can be observed with the addition of fresh yeast or extension of the fermentation schedule (22). Given that AAL is rarely achieved in the brewery, the ADF can thus be measured and compared to lab results in order to gauge the amount of fermentable extract remaining in the beer (22), giving the brewer a means to assess fermentation performance and to judge the extent of fermentation:

$$ADF = \frac{OE-AE}{OE} * 100 \quad (2)$$

In order to directly measure the real degree of fermentation (RDF), all ethanol must first be removed by distillation from the fermented wort and replaced with an equal volume of water. Prior to 1979 the RDF was expressed as a percentage describing the difference between the extract of the original wort and the real extract of the final fermented sample divided by the original extract (69), as described in equation 3a:

$$RDF = \frac{OE-RE}{OE} * 100 \quad (3a)$$

and was later corrected by Cutaia and Munroe (26), shown in equation 3b:

$$\%RDF = \left[\frac{100*(OE-RE)}{OE}\right] * \left[\frac{1}{(1-0.005161*RE)}\right] \quad (3b)$$

More recently, Cutaia, Reid, and Speers (27) published a number of updated equations to more readily, and accurately interconvert between common brewing measures, including SG, AE, RE, RDF, ADF, and alcohol content. Of particular utility in this study was the relationship described in equation 3c (27), which, upon rearranging, allowed for consistent (i.e., resulting in low variation per data set) conversion of AE to RE using measured values of alcohol content, thus enabling subsequent non-linear modeling of RE throughout fermentation. Measured values of alcohol content were converted from a volumetric basis ($A_{v/v}$) to a weight/weight basis ($A_{w/w}$) using equation 14 (not shown) from Cutaia, Reid, and Speers (27):

$$\frac{A_{w/w}}{(RE-AE)} = b_{c0} + b_{c1} * OE \quad (3c)$$

where b_{c0} and b_{c1} are parameter estimates for the intercept and slope respectively of the linear regression conducted by Cutaia, Reid, and Speers (27).

However, the extent of carbohydrate fermentation will not only depend on the composition of the wort, but also the specific strain and activity of the fermenting yeast (21, 70). As noted by McCabe (70), brewer's yeast is often classified according to a defined

complement of specific quality traits desired by the brewer, as opposed to the usual biological taxonomical method. According to McCabe (70) brewer's yeast must be:

i. "Genetically stable over repeated growth cycles"
ii. "Capable of fermenting wort in an acceptable period of time"
iii. "Capable of producing a fermented medium devoid of undesirable amounts of metabolites responsible for… off flavors"
iv. "Easily removed from the medium at the end of fermentation"
v. " Sufficiently viable after cropping that it could be re-pitched into wort with minimal autolysis and maximum confidence"

It has been found that such quality characteristics are present within species/strains of the genus *Saccharomyces* (70). Within this group of unique organisms, significant differences can be observed in the type of beer each variety will produce. Of the 14 species of *Saccharomyces* yeast, only *Saccharomyces cerevisiae* contributes to the three major industrial yeast-based applications including beverage production (both beer and wine), baking, and the production of biomass (17). However, specific to the process of brewing beer, the term *S. cerevisiae* has been reserved for the use as a top-fermenting yeast to produce ales (70), while *S. pastorianus* (formerly *S. carlsbergensis*) are specifically used as a bottom-fermenting yeast in the production of lager (17, 70). This is, however, a traditional classification system which has undergone a certain degree of modification (70), resulting in some authors of published literature retaining older taxonomical classifications while others do not. It must be noted that recent reclassification of *Saccharomyces* spp. by taxonomists has resulted in the categorization of both ale and lager yeasts as *S. cerevisiae* (70). In an effort to alleviate a small degree of

confusion, hence forth in this paper any reference to ale yeasts will refer specifically to *S. cerevisiae* while lager yeasts will refer to *S. pastorianus*, however, also acknowledging that a newer classification system is currently in place.

2. Literature Review

2.1. Yeast Classification (Lager versus Ale yeast)

Though ale and lager yeast strains are closely related genetically (97), each possesses a similar but unique complement of genetic material causing distinct differences in both fermentation performance and final product composition. Most notable is the ability of lager yeasts to utilize the sugar melibiose (76) whereas ale yeast cannot. Additionally, it has been found that lager yeasts have a greater affinity for galactose, displaying simultaneous utilization of both maltose and galactose, where ale yeasts typically ferment maltose first (25). It has also been observed that lager yeasts tend to utilize the sugar maltotriose, an important fermentable sugar in wort extract (discussed later), more readily than ale yeasts (93). Further, the synthesis of byproducts such as various sulphites, which seem to be produced more readily in *S. pastorianus* then *S. cerevisiae* (25), can impart unique flavours to the finished beer. Indeed it can be argued that due to such varietal differences, a chief factor in determining the unique characteristics of a particular beer style is the strain of yeast used. As Boulton and Quain (16) point out "almost without exception the strain or mixture of strains of yeast used to produce a particular type of beer is the single essential component, which cannot be substituted".

Traditionally, ale yeasts have been classified as those which are top-fermenting while lager yeasts are those which are bottom-fermenting (20, 70). In general, this is due to the manner in which the yeast flocculate at the end of fermentation. Ale yeasts flocculate and rise to the top of the fermentation vessel while lager yeasts flocculate and settle to the bottom (17, 20, 70). This classification method, however, is no longer adequate for today's industrial brewing processes because of the proliferate use of cylindroconical fermenting vessels which can cause ale yeasts to flocculate to the bottom (17). As well, it has been generally regarded that

ale yeasts will ferment optimally at approximately 20 ^{o}C while lager yeasts typically ferment best at a range of 8-15 ^{o}C (17). Once again this rule does not always hold true as there has been observed variance in the optimum fermentation temperatures between ale and lager yeast (47). However, it is generally agreed that ale strains have a higher maximum growth temperature than do lager strains (98, 101). Taxonomically, a better distinction of lager versus ale *Saccharomyces* yeast strains was described by Vaughan-Martini and Martini (99), stating that *S. pastorianus* cannot grow at temperatures exceeding 34^{o}C while *S. cerevisiae* can grow at temperatures as high as 37 ^{o}C without difficulty. This, combined with the ability of lager yeast strains to ferment the sugar melibiose, provides a clearer distinction between ale and lager yeast strains than had previously been employed.

2.2. Physiology of Yeast

Yeast are fundamentally a complex biological organism, and require a range of necessary environmental factors in order to thrive and ferment the liquid medium known as "sweet wort" (see section 2.4.) into an alcoholic beverage with defined character. Yeast research and biology is a well-established field of study, with substantial historical significance, and has been the cornerstone of many scientific discoveries and elucidations in a range of disciplines. This is not surprising given the extensive use of yeasts by humans for thousands of years (102, 66, 82, 12). Therefore, it is beyond the scope of this report to discuss the biology of yeast in its entirety, however key points of consideration relevant to the brewer are presented.

2.2.1. Oxygen Requirements

Despite predominant use as an anaerobic organism, brewing yeast displays an initial aerobic state, requiring a certain amount of oxygen early in fermentation in order to facilitate

reproduction and increase the population to adequate numbers. Prior to budding, yeast must synthesize additional plasma membrane which requires assimilation or production of sterols and unsaturated fatty acids. These lipid-related compounds are a key component in cellular membranes to allow fluidity and maintain cell dynamics such as permeability, enzyme regulation, ethanol tolerance, and secretion/uptake of flavour compounds (70, 104). Formation of the necessary sterols and other essential lipids requires several biosynthetic steps, utilizing molecular oxygen throughout in order to acquire the desired components (104). Though some of these components can be found in the wort as individual constituents, they are often not available in adequate supply (70). However, brewers tend to be weary when adding oxygen to their brews, worried of possible flavour defects, and premature staling. Therefore, a balance must be achieved to allow proper aeration of the initial wort to promote healthy growth and achieve optimal fermentation rates, while limiting its availability for the majority of fermentation to ensure yeast metabolism is not directed towards unnecessary population increases rather than ethanol production (70).

2.2.2. FAN

Imperative to the efficient utilization of malt extract and improved overall fermentation performance is an adequate supply of nitrogen for yeast growth and maintenance of metabolism. The predominant source of nitrogen is provided by the wort in the form of amino acids, and ammonium ions. Additionally, and to a limited extent, di- and tri-peptide by-products of protein degradation as a result of malting can also be utilized by typical brewing yeast strains (77). Together, these constitute the major sources of nitrogenous compounds utilizable by the yeast, and are colloquially and collectively referred to as free amino nitrogen (FAN) (59, 77). Further sources of nitrogen do exist in wort, however, typically only in very small amounts, and generally participate in biosynthetic pathways rather than growth (77). All-malt wort generally

contains all the necessary nitrogen requirements of brewer's yeast, however, it should be noted that specific nutrient requirements can vary between *Saccharomyces* spp. as well as between members of the same species (104). Upon introduction to the brewing medium, yeast can than employ a number of nitrogen utilization events, including direct uptake of wort amino acids, or assimilation of the required amino acid from wort constituents (104).

Similar to the utilization of fermentable carbohydrates (see section 2.6), wort assimilable nitrogen is taken up by the yeast in an ordered and sequential manner (77, 104). Previous research has classified wort amino acids into four general groups (A-D), based on the ordered preference in which (primarily ale) yeast will utilize each throughout fermentation, as shown in Table 2-1 (58, 59, 77, 104). Explicit utilization patterns for other strains, including lager strains, have not yet been fully ascertained (58, 59). It has been observed that Group A are the preferred amino acids, being utilized within the first 24 hours of fermentation, while Group B are slowly used throughout (104). Group C (which include ammonia) show a lag in utilization, being absorbed only after Group A amino acids have been exhausted. Group D are generally not utilized to any extent by brewer's yeast (77, 104). The utilization of amino acid sources through uptake via yeast cell permeases can have a profound impact upon final beer flavour and quality (77), as the chemical processes necessary for cellular uptake can cause changes to aldehyde, fusel alcohol, ester, and diketone profiles (104).

Table 2-1. Amino acid classification for brewer's yeast[1]. Groups represent order of preference for yeast utilization.

Group A (Preferred utilization)	Group B (Intermediate utilization)	Group C (Slow utilization)	Group D (Little/no utilization)
Glutamic acid	Valine	Glycine	Proline
Aspartic acid	Methionine	Phenylalanine	
Asparagine	Leucine	Tyrosine	
Glutamine	Isoleucine	Tryptophan	
Serine	Histidine	Alanine	
Threonine		Ammonia	
Lysine			
Arginine			

[1]Source: Lekkas *et al.* (59).

2.2.3. Flocculation

Flocculation is not a trivial phenomenon to describe or define and has been a central field of study among many brewing researchers, as it poses serious implication on brewing performance. A concise summary of relevant research would span some 60 years of published literature, and thus is beyond the scope of this paper. Therefore, only key points of consideration and current theory are discussed. For a detailed report, see Speers (88). Flocculation describes agglutination, or aggregation of a substance. In the case of brewing science, this often refers to the propensity of yeast cells to form non-sexual, reversible aggregates under certain environmental and physiologic conditions, and thereby sediment out of suspension (87, 88). Typically these conditions present themselves near the end of fermentation following the consumption of, preferably all, available fermentable carbohydrates and their resultant conversion to ethanol (91). Ideally, it is only when these conditions occur that yeast cells flocculate and fall out of suspension, aiding beer clarification, and readily allowing cropping of yeast sediment. However, atypical brewing yeast strains exhibiting aberrant flocculation activity pose serious concern to the brewer, as early precipitation of yeast cells from the medium prior to completion of fermentation results in under attenuated wort, ultimately costing the brewer in lost or poor quality product. As such, understanding yeast

flocculation has been a primary focus of brewing researchers for many years in order to elucidate the mechanisms involved. Efforts to define key parameters and distinguish those which are likely to cause the problematic early flocculent behavior, commonly known as premature yeast flocculation (PYF) have been central to this focus. Identification of such factors would allow the brewer a greater measure of control prior to fermentation than currently is possible, due to the lack of identified variables (in both yeast and malt physiology) that may confidently suggest inadequate yeast performance prior to pitching.

Currently, the degree of flocculation experienced by the yeast can have a major impact on the fermentability of the wort. Early flocculating yeast strains will leave the final product lacking necessary ethanol levels, reduced uptake/production of flavour compounds causing sensory defects, and a possible risk of microbiological spoilage due to a lack of competitive inhibition (88, 91). Similarly, weakly flocculating yeast strains can also impart flavour defects with the added problem of remaining in suspension within the final product causing subsequent filtration issues as a result (88, 91). This has led researchers to determine mechanisms involved in yeast flocculation, and over the course of nearly 60 years have identified a number of key variables highly implicated in its induction.

Environmental changes such as pH, carbohydrate, and alcohol content (88) are all believed to impact cell surface biochemistry in manners that either promote or interfere with cell to cell interaction. Whether flocculation is genetically induced due to environmental triggers, or results simply as a consequence of reduced inhibiting substances, changes to the surface of the cell are likely to occur, and have thus been a focus of flocculation research. Hydrophobic interactions between cells have been implicated in stabilizing floc formation, while protein synthesis has also been described as having a key impact on flocculation (88).

Calcium (Ca^{++}) ions have also been reported to promote the flocculation of yeast, and that chelating compounds such as EDTA can disrupt this influence (30, 53).

Unfortunately, only limited information exists that truly describes the composition of the yeast cell wall and its implications on flocculent behaviour, hence an adequate body of evidence fully explaining this phenomenon has yet to be realized (88). In general, the current and most plausibly accepted theory of yeast flocculation to date, set out by Miki et al. (74), assumes the existence of lectin-like proteins (coined "zymolectins" as they do not fully fit the description of lectins) which bind to neighbouring mannose moieties present on cell wall proteins of nearby cells (88). Although it is likely that more than one mechanism is at play to induce or prevent flocculation such as hydrophobic interactions, hydrogen bonding, and surface charge (electrostatic) interactions, along with the requirement for media shear forces to allow cell to cell interaction (88), the zymolectin theory does present a somewhat comprehensive understanding to account for the majority of cell to cell adhesion, taking into consideration the dependence of calcium to maintain optimal zymolectin binding profiles. The theory also provided a plausible explanation for observed carbohydrate inhibition. It is generally believed that early in the fermentation excess sugar occupies the zymolectin binding sites, rendering them unavailable to bind to neighbouring mannoproteins (53).

2.3. Malting, an Overview

Fermentability (as discussed above) is predicated on yeast metabolism of wort nutrients. Often of chief interest to brewers is the process by which yeast convert sugar into alcohol, and thus carbohydrate availability is of prime concern. In order to yield sugars capable of being utilized by the brewing yeast, barley grain must first be allowed to germinate in order to produce the enzymes necessary to break down the carbohydrate reserves of the seed, stored in

the form of starch (9). To achieve the required levels of starch degrading enzymes, the barley must first be subjected to a process known as malting, well described in the literature (10, 16, 18, 19, 67). Such enzymes are endogenous proteins used by the barley grain to facilitate break down of the starchy endosperm of the seed, which in turn provides necessary energy for the plant and facilitates its growth (16). Indeed, barley grain is the most widely utilized cereal grain in the brewing industry, in large part due to its high levels of hydrolytic enzymes necessary to break down starch (36). The resultant sugar compounds, however, should only be minimally utilized by the plant as they are the essential components needed for yeast metabolism and growth. Thus, the malting stage concludes with the stabilization or halting of barley enzyme activity.

The overall process of malting involves three predominant stages; the first is a steeping step where the barley is imbibed with water over a period of one to two days, increasing the moisture content of the barley grain to the range of 42-45 % (10, 11, 16). The second stage, known as germination, involves holding the barley grain at a particular moisture range and temperature (exact regimens will vary between maltsters), allowing it to germinate for 4-5 days, releasing or synthesizing the necessary enzymes to degrade the cell structure of the starchy endosperm (36). During this step modification occurs. A well modified malt has an extensively degraded endosperm (16). This modification prepares the starch for breakdown into simpler sugars. This in turn releases granules of starch, preparing it for degradation into simpler carbohydrates. The third stage of malting consists of kilning, often at temperatures of 60-80 ^{o}C (18, 36) in order to suspend enzymatic activity, thereby preserving the barley starch from further degradation and reducing moisture content of the grain to less than 5 % (10). Kilning can sometimes reach temperatures as high as 110 ^{o}C to impart unique flavour characteristics for specific styles of beer, while avoiding breakdown of heat labile enzymes (10). Malting also

serves to induce starch gelatinisation, which is necessary for complete enzymatic degradation of starch (10, 36).

2.4. Mashing and Starch Degradation

Following malting, the kernels undergo a process of extraction termed mashing, in order to produce a fermentable liquid known as wort. Mashing involves the combination of ground or milled malt suspended with heated water in order to solubilize nutrients from the barley grain, resulting in a malt extract known as "sweet wort"; sweet because it contains large amounts of unfermented sugars. Often the mash consists of three parts water by volume to one part ground malt (10) or four parts water to one part ground malt (36). Many of the components of wort are derived from simple extraction at this stage, while others are formed from the activity of malt derived enzymes and their substrates (10, 11 16, 43). Most notable are the starch degrading enzymes which culminate in the production of smaller fermentable sugars such as glucose, maltose and maltotriose, but also the formation of larger carbohydrates such as dextrins, which cannot be utilized by the yeast.

Many studies have been conducted on the ability of starch degrading enzymes to produce a fermentable media, which has resulted in a generally accepted theory of starch degradation (11, 36, 43). Currently, it has been elucidated that the collective action of four starch-degrading enzymes: α-amylase, β-amylase, limit dextrinase; and α-glucosidase are primarily responsible for the hydrolysis of starch into fermentable sugars (36, 43). As illustrated in Figure 2-1 by Evans (36), α-amylase is an endo-acting enzyme which randomly attacks the starch molecule within the chain to produce oligosaccharides and limit dextrins. β-amylase is an exo-acting enzyme cleaving the starch molecule at the non-reducing ends to produce maltose, limit dextrinase is an endo-acting enzyme cleaving branched chains of

amylopectin allowing for further degradation by β-amylase, and finally α-glucosidase is an exo-acting enzyme producing glucose (11, 36). The cumulative activity of all four enzymes is known as the diastatic power (DP) of the malt, and has traditionally been a key parameter in the brewing industry as it gauges the relative fermentability of the malt as a function of its ability to convert starch into fermentable sugars (36). Recently, however, DP is beginning to lose acceptance among the scientific community as being considered the dominant aspect for predicting starch degradation, and is rather being modified to include a parameter more highly correlated with fermentation performance (or starch breakdown): enzyme thermostability (35, 37, 38, 39, 40).

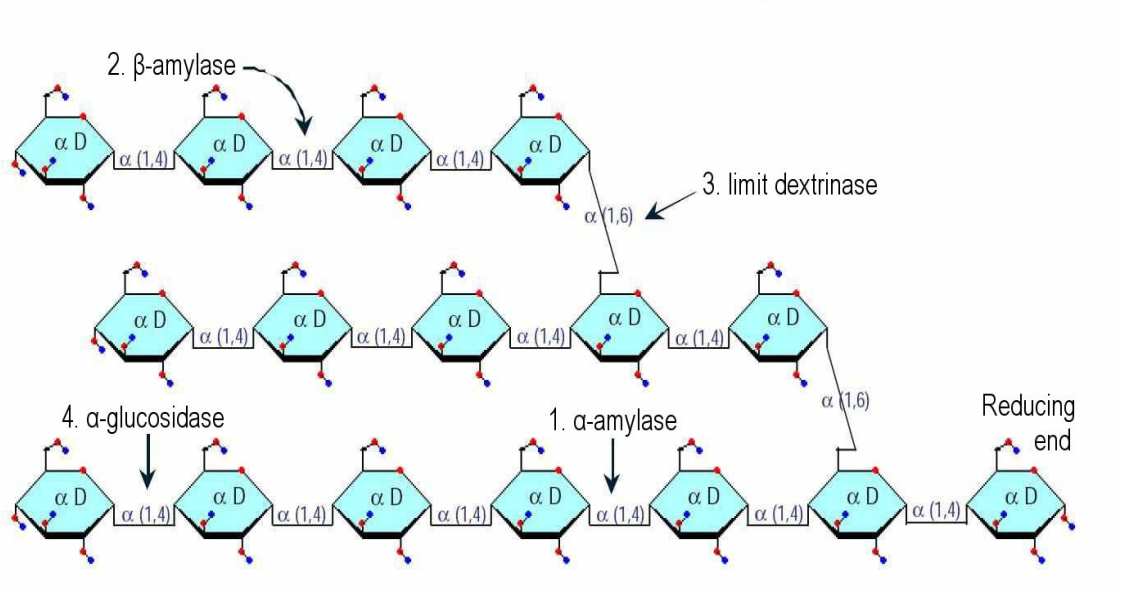

Figure 2-1. Simplified starch molecule depicting the actions of the primary starch degrading enzymes; 1. α-amylase (endo-acting, producing shorter chain carbohydrates by hydrolysis of glycosidic bonds from within the starch molecule), 2. β-amylase (exo-acting, producing units of maltose by hydrolysis of glycosidic bonds on the periphery), 3. limit dextrinase (endo-acting, cleaving branched chains from within), and 4. α-glucosidase (exo-acting, producing glucose units on the periphery) (from Evans *et al.* (36)).

2.5. Diastatic Power and Enzyme Thermostability

Despite the collective contribution of all four enzymes (mentioned above), only β-amylase has consistently and significantly been correlated with DP (37, 38, 43). This is due, in large part, to the efficiency of starch hydrolysis as dependent upon the ability of starch

degrading enzymes to withstand temperatures typical of mashing. As a result, it is necessary for α / β-amylase, limit dextrinase, and α-glucosidase to remain active while exposed to temperatures as high as 70-75^{o}C; a characteristic known as enzyme thermostability. Currently a significant focus in brewing research, the thermal stability of malt enzymes is proving to be a crucial aspect in the development of new barley varieties which express enhanced fermentability. Given the significant contribution of β-amylase to DP, a sufficiently high level of activity would be required for the adequate breakdown of starch into fermentable sugars during the mashing stage of brewing. The importance of β-amylase activity is supported by the high level of maltose (50-60% of total sugars) present in the wort following mashing (43).

Studies conducted on the thermostability of the chief starch degrading enzymes have resulted in a sound understanding of the complex process of starch hydrolysis, which also accounts for differences observed in enzyme activity. It must be noted that in order to achieve adequate starch hydrolysis via the activity of starch-degrading enzymes, gelatinization of the starch (the point at which starch is solubilized) must occur in order to optimize the formation of enzyme-substrate complexes. In the absence of starch gelatinization, degrading enzymes cannot form adequate interactions necessary to hydrolyze the molecule (67, 69) due to the obstructive crystalline structure of the sugar. In order to overcome this obstacle, gelatinization of the starch is induced by exposing the solution mixture to temperatures of 60-70 ^{o}C during the mashing protocol. This causes the starch molecule to absorb water and swell, disrupting the ridged sugar structure (9, 45) and allowing the starch degrading enzymes access to their substrate.

Once adequate gelatinization has been achieved, enzymes breakdown the complex carbohydrate structure of starch (see above) into smaller sugars, some of which can be metabolized by yeast. However, not all of the hydrolysable sugar gets broken down into usable forms due to the incomplete activity of the starch degrading enzymes, and these dextrins and

soluble starches remain in the finished wort as unfermentable material. This has been an area of intense focus primarily as a means to develop malt with higher fermentability, thereby increasing yield and profitability.

Enzyme activity is proving a key parameter to be modulated in order to enhance the DP of a particular malt, specifically that of β-amylase and limit dextrinase. Due to its high thermostability and abundance, α-amylase is not considered a limiting enzyme in the production of fermentable sugar (9), as is evident from its negative correlation with DP (37, 38). Similarly, α-glucosidase seems to also play a minor role, given the low level of glucose present in a typical wort sugar profile (43). β-amylase and limit dextrinase, however, have been shown to express varying degrees of thermal instability, with β-amylase being more thermolabile than limit dextrinase (37, 43). Eglinton *et al.* (35) has identified three unique forms of cultivated barley (*Hordeum vulgare L.)* β-amylase alleles and a fourth from a wild barley variety *Hordeum vulgare* ssp. *spontaneum*, each encoding a distinct form of β-amylase with associated variation in thermostability.

Alleles Bmyl-Sd1, Bmyl-Sd2L, and Bmyl-Sd2H were identified within cultivated barley, while Bmyl-Sd3 was unique to the wild barley variety. T_{50} temperatures (the temperatures at which 50% of enzyme activity is retained) were then determined for Bmyl-Sd1, Bmyl-Sd2L, and Bmyl-Sd3 and found to be 58.5, 56.8 and 60.8 ^{o}C respectively (35) suggesting that β-amylase, having the highest thermal instability of the DP enzymes, may be the rate limiting enzyme in the conversion of starch to fermentable sugars. Similarly, Ovesna *et al.* (78) evaluated 84 varieties of Czechoslovakian spring malting barley varieties and determined a similar series of β-amylase alleles, identifying Sd2L having low, Sd1 having intermediate, and Sd3 having high thermostability. Indeed it was found that malt possessing alleles for β-amylase

exhibiting higher thermal stability resulted in a more efficient degradation of starch when exposed to temperatures typical of mashing (35).

Additionally, it has been noted that sub-optimal limit dextrinase activity is associated with poor fermentability due to an accumulation of unfermentable branched limit dextrins (43). Limit dextrins are the result of enzymatic activity of both α- and β-amylase (discussed above); here enzymatic degradation only proceeds to a point, after which the activity of limit dextrinase is required in order to produce further substrate for β-amylase (61). As such, the overall activity of β-amylase is partially dependent upon the activity of limit dextrinase, and vice versa. However, unlike β-amylase, limit dextrinase seems to be restricted in activity primarily because of inhibitor proteins, rather than thermal instability (9). Macri *et al.* (65) have identified two such inhibitor proteins, and have classified them, according to their apparent isoelectric points (pI), as high (pI 7.2) and low (pI 6.7) pI inhibitors. During the malting process, sufficient amounts of pI inhibitors undergo degradation to allow a certain degree of activity, however enough remain to inhibit a large proportion of the limit dextrinase (62). Therefore, improvement in the availability of free limit dextrinase suggests an increased conversion of unfermentable carbohydrates into fermentable sugars (increased DP), with a parallel increase in fermentability.

2.6. Carbohydrate Utilization

Many studies have been conducted with the aim of elucidating carbohydrate utilization in brewing yeast strains (29, 31, 46, 49, 94). As such, there is a plethora of information available which discusses a generally accepted mechanism of carbohydrate uptake and utilization by *Saccharomyces* spp. (21, 70, 93, 103). Indeed, there are a number of fundamental differences exhibited between species of the *Saccharomyces* genus (see section 2.1 for comparison of ale and lager yeast), however, common to the brewing strains (*S. cerevisiae* and

S. pastorianus) is the ability to ferment a unique series of wort carbohydrates in a regulated, sequential order.

As a result of the actions of starch degrading enzymes (see section 2.4), wort contains sugars such as sucrose, glucose, fructose, maltose, maltotriose, and larger unfermentable dextrins. It is generally agreed that brewing yeast will utilize sucrose, glucose, fructose, maltose, and maltotriose in that general order of preference, noting that some degree of overlap will occur (i.e., yeast may complete metabolism of glucose while beginning the uptake of fructose). Maltotetraose and other higher (i.e., longer chain) dextrins are left unfermented due to a lack of appropriate hydrolytic enzymes necessary for utilization of such longer chain sugars (70, 93).

Differences in the mechanisms of carbohydrate uptake have been observed among the sugars utilized by brewing yeast strains. Primarily, monosaccharides such as glucose and fructose are transported across the cell membrane via facilitated diffusion, whereas both maltose and maltotriose require energy in the form of ATP in order to be transported into the cell (15, 93). Additionally, sugar uptake is regulated through a mechanism of carbohydrate repression which is dependent upon the environment in which the yeast is growing. Of the fermentable sugars comprising wort, maltose and maltotriose are found in highest concentration (often 50-60%, and 15-20% of total carbohydrate respectively), and are therefore the most significant sugars to the brewer. Both sugars are regulated through independent cell membrane permeases, and once inside the cell, are hydrolyzed to glucose units by the α-glucosidase system (93). Maltose fermentation is under the genetic regulation of any one of five known MAL loci which cannot become activated when environmental glucose levels exceed 1% (w/v), a phenomenon known as glucose repression. It is generally regarded that only after a threshold level of glucose

has been removed, in the range of 40-50% from the wort, will the uptake of both maltose and maltotriose proceed (93).

2.7. Development of Miniaturized Fermentation Assay

Since the early 1960's, research has been conducted on the development of a small scale fermentation assay easily performed in a laboratory environment, but which can accurately simulate conditions typical of large scale brewery fermentations. Early work by the European Brewery Convention (EBC) yeast group indicated that a laboratory fermenting vessel of 5 cm diameter and a working height of 102 cm accurately simulated yeast performance at the brewery scale, while later work in the Kirin Brewery indicated that fermenting vessels with height to diameter ratios greater than 15, and heights greater than 52 cm were sufficient (56). Kruger *et al.* (54) reported successful simulation using a fermenting vessel of 4.7 cm inner diameter and 150 cm height, with a working volume of 2 L, corresponding to a fill height of 117 cm (often referred to as "tall-tube" fermentations). More recently, a method of fermentation using 50 mL measurement cylinders was successfully developed to detect premature yeast flocculation (PYF), after incubation at 21 ^{o}C in a water bath for only 48 hours (52). This method allowed quick, accurate fermentations to be performed in the laboratory with relative ease, requiring less time and smaller sample sizes than previous assays. However, it must be noted that in order to achieve satisfactory results, this method incorporated the addition of glucose to the wort at a concentration of 4%, as well as higher aeration rates in order to improve yeast growth and yield results amenable to brewery scale fermentations (52).

Recently, Lake *et al.* (56) developed a novel miniaturized fermentation assay capable of simulating brewery scale fermentations. The method employs the use of standard laboratory test tubes as fermenting vessels with a height of 12.5 cm and diameter of 1.3 cm, the smallest

known method (to this author's knowledge) of laboratory scale fermentations to date. Similar to the techniques outlined by Jibiki *et al.* (52), addition of 4% (w/v) glucose and adequate aeration is required in order to increase yeast cell activity necessary for more accurate simulation of brewery fermentations (56). Optimum conditions outlined included the use of 11.1 ^{o}P wort, pitched (inoculated) with SMA yeast to an initial cell concentration of 1.5 x10^{7} cells/mL, with a minimum volume of 15 mL for each fermenting vessel (56). Recently, MacIntosh and colleagues (63) determined the above assay was adequate for mimicking brewery scale fermentations, however, the absolute accuracy of the predicted values were found to be dependent on fermentor size, and a correction factor may be required to yield accurate estimates of larger scale brewery operations. However, discrepancies in the absolute values of AE due to fermentor size were found to be consistent over multiple trials, and therefore still of practical utility to the brewing industry.

2.8. Non-linear Modeling

Much of the current literature examining fermentation parameters, such as the decline in wort density over time, do so without a standard method of statistical analysis to assess the data. Often, comparisons of variables will be examined visually, or perhaps using an analysis of variance (ANOVA) of data at fixed times throughout the fermentation, neither of which are ideal (90). Recently, however, Speers *et al.* (90) have described an effective method of mathematically modeling non-linear data resulting from typical fermentation processes (i.e., yeast consumption of fermentable carbohydrates, decline in wort density, ethanol production, etc.), allowing for more comprehensive statistical analyses to be performed, and therefore more direct comparisons of fermentation performance between test samples than has previously been cited in the literature. Additionally, with the added benefit of model parameters, and greater

overall fermentation description, more data is readily available for comparison between key brewing parameters (e.g., malting quality parameters, genetic markers, ADF, yeast strain utilized etc.).

As wort density is a common and simple measure of brewing performance in industry, use of a simple and effective mathematical model incorporating such data would prove useful in constructing coherent representations of fermentation performance. Speers et al. (90) discussed an established equation, known as the logistic equation, which best models this relationship in terms of the decline in Plato over time, and is illustrated in Equation 4. Fermentation parameters are calculated from a "best fit" curve of the data, and include "P_∞" which is the density of the wort upon reaching equilibrium (fermentation has completed) measured in degrees Plato (^oP), "B" (hr^{-1}) which is the maximum rate of fermentation and is a function of the slope at "M" (hr), the inflection point of the curve (90):

$$P_t = \frac{P_D}{\{1 + e^{[-B * (t-M)]}\}} + P_\infty \quad (4)$$

Here, "P_t" is the Plato value at any time, "t", and "P_D" is the change in density during the fermentation, also expressed as P_i-P_∞, where "P_i" is the initial Plato reading before fermentation begins (90). The equation can be expressed as:

$$P_t = \frac{P_i - P_\infty}{\{1 + e^{[-B * (t-M)]}\}} + P_\infty \quad (5)$$

Such a model allows statistical comparison between all fermentation parameters P_∞, P_i, B, and M, thus enabling the detection of deviations from an expected decline in wort density exhibited by a typical fermentation (90).

Additionally, during the course of this study a new model was developed which was aimed at predicting and describing yeast in suspension (YIS) resulting from absorbance measures throughout fermentation. This was applied to all measures of absorbance from the current study, and provides not only a visual description, but also quantitative measures of the trends of YIS throughout fermentation (see section 4.13.3). Noting that absorbance data resulting from changes in YIS generally followed a pattern similar to the normal curve, parameter values of the Gaussian equation were modified to introduce a "tilt" to the overall curvature of the regression line, fitting YIS absorbance data suitably:

$$Abs_{600} = R * t + A * e^{-\frac{1}{2}\left(\frac{t-\mu}{\sigma}\right)^2} \quad (6)$$

Where "Abs600" refers to absorbance at a wavelength of 600 nm at any time, "t", "R" is the slope term (AU/hr) which introduces a tilt (rotation) to the overall curve, "A" is the amplitude (AU) or height of the curve, "μ" is the mean and defines the midpoint of the curve (hr), and "σ" refers to the width (hr) (6). The above function is henceforth referred to as the Tilted Gaussian equation/function.

3. Objectives

The objectives of the current study were conceptually simple: measure and assess the relative fermentation performance achieved between select varieties of Canadian malting barley. The need for continual research and development of new barley varieties has been outlined, with identification of genetically superior breeds being a chief concern for the sustained growth and productivity of the Canadian malting barley market. Identification of some such varieties was achieved, in part, through the collaboration of three brewing research institutes. Crop varieties were bred, grown, and specifically chosen for testing through the Crop Development Centre (CDC) of the University of Saskatchewan, Saskatoon, SK. Selected varieties were then malted, and preliminary testing was conducted through the Canadian Grain Commission (CGC), Winnipeg, MB. Finally, malted barley was then mashed, fermented, and analyzed for fermentation performance by the miniature fermentation assay outlined by Lake *et al.* (56) at Dalhousie University, Halifax, NS (see section 4.9). However, the concept of fermentability is not a trivial parameter of measure, as it encompasses complex biological systems working in synergy, and each with an effect upon the other. Therefore, the goal of the current study was to measure, assess, and correlate an array of crucial fermentation parameters in an effort to gauge the overall fermentability of the selected malting barley varieties, and assess their performance relative to each other.

4. Experimental Methods

4.1. Study Overview

Figure 4-1 briefly describes the major aspects of the current study, and the predominant measures therein. The major focus of the current study was to gain a better understanding of the fermentation capabilities inherent within predominant varieties of Canadian two-row malting barley. Therefore, chief focus was applied to the measurement of wort attenuation, and YIS, crucial parameters that are indicative of fermentation performance. Additionally, fermentable carbohydrates and ethanol were monitored throughout fermentation to gain better insight into the typical constituent profiles displayed in the selected varieties, along with a broad understanding of the expected biochemical mechanisms involved in carbohydrate metabolism under conditions of the miniature fermentation assay. As well, data was also collected prior to malt acquisition by other study collaborators (section 4.12). This provided further valuable information regarding malt quality, including an array of endogenous malt enzyme activity assays, allowing correlation with previously mentioned measures of fermentation performance.

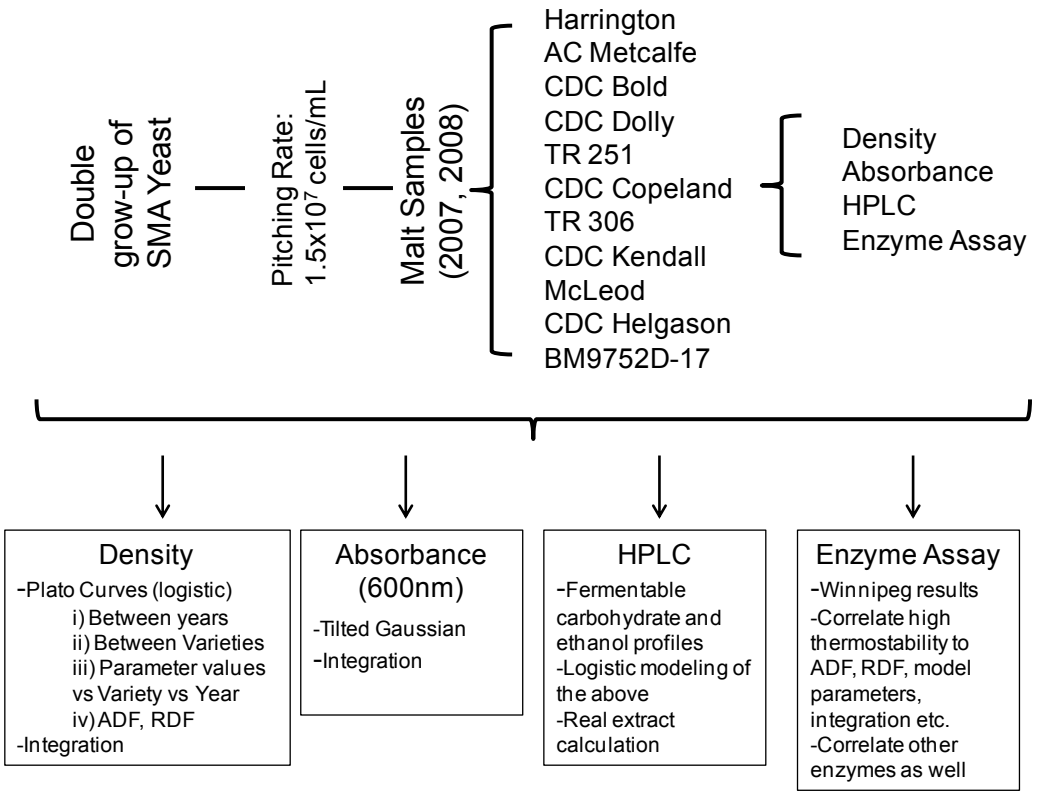

Figure 4-1. Study outline displaying predominant measures and objectives of the current fermentation analyses, illustrating the overall study design. N.B. Varieties listed were sampled over two crop seasons, together constituting 22 malt varieties.

4.2. Malt Varieties

Eleven two-row barley varieties were selected from two locations in Western Canada, over two crop seasons. Common malting varieties AC Metcalfe, CDC Copeland, and Harrington were chosen based on recommendations from the Canadian Malting Barley Technical Centre (CMBTC) as possessing high fermentability. AC Metcalfe accounts for the majority of seeded acreage of two-row malting barley cultivars in Canada, averaging 50.3% from 2007 to 2011 (64). This was followed by CDC Copeland which averaged 19.1% of seeded acreage, and Harrington which averaged only 1.7% from 2007-2011 (64). Though less common, Harrington possesses ideal fermentation characteristics and is still largely considered the de facto "gold standard" among malting barley varieties. AC Metcalfe has been noted to have high

fermentability with high enzyme content, while CDC Copeland has also been deemed highly fermentable despite relatively lower enzyme content (Personal communication, Aaron McLeod, CGC, Winnipeg, MB).

CDC Bold, a feed variety, was selected because it possesses moderate malting quality with low enzyme content. CDC Dolly, another feed variety, was selected as a negative fermentability control as it possesses relatively low malting quality. Experimental variety BM9752D-17 was selected due to its expression of high β-amylase thermal stability. In addition, experimental variety TR251, parent of BM9752D-17, was included in this study due to its immediate genetic heritage with BM9752D-17. CDC Kendall is a malting barley variety which was selected due to its high enzyme content. Finally, feed varieties TR306, McLeod, and CDC Helgason were also included in this study for comparison to that of the malting varieties. Table 4-1 summarizes the malt varieties utilized for this study. Collaborators from the Canadian Grain Commission (CGC, Winnipeg, MB) subjected all barley samples to a malting regimen similar to that utilized in industry (see section 4.2.1), using a pilot scale malting system. Varieties consisted each of two samples, grown over two crop seasons and locations. Barley varieties harvested in 2007 were grown in the Kernen Crop Research Farm, University of Saskatchewan, SK, while barley harvested in 2008 were grown in the CDC Crop Science Seed Farm, Saskatoon, SK (see Table 4-1).

4.2.1. Malting Regimen

All varieties were malted according to the following malting regimen in a custom pilot scale malting system located at the CGC in Winnipeg, MB. The system itself consisted of independent metal steep tanks, drum germinators, and continuous-rotational kilns. Four kilograms of each barley variety was steeped in 13 ^{o}C water for 48 hours, periodically draining

the water to allow for air rests. The steeping schedule consisted of 10 hours of wet steeping, followed by an 18 hour air rest, 10 hour wet steep, six hour air rest, two hour wet steep, and a two hour air rest. Following this, germination proceeded for 96 hours at 15 ^{o}C with a continuous 100% relative humidity maintained throughout. Finally, the process was concluded with kilning over the course of 24 hours. A ramped temperature schedule was used, beginning with 12 hours at 55 ^{o}C, followed by six hours at 65 ^{o}C, two hours at 75 ^{o}C, and four hours at 85 ^{o}C (Personal communication, Aaron McLeod, CGC, Winnipeg, MB).

Table 4-1. Brief description of malt varieties utilized in the current study, including growing location and crop year.

Variety	Year (Grown)/ Location	Code	Description
CDC Bold	2007 / Kernen	SP-09-01	Feed variety, moderate ferm.
AC Metcalfe	2007 / Kernen	SP-09-02	Common malting variety
Harrington	2007 / Kernen	SP-09-03	"Gold Standard" malting variety
CDC Dolly	2007 / Kernen	SP-09-04	Feed variety, poor ferm.
TR251	2007 / Kernen	SP-09-05	Experimental variety, parent of BM9752D-17
CDC Copeland	2007 / Kernen	SP-09-06	Common malting variety
TR306	2007 / Kernen	SP-09-07	Feed Variety
CDC Kendall	2007 / Kernen	SP-09-08	Malting variety
McLeod	2007 / Kernen	SP-09-09	Feed variety
CDC Helgason	2007 / Kernen	SP-09-10	Feed variety
BM9752D-17	2007 / Kernen	SP-09-11	Experimental variety, high β-amylase thermostability
CDC Bold	2008 / SK	SP-09-12	Feed variety, moderate ferm.
AC Metcalfe	2008 / SK	SP-09-13	Common malting variety
Harrington	2008 / SK	SP-09-14	"Gold Standard" malting variety
CDC Dolly	2008 / SK	SP-09-15	Feed variety, poor ferm.
TR251	2008 / SK	SP-09-16	Experimental variety, parent of BM9752D-17
CDC Copeland	2008 / SK	SP-09-17	Common malting variety

Table 4-1. (continued)

Variety	Year (Grown)/ Location	Code	Description
TR306	2008 / SK	SP-09-18	Feed variety
CDC Kendall	2008 / SK	SP-09-19	Malting variety
McLeod	2008 / SK	SP-09-20	Feed variety
CDC Helgason	2008 / SK	SP-09-21	Feed variety
BM9752D-17	2008 / SK	SP-09-22	Experimental variety, high β-amylase thermostability

N.B. Ferm.= fermentability; CDC= Crop Development Centre (Canada); AC= Agriculture Canada; Kernen= Kernen Crop Research Farm, University of Saskatchewan; SK= CDC Crop Science Seed Farm, Saskatoon, SK.

4.3. Yeast strain

All fermentations were carried out using the SMA yeast strain obtained from Wyeast Laboratories, Inc. (Odell, OR) SMA yeast has been adapted by ASBC Methods of Analysis Yeast-14 (6) for use as a standard strain for brewing research. As well, it has been specified by Japanese brewers for use in Canadian malting tests, and is additionally a standard commercial lager strain in Germany (Speers, 2012 personal communication). Yamauchi *et al.* (106) described the SMA yeast strain as "a typical bottom fermentation brewer's yeast for pilsner type beer". Similarly, yeast manufacturer's, Versuchs-und-Lehranstalt für Brauerei (VLB) in Berlin (DEU), specifications describe the strain as bottom fermenting and almost completely flocculent towards the end of fermentation, with a high rate of fermentation throughout. In general, the variety is regarded as the current de facto standard yeast strain used in brewing research (Edney, Rossnagel, Speers, 2011 personal communications), however, it should be noted that many varieties of brewing yeast are still utilized between research organizations for a multitude of reasons.

4.4. Preparation of Yeast Cultures

Yeast handling and culture methods were followed according to Lake (55). Yeast was received in slurry form from Wyeast Laboratories, Inc. and transferred to Yeast Extract Peptone Dextrose (YEPD) agar slants consisting of 20 g/L dextrose (BioShop Canada Inc., Burlington, ON), 20 g/L peptone (Bacto, Sparks, MD), and 10 g/L yeast extract (Bacto, Sparks, MD) and between 25-30 g/L of agar (Bacto, Sparks, MD). Slants were aerobically incubated at 30 ^{o}C for 48 hours, and continually agitated at 100 revolutions per minute (rpm) using a New Brunswick Scientific classic series C24 incubator shaker (Classic C24, New Brunswick Scientific Co., Inc., Edison, NJ), followed by removal, sealing, and storage at 4^{o}C. All yeast were re-cultured on new slants every 3-4 months to ensure high vitality, viability, and to minimize mutations.

4.5. Yeast Propagation and Collection

Yeast propagation methods were followed according to Lake (55). One loop-full of yeast from YEPD agar slants were transferred aseptically to a 125 mL Erlenmeyer flask containing 50 mL of YEPD broth consisting of 20 g/L of dextrose, 20 g/L peptone, and 10 g/L yeast extract. Flasks were incubated aerobically at 30 ^{o}C, and shaken at 100 rpm for 24 hours in a New Brunswick Scientific classic series C24 incubator shaker (Classic C24, New Brunswick Scientific Co., Inc., Edison, NJ). The yeast slurry was then centrifuged at 3000 rpm for three minutes, and the supernatant discarded. The resultant yeast pellet was then re-suspended in sterile, distilled water using a vortex mixer, centrifuged as described previously and the supernatant discarded. This procedure served to wash the yeast of any remaining broth or particulate matter, and was repeated twice more for a total of three washes. Upon final washing, the yeast pellet was re-suspended in ~20 mL of sterile, distilled water for counting and determination of slurry concentration (see section 4.6). Yeast was then pitched (inoculated) to

an initial cell concentration of 1.5 x 10^7 cells/mL into a further 100 mL of YEPD broth in a 250 mL Erlenmeyer flask to commence the secondary grow up. In this secondary grow up the above incubation and washing procedures were repeated. This resulted in a final slurry with a high cell concentration. The procedure can be simplified to the following steps:

Day 1

i) Inoculate 50mL of YEPD broth with one loop full of stock yeast from slants, incubate with shaking overnight (1st grow-up).

Day 2

ii) Spin yeast broth down, wash cells, and re-suspend in ~20mL of water.

iii) Count yeast using a haemocytometer, and calculate slurry concentration (see section 4.6).

iv) Use calculated concentration to inoculate a further 100mL of YEPD broth to an initial cell concentration of 1.5 x 10^7 cells/mL, incubate with shaking overnight (2nd grow-up).

Day 3

v) Repeat as in Day 2 to calculate new slurry concentration (high concentration slurry due to increased yeast growth over two days).

vi) Use calculated concentration to inoculate wort samples to an initial cell concentration of 1.5 x 10^7 cells/mL (see section 4.7).

4.6. Cell Counting

Counting of microscopic yeast cells followed general procedures described in ASBC Methods of Analysis Yeast-4 (5). The water washed yeast slurry was diluted with sodium acetate buffer (pH 4.5) consisting of 328 mL of 0.1 M sodium acetate solution, 456 mL of 0.1 M acetic acid solution, and 43.56 mL of 95 % ethanol. Dilution was carried out in proportions of one part slurry to 199 parts buffer, resulting in a 1:200 dilution factor. This was achieved by adding 10 µL of homogenized yeast slurry to 1990 µL of buffer. Individual cells were then counted under a microscope using a haemocytometer (Bright-Line Hemacytometer, Hausser Scientific, Horsham, PA), averaging the total count from five squares. This was done for both sides of the haemocytometer (total of ten squares counted altogether; See Appendix A3). Following this, the total count from five

squares on side #2, divided by two constituted the average cell count (AVC). Slurry concentration in cells/mL was then calculated using equation 7:

$$Slurry\ Concentration = (AVC) \times 5 \times 10{,}000 \times 200 \quad (7)$$

Where "AVC" is the average cell count from the haemocytometer discussed above, "5" accounts for the total number of squares on each side of the haemocytometer, "10,000" accounts for the volume fraction of slurry contained within the counted area (one ten thousandth of a mL), and "200" is the dilution factor.

4.7. Yeast Pitching

The yeast was pitched using a yeast/water mixture (see section 4.6) to yield an initial cell concentration of 1.5 x 10^7 cells/mL. Details of the wort used are described in section 4.8. The volume of yeast slurry to add was calculated according to equation 8:

$$Volume\ to\ Pitch = \frac{(1.5\ x10^7 \times Media\ Volume)}{Slurry\ Concentration} \quad (8)$$

where the "Volume to Pitch" refers to the volume of concentrated yeast slurry necessary to inoculate each wort sample to an initial cell concentration of 1.5 x 10^7 cells/mL, the so called "standard pitching rate", the "Media Volume" refers to the final volume of pitched wort required to fill 30 test tubes each to 15 mL, in this case 450 mL, and "Slurry Concentration" calculated as discussed previously (equation 7). The yeast was distributed evenly to all samples

ensuring that no more than three minutes elapsed from the time of the first inoculation of sample one, to the final inoculation of the last sample.

4.8. Wort Preparation

Preparation of wort samples was carried out according to the standard protocol outlined in the ASBC Methods of Analysis (2009), Malt- 4 (1). Approximately 55 g of malt kernels were finely ground in a standardized, laboratory scale malt mill (Universal Laboratory Disc Mill DFLU, Buhler GmbH, Braunschweig, DEU), collecting any powdered particulate left in the mill by means of brushing into the attached beaker. Calibration of the mill to produce finely ground malt was carried out according to the procedures outlined in ASBC Methods of Analysis (2009) Malt-4 Standardization of Mill Setting (1). From the finely ground malt flour, a sample of 50g (±0.05g) was weighed and placed immediately into an empty mash beaker of a laboratory scale, bench-top mash bath (IEC Mash Bath, Industrial Equipment & Control PTY LTD., Melbourne, AUS), along with a two inch magnetic stir bar. In general, experiments carried out each week utilized all twelve beakers of the mash bath, constituting four malt samples prepared in concert. In order to obtain an adequate final volume of wort, a total of 150 g of ground malt was utilized for each malt variety, necessitating the use of three mash beakers per variety to be fermented. Beakers of dry, ground malt were secured inside the mash bath and preheated to 45 ^{o}C. To each malt-containing mash beaker was added 200 mL of 45 ^{o}C, distilled water, previously heated in the accompanying sparge pots. Initiating magnetic stir bars immediately following water addition commenced the mash cycle. Bath temperature of 45 ^{o}C was maintained for 30 minutes, followed by a gradual increase in bath temperature of 1 ^{o}C every minute until a final temperature of 70 ^{o}C was reached. Upon reaching final temperature an additional 100 mL of distilled sparge water, simultaneously preheated in the mash bath to 70 ^{o}C,

was added to each mash beaker. Temperature of bath was maintained at 70 ^{o}C for a further 60 minutes. This process follows the method of mashing designed by the European Brewery Convention (EBC), and subsequently adapted by the American Society of Brewing Chemists (ASBC). The process is fully described in the ASBC methods of analysis (2009) Malt-4 (1) and the resulting wort is often referred to as a 'Congress Wort'.

Following completion of mash cycle, hot water from the bath was drained, allowing the beakers to cool at room temperature for not more than 15 minutes. The resulting mixture from each mash beaker was then poured into a 500 ml Erlenmeyer flask equipped with a funnel fitted with fluted filter paper (Reeve Angel 802 Fluted Filter Paper 32 cm, Whatman Inc., Fairfield, NJ), and allowed to gravity filter for approximately one hour. Due to possible inadequacies in separation when filter paper is initially dry, the first 100 mL of filtrate was returned to the filtration apparatus, allowing for greater separation of malt extract from residual grist. Filtration was deemed complete when the retained solid (retentate) resembled a cracked, dense cake. Flasks were then capped with aluminum foil, autoclaved, and immediately placed in cold storage until ready to pitch. Autoclaving the filtered wort served the dual purpose of sterilization, and simulation of a kettle boil, thereby forcing "hot break" proteins (trub) to coagulate and separate out of suspension.

4.9. Fermentation Parameters

Fermentations were carried out according to Lake *et al.* (56), using the miniaturized fermentation assay. Following cold storage for 24 hours to allow for "cold break" proteins to coagulate and fall out of suspension, sterilized wort was centrifuged at 4400 rpm for 15 minutes to separate wort from residual trub. Total clear wort volume was then determined using a 500 mL graduated cylinder, and density was measured in degrees Plato (see below). Clear wort was

then adjusted to 12.6 ^{o}P through the addition of sterile, distilled water, and measured to 410 mL. Wort was then returned to a 500 mL Erlenmeyer flask, equipped with a two inch stir bar and oxygenated at 6-7 psi using medical grade compressed oxygen (VitalAire Canada Inc., Halifax, NS) for five minutes with stirring (medium speed). Oxygen pressure was slightly adjusted for each sample in order to control foaming. Simultaneously, 4% (w/v) laboratory grade D-Glucose (BioShop Canada Inc., Burlington, ON) was added to each sample. Upon completion of oxygenation and sugar addition, previously counted yeast slurry (See section 4.7) was inoculated (pitched) into the wort, and volume brought up to 450 mL through the addition of sterile, distilled water.

Immediately following pitching, initial density was measured from the bulk solution (t=0 hr), and finally 15 mL samples were decanted into each of 30 standard laboratory test tubes (12.5 cm (height), 1.3 cm (inside diameter)) equipped with a large PTFE boiling chip (PTFE Boiling Stones, Sigma-Aldrich Inc., Missouri, USA). All test tubes were fitted with a foam stopper and allowed to ferment at 21 ^{o}C for 78 hours while submerged to sample height in a water bath. Time zero (hr) density readings were collected unfiltered and in singlet, while time one readings were measured from pitched wort remaining after decanting, sometimes in triplicate, but often in duplicate (quantity of wort remaining after decanting varied slightly). All remaining samples were measured in triplicate to determine density, relative absorbance, fermentable carbohydrate, and ethanol profiles (See sections 4.10 and 4.11). The above discussed procedure was carried out for each malt variety.

Varieties were tested in three distinct fermentation rounds. The first round of testing was completed prior to the laboratory's capability for HPLC sugar analysis. Therefore, no fermentable carbohydrate data is available for the initial round of fermentations. Additionally, complications regarding density measurement for all first-round fermentations caused the data

to be unreliable, and were therefore not included in this document. Second-round fermentations included all varieties, with density, turbidity, and fermentable carbohydrates measured at each time interval.

Third round fermentations were conducted chiefly to observe carbohydrate consumption rates throughout fermentation. These included three artificially constructed wort variants using only the malt variety AC Metcalfe. Resulting wort was prepared using three distinct carbohydrate spikes in effort to elucidate possible bias that may have resulted due to the standard 4% (w/v) glucose addition described in Yeast-14 (6) prior to inoculation. The three carbohydrate spikes consisted of the following: standard 4% (w/v) addition of glucose which acted as a control, 4% (w/v) addition of maltose only, and 4% (w/v) addition of mixed fermentable carbohydrates to maintain natural profiles. In order to determine the composition of the latter carbohydrate spike, it was first necessary to determine typical relative concentrations of each sugar found naturally in all-malt wort. This was achieved following mashing by immediately withdrawing a sample of fresh wort and conducting HPLC analysis. Though effective in determining relative concentrations of maltotriose, maltose, glucose and fructose, sucrose remained undefined using the available HPLC method. Therefore, a theoretical percentage of sucrose relative to that of glucose was used to approximate the required amount to add. Stewart (93) outlines typical wort sugar profiles, therefore initial sucrose concentration, using typical maximum values, was calculated as two fifteenths ($^{2}/_{15}$) the amount of initial glucose. Upon HPLC analysis of initial wort sugar levels, it was determined that glucose was present at 1.36 g/100mL (percent), and therefore initial sucrose would theoretically, and maximally exist as ($^{2}/_{15}$) * 1.36, yielding 0.18 g/100mL. Thus maltotriose, maltose, sucrose, glucose, and fructose were added, according to their relative concentrations, as a mixed 4% spike to the final preparation. Due to the relatively large amount of malt required to conduct

such analyses, each wort was prepared in volumes large enough to accommodate only duplicate measures throughout fermentation. Measures for HPLC analysis were taken in greater quantities early in the fermentation in order to capture carbohydrate consumption during the most highly active times, thus samples were taken at 0, 2, 6, 15, 18, 21, 25, 30, 42, 48, and 66 hours post inoculation.

4.10. Turbidity and Density Measures

Samples of fermenting wort were measured at times 1, 6, 22, 26, 30, 46, 50, 54, 70, 74, and 78 hours using a UV-vis spectrophotometer (HP 8453 UV-Visible Spectrophotometer, Hewlett-Packard GmbH, Waldbronn, DEU) to measure relative absorbance, and hence wort turbidity as an indicator of YIS (57). Timing of samples was based upon standard methods described in ASBC Methods of Analysis Yeast-14 (6), and was developed to accommodate technician availability during a regular eight hour work day, thus easing implementation within industry. Approximately three mL of wort from each fermenting vessel (test tube) was extracted using a macro pipette at a depth of roughly 1/2 the sample height. Wort was transferred to a 3.5 mL disposable cuvette and relative absorbance measured at 600 nm and reported as absorbance units (AU). Each measurement employed a destructive sampling technique, utilizing a full test tube for each replicate.

Upon completion of turbidity measures, wort was immediately filtered by decanting directly from the cuvette into a clean test tube equipped with a funnel and a folded Whatman #4 filter paper (Grade 4 Qualitative Circles 70mm, Whatman, GE Healthcare UK Limited, Buckinghamshire, GBR). Additional wort was filtered directly from the remaining sample to ensure adequate volume. Density was then measured using a digital density meter (DMA 35N Digital Density Meter, Anton Paar GmbH, Graz, AUT), and reported in degrees Plato (^{o}P). With

the exception of times zero and one, all samples from each time interval were measured in triplicate for both absorbance and density. It should be noted that only time zero density readings were measured unfiltered, having only access to the initial bulk solution of pitched wort at that time. Thus, absorbance readings were impractical, and therefore not collected. Upon completion of density measures and prior to discarding, approximately 1.5 mL of each sample was retained in an Eppendorf tube and immediately frozen pending further carbohydrate analysis.

4.11. Carbohydrate and Ethanol Analysis

Fermenting wort carbohydrate composition was analysed by high performance liquid chromatography (HPLC) at each time interval using a Waters separation system (Waters 2695 Separations Module, Waters Corporation, Milford, MA) equipped with a refractive index (RI) detector (Waters 2414 Refractive Index Detector, Waters Corporation, Milford, MA). HPLC methods including sample preparation and instrument analyses, were adopted from study collaborators at the CMBTC. A heated Benson Polymeric Ag+ form column (806 BP-100 Ag+ Carbohydrate Column, Benson Polymeric Inc., Sparks, NV), fitted with a guard column (806G BP-100 Ag+ Carbohydrate Guard Column, Benson Polymeric Inc., Sparks, NV), was used for the separation of four primary wort fermentable carbohydrates: maltose, glucose, maltotriose, and fructose. Ethanol also separated efficiently with this column, and was thus quantified concurrently with each sample run. Approximately 1.5 mL of all filtered samples measured from section 4.10 were collected and sealed in 1.5 mL Eppendorf tubes, and stored frozen at -30 ^{o}C until HPLC analyses could be performed. Due to the high concentration of carbohydrates early in fermentation, and the inherent sensitivity of HPLC detection, all samples collected at time zero, one, and six hours were diluted using distilled water, by a factor of 1:10 (one part

sample plus nine parts water) prior to injection into the HPLC system. Similarly, all samples collected at times 22, 26, and 30 hours were diluted by a factor of 1:5 (one part sample plus four parts water). Samples collected at time 46 hours and onward were left undiluted. Prior to injection of carbohydrate solutions into the HPLC system, regardless of dilution factor, each sample was filtered directly into a two mL glass auto sampler vial (Supelco Analytical 8/425 Clear Glass Vial, Bellefonte, PA) using a disposable syringe filter with a 0.2 μm pore size (Nylon Disposable Syringe Filter 13 mm, Whatman, GE Healthcare UK Limited, Buckinghamshire, GBR) fitted onto a ten mL disposable syringe (Latex Free Syringe 10 mL, Becton Dickinson, Franklin Lakes, NJ). Each vial was capped and sealed with a polypropylene cap fitted with a PTFE/Silicone septa, and placed into the HPLC carousel to await auto-injection.

Glucose, fructose, maltose, maltotriose, and ethanol concentrations were analysed for each malt variety at each time interval throughout fermentation. As per the HPLC column manufacturer's operating instructions, the column was heated and maintained at a 90 ^{o}C operating temperature throughout separation, filtered/degassed water was used as the liquid carrier phase (eluent), and flow rate was set to 0.3 mL per minute. Eluent was prepared using de-ionized water, filtered through a nylon membrane filter with a 0.45 μm pore size (NL 17 Membrane Filters Polyamide 47 mm, Whatman GmbH, Dassel, DEU) using an aspirator, and remained under vacuum for approximately one hour to degas. RI detector was set to 410 (standalone) mode with a sensitivity of 4, and detection chamber was maintained at 30 ^{o}C. Output signal from the detector was sent directly to a desktop computer running Waters Millennium$_{®}^{32}$ Data Acquisition/Processing Software (Millennium$_{®}^{32}$ Software v3.20, Waters Corporation, Milford, MA), which displayed, recorded, and processed the data.

4.11.1. HPLC Calibration and Carbohydrate Calculation

Individual standard solutions were prepared, each consisting of a known concentration of a single analyte: glucose (D-Glucose Anhydrous, BioShop Canada Inc., Burlington, ON), fructose (D-Fructose, BioShop Canada Inc., Burlington, ON), maltose (D-(+)-Maltose monohydrate, FisherBiotech, Bridgewater, NJ), maltotriose (Maltotriose, Sigma-Aldrich Inc., St. Louis, MO), sucrose (D-Sucrose, Fisher Scientific, Bridgewater, NJ), or ethanol (Ethyl Alcohol, Fisher Scientific, Bridgewater, NJ). Standards were dissolved in de-ionized water, filtered, and subsequently injected into the system. From this, a processing method was created within the Millennium®[32] software, allowing automatic processing of collected data.

Injection volumes of ten μL for each sample were used, with a duration of 40 minutes between injections to ensure all previous sample had exited the column. Elution times were determined for each standard solution and defined within the processing method to facilitate automatic peak detection. Peak response was then integrated by the Millennium®[32] software. Using known concentrations of standard solutions, standard curves of peak integration versus analyte concentration were constructed and found to be strongly linear (r^2>0.99) and positive, as expected. All subsequent concentration calculations utilized the ASBC response method described in ASBC Methods of Analysis (2009), Wort-14B (4). Each wort sample collected was similarly analysed by the methods discussed above; data was automatically processed by the user-defined processing method in the Waters Millennium®[32] software, and raw integration data collated by hand and entered into Microsoft Excel for subsequent calculation of concentration. Samples were prepared for HPLC analysis in large groups each day, loaded into the system, and programmed to run over night, as a grouped set. A standard solution was included with each sample set and was injected intermittently throughout the programmed run to ensure continued

calibration of the system and allow monitoring of possible aberrant behavior (no deviations from equilibrium were noted during analysis of samples).

4.12. Enzyme Activity

All enzyme assays were carried out by colleagues at the Canadian Grain Commission (CGC, Winnipeg, MB). Malt diastatic power, which represents the activity of the four starch degrading enzymes, α-amylase, β-amylase, limit dextrinase and α-glucosidase, was assayed using a Skalar segmented flow auto analyzer, and reported in degrees Lintner (^{o}L). Enzymes were first extracted in salt water from ground malt flour, and the mixture allowed to react with a standard starch solution under controlled conditions. Starch hydrolysis products were measured using a copper-neocuprione assay. This method was calibrated with malt standards which were analysed according to the methods outlined in ASBC Methods of Analysis (2009), Malt-6A (2). For more detail regarding the assays conducted at the CGC, refer to their online resources (98).

In addition, each malt variety was assayed individually for α-/β-amylase, and limit dextrinase activity, as well as β-amylase thermostability. Activity of α-amylase was measured according to the methods outlined in ASBC Methods of Analysis (2009), Malt-7B (3), adapted to a segmented flow auto analyser, and reported as Dextrinizing Units (D.U.). The method was calibrated with standards that were assayed by ASBC Methods of Analysis (2009), Malt-7A (3). Activity of β-amylase was measured according to the Megazyme Betamyl-3 method (Megazyme International Ireland, Wicklow, IRL) and reported as enzyme units per g of ground malt (U/g). Limit dextrinase activity was determined by the Limit Dextrizyme assay (Megazyme International Ireland, Wicklow, IRL), and reported as enzyme units per g of ground malt (U/g). See Appendix A2 for all collaborator data provided. Thermostability was determined by the previous method after heat treatment at 55^{o}C for ten minutes, reported as a

percentage of the original activity. For further detail regarding the Megazyme enzyme assays, refer to their website for the most current information and methodology (72).

4.13. Non Linear Modeling

4.13.1. Attenuation

Decline in density over the course of fermentation, known as wort attenuation, was modeled according to the methods outlined by Speers et al. (90) using the logistic equation (equation 5), which describes a declining sigmoid curve. Data was fitted using the non-linear module of Graphpad Prism software (Version 5.03, GraphPad Software Inc., San Diego, CA) by estimating equation parameter values (Pi, P_∞, B, and M), in an iterative manner, that maximally reduces the sum of squared deviations of data points from the curve. Vertical deviations of raw data from the curve are known as residuals, and Prism software determines a best fit curve to the data by reducing the sum of these squared residuals (known as reducing the "residual sum of squares"; RSS) such that additional changes no longer alter the RSS significantly. This is achieved by repeatedly altering equation parameters, and calculating the resulting RSS until a minimum has been reached. This effectively produces a curve which is maximally associated with the given data. Attenuation curves from each variety were then compared to that of Harrington, of the same year, using a global F test to determine if the fitted models were statistically different, or if one curve best described both data sets. This allowed gross examination of fermentation performance among barley varieties, including visual analysis, be it similar or disparate relative to Harrington.

4.13.2. Fermentable Carbohydrates and Ethanol

Samples at each time interval throughout fermentation were analysed for glucose, fructose, maltose, maltotriose, and ethanol for each malt variety as discussed in section 4.11. As a result, percent analyte concentration was determined and reported as g per 100mL (fermentable carbohydrates; g/100mL) or on a volumetric basis (ethanol; v/v). Data was modeled according to the logistic equation (equation 5) and individual curves were fitted to each analyte using Graphpad Prism. In general, the logistic model fitted carbohydrate and ethanol data well, as evidenced from the resulting high coefficient of determination (predominately achieving $r^2>0.98$ from each curve) calculated for each model, suggesting that in addition to modeling wort attenuation throughout fermentation, the logistic model is also an effective method for modeling carbohydrate and ethanol data. Model parameters are summarized for each carbohydrate analysed, and ethanol in Table 5-4 to Table 5-8.

4.13.3. Yeast in Suspension

As described in section 4.10, turbidity can be regarded as an indicator of YIS (57, 75). Throughout the course of this study, turbidity measurements using a spectrophotometer set to a wavelength of 600 nm was found to yield less variable results once "cold break" proteins (trub) were removed by centrifugation of the wort prior to pitching (see section 4.9). Seemingly, this was due to the removal of particulates which would otherwise have become suspended in solution once fermentation produced adequate shear (agitation), thereby interfering with absorbance measures. In order to visualize and quantify the YIS more effectively, all absorbance data was modeled using the modified Gaussian function (equation 6), referred to as the Tilted Gaussian equation (6), described in section 2.8.

4.13.4. Statistical Analysis

Fermentation model parameters for attenuation (B, M, Pi, and P_{∞}), and absorbance (R, A, μ, and σ) for each malt variety were calculated and compared using the Prism software package (GraphPad Software Inc., San Diego, CA), which has the ability to test for significant differences between non-linear data sets derived from the experimental fermentations (63).

5. Results

5.1. Non-Linear Modeling of Attenuation and YIS

Malt samples listed in Table 4-1 were each analyzed using the miniaturized fermentation assay outlined by Lake *et al.* (56). Density was measured throughout fermentation, in degrees Plato, using a digital density meter at time intervals discussed in section 4.10. This constituted a total of twelve time points, eleven of which were measured in triplicate, resulting in 34 data points available for non-linear modeling using the logistic equation (equation 5) described in section 4.13. Plato values at time zero (immediately following pitching and prior to decanting into test tubes) were measured once as only one large sample vessel was available for sampling at that time, precluding sampling of companion fermentation replicates. Additionally, density measures at this time were taken unfiltered. Therefore, time zero readings for each fermentation were excluded from model calculations as this time point has no replicates and consequently no associated error, coupled with possible measurement variation due to unfiltered media.

Results of attenuation modeling indicate that in general each malt variety fermented as expected, displaying typical attenuation as described by a negative sigmoid curve. With the anticipated exception of CDC Dolly, attenuation began relatively slowly, rapidly decreasing after approximately ten hours, slowing once again after 40 hours, and subsequently reaching a final plateau after 78 hours, the equilibrium Plato, where additional fermentation time produced no further change in density. Recall CDC Dolly is a poorly fermenting feed variety, evident from its loosely defined sigmoid curve and lack of comparable final attenuation to that of the malting varieties. Table 5-1 summarizes the results of the estimated fermentation parameters from the “best fit” curve of collected AE data, described by the logistic equation for each malt variety. Additionally, all AE were converted to RE using equation 5b (see Appendix A4.)

described by Cutaia *et al.* (27), and resulting values similarly fitted using the logistic model. Fermentations took place over the course of one month, fermenting on average six malt varieties each week. Special care was taken to mash and ferment each variety in pairs (e.g., Harrington 2007 and Harrington 2008) in order to minimize experimental error between duplicate varieties grown in separate years. In doing so, differences observed in fermentation performance can more accurately be attributed to growth conditions and malting performance among samples of the same variety.

Table 5-1. AE parameter values estimated by the logistic model for each malt variety. Values and associated error were calculated from a non-linear regression model described by the logistic equation (Eq. 5) and fitted to individual fermentations. Results shown from second round fermentations only.

Malt Variety	$\mathbf{P_i}$ (^oP)	$\mathbf{P_\infty}$ (^oP)	**B** (hr^{-1})	**M** (hr)	r^2	***n***
			2007			
CDC Bold	15.6 (0.673)	2.07 (0.104)	-0.1478 (0.019)	18.77 (1.49)	0.994	30
AC Metcalfe	15.36 (0.21)	1.9 (0.051)	-0.1559 (0.009)	20.35 (0.416)	0.998	33
Harrington	15.14 (0.095)	1.75 (0.026)	-0.1798 (0.005)	19.42 (0.192)	0.999	33
CDC Dolly	18.92 (0.963)	2.27 (0.096)	-0.0799 (0.006)	15.46 (1.744)	0.998	30
TR251	15.39 (0.189)	2.15 (0.04)	-0.155 (0.007)	19.44 (0.382)	0.999	33
CDC Copeland	15.74 (0.317)	1.9 (0.066)	-0.1533 (0.01)	18.84 (0.588)	0.997	33
TR306	15.61 (0.365)	2.15 (0.075)	-0.1574 (0.012)	18.53 (0.7)	0.996	33
CDC Kendall	15.76 (0.17)	1.78 (0.035)	-0.1466 (0.005)	19.07 (0.31)	0.999	33
McLeod	15.85 (0.447)	2.86 (0.094)	-0.1301 (0.012)	20.04 (0.859)	0.995	33
CDC Helgason	16.4 (0.72)	2 (0.126)	-0.1182 (0.013)	19.12 (1.263)	0.993	33
BM9752D-17	16.72 (0.693)	2.14 (0.097)	-0.1092 (0.01)	17.97 (1.236)	0.996	33
			2008			
CDC Bold	16.15 (0.63)	2.12 (0.091)	-0.1342 (0.014)	18.79 (1.294)	0.996	30
AC Metcalfe	15.65 (0.122)	2.19 (0.024)	-0.1412 (0.004)	19.48 (0.237)	0.999	33
Harrington	15.89 (0.629)	2.5 (0.115)	-0.1358 (0.016)	18.84 (1.181)	0.992	33
CDC Dolly	22.29 (3.667)	2.3 (0.272)	-0.0618 (0.01)	10.63 (6.229)	0.992	30
TR251	16.45 (0.317)	2.26 (0.049)	-0.1203 (0.006)	18.46 (0.575)	0.999	33
CDC Copeland	16.79 (0.812)	2.06 (0.118)	-0.1134 (0.012)	17.88 (1.4)	0.994	33
TR306	16.09 (0.454)	2.31 (0.073)	-0.1358 (0.014)	18.48 (0.811)	0.996	31
CDC Kendall	16.42 (0.347)	1.9 (0.056)	-0.1236 (0.006)	18.33 (0.601)	0.998	33
McLeod	18.28 (1.429)	3.14 (0.182)	-0.0799 (0.01)	16.49 (2.733)	0.991	33
CDC Helgason	15.47 (0.225)	2.03 (0.057)	-0.1325 (0.007)	21.29 (0.41)	0.998	33
BM9752D-17	16.28 (0.281)	2.15 (0.047)	-0.1211 (0.005)	19.14 (0.512)	0.999	33

N.B. Bracketed values denote the asymptotic standard error (ASE); with few exceptions data from each fermentation were collected in triplicate at 11 time intervals (n=33).

Using the logistic (equation 5) and substituting predicted parameter values thereof (Table 5-1), original extract (OE) was calculated for each variety where OE = P_t at t=0 hr. Similarly, final apparent extract (AE_{75}) was also calculated where AE_{75} = P_t at t=75 hr. Using OE and AE_{75} values obtained, Apparent Degree of Fermentation (or Apparent Degree of Fermentability; ADF) was then calculated for each fermentation according to a variant of equation 2 (equation 9). Results from each fermentation are summarized in Table 5-2. In addition, corresponding p-values resulting from a global F-test denote significance of each fitted model to that of the control (Harrington, of the same year), and were included as a means of comparison to that of the single indicator of fermentation performance, ADF. Statistical analysis comparing each data set to the control was achieved by fitting each with the logistic model (equation 5; see Appendix A8 for all attenuation curves) and comparing predicted parameters. A global F-test was used to determine whether the two models differed significantly ($p<0.05$) or, given the associated error with each predicted parameter, if one curve could describe both data sets ($p>0.05$). That is to say, a $p<0.05$ indicates varied fermentation performance between two varieties, and that model curves predicted by each are significantly different:

$$ADF = \frac{(OE - AE_{75})}{OE} * 100 \quad (9)$$

Table 5-2. OE, AE, and ADF values resulting from second round fermentations of each malt variety. Included p-values indicate significance of modeled AE data (attenuation curves; see Table 5-1), comparing logistic curves from each variety to that of the control malt, Harrington, of the same year.

Control	*Malt*	*Year (Grown)*	*OE*	*AE*	*ADF*	*Probability (F-Test)*[1]
Harrington 2007	Harrington	2007	14.744	1.752	0.881	N/A
	CDC Bold	2007	14.805	2.073	0.860	<0.0001
	AC Metcalfe	2007	14.819	1.899	0.871	<0.0001
	CDC Dolly	2007	15.170	2.414	0.841	<0.0001
	TR251	2007	14.770	2.147	0.855	<0.0001
	CDC Copeland	2007	15.010	1.904	0.873	<0.0001
	TR306	2007	14.920	2.149	0.856	<0.0001
	CDC Kendall	2007	14.955	1.781	0.881	<0.0001
	McLeod	2007	14.958	2.873	0.808	<0.0001
	CDC Helgason	2007	15.040	2.023	0.865	<0.0001
	BM9752D-17	2007	14.924	2.171	0.854	<0.0001
Harrington 2008	Harrington	2008	14.928	2.504	0.832	N/A
	CDC Bold	**2008**	**15.107**	**2.126**	**0.859**	**0.0547**
	AC Metcalfe	2008	14.842	2.192	0.852	0.0336
	CDC Dolly	2008	15.465	2.664	0.828	<0.0001
	TR251	**2008**	**15.060**	**2.273**	**0.849**	**0.4023**
	CDC Copeland	**2008**	**15.076**	**2.078**	**0.862**	**0.1294**
	TR306	**2008**	**15.054**	**2.315**	**0.846**	**0.3735**
	CDC Kendall	2008	15.055	1.913	0.873	0.0001
	McLeod	2008	15.081	3.281	0.782	<0.0001
	CDC Helgason	2008	14.714	2.037	0.862	0.0002
	BM9752D-17	**2008**	**15.013**	**2.166**	**0.856**	**0.0630**

1. Values indicate significance of modeled data (shown in Table 5-1) resulting from global F-tests between each variety and Harrington.
N.B. OE= Original Extract, AE= Apparent Extract, and ADF= Apparent Degree of Fermentability; Bolded varieties indicate a non significant difference to the control malt (α= 0.05).

Comparison of fermentation data resulting from AC Metcalfe 2007 and 2008 samples illustrate typical Plato curves, and show only minor differences in attenuation between growing years. Explanatory factors causing such fermentation variability would be speculative, however it is probable that variation in environmental growing conditions would have a major impact on kernel development, and therefore malting quality/fermentability, between seasons. As illustrated in Figure 5-1, two independent curves were fit to both sets of data, indicating significant difference in fermentation performance between AC Metcalfe 2007 (SP-09-02) and AC Metcalfe 2008 (SP-09-13). Though malting regimens could introduce a source of variation in fermentation characteristics, these results assume insignificant malting variation, as each

variety was subject to automated regulation and control of malting conditions via a custom pilot scale malting system.

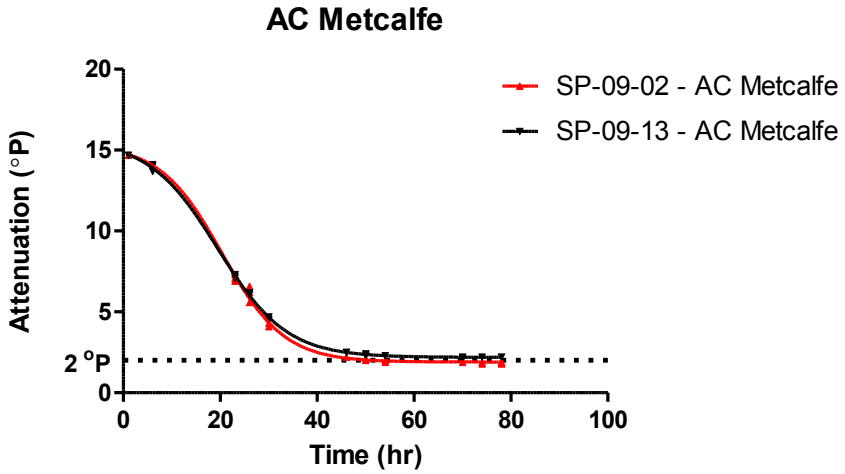

Figure 5-1. Comparison of attenuation curves between AC Metcalfe 2007 (SP-09-02) and AC Metcalfe 2008 (SP-09-13). Both sets of data were fit using the logistic equation and parameters P_i, P_∞, B, and M were compared using an F-test. Significant difference was found ($p<0.05$), therefore separate curves were fit to each set of data. Complete modeling of this graph is included in Table 5-1.

However, despite statistical significance between the two samples, visual inspection of Figure 5-1 clearly illustrates similar fermentation performance between sample years. That is to say, the fermentations were statistically ($p<0.05$) but not substantially different. Additionally, a predicted equilibrium attenuation of 1.899 ^{o}P and ADF of 87.1% achieved by AC Metcalfe 2007 is reasonably comparable to a predicted equilibrium attenuation of 2.192 ^{o}P and ADF of 85.2% achieved by AC Metcalfe 2008. Such results indicate highly conserved fermentation quality of AC Metcalfe barley between growing years. Concurrent relative absorbance data modeled using the Tilted Gaussian (equation 6) show approximate YIS throughout fermentation. A marked visual distinction can be seen between seasons, with AC Metcalfe 2007 more rapidly reaching a larger peak yeast activity, and declining sharply as compared to its 2008 counterpart (Figure 5-2). Pragmatically this suggests higher fermentation performance by

the 2007 variant, that is mirrored in the speed to reaching and magnitude of the final attenuation (Figure 5-1) achieved, and is also supported by the overall higher ADF values.

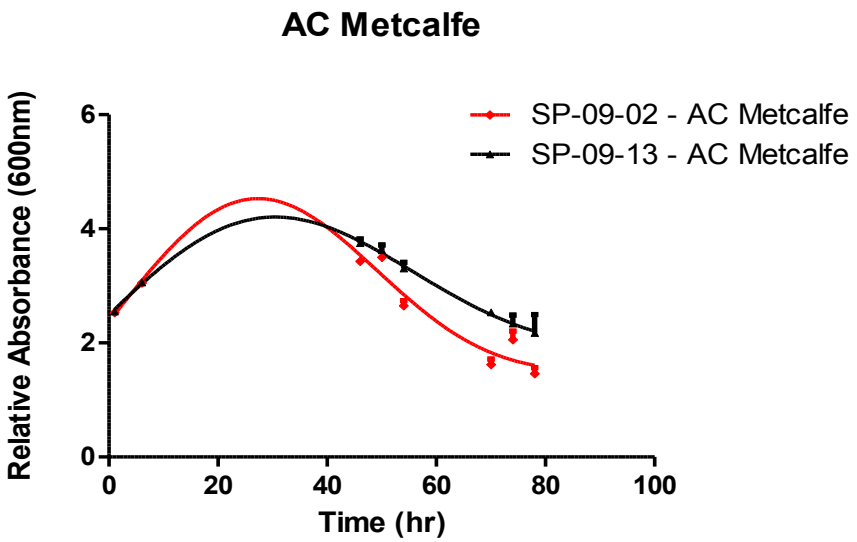

Figure 5-2. Comparison of relative absorbance curves for AC Metcalfe 2007 (SP-09-02) and AC Metcalfe 2008 (SP-09-13). Each set of data was fit using the Tilted Gaussian (equation 6) for comparison of YIS.

Similarly, CDC Copeland experienced varied fermentation performance between the two sample years (Figure 5-3). Resultant attenuation curves for CDC Copeland appear more visually distinct from that of AC Metcalfe, illustrating an appreciable difference in fermentation behaviour. This is partially reflected in the AE_{75} and ADF values achieved by each sample. Where CDC Copeland 2007 resulted in a predicted equilibrium attenuation of 1.904 ^{o}P and an ADF of 87.3%, CDC Copeland 2008 achieved a predicted equilibrium attenuation of 2.078 ^{o}P and an overall ADF of 86.2%. Although these differences in final attenuation values are in fact smaller than differences observed between samples of AC Metcalfe, the distinction between curves of CDC Copeland resulted from the variance in predicted values of *B*, the slope function at inflection. However, this deviation appeared to have minimal impact on the overall

fermentability of CDC Copeland between growing years, as both samples achieved low predicted equilibrium attenuation along with high ADF within the allotted fermentation time, resulting in greater consistency than AC Metcalfe in terms of final fermentation values. It is noteworthy that although showing greater consistency in final attenuation, a larger variance in *B* resulted in CDC Copeland 2008 exhibiting greater time to reaching final extract values, a phenomenon not as pronounced between samples of AC Metcalfe. Once again, as with AC Metcalfe, it can be seen that the 2007 variant of CDC Copeland reached peak values of YIS more rapidly than did the 2008 counterpart (Figure 5-4), and thus experienced an earlier decline in yeast activity. Not surprisingly, the attenuation curves also reflect this fermentation behaviour, where the decline in wort density is noticeably quicker in the 2007 malt compared to that of the 2008 (Figure 5-3), coupled with lower overall attenuation, and higher ADF achieved.

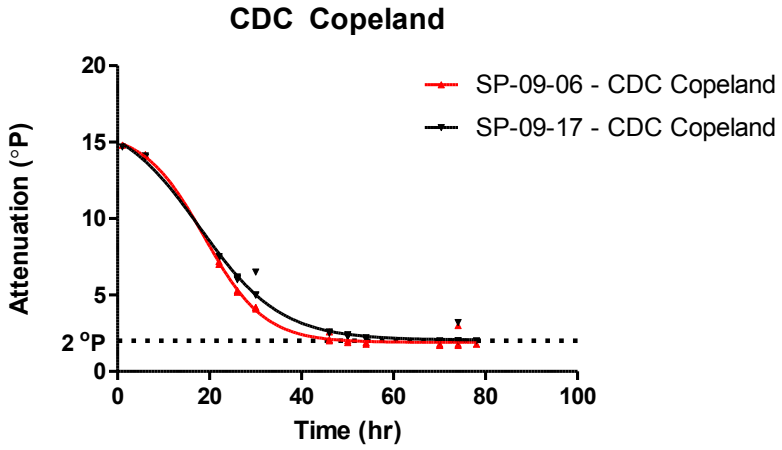

Figure 5-3. Comparison of attenuation curves between CDC Copeland 2007 (SP-09-06) and CDC Copeland 2008 (SP-09-13). Both sets of data were fit using the logistic equation and parameters P_0, P_∞, B, and M were compared using an F-test. Significant difference was found ($p<0.05$), therefore separate curves were fit to each set of data. Complete modeling of this graph is included in Table 5-1.

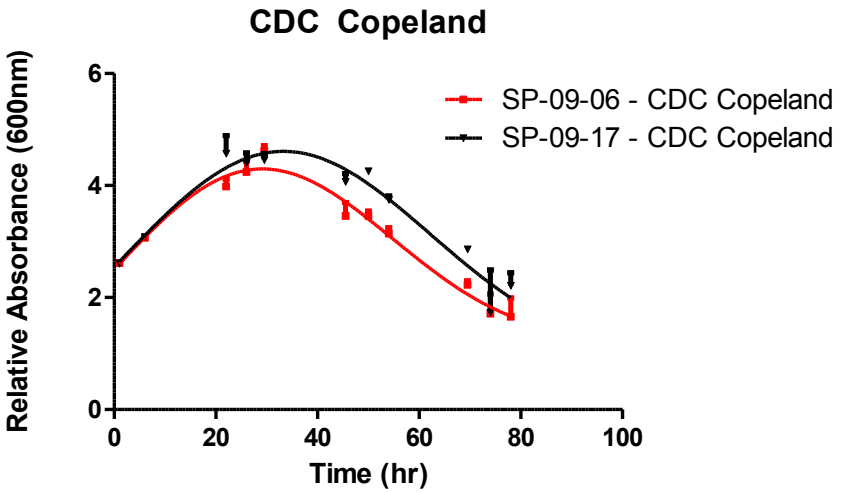

Figure 5-4. Comparison of relative absorbance curves for CDC Copeland 2007 (SP-09-06) and CDC Copeland 2008 (SP-09-17). Each set of data was fit using the Tilted Gaussian (equation 6) for comparison of YIS.

Interestingly, results exhibited by Harrington between years are relatively variable (Figure 5-5), with the 2007 sample achieving the highest degree of fermentability among tested varieties of that year, to the 2008 sample declining to the third lowest amongst tested varieties for that season. Harrington 2007 achieved a predicted equilibrium attenuation of 1.752 ^{o}P and an ADF of 88.1%, while Harrington 2008 only achieved a predicted equilibrium attenuation of 2.504 ^{o}P and an ADF of 83.2%. Possible reasons for this decline in fermentability between years is speculative, however, it is assumed that while all barley varieties exhibited a decrease in fermentability from 2007 to 2008, growing conditions would have imparted major impact on kernel development, and therefore malting quality of the Harrington barley variety, resulting in variable fermentation performance. Such variation between seasons is also distinguished through visual analysis of YIS (Figure 5-6), noting that Harrington 2007 experienced an earlier peak in YIS compared to that of Harrington 2008, followed by an abrupt decline. As previously observed, reduced time to reaching peak YIS, with a subsequent rapid decline in suspended yeast cell density, results in optimal fermentation performance compared to those varieties

expressing opposite character. Substantial differences in both absorbance and attenuation curves are evident between seasonal crops of Harrington, once again providing further evidence to improved malting and brewing performance of barley grown in the 2007 crop season, from that of the 2008 harvest.

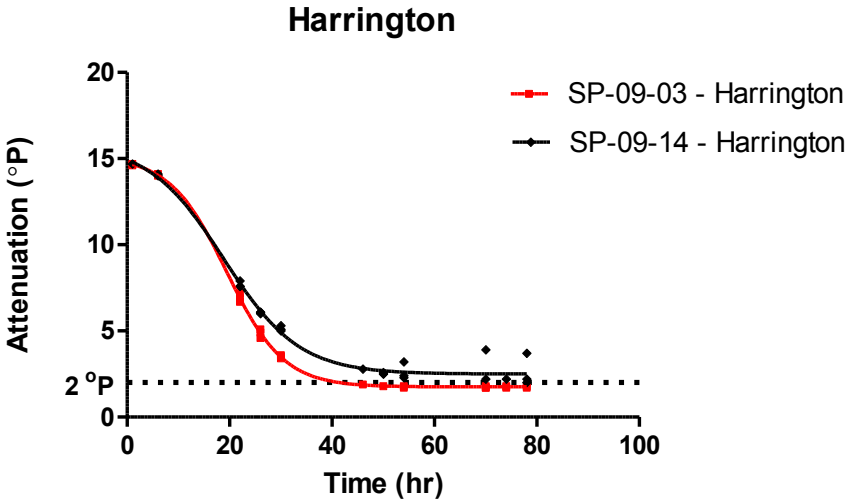

Figure 5-5. Comparison of attenuation curves between Harrington 2007 (SP-09-03) and Harrington 2008 (SP-09-14). Both sets of data were fit using the logistic equation and parameters P_0, P_∞, B, and M were compared using an F-test. Significant difference was found ($p<0.05$), therefore separate curves were fit to each set of data. Complete modeling of this graph is included in Table 5-1.

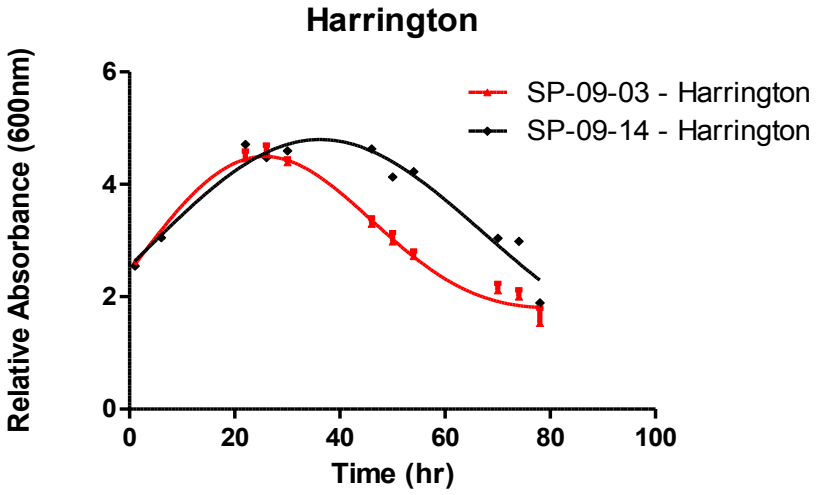

Figure 5-6. Comparison of relative absorbance curves for Harrington 2007 (SP-09-03) and Harrington 2008 (SP-09-14). Each set of data was fit using the Tilted Gaussian (equation 6) for comparison of YIS.

However, comparison of data resulting from CDC Bold fermentations indicate that both 2007 and 2008 growing years produced consistently stable fermentation characteristics as evident from their comparable attenuation curves (Figure 5-7). Non-linear modelling of AE data using the logistic equation predicted relatively low equilibrium (final) Plato values with an associated low margin of error (Table 5-1); commendable fermentation performance from a feed variety of barley. As well, statistical analysis of the fermentation parameters, P_0, P_∞, B, and M, showed no significant difference between attenuation curves of the two samples (Figure 5-7), indicating stable and consistent fermentation quality between growing years. This is further substantiated by the highly comparable ADF and predicted equilibrium attenuation (AE_{75}) achieved by both samples; 86.0% and 2.073 ^{o}P by CDC Bold 2007, and 85.9% and 2.126 ^{o}P by CDC Bold 2008, respectively. Interestingly, of the varieties tested in the current study, CDC Bold was one of three malt varieties which displayed very consistent fermentation quality between years (CDC Helgason and BM9752D-17 also exhibited highly conserved performance), yet is not a variety of barley which lends itself to ideal malting and brewing quality, and is grown predominantly as a feed variety. Unfortunately, due to a technician error in absorbance data collection in some varieties (e.g., CDC Bold 2007 & 2008), curve fitting models of YIS for many of the data sets resulted in poor fitting (see Figure 5-8), with high residual error (absolute sum of squares). Therefore modelling of YIS for this variety must be viewed with prudence, noting that only approximations and limited conclusions can be drawn from the resultant curves.

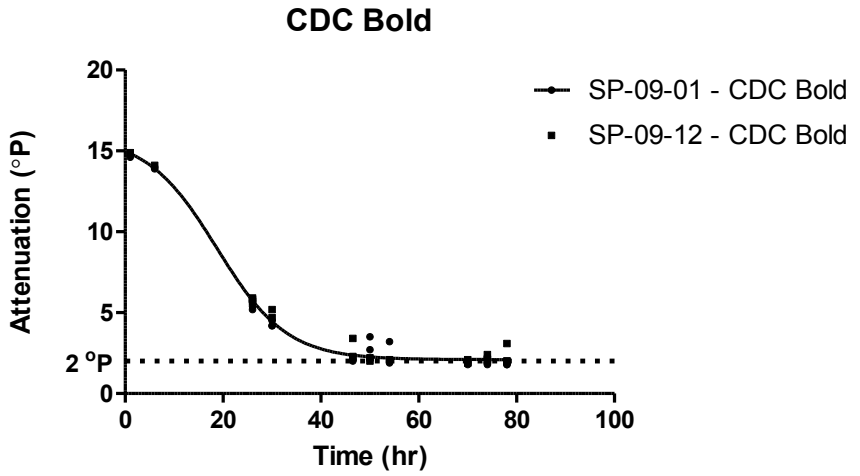

Figure 5-7. Comparison of attenuation curves between CDC Bold 2007 (SP-09-01) and CDC Bold 2008 (SP-09-12). Both sets of data were fit using the logistic equation and parameters P_0, P_∞, B, and M were compared using an F-test. No significant difference was found (p= 0.1954), therefore a single curve was fit to describe both sets of data. Complete modeling of this graph is included in Table 5-1.

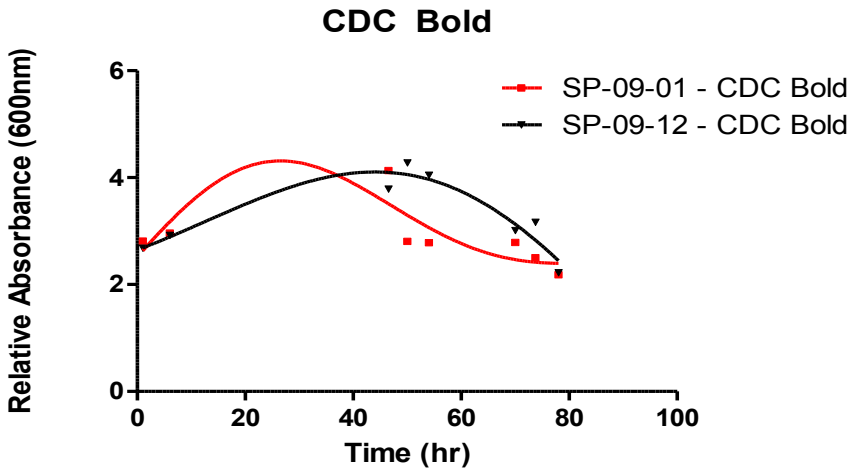

Figure 5-8. Comparison of relative absorbance curves for CDC Bold 2007 (SP-09-01) and CDC Bold 2008 (SP-09-12). Each set of data was fit using the Tilted Gaussian (equation 6) for comparison of YIS.
N.B. Coefficient of determination (R^2) = 0.2426 for CDC Bold 2007, suggesting insufficient data (and/or data quality) to confidently predict true YIS.

Interestingly, all 2007 malts resulted in statistically significant attenuation curves when compared to the control ($p<0.05$), illustrated in Table 5-2. ADF did vary considerably between varieties, ranging from 80.8% attenuation exhibited by McLeod (SP-09-09) to 88.1% displayed by both Harrington and CDC Kendall. In contrast, 2008 malt varieties exhibited varying levels of significance to that of the control, along with a general trend towards lower ADF for all

samples fermented as compared to the previous year. Of particular note is the decline in ADF displayed by Harrington between years, from 88.1 to 83.2% attenuation. Perhaps a result of poorer growing conditions, the decline in ADF experienced by Harrington 2008 could offer a possible explanation for the attendant increase in non-significant fermentation curves between varieties of that year.

Collectively, with the expected exception of CDC Dolly, and unexpected exception of McLeod, all malt varieties fermented relatively well, achieving low final extract levels. Of the 2007 malt samples, both Harrington and CDC Kendall fermented to the highest degree resulting in predicted equilibrium apparent extracts of 1.752 ^{o}P and 1.781 ^{o}P respectively. Closely following these were AC Metcalfe, CDC Copeland, and CDC Helgason reaching equilibrium apparent extracts of 1.899 ^{o}P, 1.904 ^{o}P, and 2.023 ^{o}P respectively, and overall ADF of 87.1 % from AC Metcalfe, 87.3 % from CDC Copeland, and 86.5% from CDC Helgason. Low to moderate fermenting varieties included CDC Bold, reaching an equilibrium attenuation 2.073 ^{o}P and an ADF of 86.0 %, TR251achieving an equilibrium attenuation of 2.147 ^{o}P and ADF of 85.5 %, TR306 reaching an equilibrium attenuation of 2.149 ^{o}P and ADF 85.6 %, BM9752D-17 achieving an equilibrium attenuation of 2.171 ^{o}P and ADF of 85.4 %, CDC Dolly reaching an equilibrium attenuation of 2.414 ^{o}P and ADF of 84.1 %, and finally McLeod achieving an equilibrium attenuation of 2.873 ^{o}P and ADF of 80.8 %.

Overall the 2008 varieties showed fewer significant differences to that of the control. As mentioned above, poorer fermentation performance by that of Harrington, 2008 could account for the obvious fluctuations in significance among varieties of that year. Indeed the unexpected decline in fermenting quality by that of the control accounts for the lack of statistically significant differences to those malts typically regarded as inferior fermenters. One might argue this to be an unfair comparison as reduced malting and fermentation performance could simply

be a result of atypical growing conditions. However, it should also be noted that such variability in fermentation characteristics could prove as further evidence to the unpredictable nature and lack of controlled quality now expressed by Harrington, a relatively old malting barley variety noted as showing declining quality due to an aging genotype (71). Nonetheless, variable fermentation quality is expected among barley varieties grown over different years, and despite this unexpected decline in fermentability, Harrington still serves as an adequate control malt by highlighting those varieties which exhibit comparatively less variation between years.

The highest degree of fermentation expressed in the 2008 samples was achieved by CDC Kendall, reaching a predicted equilibrium attenuation of 1.913 ^{o}P and an overall ADF of 87.3%. Statistical analysis of predicted models ("best fit" curves) between CDC Kendall and the control resulted in significant difference ($p<0.05$), and consequently separate curves were fit to both sets of data. CDC Helgason and CDC Copeland achieved the next highest degree of fermentability, reaching a predicted apparent attenuation of 2.037 ^{o}P and 2.078 ^{o}P respectively, and each achieving an ADF of 86.2 %. Statistical analysis of both models to that of the control resulted in significance ($p<0.05$) with CDC Helgason, and non-significance ($p=0.1294$) with CDC Copeland. Closely following these were CDC Bold, BM9752D-17, and AC Metcalfe reaching predicted equilibrium attenuations of 2.126 ^{o}P, 2.166 ^{o}P, and 2.192 ^{o}P respectively. Resulting ADF values were 85.9 % for CDC Bold, 85.6 % for BM9752D-17, and 85.2 % for AC Metcalfe. Statistical analyses (F-tests) of predicted models to that of the control resulted in non-significance for both CDC Bold ($p=0.0547$) and BM9752D-17 ($p=0.0630$), while AC Metcalfe was determined to be significantly different ($p<0.05$).

It should be noted, however, that statistical non-significance with CDC Bold was only just attained, evident from the very low (borderline) p-value of 0.0547. Variation in fermentation performance can still be distinguished visually by examining the resultant

attenuation curves between the two (data not shown). Finally, the low to moderate fermenting varieties included TR251, reaching a predicted equilibrium attenuation of 2.273 ^{o}P, ADF of 84.9 %, and statistically non-significant (p=0.4023) to that of the control malt; TR306, reaching a predicted equilibrium attenuation of 2.315 ^{o}P, ADF of 84.6 %, and non-significance (p=0.3735) to the control; CDC Dolly reaching a predicted equilibrium attenuation of 2.664 ^{o}P, ADF of 82.8 %, and significance (p<0.05) to the control; and McLeod, reaching a predicted equilibrium attenuation of 3.281 ^{o}P, and an ADF of 78.2 % resulting in significance (p<0.05) to the control.

In addition to assessing the performance of each variety relative to the control of the same year, it is prudent to also comment on the inherent changes (natural fluctuations) in fermentation characteristics observed between crop seasons. In an effort to account for the natural (and unavoidable) variation in growing conditions from one year to the next, individual varieties were each compared to their yearly counterpart (Table 5-3).

Table 5-3. OE, AE, and ADF values resulting from second round fermentations of each malt variety. Included p-values indicate significance of modeled AE data (attenuation curves; see Table 2-1), comparing logistic curves from each variety to its yearly counterpart.

Malt	***Year (Grown)***	***OE***	***AE***	***ADF***	***Probability[1] (F-Test)***
Harrington	2007	14.744	1.752	0.881	
Harrington	2008	14.928	2.504	0.832	<0.0001
CDC Bold	**2007**	**14.805**	**2.073**	**0.860**	
CDC Bold	**2008**	**15.107**	**2.126**	**0.859**	**0.1954**
AC Metcalfe	2007	14.819	1.899	0.871	
AC Metcalfe	2008	14.842	2.192	0.852	<0.0001
CDC Dolly	2007	15.170	2.414	0.841	
CDC Dolly	2008	15.465	2.664	0.828	<0.0001
TR251	2007	14.920	2.147	0.855	
TR251	2008	15.060	2.273	0.849	<0.0001
CDC Copeland	2007	15.010	1.904	0.873	
CDC Copeland	2008	15.076	2.078	0.862	<0.0001

Table 5-3. (continued)

Malt	*Year (Grown)*	*OE*	*AE*	*ADF*	*Probability[1] (F-Test)*
TR306	2007	14.920	2.149	0.856	
TR306	2008	15.054	2.315	0.846	0.0002
CDC Kendall	2007	14.955	1.781	0.881	
CDC Kendall	2008	15.055	1.913	0.873	<0.0001
McLeod	2007	14.958	2.873	0.808	
McLeod	2008	15.081	3.281	0.782	<0.0001
CDC Helgason	**2007**	**15.040**	**2.023**	**0.865**	
CDC Helgason	**2008**	**14.714**	**2.037**	**0.862**	**0.2339**
BM9752D-17	**2007**	**14.924**	**2.171**	**0.854**	
BM9752D-17	**2008**	**15.013**	**2.166**	**0.856**	**0.4828**

1. Values indicate significance between logistic models (Table 5-1) resulting from global F-tests between crop years of each variety.
N.B. OE =Original Extract, AE =Apparent Extract, and ADF =Apparent Degree of Fermentability, Bolded text indicate a non significant difference (α= 0.05).

As illustrated in Table 5-3, only three of the eleven varieties displayed non-significantly different fermentation performance between years, CDC Bold, CDC Helgason, and BM9752D017, according to the global F-tests applied to the logistic model for each fermentation. This is especially evident when comparing ADF between years of these varieties, each reaching very similar respective final attenuation values. Indeed, greater variance in ADF can be seen between other varieties deemed significant between years when analysed solely through model comparisons (F-test). Similarly, calculated values of both OE and AE remained strongly conserved between years, suggesting similar fermentation performance overall. Such homogeneity is not generally reflected in those malts which performed dissimilarly between growing years.

5.2. Fermentable Carbohydrate and Ethanol Profiles

In addition to monitoring Plato and absorbance throughout fermentation, samples of the fermenting medium were analysed for accompanying fermentable carbohydrate and ethanol

levels by HPLC (see section 4.11). Figure 5-9 shows a typical HPLC chromatogram produced at 22 hours post inoculation. This time was chosen for illustrative purposes as it represents a suitable time during fermentation whereby all analytes of interest were detected in a single run. Due to limitations of the HPLC method available, only four of the five primary fermentable carbohydrates could be quantified: maltotriose, maltose, glucose, and fructose. Sucrose, though not a prominent component of fermentable extract, was unable to be quantified due to unavoidable co-elution (and resultant signal hindrance) with that of maltose. Specifically, maltose was determined to have an average elution time of approximately 21.2 minutes, compared to sucrose, which elutes at approximately 20.6 minutes using the method described in section 4.11. Due to these similar elution times, and dramatically larger quantity of maltose relative to sucrose in typical fermentation extract, signal peaks resulting from refractive index (RI) detection of maltose vastly overshadow (i.e., hide) the much smaller sucrose peaks, thereby preventing quantification of the latter. Regardless, sucrose constitutes only 1-2 % of total carbohydrate extract of typical wort (93), while the remaining fermenting sugars, and by extension the major components of yeast metabolism, have been reported. Maltose peaks identified and the resulting concentrations predicated upon them should be regarded as both maltose + sucrose, noting that the majority constitutes maltose, and a very small margin of error exists due to the additional signal strength conferred by the low concentration of sucrose present. Hereafter, maltose + sucrose peaks are regarded simply as maltose.

In addition to sugar concentrations, ethanol levels were quantified concurrently as residual solvent peaks at the end of each chromatographic run. Longer chain carbohydrates, such as maltotetraose and higher, were retained poorly on the column, and therefore eluted quickly with little resistance, resulting in a large tailing peak near the beginning of the run. Individual longer chain sugars were poorly resolved, and thus not quantifiable. Therefore early

peak detection (i.e., prior to 17 minutes elution time) resembling a large, unresolved, tailing peak has been ubiquitously regarded simply as unfermentable dextrin material (Personal communication, Aleksandar Egi, CMBTC; Manufacturer's specifications, Benson-Polymeric), but should be noted that this constitutes maltotetraose and longer chain carbohydrates (see Figure 5-9).

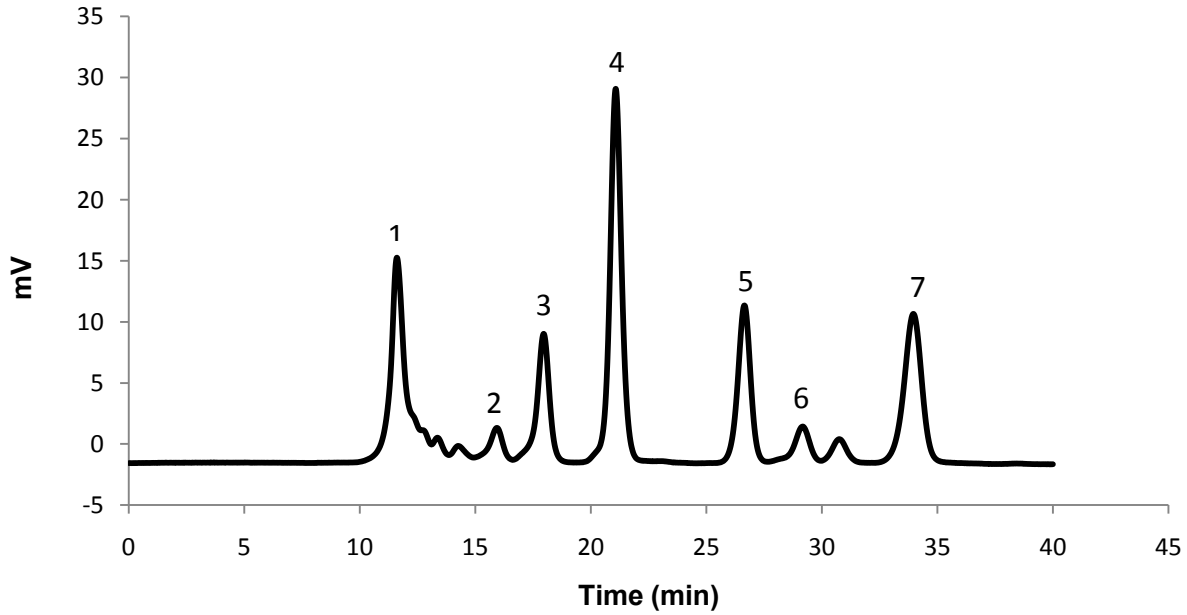

Figure 5-9. Carbohydrate chromatogram resulting from HPLC analysis, RI detection, illustrating a typical sugar profile in fermenting wort. Wort samples constituting this chromatogram were taken at time=22 hours post inoculation during fermentation of Harrington (2007). Peak 1 is composed of many longer chain sugars (dextrin material) not retained on the column resulting in early elution, long tailing, and poor resolution. Peak 2 is poorly resolved but detectable as maltotetraose. Peak 3 is maltotriose, peak 4 is maltose/sucrose (where maltose is the largely dominant sugar, effectively obstructing the substantially smaller sucrose peak), peak 5 is glucose, peak 6 is fructose, and peak 7 is ethanol.
N.B. Ordinate label "mV" refers to RI millivolts signal. The signal response was subsequently quantified to sugar and ethanol levels.

Typical wort carbohydrate profiles were observed throughout fermentation from all of the malt varieties tested, and over both testing years. Maltose constituted the highest naturally occurring fermentable sugar in all varieties, reaching an average predicted initial concentration of 5.85 g/100mL, followed by maltotriose which reached an average predicted concentration of 1.38 g/100mL, and fructose reaching on average 0.41 g/100mL. As a result of the 4% (w/v)

addition prior to pitching, glucose artificially displayed similar initial levels to that of maltose at the commencement of fermentation, with a predicted average of 5.21 g/100mL. Similarly, final ethanol levels were in the expected range, averaging 5.13 % (v/v). In order to better visualize and describe fermentable carbohydrate and ethanol profiles for each variety, and to predict levels at any time throughout fermentation, the logistic equation was used to model changes in ethanol and each respective sugar using the Prism software. The following (Table 5-4 to Table 5-8) summarize equation parameters, and initial/final analyte concentrations, predicted by the models for ethanol and carbohydrates for each variety tested. From this analysis, each sugar was predicted with its own curve for visual assessment of consumption throughout the fermentation process.

Table 5-4. Maltose data was modeled using the logistic equation and profiles throughout fermentation from each variety were analysed. Estimates of equation parameter values with associated error are listed, and initial (Mal_0) and final (Mal_{75}) maltose concentrations are predicted (g/100mL).

Variety (Year Grown)	Mal_0	Mal_{75}	P_i (^oP)	P_∞ (^oP)	B (hr^{-1})	M (hr)	r^2	n
CDC Bold (2007)	5.83	0.53	5.88 (0.427)	0.53 (0.106)	-0.2244 (0.147)	20.8 (3.846)	0.973	24
AC Metcalfe (2007)	5.99	0.17	6.25 (0.154)	0.17 (0.04)	-0.1429 (0.012)	22.03 (0.603)	0.996	32
Harrington (2007)	5.72	0.17	5.84 (0.118)	0.17 (0.039)	-0.1838 (0.016)	21.17 (0.505)	0.995	31
CDC Dolly (2007)	5.56	0.21	6.57 (0.505)	0.01 (0.184)	-0.06981 (0.01)	25.8 (2.443)	0.986	26
TR251 (2007)	5.84	0.35	6.3 (0.16)	0.34 (0.032)	-0.1191 (0.008)	20.96 (0.662)	0.997	32
CDC Copeland (2007)	5.93	0.27	6.27 (0.257)	0.26 (0.061)	-0.1301 (0.016)	21.48 (1.01)	0.990	32
TR 306 (2007)	5.64	0.30	6.08 (0.445)	0.3 (0.074)	-0.1317 (0.023)	18.9 (1.872)	0.981	32
CDC Kendall (2007)	4.99	0.14	5.16 (0.172)	0.14 (0.066)	-0.1349 (0.017)	24.88 (0.74)	0.988	31
McLeod (2007)	5.44	0.38	6.06 (0.418)	0.36 (0.09)	-0.0983 (0.015)	21.47 (1.876)	0.983	32
CDC Helgason (2007)	5.80	0.28	6.68 (0.64)	0.23 (0.122)	-0.0904 (0.017)	20.35 (2.631)	0.981	29
BM9752D-17 (2007)	6.11	0.31	7.8 (0.975)	0.19 (0.142)	-0.0708 (0.012)	17.71 (3.944)	0.984	32
CDC Bold (2008)	6.31	0.28	6.7 (0.512)	0.33 (0.111)	-0.1469 (0.04)	19.84 (1.876)	0.980	27
AC Metcalfe (2008)	5.87	0.16	6.24 (0.111)	0.15 (0.027)	-0.1239 (0.006)	22.19 (0.435)	0.998	32
Harrington (2008)	5.79	0.40	6.06 (0.37)	0.39 (0.108)	-0.1298 (0.027)	23.05 (1.487)	0.970	32
CDC Dolly (2008)	6.25	0.07	7.4 (0.899)	-0.44 (0.475)	-0.0593 (0.014)	30.55 (3.979)	0.968	27
TR251 (2008)	6.03	0.19	6.92 (0.387)	0.15 (0.073)	-0.09261 (0.01)	20.44 (1.509)	0.992	32
CDC Copeland (2008)	6.17	0.24	7.47 (1.23)	0.2 (0.15)	-0.0900 (0.022)	16.98 (4.562)	0.966	32
TR 306 (2008)	5.80	0.16	6.2 (0.298)	0.15 (0.08)	-0.1163 (0.016)	22.78 (1.164)	0.987	32

Table 5-4. (continued)

Variety (Year Grown)	Mal_0	Mal_{75}	P_i (^oP)	P_∞ (^oP)	B (hr^{-1})	M (hr)	r^2	n
CDC Kendall (2008)	5.93	0.13	6.35 (0.184)	0.12 (0.051)	-0.1124 (0.009)	23.2 (0.7)	0.995	32
McLeod (2008)	5.67	0.59	7.98 (1.626)	0.11 (0.322)	-0.0482 (0.012)	18.24 (7.999)	0.979	32
CDC Helgason (2008)	5.77	0.27	6.09 (0.107)	0.26 (0.032)	-0.1206 (0.007)	23.5 (0.406)	0.998	29
BM9752D-17 (2008)	6.27	0.15	6.74 (0.21)	0.12 (0.067)	-0.1028 (0.009)	25.02 (0.775)	0.995	29

N.B. Bracketed values denote asymptotic standard error (ASE).

Table 5-5. Maltotriose data was modeled using the logistic equation and profiles throughout fermentation from each variety were analysed. Estimates of equation parameter values with associated error are listed, and initial ($MalTri_0$) and final ($MalTri_{75}$) maltotriose concentrations were predicted.

Variety (Year Grown)	$MalTri_0$	$MalTri_{75}$	P_i (^oP)	P_∞ (^oP)	B (hr^{-1})	M (hr)	r^2	n
CDC Bold (2007)	1.63	0.33	2.13 (0.669)	0.31 (0.058)	-0.0756 (0.028)	12.66 (11)	0.963	24
AC Metcalfe (2007)	1.50	0.25	1.55 (0.031)	0.25 (0.011)	-0.1381 (0.013)	24.54 (0.516)	0.994	32
Harrington (2007)	1.53	0.26	1.55 (0.023)	0.26 (0.009)	-0.1778 (0.014)	23.12 (0.39)	0.995	31
CDC Dolly (2007)	1.29	0.12	1.35 (0.037)	0.12 (0.022)	-0.1086 (0.01)	29.12 (0.935)	0.991	26
TR251 (2007)	1.54	0.33	1.63 (0.025)	0.33 (0.008)	-0.1091 (0.006)	24.28 (0.465)	0.998	32
CDC Copeland (2007)	1.49	0.29	1.53 (0.041)	0.28 (0.014)	-0.1372 (0.016)	24.28 (0.715)	0.989	32
TR 306 (2007)	1.44	0.25	1.52 (0.093)	0.25 (0.02)	-0.1296 (0.025)	20.73 (1.762)	0.976	32
CDC Kendall (2007)	1.20	0.24	1.21 (0.027)	0.24 (0.015)	-0.1478 (0.018)	28.83 (0.679)	0.986	31
McLeod (2007)	1.41	0.25	1.48 (0.063)	0.24 (0.024)	-0.1092 (0.016)	26.22 (1.23)	0.978	32
CDC Helgason (2007)	1.47	0.29	1.58 (0.094)	0.28 (0.03)	-0.0954 (0.017)	24.59 (1.935)	0.976	29
BM9752D-17 (2007)	1.42	0.28	1.57 (0.085)	0.26 (0.026)	-0.0816 (0.011)	24.85 (1.815)	0.984	32
CDC Bold (2008)	1.41	0.28	1.49 (0.083)	0.29 (0.025)	-0.1316 (0.033)	22.08 (1.492)	0.980	27
AC Metcalfe (2008)	1.35	0.26	1.39 (0.02)	0.26 (0.008)	-0.1307 (0.009)	25.84 (0.391)	0.996	32
Harrington (2008)	1.31	0.27	1.36 (0.063)	0.27 (0.022)	-0.1284 (0.025)	24.51 (1.283)	0.971	32
CDC Dolly (2008)	1.25	0.12	1.31 (0.073)	0.1 (0.051)	-0.0926 (0.017)	31.78 (2.285)	0.963	27
TR251 (2008)	1.41	0.27	1.53 (0.062)	0.26 (0.018)	-0.0989 (0.012)	23.75 (1.231)	0.989	32
CDC Copeland (2008)	1.46	0.26	1.78 (0.354)	0.25 (0.038)	-0.085 (0.025)	15.72 (6.397)	0.953	32
TR 306 (2008)	1.25	0.20	1.29 (0.038)	0.2 (0.014)	-0.1244 (0.015)	25.35 (0.778)	0.988	32
CDC Kendall (2008)	1.32	0.26	1.35 (0.025)	0.26 (0.012)	-0.132 (0.012)	27.56 (0.529)	0.993	32
McLeod (2008)	1.09	0.21	1.17 (0.051)	0.19 (0.023)	-0.0825 (0.01)	29.66 (1.563)	0.982	32
CDC Helgason (2008)	1.38	0.30	1.42 (0.016)	0.3 (0.007)	-0.1248 (0.007)	25.86 (0.342)	0.998	29
BM9752D-17 (2008)	1.29	0.24	1.32 (0.026)	0.24 (0.013)	-0.1249 (0.011)	28.74 (0.596)	0.992	29

N.B. Bracketed values denote asymptotic standard error (ASE).

Table 5-6. Glucose data was modeled using the logistic equation and profiles throughout fermentation from each variety were analysed. Equation parameter values are listed, and initial (Glu_0) and final (Glu_{75}) glucose concentrations were predicted.

Variety (Year Grown)	Glu_0	Glu_{75}	P_i (°P)	P_∞ (°P)	**B** (hr^{-1})	**M** (hr)	r^2	**n**
CDC Bold (2007)	5.67	0.01	5.96 (0.114)	0.01 (0.023)	-0.2278 (0.013)	13.06 (0.501)	0.999	24
AC Metcalfe (2007)	5.79	-0.05	6.84 (0.633)	-0.05 (0.053)	-0.1326 (0.015)	12.92 (2.022)	0.991	30
Harrington (2007)	5.23	0.00	5.31 (0.072)	0 (0.017)	-0.2459 (0.014)	17.02 (0.419)	0.999	31
CDC Dolly (2007)	4.50	0.00	4.51 (0.028)	0 (0.011)	-0.5204 (0.124)	16.81 (1.255)	1.000	26
TR251 (2007)	5.50	-0.02	5.71 (0.141)	-0.02 (0.024)	-0.1986 (0.013)	16.42 (0.666)	0.997	32
CDC Copeland (2007)	5.38	-0.02	5.64 (0.106)	-0.02 (0.017)	-0.2089 (0.009)	14.64 (0.446)	0.999	32
TR 306 (2007)	5.15	-0.01	5.4 (0.085)	-0.01 (0.013)	-0.2159 (0.007)	14.08 (0.362)	0.999	32
CDC Kendall (2007)	4.59	0.00	4.64 (0.055)	0 (0.016)	-0.2597 (0.017)	17.9 (0.395)	0.998	31
McLeod (2007)	5.18	-0.01	5.42 (0.098)	-0.01 (0.017)	-0.1837 (0.009)	16.87 (0.456)	0.999	32
CDC Helgason (2007)	5.21	-0.01	5.6 (0.131)	-0.01 (0.016)	-0.1674 (0.008)	15.48 (0.535)	0.999	29
BM9752D-17 (2007)	5.25	-0.01	5.46 (0.122)	-0.01 (0.021)	-0.1943 (0.012)	16.63 (0.601)	0.998	32
CDC Bold (2008)	5.50	0.00	5.61 (0.053)	0.01 (0.014)	-0.277 (0.009)	14.16 (0.287)	0.999	27
AC Metcalfe (2008)	5.43	-0.03	6.15 (0.345)	-0.03 (0.033)	-0.1462 (0.012)	13.9 (1.248)	0.995	32
Harrington (2008)	5.04	-0.01	5.33 (0.118)	-0.01 (0.017)	-0.1891 (0.009)	15.12 (0.52)	0.998	32
CDC Dolly (2008)	4.93	0.00	4.94 (0.038)	0 (0.013)	-0.3936 (0.027)	15.31 (0.5)	0.999	27
TR251 (2008)	5.33	-0.01	5.6 (0.122)	-0.01 (0.018)	-0.1925 (0.01)	15.55 (0.542)	0.998	32
CDC Copeland (2008)	5.35	0.02	5.69 (0.226)	0.02 (0.031)	-0.1981 (0.015)	13.8 (0.876)	0.995	32
TR 306 (2008)	5.25	-0.02	5.63 (0.151)	-0.02 (0.02)	-0.1965 (0.009)	13.38 (0.571)	0.998	32
CDC Kendall (2008)	4.98	0.00	5.13 (0.061)	0 (0.011)	-0.2225 (0.008)	15.77 (0.314)	0.999	32
McLeod (2008)	4.90	-0.01	5.28 (0.171)	-0.01 (0.026)	-0.1591 (0.01)	16.05 (0.769)	0.997	27
CDC Helgason (2008)	5.19	-0.02	5.67 (0.126)	-0.02 (0.014)	-0.1467 (0.006)	16.18 (0.506)	0.999	29
BM9752D-17 (2008)	5.37	-0.02	5.82 (0.223)	-0.02 (0.03)	-0.1786 (0.012)	13.84 (0.827)	0.997	29

N.B. Bracketed values denote asymptotic standard error (ASE).

Table 5-7. Fructose data was modeled using the logistic equation and profiles throughout fermentation from each variety were analysed. Equation parameter values are listed, and initial (Fru_0) and final (Fru_{75}) fructose concentrations were predicted.

Variety (Year Grown)	Fru_0	Fru_{75}	P_i (°P)	P_∞ (°P)	**B** (hr^{-1})	**M** (hr)	r^2	**n**
CDC Bold (2007)	0.45	0.03	0.46 (0.02)	0.03 (0.006)	-0.1803 (0.064)	23.21 (1.417)	0.991	24
AC Metcalfe (2007)	0.48	0.03	0.48 (0.008)	0.03 (0.004)	-0.2343 (0.021)	27.23 (0.301)	0.992	32
Harrington (2007)	0.49	0.03	0.5 (0.008)	0.03 (0.004)	-0.2181 (0.018)	24.63 (0.316)	0.993	31
CDC Dolly (2007)	0.40	0.02	0.4 (0.006)	0.02 (0.003)	-0.3318 (0.043)	20.45 (0.386)	0.995	26
TR251 (2007)	0.50	0.03	0.5 (0.003)	0.03 (0.001)	-0.2125 (0.007)	26.1 (0.104)	0.999	32
CDC Copeland (2007)	0.45	0.02	0.45 (0.004)	0.02 (0.002)	-0.2432 (0.011)	24.14 (0.158)	0.998	32
TR 306 (2007)	0.41	0.02	0.41 (0.005)	0.02 (0.003)	-0.2244 (0.015)	23.64 (0.259)	0.995	32
CDC Kendall (2007)	0.40	0.02	0.4 (0.008)	0.02 (0.004)	-0.202 (0.02)	26.54 (0.373)	0.991	31

Table 5-7. (continued)

Variety (Year Grown)	Fru_0	Fru_{75}	P_i (ASE)	P_∞ (ASE)	B (ASE)	M (ASE)	r^2	n
McLeod (2007)	0.44	0.02	0.44 (0.008)	0.02 (0.004)	-0.1796 (0.018)	27.04 (0.401)	0.992	32
CDC Helgason (2007)	0.37	0.02	0.37 (0.007)	0.02 (0.004)	-0.2391 (0.044)	27.24 (0.578)	0.991	29
BM9752D-17 (2007)	0.43	0.02	0.43 (0.007)	0.02 (0.004)	-0.173 (0.016)	26.74 (0.356)	0.994	32
CDC Bold (2008)	0.43	0.02	0.44 (0.012)	0.02 (0.005)	-0.203 (0.038)	21.72 (0.662)	0.990	27
AC Metcalfe (2008)	0.43	0.03	0.44 (0.006)	0.03 (0.003)	-0.238 (0.019)	26.97 (0.257)	0.994	32
Harrington (2008)	0.37	0.02	0.38 (0.005)	0.02 (0.002)	-0.1861 (0.013)	25.22 (0.281)	0.996	32
CDC Dolly (2008)	0.36	0.02	0.37 (0.006)	0.02 (0.003)	-0.2644 (0.038)	20.21 (0.536)	0.994	27
TR251 (2008)	0.41	0.03	0.42 (0.006)	0.03 (0.003)	-0.1744 (0.013)	26.6 (0.292)	0.996	32
CDC Copeland (2008)	0.38	0.02	0.39 (0.02)	0.02 (0.007)	-0.1306 (0.024)	24.37 (1.203)	0.974	32
TR 306 (2008)	0.36	0.02	0.36 (0.005)	0.02 (0.003)	-0.2961 (0.022)	24.54 (0.243)	0.993	32
CDC Kendall (2008)	0.36	0.03	0.36 (0.006)	0.03 (0.003)	-0.1931 (0.016)	26.07 (0.325)	0.994	32
McLeod (2008)	0.36	0.02	0.36 (0.004)	0.02 (0.002)	-0.1958 (0.012)	29.36 (0.264)	0.997	32
CDC Helgason (2008)	0.38	0.02	0.38 (0.002)	0.02 (0.001)	-0.2219 (0.014)	28.83 (0.27)	0.999	29
BM9752D-17 (2008)	0.39	0.03	0.4 (0.008)	0.03 (0.004)	-0.1998 (0.021)	26.39 (0.405)	0.990	29

N.B. Bracketed values denote asymptotic standard error (ASE).

Table 5-8. Ethanol data was modeled using the logistic equation and profiles throughout fermentation from each variety were analysed. Equation parameter values are listed, and initial ($EtOH_0$) and final ($EtOH_{75}$) ethanol concentrations were predicted.

Variety (Year Grown)	$EtOH_0$	$EtOH_{75}$	P_i (% w/w)	P_∞ (% w/w)	B (hr^{-1})	M (hr)	r^2	n
CDC Bold (2007)	0.59	5.48	0 (0)	5.48 (0.06)	-0.1553 (0.013)	13.68 (0.794)	0.980	21
AC Metcalfe (2007)	0.30	5.53	0 (0)	5.53 (0.032)	-0.1623 (0.007)	17.57 (0.345)	0.992	30
Harrington (2007)	0.33	5.21	0 (0)	5.21 (0.022)	-0.1541 (0.004)	17.41 (0.233)	0.997	28
CDC Dolly (2007)	0.28	4.54	0 (0)	4.54 (0.041)	-0.1543 (0.01)	17.74 (0.627)	0.990	21
TR251 (2007)	0.15	5.26	0 (0)	5.26 (0.045)	-0.1732 (0.013)	20.37 (0.453)	0.987	30
CDC Copeland (2007)	0.18	5.14	0 (0)	5.14 (0.04)	-0.1643 (0.01)	20.18 (0.388)	0.989	30
TR 306 (2007)	0.12	5.01	0 (0)	5.01 (0.039)	-0.1801 (0.012)	20.76 (0.369)	0.990	30
CDC Kendall (2007)	0.10	4.88	0 (0)	4.88 (0.116)	-0.1963 (0.043)	19.86 (1.199)	0.911	29
McLeod (2007)	0.15	4.71	0 (0)	4.72 (0.041)	-0.1525 (0.011)	22.47 (0.374)	0.989	30
CDC Helgason (2007)	0.08	4.96	0 (0)	4.96 (0.06)	-0.194 (0.028)	21.51 (0.533)	0.978	27
BM9752D-17 (2007)	0.29	5.39	0 (0)	5.39 (0.049)	-0.1358 (0.009)	21.02 (0.441)	0.988	31
CDC Bold (2008)	0.10	5.26	0 (0)	5.26 (0.064)	-0.2035 (0.024)	19.25 (0.944)	0.983	21
AC Metcalfe (2008)	0.27	5.30	0 (0)	5.3 (0.037)	-0.1592 (0.007)	18.42 (0.384)	0.993	32
Harrington (2008)	0.19	4.97	0 (0)	4.97 (0.062)	-0.155 (0.015)	20.75 (0.602)	0.974	30
CDC Dolly (2008)	0.17	4.80	0 (0)	4.8 (0.086)	-0.1634 (0.025)	20.18 (1.192)	0.965	21
TR251 (2008)	0.15	5.31	0 (0)	5.31 (0.04)	-0.1662 (0.011)	21.29 (0.375)	0.990	30
CDC Copeland (2008)	0.20	5.18	0 (0)	5.19 (0.056)	-0.1535 (0.013)	20.97 (0.508)	0.984	31
TR 306 (2008)	0.17	4.91	0 (0)	4.91 (0.041)	-0.1622 (0.012)	20.44 (0.44)	0.985	29

Table 5-8. (continued)

Variety (Year Grown)	$EtOH_0$	$EtOH_{75}$	P_i (ASE)	P_∞ (ASE)	B (ASE)	M (ASE)	r^2	n
CDC Kendall (2008)	0.20	5.04	0 (0)	5.04 (0.037)	-0.1537 (0.009)	20.66 (0.357)	0.991	30
McLeod (2008)	0.32	4.59	0 (0)	4.6 (0.07)	-0.1127 (0.011)	22.92 (0.691)	0.973	30
CDC Helgason (2008)	0.26	5.27	0 (0)	5.28 (0.051)	-0.1375 (0.01)	21.65 (0.471)	0.990	29
BM9752D-17 (2008)	0.18	5.26	0 (0)	5.26 (0.057)	-0.1644 (0.014)	20.42 (0.5)	0.984	27

N.B. Bracketed values denote asymptotic standard error (ASE); P_i and P_∞ describe percent ethanol concentration (w/w); Pi constrained to zero in order to better illustrate ethanol levels early in fermentation.

Figure 5-10 exemplifies a typical carbohydrate and ethanol profile for the 2007 Harrington variety (raw data not shown). As can be seen from the graph, maltose and glucose accounted for the majority of the fermentable sugars available early in fermentation, and showed a rapid decline in concentration after nearly 20 hours post inoculation. As expected, glucose levels declined quicker than maltose due to the immediate and rapid utilization of this simple monosaccharide by the yeast (93). After approximately 30 hours of fermentation, nearly all of the glucose had been exhausted, with maltose rapidly approaching similar levels. Maltotriose and fructose concentrations displayed much lower initial concentrations but followed similar trends, however, exhibiting delayed peak consumption due to the preferred initial utilization of readily available glucose and maltose (93). Following 46 hours post inoculation, no substantial changes in sugar concentrations were observed, and the fermentation was deemed complete. Small but detectable amounts of maltose and maltotriose remained, while glucose and fructose were deemed to be below the limit of detection upon completion of fermentation. This is in support of the theory of yeast carbohydrate utilization whereby the simpler monosaccharides are readily taken up by the cell through facilitated diffusion, and are therefore preferentially consumed before larger sugars such as maltose and maltotriose, which require active transport into the cell (93).

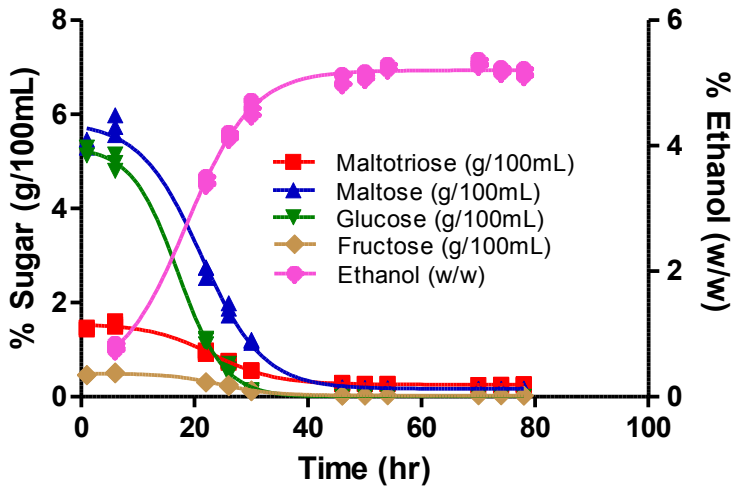

Figure 5-10. Carbohydrate and ethanol profiles of Harrington (2007) throughout fermentation

With the onset of flocculation, yeast then fall out of suspension, leaving behind any remaining fermentable sugars and dextrin. If given a larger window of fermentation, it is likely that the remaining maltose and maltotriose would eventually be consumed by those yeast cells still exposed to the fermenting medium, however, such extensions would require lengthy and time consuming additional measures, and result in little informational gain, and therefore were deemed out of scope of the present study. Finally, ethanol production was also fitted using the logistic model, and displayed an expected mirrored (inverse) curve to that of sugar consumption, increasing maximally during peak carbohydrate consumption, and reaching a typical final plateau concentration of approximately 5% (w/w) by the end of fermentation.

5.3. Carbohydrate Consumption Rates

Of particular interest is the phenomenon exhibited in Figure 5-11, exemplified through data collected on Harrington (2007), where it can be seen that despite high levels of glucose early in the fermentation (4% addition + endogenous glucose present), maltose is immediately consumed. This is in contrast to the widely held view that maltose utilization typically does not

occur until roughly 50 % of glucose has first been consumed (93). Similar findings were seen with all varieties (data not shown). The fact that the logistic model fitted all of the sugar consumption data well, indicates that the consumption of sugars began at t=0.

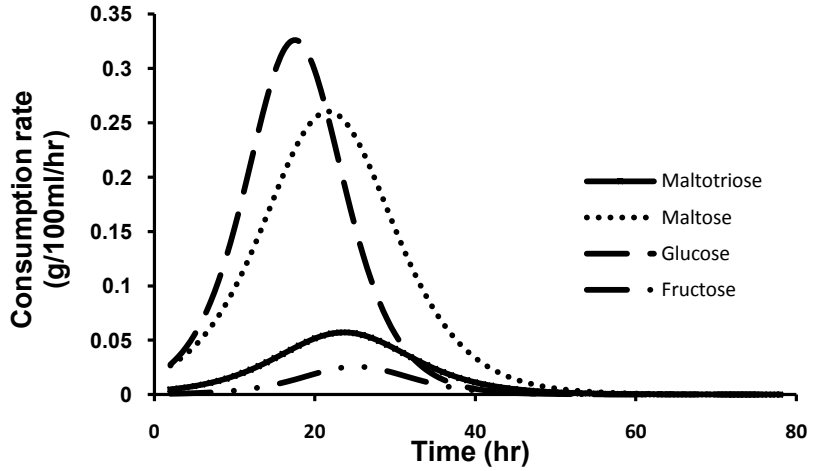

Figure 5-11. Carbohydrate consumption rates displayed in Harrington (2007).

5.4. Absorbance

Turbidity of fermenting wort was monitored through measures of absorbance at 600 nm for each sample and at all time intervals. Resultant data indicates levels of YIS, and serves as an indirect measure of yeast activity in the fermenting medium. Absorbance data was fitted using a recently developed Tilted Gaussian function (equation 6), allowing a representative model to describe the data. Table 5-9 summarizes the predicted parameter values for the model (see Appendix A5 for raw data). Unfortunately, due to a technician error throughout the fermentation of certain varieties, some samples (i.e., time points) were mistakenly measured at 480nm (the default start-up setting of the spectrophotometer used) instead of the standard 600nm, a setting which must be manually programmed prior to each use. Therefore, these

points were removed from regression analyses, resulting in generally higher residual error and poor curve fits (evident from the low coefficient of determination; r^2). Refer to Table 5-9 for those varieties which had data omitted from regression analysis, indicated by having fewer than 33 data points. Remaining data is limited, but does indicate YIS trends.

Table 5-9. Summary of Tilted Gaussian parameter values predicted for all absorbance data.

Variety (Year Grown)	A (AU)	μ (hr)	σ (hr)	R (AU/hr)	r^2	n
CDC Bold (2007)	3.664 (1.08)	21.79 (3.536)	25.17 (11.99)	0.027 (0.011)	0.243	24
AC Metcalfe (2007)	4.109 (0.352)	25.08 (0.944)	23.96 (3.01)	0.016 (0.004)	0.895	24
Harrington (2007)	4.004 (0.077)	22.96 (0.73)	23.09 (0.873)	0.02 (0.002)	0.953	33
CDC Dolly (2007)	4.438 (0.637)	39.74 (9.38)	37.54 (5.051)	-0.003 (0.019)	0.931	24
TR251 (2007)	4.542 (0.404)	32.47 (4.38)	29.01 (2.453)	-0.001 (0.01)	0.960	24
CDC Copeland (2007)	3.963 (0.119)	26.8 (1.67)	27.9 (1.948)	0.012 (0.004)	0.903	33
TR 306 (2007)	4.285 (0.189)	26.85 (2.047)	25.45 (2.375)	0.008 (0.006)	0.838	33
CDC Kendall (2007)	4.135 (0.07)	27.32 (0.838)	26.21 (0.966)	0.02 (0.002)	0.961	33
McLeod (2007)	4.941 (2.497)	32.44 (13.67)	25.84 (4.687)	-0.008 (0.072)	0.622	24
CDC Helgason (2007)	3.85 (0.165)	25.89 (1.892)	24.86 (2.241)	0.022 (0.005)	0.783	33
BM9752D-17 (2007)	3.84 (0.209)	28.61 (3.439)	29.66 (2.369)	0.014 (0.006)	0.963	24
CDC Bold (2008)	~6 (N/A)[1]	56.97 (30.15)	44.51 (5.622)	-0.037 (0.077)	0.703	24
AC Metcalfe (2008)	3.693 (0.156)	26.21 (2.138)	29.54 (4.344)	0.018 (0.006)	0.892	24
Harrington (2008)	4.614 (0.356)	35.11 (3.972)	32.16 (2.955)	0.005 (0.011)	0.854	33
CDC Dolly (2008)	5.797 (4.447)	~60 (N/A)[1]	48.82 (4.412)	-0.028 (0.072)	0.747	24
TR251 (2008)	4.444 (0.398)	34.67 (4.268)	31.69 (2.076)	0.006 (0.01)	0.961	24
CDC Copeland (2008)	4.353 (0.283)	31.64 (3.625)	30.56 (3.271)	0.008 (0.01)	0.812	33
TR 306 (2008)	3.928 (0.156)	26.77 (2.242)	28.02 (2.616)	0.019 (0.006)	0.816	32
CDC Kendall (2008)	4.288 (0.197)	33.07 (2.546)	31.88 (2.089)	0.012 (0.006)	0.915	33
McLeod (2008)	3.881 (0.899)	27.8 (8.792)	27.21 (3.825)	0.025 (0.032)	0.872	24
CDC Helgason (2008)	3.745 (0.133)	29.24 (2.159)	30.52 (2.174)	0.022 (0.005)	0.885	33
BM9752D-17 (2008)	4.346 (0.531)	34.73 (6.053)	32.87 (2.886)	0.005 (0.013)	0.933	24

1. Values calculated are approximate due to software calculations hitting the limits of parameter constraints.
N.B. Bracketed values denote standard error (SE).

5.5. Modified Wort Fermentation

In an effort to elucidate a probable cause of maltose utilization early in fermentation, a third round of fermentation was completed using only one malt variety, and artificially varying the initial concentrations of maltose and glucose. As the miniature fermentation assay requires a

glucose spike of 4% (w/v) prior to pitching (6), initial levels were modified to observe the effect of initial carbohydrate profiles on yeast utilization of maltose early in fermentation. Results of HPLC analysis of fermentable carbohydrates and ethanol are summarized in Appendix A6, and indicate that sugar consumption is not substantially altered by initial carbohydrate spike. It can be seen that all sugars display a non-zero consumption rate early in fermentation (observed as little as one hour post inoculation), particularly evident in glucose and maltose, whereby the latter experience substantial utilization concurrently.

5.6. Effect of Malt Variety and Crop Year

In order to gauge the error magnitude of utilizing distinct yeast batch grow-ups per week on the overall fermentation performance of the malt varieties, parameter values from logistic modeling of RE, maltotriose, maltose, glucose, fructose, and AE were analysed using a two-way analysis of variance (ANOVA) by year and variety to distinguish significant effects upon each of the function parameters. Varieties were first analyzed by grouping all data together. However, due to the unavoidable utilization of multiple yeast grow-ups between fermentation rounds (discussed above) data was then categorized by the week in which they were fermented, to remove this possible confounding effect. Statistical significance values (p-values) are summarized in tables A7-1 through A7-6. Each set of analyses surmised the percentage of the variance contributed by each parameter (data not shown), and provided associated significance for three levels of comparison: statistical interaction, year, and variety. That is, each logistic model parameter for each variety was compared with the aim of discerning which factor had a greater and/or significant effect upon it: growing year or malt variety. Indeed, the level of statistical interaction also impacts the interpretation and validity of the results, obviating any direct conclusions in cases where interaction played a statistically significant role. Tables 5-10

and 5-11 summarize the results of both sets of ANOVA analyses simply on the basis of total significant results between variety and year. Detailed results are summarized in Appendix A7.

Table 5-10. Summary of two-way ANOVA with all measurement data grouped together. Two-way ANOVA was conducted by year and variety against each of the predicted parameter values of the logistic function describing each measurement. Results summarize the number of statistically significant results for each measurement, out of a total of four possible (i.e., P_i, P_∞, B, and M per measurement)

Measurement	Interaction[1]	Year	Variety
RE	1	3	4
Maltotriose	1	1	4
Maltose	0	0	2
Glucose	0	2	3
Fructose	3	1	4
AE	3	4	4
Total	8	11	21

1. Interaction is a statistical function describing the level of variability introduced due to opposing parameter interactions between year and variety.

Table 5-11. Summary of two-way ANOVA with measurement data grouped by week fermented. Two-way ANOVA was conducted by year and variety against each of the predicted parameter values of the logistic function describing each measurement. Results summarize the number of statistically significant results for each measurement, out of a total of 24 possible (i.e., P_i, P_∞, B, and M for each measurement RE, maltotriose, maltose, glucose, fructose, AE)

	Total Significant results ($p<0.05$)		
Fermentation Schedule	Interaction[1]	Year	Variety
Week 1	0	1	11
Week 2	6	8	17
Week 3	6	14	13
Week 4	3	4	9
Total	15	27	50

1. Interaction is a statistical function describing the level of variability introduced due to opposing parameter interactions between year and variety.

6. Discussion

6.1. The Current Paradigm

Despite the seemingly large abundance of data collected throughout the course of this study, the end goal was ultimately quite simple: observe and attempt to quantify the relative fermentability of a variety of prominent Canadian two-row barley malt varieties through measurement of an array of crucial fermentation parameters. This constituted a broad spectrum, multi-factorial approach, aimed at elucidating the overall relative fermentation performance among select malting barley varieties that have previous and/or potential significance in the Canadian grain market, while also exerting some measure of explorative action. Measurement of a number of fermentation parameters to observe possible correlation among variables previously thought unrelated was an important aspect of this study. Indeed much research suffers from a lack of encompassing data collection, whereby conclusions will often be derived from few measured parameters. Though not detrimental to our furthered understanding of fundamental principles, such research styles in brewing can easily oversimplify complex systems, with the potential of losing sight of the "bigger picture" paradigm, in favour of drawing select conclusions on specific and often isolated situations, perhaps having only limited practical significance.

However, in light of this broad experimental approach, a number of controls were incorporated as part of this larger design, to aid the confident establishment of fermentation activity. Such aspects included the incorporation of select barley varieties that are typically used as feed and generally display poor fermentation quality (e.g., CDC Dolly, CDC Bold) to use as negative fermentability controls, while also incorporating those varieties which generally display high fermentation performance (e.g., Harrington, CDC Copeland). Additionally, a key control in the present study was the inclusion of a barley variety specifically bred for its display

of thermally stabile β-amylase (BM9752D-17), a key starch degrading enzyme of malt. Comparison to that of its thermo labile counterparts was aimed to give credence to the widely held belief that the sustained activity of β-amylase throughout the latter stages of mashing could greatly influence available levels of fermentable carbohydrates of the wort (37, 38, 39), and therefore afford direct control of fermentation potential.

Ongoing breeding programs and hybridization of malting barley varieties has been at the forefront of modern brewing research in light of not only the possibility to accentuate certain desirable malting quality traits, but also to help mitigate variable agronomic performance often displayed in traditional crops. Such studies aim to not only maintain, but to optimize and accentuate those intrinsic features of certain barley cultivars that display ideal malting and fermenting qualities, such as enhanced enzyme activity, increased resistance to common infectious agents, maintenance of consistent crop quality between (sometimes highly) variable growing seasons, increased yearly crop yields through enhanced crop viability and vitality (hardiness), and improved malting quality factors (13, 14, 33, 83, 92).

However, in order to truly assess malting and fermentation performance resulting from the various fields of brewing research, many researchers throughout history have attempted to assign specific fermentation values and tolerances to complex systems. Unfortunately, few studies have successfully modeled fermentation performance quantitatively. Recently, several reports and official methods of analyses have established methods for mathematically modeling fermentation activity through the use of non-linear regression modeling (6, 55, 56, 57, 63, 90). Applying the logistic model (equation 5), which has well established use in the biological fields for monitoring and/or predicting growth curves (90), to measurements of density taken throughout fermentation has yielded highly predictable models with only a (relatively) small number of data points. This has resulted in the establishment of an extremely useful tool for

visualization, and description of fermentation performance, where none previously had existed. Such curve fitting is now routine among many brewing researchers, comparing fermentation performance between and among various barley varieties, and under variable conditions. Particularly of interest in the current study, was the possible application of the same model to individual carbohydrate consumption, and whether fermentation could be quantified on the basis of specific sugar utilization. Similarly, application of the logistic model to the corresponding production of ethanol was also investigated, as it essentially represents the mirrored action of carbohydrate consumption by yeast (equation 1).

Regardless of the data collected, and conclusions drawn from it, there remains a fundamental problem with the definition of malt fermentability. Due to the high variability expressed in a complex biological system, predicting and defining fermentability has posed serious challenge to brewing scientists. To the point where there is no single, formal agreement as to the exact definition of "model fermentability", given that final sensory factors such as aroma, mouthfeel (e.g., unfermentable constituents remaining), alcohol content, foam retention, laciness, colour, etc., will ultimately dictate the measure of a successful fermentation, and that these factors can greatly vary between accepted brewing regimens. Pragmatically, however, brewing researchers must adopt a convenient yet representative adaption, which can lead to varied and (unfortunately) incomparable data sets between labs. This is not to say that any one method is fundamentally wrong, in fact quite the opposite, rather each method illustrates different aspects of a similar phenomenon. Though a somewhat regrettable circumstance, it at least poses some measure of flexibility to the varied fields of brewing research, and certainly encourages the observation of multi-factorial designs.

The definition of fermentability throughout brewing research is analogous to the determination of enzyme activity, whereby the activity is described only in relation to a defined

set of reaction conditions and detection methods employed. Indeed different manufacturers and producers of purified enzyme reagents define enzyme activity in different manners, depending on the method preferred. For example, enzyme activity is often defined by the rate of product formation, however, it is dependent upon the substrate used, method of detection for quantification of product, and the reaction environment facilitating the process. Similarly, though fermentability is often expressed as a percentage of the original extract consumed throughout fermentation (ADF), the process of malting, mashing, and fermenting can vary greatly between laboratories. For example, Gomez and Edney (48) describe several laboratory scale fermentation tests, each aimed to assess fermentability as the total amount of extract fermented under continuously agitated (stirred) conditions, using a variety of malts, at a variety of pitching rates, using a defined yeast strain, fermentation temperature, and time. The use of a stirred fermentation artificially promotes YIS, and discourages floc formation, which in turn could increase the final attenuation of the wort more than would otherwise be achieved naturally. Such a test emphasizes the best possible attenuation for a given wort extract level if all nutrients have been maximally utilized by the yeast, yielding the apparent attenuation limit of a given extract.

As well, a number of laboratory scale assays have originally been developed and optimized over the years as a means to rapidly determine the flocculation behaviour of yeast, specifically to detect premature yeast flocculation, each with variations on fermentation parameters (79). These compared to the present study which defines fermentability as the percentage of extract lost throughout the miniaturized fermentation assay, a measure aimed to more closely resemble the natural fermentation environment facilitated in a brewery scale production, where pitching rates remain constant, and fermentation occurs over a longer period. Common to most laboratory scale assays is the quantification of fermentability through calculation of wort

attenuation; the loss of gravity due to sugar metabolism and alcohol formation. The degree to which the extract ferments is largely dependent on the conditions utilized. Additionally, Evans (40) points out that the use of AAL alone may not be the best predictor of subsequent malt fermentability upon commercial utilization, and that performance predictions may better be defined through the measurement of malt diastatic power enzymes (40, 41).

6.2. Comparison of Fermentation Performance between Varieties

One of the goals of this study was to compare the relative fermentability of a malt variety displaying high β-amylase thermostability, to varieties expressing the typical lower thermostable variants. As anticipated, malt variety BM9752D-17 showed a substantially larger, and consistently higher β-amylase thermostability compared to any of the other varieties, as determined by the enzyme stability assays conducted directly on all the malt varieties (Appendix A2). This is due to its known expression of the high enzyme stability form of the β-amylase gene, Sd2-H (McLeod, A., Grain Research Laboratory, CGC, Winnipeg, MB, Personal Communication). Although BM9752D-17 performed very well, achieving a high ADF and displaying consistent results between seasons, it was not the highest fermenting variety either crop year when comparing ADF results from the miniature fermentation assay (see Table 5-3). Though it achieved a respectable level of attenuation, approximately 85% of the original extract each year, many of the other varieties, including the feed variety CDC Bold, achieved equal or higher attenuation in at least one of their respective crop seasons. Only CDC Dolly and McLeod performed consistently lower, unsurprising given their predominant use as feed varieties. Interestingly, Harrington 2008 also performed considerably poorer when compared to most of the other varieties, both in absolute ADF, and with respect to varietal consistency between seasons. Reaching an ADF of approximately 88% in 2007, and substantially dropping to

roughly 83% the following year, perhaps lends evidence to the theory of declining genotypic stability in the Harrington breed line (71). However, it should be noted that variation between crop years could have been influenced not only by variation in seasonal conditions, but also growing location, given that the 2007 and 2008 crop varieties were grown in separate areas. Regardless, Harrington displayed the largest decline amongst tested varieties, which may indicate a greater susceptibility to a particular stressor. In light of this, BM9752D-17 could be regarded as a superior malting barley variety to that of Harrington, as yearly fermentation performance consistency is a desirable quality trait for maltsters and brewers (BMBRI, 2008), with whom a chief concern is the stable production of consistent beverages, displaying nearly identical quality characteristics each year, regardless of environmental variability and its impact on raw material. Additionally, BM9752D-17 displayed reliable malting quality despite two seemingly distinct growing seasons and sites, evident from the varied results of the other varieties between years. The 2007 crop season unanimously produced malt which achieved higher degrees of attenuation to that of the 2008 season. However, BM9752D-17 displayed only a slight change between years, retaining similar fermentation performance regardless of growing conditions.

Pragmatically, these results neither confirm nor refute the hypothesis that greater β-amylase thermostability effects greater fermentation performance. As evident from the final ADF achieved by BM9752D-17, crop seasons 2007 and 2008 both produced only a moderately fermenting malt, when compared to the other varieties of the same year. Harrington, AC Metcalfe, CDC Copeland, CDC Kendall, along with the feed varieties CDC Bold, and CDC Helgason (2007) all produced malt which attained a visibly higher degree of fermentation than BM9752D-17 (2007). Similarly, the following season (2008), CDC Helgason, CDC Kendall, and CDC Copeland all achieved higher degrees of fermentability when compared to BM9752D-

17 of the same year, though it should be noted the differences attained were smaller than observed the previous year. This is likely due in large part to the apparent inferior growing conditions generally exhibited during the 2008 crop season, as evident from the lower over-all fermentation performance displayed by each malted variety when compared to the previous season. On average, varieties grown in 2007 achieved an ADF of 85.9% with a standard deviation (SD) of ±2.1%, compared with 84.6% SD ±2.5% achieved in the 2008 crop.

6.2.1. Multiple Yeast Croppings

Not all of the above mentioned variation can be accounted through seasonal aberration, indeed significant variation could also have arisen through the use of multiple yeast grow-ups (croppings) utilized over consecutive fermentations (see Appendix A7.). Given that the present study documented fermentability at the end of fermentation, any and all environmental fluctuations occurring as a result of assay preparation/implementation will have contributed to the overall variability experienced in the data. This, perhaps quite significantly, would include yeast crop variability, however may also arise from the myriad of additional factors typical of operator error, including (but not limited to) measurement accuracy of assay reagents, yeast cell counting, age of malt utilized, and instrument error. Consequence of limited laboratory resources, six fermentations were the maximum possible per week for the present experiment, necessitating the need for repeated yeast grow-ups prior to each fermentation, as described in section 4.5. It has been documented (51, 70, 80, 81, 84, 85, 100) that yeast viability, vitality, and fermentation activity can change over time as yeast cells are subject to repeated use, often with regard to serial re-pitching on an industrial scale.

Although there is convincing evidence that this does not constitutively occur with every yeast strain (80, 89, 90), it is common practice in industry to reuse a single batch of yeast for

approximately 8-15 sequential generations (81). Therefore yeast growing conditions were closely regulated and controlled in the present experiment (sections 4.3. to 4.5.), and yeast re-cropping onto stock slants was not performed between fermentations (i.e., each yeast grow-up was inoculated from a single set of stock slants). Yeast crop variability between batches is possible to have occurred nonetheless, given the established body of evidence suggesting error over successive and multiple generations. Currently, there is no data specific to the laboratory yeast strain employed which can gauge the error magnitude attributed to the use of multiple crops. Therefore, it is the recommendation of the author that future studies include such analyses in an effort to quantify the possible variation caused by sequential yeast batch grow-ups. In light of this, two-way ANOVAs were conducted on fermentation parameters in effort to quantify any significant effects of both malt variety and crop year (see section 5.6). Two separate analyses were conducted: first by grouping all data together (Table 5-10), and then grouped according to the week in which the varieties were fermented (Table 5-11).

As expected, varietal differences accounted for the majority of the variation observed between logistic parameter values RE, maltotriose, maltose, glucose, fructose, and AE. However, crop year accounts for more than 50% these values, and therefore cannot be disregarded as a substantial source of variation in fermentation character across crop seasons. Parameter values showing a significant level of interaction ($p<0.05$), are difficult to interpret, as this suggests they did not behave similarly (i.e., consistently) between varieties as they did between crop years. As stated previously, and due to the level of variation conferred by statistical interaction and crop season, it would be highly recommended that future endeavors to capture varietal variation incorporate more data points, across several crop seasons, and with replicate analyses in order to mitigate possible error due to the limited sample size available for each malt variety in the current study.

6.2.2. Further Sources of Variance

Variance in fermentation parameters noted above can be greatly influenced by the growing conditions provided for the barley which can introduce substantial variability to the fermentation quality of the malt. Factors such as seeding rate can have an effect upon kernel development, where higher seeding rates have been generally regarded as detrimental on kernel size; some studies have shown it to be beneficial in some cultivars, producing less variable sized kernels. This has shown improved malting quality through increased endosperm modification efficiency as a result of kernel uniformity which resulted in greater extract recovery (34). Varieties tested such as CDC Copeland and AC Metcalfe have shown greater overall fermentation quality between years, as measured by the logistic function of AE over time, with less variation than the other nine varieties included in the present study. It is possible that kernel development under specified environmental conditions would have effected some measure of improved malting quality in the aforementioned barley cultivars compared to the other varieties, as it has been implicated that growing conditions impart significant effect upon subsequent malting quality (96). Additionally, application of fertilizer has been shown to influence the growth and development of the barley grain, and such conditions would assume to be preferred in these cultivars (96). Harrington, once again, has shown varied malting performance between crop seasons, as has been documented (71), suggesting that this variety has become less stable, and perhaps less capable of adapting to changing environmental conditions between seasons.

6.2.3. CDC Copeland versus AC Metcalfe

Using the data from both harvest years, CDC Copeland slightly outperformed AC Metcalfe according to a final predicted ADF (Tables 5-2 and 5-3), which is supported by the

findings of Smith *et al.* (86) who studied the relative malting quality between the two barley cultivars while modeling the financial risk for growers associated with each. Using a variety of production locations and strategies, CDC Copeland was found to possess overall higher average levels of kernel plumpness and offered greater yield, with a lower overall protein content, and smaller degree of variability when compared to AC Metcalfe, which conferred to it a higher profitability (86). However, it should be noted that relative differences in ADF values between the two varieties were very small, and that each possessed highly idealized fermentation characteristics. Analysis of malting parameters (Appendix A2) shows that each variety displayed a moderately high level of kernel weight, germination index, and starch degrading ability, as evident from the measures of diastatic power, and enzyme activity. As levels of starch degrading enzymes have been determined as a key parameter of fermentability (36, 37, 38, 39, 40) it is not surprising that CDC Copeland and AC Metcalfe outperformed their counterparts, having high weight (indicative of higher starch content) and high enzyme activity. Interestingly, though kernel weight increased from the crop year of 2007 to 2008, overall fermentability decreased, a seemingly opposite reaction to increased starch content. However, measures of enzyme activity (Diastatic Power, α-amylase, β-amylase, β-amylase thermostability, and limit dextrinase) of the 2008 varieties show a substantial decrease from that of the 2007 crop season. Thus, despite higher carbohydrate reserves in the kernel, fermentability was noticeably hindered, likely due to reduced starch degrading capability. These results lend further credence to the importance of malt enzyme-induced starch degradation in producing a highly fermentable media.

6.2.4. Fermentation Performance of the Feed Varieties

The surprisingly high degree of fermentability afforded by the feed varieties, CDC Bold, CDC Dolly, TR306, McLeod, and CDC Helgason, does not always equate high final quality in beer. Many barley/malt factors beyond the scope of this report can affect the aroma, mouth feel, and flavour of the finished product (50), while still achieving a respectable degree of fermentability and adequate resultant ethanol production; the principle measurement of interest in this report. The process of malting can allow a large measure of control and modulation of fermentation activity to a variety of barley cultivars, allowing adoption of preferred fermentation performance to otherwise undesirable feed barley. Changes and adjustments throughout the malting regimen based on the expertise of the maltster, can improve malting quality parameters and allow typical low fermenting varieties to perform seemingly well. However, this is not always an ideal situation, as changes made during the crucial stages of enzyme development and endosperm modification can have substantial impact on the myriad of downstream events leading to final product evaluation.

Additionally, the commonplace method in industry of comparing end results to dictate fermentation performance can be misleading, as low final attenuation values can be achieved through aberrant fermentation behaviour. CDC Dolly displays respectable final attenuation, and yet visualization of the fermentation curve clearly indicates a deviation from the ideal (Appendix A8). Such results show clear indication for the need to analyze fermentations as a whole, rather than focus on single final measurements, which is achieved through the (officially recognized) method of non-linear modeling proposed by Speers *et al.* (90). CDC Dolly suffers from a distinct lack of starch degrading capacity, as evident from the substantially lower measures of starch degrading enzyme activity, despite having a high kernel weight, suggesting rather large extract potential (Appendix A2).

6.3. Thermostability of β-amylase

Unfortunately, few conclusions can be drawn from the results of barley variety BM9752D-17, which displays dramatically higher β-amylase thermostability compared to any of the other varieties (Appendix A2). According to Evans *et al.* (37), a malt variety expressing a high degree of β-amylase thermostability would confer greater starch degrading ability due to the prolonged enzyme stability at higher temperatures typical of mashing, thereby increasing the level of extract produced for a given amount of total starch. The general hypothesis was that β-amylase thermostability, and indeed the combined stability of all the starch degrading enzymes, would yield a more accurate predictor of fermentation performance of a given malt than has previously been established (39). Having achieved generally comparable results to that of the highest fermenters in this study, and all other malt parameters being reasonably equal, the assumption that higher levels of attenuation will be attained solely through the increased thermostability of a single starch degrading enzyme, specifically β-amylase, was not supported in the current study. However, nor has it been refuted, as barley variety BM9752D-17 did retain one of the highest levels of fermentation stability between crop seasons amongst tested varieties, indeed achieving very high final attenuation with a typical fermentation profile (Appendix A8). This suggests some level of continuity afforded by this experimental malt variety, despite seemingly disparate growing conditions between years, as is generally indicated from the decrease in fermentability displayed by the other varieties from the 2007 to 2008 crop season. Such stability may be indicative of superior enzyme activity, granting preferred fermentation quality despite discrepancies in other malting parameters.

6.4. Turbidity (Absorbance) Monitoring

Absorbance measures were modeled using the newly developed Tilted Gaussian function, described in section 2.8 and outlined in the ASBC Official Methods of Analysis (6). The function introduces a tilt to the overall curve described by the Gaussian function, which visually represents YIS throughout fermentation well. Modeling such data allows quantitative comparison of yeast activity, rather than relying on visual analysis alone, an issue noted by Lake *et al.* (57), and a similar problem which previously afflicted attenuation data prior to the use of logistic modeling (see section 2.8). Results yield not only visual trends, but also equation parameter values, which can be compared and correlated with other barley/malt and fermentation quality measures using methods of statistical analysis. Having been recently developed, parameter values have yet to be compared to a substantial data set of previous brewing parameters, and key function parameters which are likely to achieve significant correlations have yet to be discerned. In the current study, equation peak parameters from the Tilted Gaussian (A; amplitude) along with integration of the model (i.e., area under the curve of the Tilted Gaussian) were correlated with RDF and initial carbohydrate values in effort to establish correlation between maximum YIS and final attenuation achieved (data not shown). Unfortunately, little correlation was found, suggesting either the need for incorporation of a greater number of data points, or that another parameter value (or multiple values) of the Tilted Gaussian function should be utilized as a predictor of fermentability.

Promising work continues in effort to optimize this function as a tool for assessing fermentation performance. Interestingly, from visual analysis alone, it would appear that the highest fermenting varieties (CDC Copeland, AC Metcalfe, and Harrington) display similar turbidity trends, where YIS both rapidly increased and quickly fell out of suspension (Figure 5-2, Figure 5-4, Figure 5-6). These results indicate a logical chain of events, whereby optimum

conditions (i.e., nutrients) for yeast fermentation are provided immediately by the malt, and are composed of highly fermentable extract which allowed for rapid and complete consumption, suggesting an efficient and idealized fermentation.

6.5. Malt Quality Correlations

Results of select fermentation parameters were correlated to measurements of malting quality provided by colleagues at the CGC, Winnipeg, MB (Appendix A2), and complete results of all linear correlations shown in Appendix A1. Though many parameters showed only moderate, to little correlation (as measured by the reported correlation coefficient), or displayed expectedly high correlation, a specified few did show interesting results. Table 6-1 and Table 6-2 highlight those measures which displayed a correlation coefficient of 0.79 or higher, indicating a noteworthy level of correlation.

Table 6-1. Summary of correlation coefficients resulting from selected fermentation parameters correlated with measure of malt quality. Only measures displaying a correlation of 0.79 or higher, and $p<0.05$ are illustrated.

	Barley Protein (%)	**Soluble Protein (%)**	**S/T Ratio (%)**	**β-Glucan (mg/L)**	**Viscosity (mPa s)**	**Diastatic Power (°L)**	**FAN (mg/L)**
Malt Protein (%)	0.9589						
S/T Ratio (%)		0.8518					
β-Glucan (mg/L)		-0.7987					
Viscosity (mPa s)		-0.7995		0.9474			
Alpha Amylase (D.U.)		0.8044	0.7977				
FAN mg/L		0.9752	0.8812				
A.A.L.		0.8587				0.7931	0.8064
Broth Ferm. (%)		0.9103	0.8310	-0.8314	-0.8175		0.9288
RDF		0.7917					
ADF		0.7965					
RE Integration		-0.9062		0.7973	0.8393		-0.8926
Friability (%)				-0.8420	-0.8822		
Beta Amylase (U/g)						0.9239	

Table 6-2. Summary of correlation coefficients (continued from Table 6-1) resulting from selected fermentation parameters correlated with measure of malt quality. Only measures displaying a correlation of 0.79 or higher, and $p<0.05$ are illustrated.

	Friability (%)	A.A.L.	Broth Ferm. (%)	Maltotriose (g/L)	RDF	ADF
Broth Ferm. (%)		0.7903				
RDF		0.8665				
ADF		0.8681			0.9968	
RE Integration		-0.9100	-0.8435		-0.9103	-0.9014
MalTri0	0.8645					
Maltotetraose (g/L)				0.8374		

Some parameters displayed expected high levels of correlation with one another, e.g., levels of malt protein would be expected to have resulted from initial levels of barley protein. However, some notable exceptions resulted. High correlation between malt soluble protein and measures of fermentation performance RDF, ADF, and RE Integration were observed. This suggests that preliminary measures of soluble protein in malt may act as convincing predictors of fermentation performance, or incorporated into model predictions, as suggested by Evans *et al.* (42) whereby fermentation performance is predicted on not only biochemical aspects of malt fermentability (i.e., enzyme activity), but also using traditional measures of malt quality to improve model predictions. Given that FAN and KI (Kolbach Index; the soluble to total protein ratio) has been implicated in fermentation performance (42) it is reasonable to also observe soluble protein as a potential measure of fermentation performance. As well, friability of the malt was correlated to initial levels of maltotriose (MalTri0), predicted by the logistic equation. This suggests perhaps higher initial levels of malt maltotriose indicate a more highly modified grain, making it more susceptible to crushing or crumbling. Finally, integration of the logistic function seems to provide a reasonably high level of correlation with both RDF and ADF. Though not an unexpected result, it does provide promising evidence for the technique of

integrating logistic curves (e.g., RE, AE, carbohydrates curves etc.) in order to provide further fermentation parameters with which to gauge performance.

6.6. Third Round Fermentations

Finally, third round fermentations were carried out in order to ascertain the possible confounding effect of a 4% glucose "spike" prior to yeast inoculation, required as part of the miniaturized fermentation assay to establish a strong, initial fermentation (6, 56). It was noted from the results of logistic modeling of carbohydrate profiles throughout second round fermentations, that tested varieties displayed concomitant consumption of glucose and maltose early in the fermentation (complete data not shown; see Figure 5-11 for example). This is in contrast to traditional knowledge, which states that maltose fermentation does not typically commence until approximately 50% of the glucose present in the fermenting medium has first been consumed (93, 94). This was assumed to be due to the mechanism of carbon catabolite repression, where even in the presence of large quantities of other fermentable carbohydrates (primarily maltose, the most abundant fermentable carbohydrate found in malt), yeast will preferentially utilize glucose in the early stages of fermentation (93, 94). Interestingly, this was not displayed in the current study, and maltose consumption rates were detected between the earliest measures of HPLC analysis, 0 and 2 hours post inoculation.

Though generally regarded as an atypical result, this behaviour has previously been documented. Meneses *et al.* (73) studied a number of industrial strains of *Saccharomyces cerevisiae* for their patterns of sugar uptake and metabolism, and identified a number of variants which indeed showed little or no carbon catabolite repressive activity, displaying maltose utilization from the onset of fermentation. Similar results were obtained in the current study, and third round fermentations using three distinct carbohydrate spikes did not deviate from this

behaviour. Thus, the use of a 4% glucose spike can be confidently disregarded as a possible cause for altered carbohydrate uptake, and conclude that the standard SMA yeast culture utilized in the current study displays a certain lack of carbon catabolite repression, allowing all available fermentable sugars to be utilized in concert.

This is an important observation, as it has been noted that a lagging onset of maltose utilization is an undesirable fermentation trait, as it can lead to lengthy fermentations, and possibly obstruct the further utilization of other fermentable carbohydrates due to the inhibitory effect of increasing levels of ethanol on sugar transport, and the overall decline in yeast activity in the latter stages of fermentation (73). Therefore an early utilization of maltose has been deemed a desirable trait, leading to faster, consistent, and more efficient fermentations (73). It would be highly recommended that industry and research consider SMA yeast as an optimum fermentation strain which can be effectively utilized as a standard or control.

7. Conclusion

7.1. Summary of Work

The current study employed a broad spectrum analytical approach to the complex system of barley malt fermentation, specifically to a select group of barley varieties which either have significance in the Canadian grain market, or were of experimental interest. The goal was to quantify and describe the overall fermentation performance of the barley varieties selected, while also providing further testing to newly developed methods of analysis. This included an assessment of the validity of the miniaturized fermentation assay, application of the logistic function to measures of wort attenuation along with fermentable carbohydrate and ethanol concentrations throughout fermentation, and modeling of relative absorbance measures using a newly developed parabolic function coined the "Tilted Gaussian".

Altogether, it was surmised that the miniaturized fermentation assay performed as expected, yielding wort fermentation curves typical of yeast mediated attenuation of the surrounding media, and that these results were in accordance with correlations conducted on brewery scale fermentation data (63). As well, the logistic function has proven invaluable to accurately describe not only wort attenuation, but also the decline of fermentable carbohydrates, using relatively few data points. In addition, with only slight modifications to the initial estimates of parameter values, the logistic model was also successfully applied to measures of ethanol, effectively describing the primary result of carbohydrate fermentation with the corresponding increase in alcohol content throughout time. Parallel measures of relative absorbance to estimate YIS were also collected at each time interval. Prior to even the commencement of this study, YIS curves were typically only analysed visually, and were therefore difficult to utilize as precise measures indicative of fermentation quality. However, recently a new function was developed which resembled the Gaussian curve but introduced a tilt to the overall function.

Such a modification has allowed modeling of absorbance measures and assignment of parameter values, thereby transitioning qualitative measures of YIS, to a quantitative form. Although specific parameter values have yet to be substantially correlated with measures of malt quality and fermentation performance, this new method of modeling YIS has yielded promising results and should prove of great utility to brewing research once further testing is conducted.

The relative fermentability of all varieties were tested and compared, with a number of controls in place, namely the inclusion of both poorly and highly fermenting varieties, and a barley variety bred specifically to express a highly thermostable variant of the enzyme β-amylase. Ultimately, the final ADF achieved through miniaturized fermentation of each malt was the primary indicator of fermentability, given this is an industry standard and a comparable metric to other brewing studies. In general, results indicated that the fermented varieties displayed typical fermentation activity, whereby the feed varieties displayed poorer ADF and altered attenuation curves, and the malting varieties achieved relatively high levels of final attenuation, with a typical sigmoid curve describing the decline in density. Unfortunately, similar ADF collected from different laboratories offer only limited information when comparing values obtained in the current study. This is due to the unavoidable situation common in brewing research whereby many laboratories employ their own unique method of fermentation. Indeed, ascertaining data which incorporated not only similar methods of fermenting (i.e., the newly developed miniaturized fermentation assay), but also the same barley varieties with comparable growing conditions proved exceedingly challenging. Due to this limiting body of research, the dominant research focus of the current study was to quantify the fermentability of such varieties common in the Canadian market, and do so using a newly developed, and recently standardized, fermentation method (i.e., the miniaturized fermentation

assay; 6). For further comparative data using the current methods described, refer to the collaborative studies conducted on the miniaturized fermentation method (7, 8).

Results supported previous conclusions that CDC Copeland would slightly outperform AC Metcalfe, and that both would outperform Harrington. As well previous indication that Harrington displays inconsistent fermentation performance between crop years was also observed. However, both seasonal fluctuations and variations in growing location between years must be carefully considered before confirmation of such variable fermentation performance can be confidently determined. Interestingly, the variety expressing high β-amylase thermostability, BM9752D-17, did not show enhanced fermentation performance compared to the other top fermenters. However, it did display the most consistent fermentation behaviour between different seasons and farms.

CDC Copeland, AC Metcalfe and Harrington were among the top fomenters tested, with the feed varieties displaying relatively lower final attenuation, as expected. All other varieties fermented well, achieving a (relative) mid-level ADF. Interestingly, the feed varieties expressed relatively good final attenuation when compared to those which fermented the best. This highlights a rather unfortunate circumstance in brewing research, whereby a single parameter (in this case ADF, which may not be wholly representative of the entire process of fermentation) is used as the chief indicator of fermentation quality (i.e., fermentability). The skilled and adaptable processes that make up malting have the potential to enhance the fermentation characteristics of traditionally poorly fermenting varieties, however, final sensory character of the product will always be the ultimate measure of success. With the advent of the logistic, and Tilted Gaussian modeling of fermentation data, more parameters encompassing a larger portion of the process have become available to brewing researchers, and these values should prove highly valuable when comparing variable fermentation characteristics.

Such parameters resulting from measures of fermentation were analysed against measures of malt quality provided by colleagues at the CGC. Using simple linear correlations a number of interesting relationships were obtained, along with confirmation of other expected outcomes (e.g., malt protein is expected to be highly correlated with barley protein). Interestingly, traditional measures of fermentability (A.A.L., RDF, ADF) were found to be strongly correlated with both soluble protein and FAN content. As well, friability was found to show a strong relationship with initial levels of maltotriose. Such results are likely to aid the construction of multifactorial models which can more accurately predict potential fermentability with given malt quality character. As the methods of non-linear modeling outlined are still in their infancy, these analyses constituted only a small portion of the information provided by the modeled data, and so only limited conclusions could be drawn. However, given potential for defining relationships among certain malt and fermentation parameters, results illustrate an enhanced understanding of the brewing process as a whole, particularly when compared to previous visual analyses.

7.2. Future Work

Future endeavors should seek to incorporate more data points to enhance prediction models. This can be achieved both through addition of multiple samples of each variety across more crop seasons, as well as increased sampling time intervals throughout the miniaturized fermentation assay, with particular emphasis early in fermentation. Additionally, further explorative action should be taken upon acquisition of model parameters in effort to discern critical points of interest, and correlations with malt and barley quality measures. Non-linear modeling does show great promise for predicting fermentability, but methods of statistical analysis, specific correlations, and data usage must still be refined in order to be truly effective.

Additionally, it was found that the SMA yeast utilized did not display typical catabolite repressive activity i.e., maltose utilization occurs concomitantly with glucose. This can be regarded as a desirable trait, promoting the efficient and rapid utilization of the fermentable medium, however, it must be understood that if carbon catabolite repression is required (e.g., comparative studies), the yeast strain should not be utilized. Aside from this, SMA has proven to be an effective fermentation yeast strain in general, and standardizing it as a control for use in research would be highly beneficial for accurate comparisons between independent laboratories. As well, it would be recommended that future studies limit the number of yeast grow-ups between fermentations in order to mitigate possible error associated with multiple yeast croppings.

Similarly, further testing should be conducted to elucidate the effect of multiple yeast crops on fermentation variability in order to assess the possible error magnitude this may impart when conducting fermentations over long periods. Although the yeast growing conditions were tightly controlled, there remains a concern of possible variation in yeast performance when utilizing multiple grow-ups over time.

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Appendix

A1. Correlation Matrix of Malt Quality and Fermentability Parameters

Linear correlation coefficients resulting from analysis between each set of measured parameters. Parameters denoted with an asterisk were measured at Dalhousie University, Halifax, NS, all other parameters measured by study collaborators at the CGC, Winnipeg, MN. (see CGC website (24) for a description of analytical methods used).

	Barley Moisture (%)					
Barley Protein (%)	-0.1571057	**Barley Protein (%)**				
1000 KWT (g)	0.4288738	-0.435974	**1000 KWT (g)**			
Germ 4mL	-0.1959869	0.2019181	0.1417062	**Germ 4mL**		
Germ 8mL	-0.2041468	0.3762604	-0.05391749	0.6836294	**Germ 8mL**	
Germ Index	-0.4638226	0.08722677	-0.5638677	0.3591215	0.3578989	**Germ Index**
Fine Ext. (%)	0.01100185	-0.7408587	0.3118753	-0.2086775	-0.4894174	-0.08219885
Malt Protein (%)	-0.2845317	0.9589242	-0.5054705	0.1933918	0.3774253	0.09927512
Soluble Protein (%)	-0.361491	0.1449404	-0.5964915	-0.03582747	-0.1837187	0.3838169
S/T Ratio (%)	-0.2039544	-0.3730151	-0.2822422	-0.1194236	-0.3719931	0.3180018
β-Glucan (mg/L)	0.4856081	-0.1835919	0.6369792	-0.1639973	0.0172301	-0.5609659
Viscosity (mPa s)	0.515121	-0.3184424	0.7431263	-0.09311265	0.007061323	-0.5805289
Diastatic Power (^{0}L)	-0.1327452	-0.007875319	-0.3770365	-0.05218346	-0.2988338	0.1594534
Alpha Amylase (D.U.)	-0.2610389	-0.100676	-0.6139949	-0.2396252	-0.2417846	0.4811198
Beta Amylase (U/g)	-0.2236181	0.2188682	-0.4043166	0.0175503	-0.1699746	0.15543
β-Amylase Stability (%)	-0.2659207	0.1518581	-0.2091051	-0.07593522	-0.220333	-0.03995584
Total L.D. (U/g)	-0.2638998	0.1766443	-0.6049646	0.1401509	-0.03800248	0.5806077
FAN (mg/L)	-0.2920704	0.05624314	-0.5189314	-0.09961402	-0.2629669	0.3291077
Friability (%)	-0.472019	0.1905453	-0.6834394	0.1506342	0.1365773	0.7159736
A.A.L.	-0.4055981	0.02827619	-0.4676946	0.07240654	-0.1639386	0.3853222
Broth Ferm. (%)	-0.3448861	0.02959874	-0.544684	-0.04005669	-0.1731727	0.421496
Glucose (g/L)	-0.1577098	-0.07150484	-0.4270342	-0.08495884	-0.05819625	0.3667216
Fructose (g/L)	-0.1442735	0.3427015	-0.5946051	0.07441511	0.2005601	0.4690548
Maltose (g/L)	0.185212	-0.3464035	0.1391575	-0.0522054	-0.1775059	-0.1516541
Maltotriose (g/L)	-0.1600774	0.09520888	-0.5223303	0.02046057	-0.0671087	0.3794586
Maltotetraose g/L)	0.03078341	0.2731351	-0.6909019	-0.07825374	-0.00691257	0.4436939
RDF*	-0.4928588	0.09884293	-0.4712858	-0.001417607	-0.1589105	0.255887
ADF*	-0.496455	0.09973819	-0.4873597	0.00674977	-0.1362752	0.2903309
RE Integration*	0.460582	-0.157439	0.5073341	0.006157497	0.2302542	-0.3200402
Abs. Integration*	-0.1192555	-0.345652	0.271486	-0.1163397	-0.4188094	-0.2010599
Glu0*	-0.1723383	-0.2061371	-0.07195331	-0.2397839	-0.1529914	0.09675608
Mal0*	0.1254868	-0.4450463	0.4089605	-0.1846968	-0.1549647	-0.2068241
MalTri0*	-0.4172094	-0.02127541	-0.4873118	-0.02396046	0.08257925	0.5689781
Fru0*	-0.3710603	0.2097946	-0.6301359	-0.3519126	-0.00117980	0.3745132

*Blue highlighted parameters measured at Dalhousie University, Halifax, NS. All others measured at the CGC, Winnipeg, MN.

A1. (continued)

	Fine Ext. (%)					
Malt Protein (%)	-0.6976783	**Malt Protein (%)**				
Soluble Protein (%)	0.298389	0.164402	**Soluble Protein (%)**			
S/T Ratio (%)	0.6586698	-0.3745558	0.851785	**S/T Ratio (%)**		
β-Glucan (mg/L)	-0.1136407	-0.2152625	-0.7986827	-0.6435312	**β-Glucan (mg/L)**	
Viscosity (mPa s)	-0.0328046	-0.3535312	-0.7994921	-0.5729307	0.9473845	**Viscosity (mPa s)**
Diastatic Power (°L)	0.4252782	0.03842338	0.7167383	0.6552739	-0.6838852	-0.6174024
Alpha Amylase (D.U.)	0.4300182	-0.07872845	0.8043943	0.7977183	-0.7124052	-0.7203295
Beta Amylase (U/g)	0.2498889	0.2426884	0.6604919	0.4911371	-0.6768783	-0.6301396
β-Amylase Stability (%)	0.2131358	0.3100177	0.3782823	0.2108897	-0.237295	-0.2994458
Total L.D. (U/g)	0.09126804	0.1457291	0.7081667	0.5902389	-0.710078	-0.7770132
FAN (mg/L)	0.3459402	0.06063543	0.9752236	0.8811803	-0.7661995	-0.7610244
Friability (%)	0.04745017	0.2376947	0.725851	0.5624076	-0.841986	-0.8822171
A.A.L.	0.4869734	0.1053546	0.8586507	0.7596449	-0.7431092	-0.7418554
Broth Ferm. (%)	0.2856184	0.04350332	0.9102662	0.8309596	-0.8313968	-0.8175168
Glucose (g/L)	0.3483879	-0.02573008	0.7361705	0.7142406	-0.6692358	-0.6663365
Fructose (g/L)	-0.1904065	0.3914936	0.4265598	0.1989683	-0.5127595	-0.6352729
Maltose (g/L)	0.3825247	-0.319046	0.1629363	0.3211226	0.01096223	0.1274036
Maltotriose (g/L)	0.204943	0.1018492	0.7283956	0.6357126	-0.6231263	-0.6610001
Maltotetraose g/L)	-0.1636131	0.2441262	0.5434034	0.374675	-0.4772567	-0.5675404
RDF*	0.4377221	0.1819368	0.7917358	0.6539409	-0.6361697	-0.7109632
ADF*	0.4341581	0.1816639	0.7965469	0.6582177	-0.6273615	-0.7073643
RE Integration*	-0.3827557	-0.1989155	-0.9062274	-0.752546	0.7972776	0.8392975
Abs. Integration*	0.6407943	-0.3119295	0.0288206	0.2095361	0.177478	0.1042478
Glu0*	0.4142146	-0.1264264	0.400642	0.446271	-0.345803	-0.3414767
Mal0*	0.5056989	-0.3306144	-0.2176485	-0.01850692	0.3065813	0.2668042
MalTri0*	0.2286645	0.05022133	0.5914248	0.5388999	-0.5821525	-0.6799954
Fru0*	-0.06850842	0.2506601	0.5069061	0.3379661	-0.5565211	-0.6461604

*Blue highlighted parameters measured at Dalhousie University, Halifax, NS. All others measured at the CGC, Winnipeg, MN.

A1. (continued)

	Diast. Power (°L)							
Alpha Amylase (D.U.)	0.7739106	**Alpha Amylase (D.U.)**						
Beta Amylase (U/g)	0.9238518	0.6323916	**Beta Amylase (U/g)**					
β-Amylase Stability (%)	0.4955683	0.3644252	0.3486228	**β-Amylase Stability (%)**				
Total L.D. (U/g)	0.6321039	0.7013503	0.6353649	0.112528	**Total L.D. (U/g)**			
FAN (mg/L)	0.6917656	0.7740833	0.6117209	0.339996	0.7052604	**FAN (mg/L)**		
Friability (%)	0.4250908	0.6460538	0.3970556	0.2196099	0.6652036	0.7101845	**Friability (%)**	
A.A.L.	0.7930528	0.7797292	0.7515719	0.4753695	0.7216027	0.8063898	0.6269312	**A.A.L.**
Broth Ferm. (%)	0.6449584	0.7258044	0.6064441	0.1685927	0.7438009	0.9287823	0.7668253	0.7903356
Glucose (g/L)	0.5869681	0.7404057	0.4470919	0.2751726	0.4815156	0.6968333	0.5990029	0.6567011
Fructose (g/L)	0.296243	0.4400184	0.262439	0.2126617	0.4700522	0.3835042	0.519705	0.3696798
Maltose (g/L)	0.1570943	0.1156116	-0.004223	0.04129241	-0.1879354	0.1317026	-0.03917054	0.09964988
Maltotriose (g/L)	0.4215961	0.6062315	0.3331002	0.1591633	0.4760796	0.6647216	0.6928911	0.5729103
Maltotetraose g/L)	0.2381722	0.486857	0.2024546	-0.04266582	0.4620006	0.4834703	0.6352909	0.2991278
RDF*	0.6495887	0.6843863	0.6273779	0.4634214	0.6460328	0.7483003	0.5856729	0.8665398
ADF*	0.6215716	0.6901378	0.5962512	0.4466005	0.6428615	0.7486014	0.6025791	0.8680637
RE Integration*	-0.7167566	-0.7237504	-0.7203096	-0.4291757	-0.7425032	-0.8925763	-0.7157658	-0.9100407
Abs. Integration*	0.1051661	0.1089273	0.05878255	0.2281223	0.03412294	-0.001576	-0.2465318	0.3128458
Glu0*	0.3890628	0.4115036	0.2856565	0.3691876	0.1673626	0.4805634	0.496139	0.3596899
Mal0*	0.04333466	-0.04709443	-0.1024458	0.3799149	-0.2108954	-0.1340868	-0.072368	-0.01062065
MalTri0*	0.2737871	0.5911445	0.1905816	0.3146752	0.4667984	0.600351	0.8645228	0.5160734
Fru0*	0.3299792	0.5781482	0.3360234	0.2428616	0.3890954	0.5241866	0.6525767	0.2904122

*Blue highlighted parameters measured at Dalhousie University, Halifax, NS. All others measured at the CGC, Winnipeg, MN.

A1. (continued)

	Broth Ferm. (%)								
Glucose (g/L)	0.741232	**Glucose (g/L)**							
Fructose (g/L)	0.4900723	0.663031	**Fructose (g/L)**						
Maltose (g/L)	0.03394282	0.2994851	-0.2654867	**Maltose (g/L)**					
Maltotriose (g/L)	0.6383862	0.7354724	0.3881987	0.5446577	**Maltotriose (g/L)**				
Maltotetraose g/L)	0.5004403	0.549426	0.5154571	0.266626	0.8373923	**Maltotetraose (g/L)**			
RDF*	0.659919	0.5566392	0.2957047	0.189379	0.6357973	0.3185722	**RDF***		
ADF*	0.663303	0.5610905	0.3100938	0.1975191	0.6573087	0.3501796	0.996781	**ADF***	
RE Integration*	-0.8434688	-0.6037479	-0.3517943	-0.01115204	-0.6178368	-0.3621179	-0.91035	-0.90141	**RE Integration***
Abs. Integration*	-0.09797539	-0.030779	-0.2791325	0.2385545	0.07067287	-0.2516525	0.456699	0.454782	-0.2455186
Glu0*	0.5294936	0.4155501	0.2150791	-0.07065643	0.1665627	0.04740618	0.280078	0.277355	-0.4133126
Mal0*	-0.1860113	-0.053509	-0.2165146	0.1664889	-0.1602228	-0.3407317	0.070859	0.060471	0.05820554
MalTri0*	0.6507946	0.5779997	0.4769329	-0.1164387	0.5471976	0.4770898	0.491758	0.513347	-0.5965853
Fru0*	0.5225622	0.5062252	0.5169138	-0.2935813	0.3132237	0.3963961	0.349472	0.338777	-0.4769901

	Abs. Integration*			
Glu0*	-0.160679	**Glu0***		
Mal0*	0.3014941	0.548929	**Mal0***	
MalTri0*	-0.1068922	0.706566	0.226671	**MalTri0***
Fru0*	-0.3683982	0.557665	-0.01095	0.702647

*Blue highlighted parameters measured at Dalhousie University, Halifax, NS. All others measured at the CGC, Winnipeg, MN.

A2. Malt Quality Data

Measurements collected from all malt varieties tested. Assays were conducted directly on malted barley samples through collaboration with members of the Canadian Grain Commission in Winnipeg, MB. See the CGC (24) and Megazyme (72) websites for further detail regarding analytical methods used.

REF #	Station	Growing Year	Variety	Barley Moisture (%)	Barley Protein (%)	1000 KWT (g)	Germ 4 mL	Germ 8mL	Germ Index	Fine Ext. (%)	Malt Protein (%)	Soluble Protein (%)	S/T Ratio (%)
SP-09-01	Kernen	2007	CDC Bold	9	12.4	41.7	99	91	8.6	80.6	12.39	5.6	45.2
SP-09-02	Kernen	2007	AC Metcalfe	8.8	13	37.6	97	91	8.4	79	12.9	5.53	42.9
SP-09-03	Kernen	2007	Harrington	9.4	13.2	36.8	98	90	8.6	78.8	12.79	6.22	48.7
SP-09-04	Kernen	2007	CDC Dolly	9	13.1	42.5	100	98	9.1	77.1	12.87	3.96	30.8
SP-09-05	Kernen	2007	TR251	9.4	12.8	39.9	96	89	8.5	80.1	12.51	5.25	42
SP-09-06	Kernen	2007	CDC Copeland	9.1	12.8	38.6	100	97	9.6	79.2	12.78	5.49	43
SP-09-07	Kernen	2007	TR 306	9.3	14.1	43.1	100	100	8.3	77.3	13.84	5.34	38.6
SP-09-08	Kernen	2007	CDC Kendall	9	13.6	37.7	99	94	7.9	78.8	13.46	5.77	42.9
SP-09-09	Kernen	2007	McLeod	9.6	13.4	42.7	100	100	8.3	76	12.94	4.57	35.3
SP-09-10	Kernen	2007	CDC Helgason	9.5	12.9	39.4	100	99	9.5	79.6	12.55	5.33	42.5
SP-09-11	Kernen	2007	BM9752D-17	9	14	42.9	100	99	8.8	78.1	14.27	4.98	34.9
SP-09-12	Saskatoon	2008	CDC Bold	9.4	12.1	49.5	99	94	8.3	81.6	11.74	4.95	42.1
SP-09-13	Saskatoon	2008	AC Metcalfe	9.5	12.2	47.2	97	82	7.8	81.2	11.77	5.24	44.5
SP-09-14	Saskatoon	2008	Harrington	9.4	11.8	46.9	97	75	8.3	80.6	11.15	4.87	43.7
SP-09-15	Saskatoon	2008	CDC Dolly	9.7	12.6	51	97	95	6.8	78.8	12.34	3.75	30.4
SP-09-16	Saskatoon	2008	TR251	9.3	11.9	52.1	100	99	8.4	81.7	11.11	4.8	43.2
SP-09-17	Saskatoon	2008	CDC Copeland	9	11.2	49.9	100	95	8.1	81.6	11.13	4.96	44.6
SP-09-18	Saskatoon	2008	TR 306	9.4	13.7	54.7	100	92	7.2	78.9	13.19	4.79	36.3
SP-09-19	Saskatoon	2008	CDC Kendall	9.6	12.4	45.9	99	84	7.9	81.5	11.93	5.36	44.9
SP-09-20	Saskatoon	2008	McLeod	9.7	12.3	50.6	99	97	7.8	77.5	11.85	3.98	33.6
SP-09-21	Saskatoon	2008	CDC Helgason	9.4	12.2	47.8	100	95	8.6	80.9	11.84	5.5	46.5
SP-09-22	Saskatoon	2008	BM9752D-17	9.6	12.6	45.4	98	84	6.8	80.1	12.73	4.7	36.9

A2 (cont.)

REF #	Station	Growing Year	Variety	β-Glucan (mg/L)	Visc. (mPa s)	Diast. Power (°L)	α-amylase (D.U.)	β-amylase (U/g)	β-amylase Stability (%)	Total L.D. (U/g)	FAN (mg/L)	Friability (%)	A.A.L (%)
SP-09-01	Kernen	2007	CDC Bold	265	1.47	113	78.6	17.9	8.1	309	227	82.9	85.2
SP-09-02	Kernen	2007	AC Metcalfe	116	1.42	145	92.1	22.5	6.6	419	227	80.8	84.8
SP-09-03	Kernen	2007	Harrington	130	1.4	144	99.8	20.3	8.9	526	276	84.2	85.7
SP-09-04	Kernen	2007	CDC Dolly	494	1.51	71	60	13.7	2.2	344	126	67.8	80.9
SP-09-05	Kernen	2007	TR251	170	1.43	132	95.4	21.7	4.6	402	202	79	85.6
SP-09-06	Kernen	2007	CDC Copeland	110	1.42	146	84.8	23.8	3.2	543	220	84.9	86.4
SP-09-07	Kernen	2007	TR 306	171	1.42	111	65.5	19.6	4.6	425	215	79.6	85
SP-09-08	Kernen	2007	CDC Kendall	138	1.44	155	86.5	26.1	4.2	449	222	62.9	87.2
SP-09-09	Kernen	2007	McLeod	306	1.47	113	66	18.8	1.0	396	167	73.6	80.7
SP-09-10	Kernen	2007	CDC Helgason	278	1.46	135	93.4	20.0	4.5	501	209	81.7	86.9
SP-09-11	Kernen	2007	BM9752D-17	385	1.48	142	77.4	22.2	14.6	355	176	72.5	85.3
SP-09-12	Saskatoon	2008	CDC Bold	403	1.51	139	74.1	22.5	4.1	398	204	69.5	84.8
SP-09-13	Saskatoon	2008	AC Metcalfe	229	1.48	138	86.5	19.5	5.6	337	217	69.8	84.2
SP-09-14	Saskatoon	2008	Harrington	638	1.56	120	76.7	18.6	4.5	443	195	59.9	84
SP-09-15	Saskatoon	2008	CDC Dolly	1530	1.77	59	48.9	11.5	2.7	207	118	45.7	79.4
SP-09-16	Saskatoon	2008	TR251	501	1.55	138	88.1	21.1	4.3	411	182	65.2	84.3
SP-09-17	Saskatoon	2008	CDC Copeland	279	1.51	116	71.8	17.0	5.8	368	195	74.6	85.5
SP-09-18	Saskatoon	2008	TR 306	627	1.58	106	52.3	19.7	4.1	351	186	60.4	83.3
SP-09-19	Saskatoon	2008	CDC Kendall	235	1.49	159	83.6	23.9	5.4	392	207	73.4	85.6
SP-09-20	Saskatoon	2008	McLeod	857	1.73	98	53.9	16.2	1.4	222	137	50.5	79.6
SP-09-21	Saskatoon	2008	CDC Helgason	380	1.52	131	84.5	18.6	6.2	383	213	66.6	85.5
SP-09-22	Saskatoon	2008	BM9752D-17	514	1.53	161	76.1	23.0	11.9	384	179	58.8	85

A2 (cont.)

REF #	Station	Growing Year	Variety	Broth Ferm. (%)	Glucose (g/L)	Fructose (g/L)	Maltose (g/L)	Maltotriose (g/L)	Maltotetraose (g/L)
SP-09-01	Kernen	2007	CDC Bold	77.1	12.9	2.5	63.7	18.2	4.4
SP-09-02	Kernen	2007	AC Metcalfe	80.2	13.3	2.2	68.7	18.3	4.2
SP-09-03	Kernen	2007	Harrington	80.4	14.4	3.1	66.6	20.4	5.4
SP-09-04	Kernen	2007	CDC Dolly	55.2	10.0	2.4	61.1	15.2	3.5
SP-09-05	Kernen	2007	TR251	75.3	14.9	3.1	61.4	17.9	4.6
SP-09-06	Kernen	2007	CDC Copeland	79.9	13.7	3.0	62.7	17.8	4.6
SP-09-07	Kernen	2007	TR 306	78.2	13.6	2.9	65.1	18.9	4.2
SP-09-08	Kernen	2007	CDC Kendall	76.8	13.7	2.9	64.5	17.7	4.1
SP-09-09	Kernen	2007	McLeod	70.6	13.4	4.2	59.7	17.6	5.5
SP-09-10	Kernen	2007	CDC Helgason	76.0	13.3	3.7	64.1	18.7	5.1
SP-09-11	Kernen	2007	BM9752D-17	63.3	13.3	3.2	63.5	16.5	3.6
SP-09-12	Saskatoon	2008	CDC Bold	73.5	13.4	3.2	65.2	15.8	3.2
SP-09-13	Saskatoon	2008	AC Metcalfe	74.5	15.3	3.2	69.1	18.1	4.0
SP-09-14	Saskatoon	2008	Harrington	72.4	11.6	1.5	61.3	14.8	3.5
SP-09-15	Saskatoon	2008	CDC Dolly	50.8	10.5	1.1	70.0	15.1	3.7
SP-09-16	Saskatoon	2008	TR251	66.2	12.4	1.0	61.7	14.7	2.4
SP-09-17	Saskatoon	2008	CDC Copeland	74.0	13.7	1.3	71.1	17.6	3.0
SP-09-18	Saskatoon	2008	TR 306	67.1	9.7	0.8	59.1	13.7	2.5
SP-09-19	Saskatoon	2008	CDC Kendall	70.1	13.1	0.5	84.0	22.8	5.5
SP-09-20	Saskatoon	2008	McLeod	56.5	9.8	0.2	69.1	11.6	2.5
SP-09-21	Saskatoon	2008	CDC Helgason	75.8	16.4	3.8	76.7	20.0	4.3
SP-09-22	Saskatoon	2008	BM9752D-17	66.2	11.9	2.7	62.1	13.7	2.6

A3. Yeast Cell Counting using a Haemocytometer

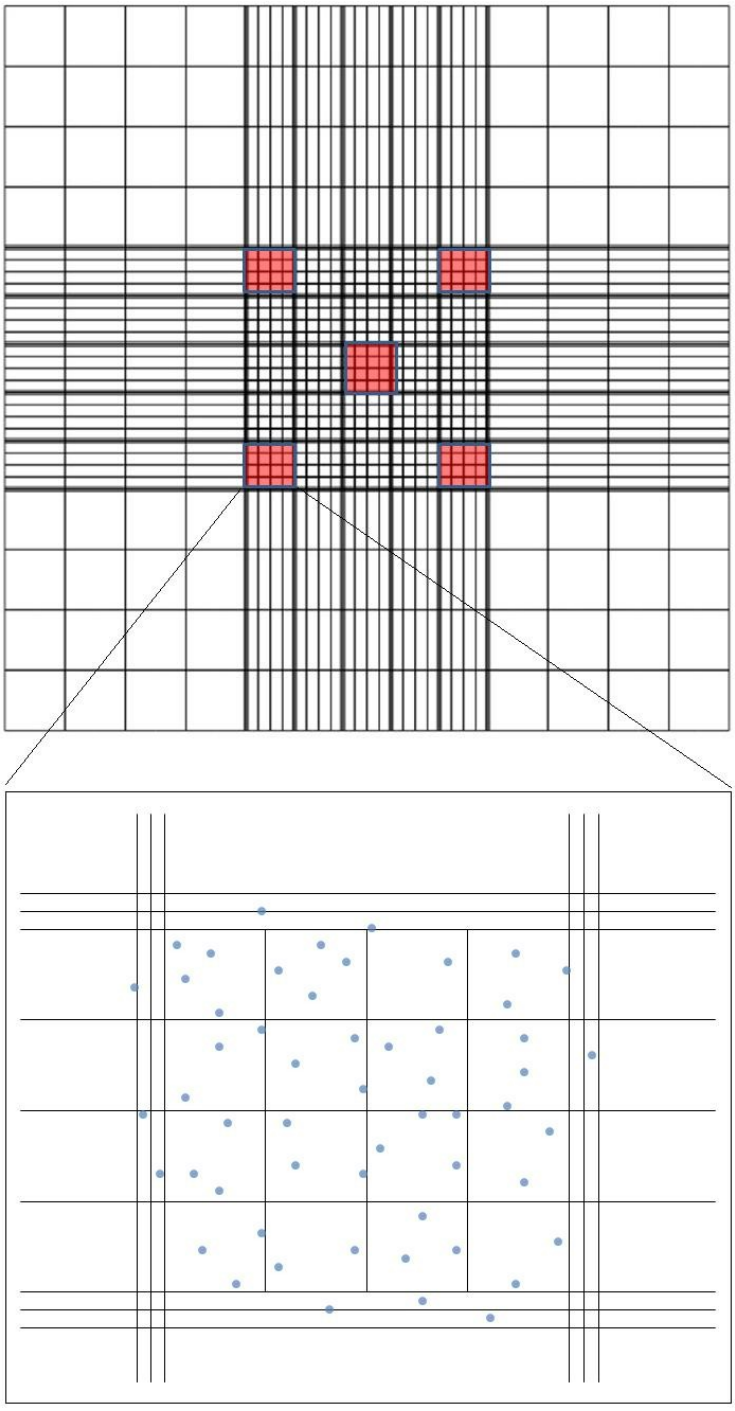

Figure A3-1 Top image adapted from Microbehunter Microscopy Magazine (Retrieved May, 2013: <www.microbehunter.com/2010/06/27/the-hemocytometer-counting-chamber>, (95)). Above depicts approximately 100x view of one side of a haemocytometer. Red highlighted boxes show counting pattern used. Below illustration is approximately 400x view of a single 4x4 square containing yeast cells.

When viewing yeast cells under the microscope using the heamocytometer, the 10x and 40x objective lenses were predominantly used. When combined with the 10x ocular lense, this constituted regular use of 100x and 400x power, respectively.

In order to count yeast cells in an effective but timely manner, five squares (Figure A3-1) in total for each side of the haemocytometer (4 corners plus the centre) were chosen and all cells contained within were counted. This was repeated on the other side of the haemocytometer and an average of both counts were calculated (e.g., (73 cells in total on one side + 65 cells in total on the other side) / 2= 69 cells).

Refer to Figure A3-1. Each side of the haemocytometer is organized as one large square broken into 25 smaller squares (5x5). Each of these are then further broken into 16 smaller squares (4x4). For the purposes of this study, the 16 small squares within the 4x4 square were ignored, and each 4x4 square was regarded as a single open space. The total number of yeast cells contained within five 4x4 squares (Figure A3-1 red highlighted boxes) were totaled. This was then repeated for the other side. Yeast cells which were touching the inside borders of the top and right edge were counted, those which were touching the inside borders of the bottom and left edges were excluded. As well, budding yeast cells were only counted if they were ≥ 50% the size of the mother cell.

A4. Calculated Real Extract

The following RE was calculated using equation 5b from Cutaia *et al.* (27) using measured values of AE (see Appendix A8.) and alcohol content. Upon conversion of percent alcohol on a volume/volume basis to percent alcohol weight/weight basis using equation 14 from Cutaia *et al.* (27), and rearranging equation 5b, RE was calculated at each time point for all varieties. N.B. SG was also required in order to employ equation 14, and was therefore calculated according to equation 10 from Cutaia *et al.* (27).

Calculated RE values using equation 5b from Cutaia *et al.* (27).

Time (Hr)	CDC Bold (2007)	AC Metcalfe (2007)	Harrington (2007)	CDC Dolly (2007)	TR251 (2007)	CDC Copeland (2007)	TR 306 (2007)	CDC Kendall (2007)	McLeod (2007)
0									
1	14.60	14.70	14.60	14.70	14.60	14.80	14.70	14.70	14.70
1	14.60	14.70	14.70	14.70	14.60	14.80	14.70	14.70	14.70
1	14.60	14.70	14.70	14.70	14.60	14.80	14.70	14.70	14.70
6	14.03	14.12	14.02	14.02	14.01	14.11	14.11	14.21	14.30
6	13.93	14.12	14.02	13.92	14.01	14.21	14.11	14.11	14.21
6	13.93	14.12	14.12	13.91	14.01	14.21	14.11	14.11	14.21
22			6.79			7.07	6.97	7.17	8.36
22			7.29			7.27	6.87	7.28	8.66
22			6.99			7.28	7.27	7.38	8.66
23		6.99			6.99				
23		7.20			7.28				
23		7.39			7.18				
26	5.52	6.31	5.01	7.08	5.40	5.29	5.39	5.39	7.08
26	5.82	6.71	4.70	7.08	5.70	5.39	5.49	5.70	6.88
26	5.32	5.71	5.20	7.08	5.50	5.40	5.39	5.49	6.88
30		4.42	3.52	6.49	4.51	4.31	4.30	4.31	5.79
30		4.23	3.71	6.29	4.51	4.21	4.31	4.31	5.89
30		4.23	3.62	6.39	4.61	4.30	4.30	4.51	5.79
46		2.33	2.03		2.54	2.22	2.23	2.13	3.42
46		2.34			2.64	2.72	2.23	2.32	3.32
46		2.34	2.03		2.64	2.13	2.33	2.31	3.22
46.5	2.24			3.61					
46.5	2.14			3.91					
46.5	2.15			3.91					
50	2.24	2.24	1.93	3.51	2.54	2.03	2.23	1.89	3.02
50	2.84	2.24	1.93	3.41	2.34	2.03	2.43	2.03	4.60
50	3.63	2.14	1.93	3.61	2.64	2.13	2.33	2.13	3.22

(continued from pg. 116)

Time (Hr)	CDC Bold (2007)	AC Metcalfe (2007)	Harrington (2007)	CDC Dolly (2007)	TR251 (2007)	CDC Copeland (2007)	TR 306 (2007)	CDC Kendall (2007)	McLeod (2007)
54	2.14	2.14	1.93	3.31	2.24	2.03	2.33	2.03	3.02
54	2.03	2.14		3.11	2.44	2.03	3.12	1.93	3.02
54	3.33	2.04	1.83	3.11	2.34	1.93	2.13	1.73	3.22
70	1.94	2.04	1.83	2.52	2.23	1.93	2.03	1.83	2.92
70	2.05	2.04	1.93	2.61	2.23	1.93	2.03	1.93	2.82
70	2.05	2.04	1.84	2.22	2.23	1.83	2.02	1.93	2.72
74		2.04	1.83		2.13	3.12	2.13	1.93	3.61
74		2.04	1.83		2.13	1.93	2.03	1.93	2.72
74		1.94	1.93		2.52	1.83	2.82	1.89	2.82
78	1.93	2.04	1.93	2.52	2.13	1.93	2.02	1.91	2.71
78	2.04	1.94	1.93	2.42	2.13	1.93	2.13		2.82
78	1.94	2.04	1.83	2.52	2.13	1.93	3.12	1.93	3.12

Time (Hr)	CDC Helgason (2007)	BM9752D-17 (2007)	CDC Bold (2008)	AC Metcalfe (2008)	Harrington (2008)	CDC Dolly (2008)	TR251 (2008)	CDC Copeland (2008)	TR 306 (2008)
0									
1	14.60	14.62	14.90	14.79	14.70	14.80	14.70	14.70	14.80
1	14.70	14.60	14.90	14.79	14.70	15.00	14.70	14.78	14.80
1	14.70	14.60	14.90	14.70	14.70	14.80	14.70	14.70	
6	14.21	13.63	14.11	14.11	14.11	14.21	14.11	14.11	14.11
6	14.20	13.91	14.11	14.01	14.01	14.21	14.11	14.01	14.11
6	14.10	14.01	14.11	13.73	14.01	14.21	14.21	14.01	
22	8.17				7.67			7.57	7.47
22	8.16				7.97			7.47	7.77
22	7.97				7.67			7.58	7.47
23		7.67		7.19			7.38		
23		7.58		7.39			7.48		
23		7.68		7.29			7.48		
26	6.09	6.29	6.00	6.21	6.19	7.57	6.30	6.30	6.09
26	6.09	6.29	5.80	6.30	6.09	7.68	6.30	6.09	6.19
26	6.19	6.29	5.90	6.20	6.19	7.68	6.29	6.19	5.89
30		5.11	4.80	4.82	5.40	6.88	5.30	5.11	4.90
30		5.70	4.80	4.82	5.20	6.89	5.40	6.59	4.80
30		5.10	5.31	4.62	5.10	6.89	5.30	5.10	4.90
46	2.63	3.03		2.63	2.92		3.03	2.73	2.62

(continued from pg. 117)

Time (Hr)	CDC Helgason (2007)	BM9752D-17 (2007)	CDC Bold (2008)	AC Metcalfe (2008)	Harrington (2008)	CDC Dolly (2008)	TR251 (2008)	CDC Copeland (2008)	TR 306 (2008)
46	2.43	2.93		2.63	2.92		3.23	2.72	2.71
46	2.72	2.74		2.63	2.92		3.03	2.63	2.82
46.5			3.52			4.51			
46.5			2.42			6.10			
46.5			2.34			4.22			
50	2.33	2.74	2.34	2.53	2.62	4.02	2.74	2.43	2.42
50	2.42	3.23	2.34	2.53	2.72	4.02	2.84	2.53	2.43
50	2.33	3.83	2.14	2.43	2.63	4.02	2.74	2.43	2.42
54	2.82	2.74	2.23	2.43	3.32	3.71	2.64	2.33	3.61
54	2.82	2.64	2.23	2.43	2.43	3.52	2.54	2.33	2.63
54	3.91	2.34	2.23	2.43	2.53	3.52	2.54	2.23	2.43
70	1.93	2.23	2.04	2.23	4.01	2.83	2.33	2.13	2.32
70	2.03	2.24	2.23	2.34	2.33	2.83	2.33	2.13	2.33
70	1.90	2.14	2.04	2.33	2.33	2.73	2.33	2.13	2.92
74	2.03	2.13		2.33	2.33		2.33	2.13	2.33
74	1.93	2.13		2.34	2.33		2.43	3.32	2.33
74	1.93	2.23		2.33	2.33		2.33	2.13	2.33
78	1.93	2.13	2.13	2.34	2.14	2.82	2.23	2.14	2.33
78	2.03	2.13	3.22	2.34	2.33	2.72	2.33	2.13	2.33
78	1.93	2.14	2.13	2.34	3.81	2.72	2.33	2.13	2.33

Time (Hr)	CDC Kendall (2008)	McLeod (2008)	CDC Helgason (2008)	BM9752D-17 (2008)
0				
1	14.70	14.60	14.59	14.70
1	14.70	14.60	14.60	14.70
1	14.70	14.60	14.50	
6	14.10	14.11	14.01	14.11
6	14.11	14.11	14.11	14.11
6	14.11	14.11	14.01	14.10
22	7.47	9.06	8.37	7.58
22	7.47	9.16	8.87	7.77
22	7.47	9.06	8.47	7.38
23				

(continued from pg. 118)

Time (Hr)	CDC Kendall (2008)	McLeod (2008)	CDC Helgason (2008)	BM9752D-17 (2008)
23				
23				
26	5.89	8.17	6.59	6.20
26	5.79	7.87	6.49	6.70
26	5.89	7.87	6.69	6.50
30	5.30	6.88		5.30
30	5.20	6.88		5.30
30	4.80	6.88		5.40
46	2.52	4.70	2.73	3.13
46	2.62	4.71	2.63	2.84
46	2.52	4.61	2.72	2.74
46.5				
46.5				
46.5				
50	2.13	4.31	2.53	
50	2.22	4.31	2.43	
50	2.42	4.31	2.53	
54	2.13	4.01	2.43	2.53
54	2.13	4.80	2.53	2.34
54	2.13	4.11	2.33	2.64
70	2.03	3.52	2.14	2.23
70	2.13	2.32	2.13	2.33
70	2.03	3.32	2.03	2.23
74	2.03	3.12	2.03	2.23
74	2.03	3.22	2.14	2.23
74	2.03	4.60	2.23	2.23
78	2.03	3.43	1.85	2.23
78	2.03	3.02	2.43	2.23
78	2.03	3.12	2.03	2.23

A5. Relative Absorbance (600nm) - Raw Data

The following summarizes all raw absorbance (600 nm) data collected for all varieties throughout the second round of fermentations.

Time (hr)	SP-09-02 AC Metcalfe			SP-09-05 TR251			SP-09-11 BM9752D-17			SP-09-13 AC Metcalfe			SP-09-16 TR251			SP-09-22 BM9752D-17		
1	2.5204	2.5354	2.5397	2.4843	2.4867	2.5055	2.4838	2.4826	2.4673	2.5539	2.5641	2.543	2.4985	2.5002	2.4947	2.4977	2.51	2.5116
6	3.0564	3.0129	3.0896	3.071	3.0082	2.9286	2.9139	2.9665	2.9926	3.0993	3.0548	3.037	3.0288	2.9938	2.931	2.9537	2.9383	3.0139
22.5				4.4824	4.3256	4.2485	4.1449	4.2742	4.0606				4.0526	4.6411	4.394	4.5502	4.6412	4.5426
26				4.2665	4.5395	4.3859	4.6144	3.9501	4.2061				4.358	4.7377	4.4297	4.5774	3.97	4.2572
30				4.2074	4.4434	4.661	4.307	4.1741	3.9269				4.7276	4.2305	4.2517	3.9432	4.0793	4.477
46	3.3488	3.5085	3.4468							3.7436	3.8745	3.642						
50	3.5182	3.5731	3.4208							3.7388	3.4533	3.694						
54	2.8308	2.5953	2.5271							3.5138	3.2102	3.195						
70	1.6471	1.4448	1.7771	1.9449	2.1108	2.1451	2.7991	2.4754	2.2406	2.5486	2.5628	2.504	2.4964	2.8996	3.0697	2.9048	2.9194	2.9688
74	2.0095	2.3343	1.8281	1.523	1.9672	0.606	2.4361	2.2033	2.1092	2.5295	2.0588	2.44	2.6265	2.5565	2.4283	2.6265	2.5565	2.4283
78	1.6505	1.4536	1.2859	1.1217	1.5708	1.3404	2.0258	2.2207	1.8617	2.59	2.3938	1.542	2.2025	2.2122	1.9758	2.2025	2.2122	1.9758
Time (hr)	**SP-09-03 - Harrington**			**SP-09-08 - CDC Kendall**			**SP-09-09 - McLeod**			**SP-09-14 - Harrington**			**SP-09-19 - CDC Kendall**			**SP-09-20 - McLeod**		
1	2.6123	2.6197	2.623	2.5679	2.5502	2.5675	2.3266	2.3414	2.3307	2.542	2.5471	2.5463	2.5385	2.5281	2.5388	2.4431	2.4402	2.4431
6	3.012	3.12	3.126	3.0136	3.0443	3.0987	2.8069	2.863	2.853	3.0705	2.9993	3.0929	3.0863	3.1348	3.0052	2.9545	2.8859	2.864
22	4.5158	4.6574	4.217	4.5304	4.4466	4.6176	4.8605	4.6382	4.8605	5.0345	4.6699	4.4355	5.0507	4.218	4.5568	4.5524	4.3161	4.7643
26	4.3715	4.6794	4.716	4.1244	4.6888	4.6006	4.4803	4.0293	4.2028	4.3415	4.1438	4.9536	4.2581	4.4065	4.2754	4.6082	4.5467	4.114
30	4.2958	4.3853	4.498	4.9653	4.8471	4.8222	4.9402	4.5691	4.1298	4.8321	4.4541	4.5008	4.4719	4.3327	4.5987	4.8301	4.4883	4.3941
46	3.4989	3.2402	3.15	4.3973	4.295	3.9932	4.6913	4.3803	4.1007	4.3497	4.7516	4.8021	4.7653	5.0341	4.5666	4.2215	4.1566	4.2913
50	2.699	2.9803	3.261	3.9762	3.6093	3.9167	4.231	1.0314	3.9362	4.389	4.1189	3.8891	4.0836	4.1961	3.9921	4.2041	4.2331	4.2627
54	2.5491	2.8346	2.789	3.5505	3.4839	3.2054	3.5462	3.3084	2.6111	4.4479	3.8362	4.3943	4.1528	4.1234	3.8882	4.1708	2.7986	4.1859
70	2.0217	2.3642	1.945	2.572	2.57	2.4305				3.1147	3.1893	2.8277	3.1602	3.1757	3.1616			
74	2.0055	1.7859	2.212	2.6691	2.3123	2.6369				2.8277	3.0427	3.0948	3.0239	2.9777	2.2907			
78	1.1453	1.4223	2.035	2.1797	2.2565	1.7679				2.0612	2.7445	0.8749	2.466	2.7211	2.1159			

A5. continued

Time (hr)	SP-09-01 - CDC Bold			SP-09-04 - CDC Dolly			SP-09-12 - CDC Bold			SP-09-15 - CDC Dolly		
1	2.7992	2.8128	2.8326	2.6056	2.6632	2.668	2.6577	2.6632	2.7254	2.7376	2.763	2.794
6	2.9351	2.9966	2.9628	2.8548	2.9887	2.8651	2.9198	2.985	2.8686	2.9712	3.0468	2.9943
26												
30												
46.5	3.9233	4.147	4.327	3.9096	4.2667	4.2997	3.2223	3.8787	4.261	4.4091	2.9	4.2094
50	3.5419	3.5921	1.2887	4.3708	4.3987	4.2147	4.5136	4.4651	3.8608	4.545	4.6794	4.3999
54	3.3447	3.845	1.151	4.0698	3.9051	3.7787	3.8588	4.2437	4.0441	3.9673	4.5362	4.4735
70	2.9535	2.7785	2.6324	2.9158	3.0387	2.855	3.179	2.8889	2.9676	3.8876	3.7407	3.7532
73.75	2.1557	3.1983	2.1566	3.0166	2.8928	2.5566	3.2642	2.5302	3.7107	3.4127	3.2827	3.3105
78	1.7222	1.9158	2.9143	2.7703	2.5941	1.9627	2.2366	1.3754	3.0502	3.1972	3.3195	3.1007
Time (hr)	**SP-09-06 - CDC Copeland**			**SP-09-07 - TR306**			**SP-09-17 - CDC Copeland**			SP-09-18 - TR306		
1	2.6234	2.6265	2.6034	2.6197	2.6101	2.6138	2.6157	2.6095	2.6022	2.6017	2.611	2.6285
6	3.08	3.0423	3.0838	3.0132	2.9775	3.0512	3.0948	3.0444	3.1014	3.1073	3.1144	
22	3.856	4.2459	3.8514	4.6971	3.9715	4.8684	3.941	5.0022	4.77	3.9189	4.293	4.1102
26	4.0172	4.0763	4.6327	4.7013	4.7353	4.0051	4.1088	4.408	4.713	4.7937	4.6387	4.7214
29.5	4.4999	4.8037	4.5463	4.3898	4.5589	4.3829	4.2327	4.5116	4.6224	4.2643	4.5301	4.2318
45.5	3.7682	2.9816	3.6212	3.5747	3.5663	3.6749	4.121	4.29	3.7929	3.7899	4.1782	3.7121
50	3.4529	3.6064	3.3203	3.706	3.6671	3.7739	4.2035	4.2764	4.2985	3.917	3.9029	4.0572
54	3.3358	3.1227	2.9683	3.0218	0.8482	2.9557	3.658	3.8777	3.7029	3.7978	3.8216	2.9211
69.5	2.268	2.3224	2.0963	2.1211	1.7107	1.9967	2.8251	2.9162	2.8392	2.732	2.8989	0.8483
74	1.0114	2.0737	2.0562	2.1627	1.826	0.69471	2.0338	0.3092	2.8758	2.7888	2.7916	2.6384
78	1.0159	1.838	2.1221	1.3221	1.302	0.11451	1.7631	2.4364	2.4439	2.2965	2.0819	2.1923

A5. continued

Time (hr)	SP-09-10 - CDC Helgason			SP-09-21 - CDC Helgason		
1	2.373	2.3888	2.4002	2.4465	2.4554	2.4537
5.5	2.8329	2.899	2.8272	2.8105	2.8853	2.923
21.5	4.7565	3.7433	4.3274	4.1593	3.8333	4.6796
26	4.4352	4.7598	4.3566	4.4619	3.3932	4.7968
30	4.1055	4.0161	4.3504	4.575	4.3679	4.2162
45.5	3.9466	4.0728	4.6976	4.1424	4.271	4.1369
50	3.8764	3.755	3.8476	4.2056	4.0029	3.7754
54	2.9102	3.1691	1.1489	4.0455	4.0307	4.1732
70	2.4145	2.6823	2.688	2.9631	3.0724	2.997
74	2.294	2.2046	2.0877	2.6839	2.9157	2.9729
78	1.76	2.0846	2.2171	3.1511	2.6145	2.703

A6. Third Round Fermentations

Third round fermentations were conducted on a single malt variety using three sugar spikes to monitor the effect of initial sugar profiles on carbohydrate consumption throughout fermentation.

Raw sugar and ethanol data collected over time using HPLC analysis of fermenting wort for malt variety AC Metcalfe using a 4% glucose spike prior to yeast inoculation.

AC Metcalfe (4% Glucose Spike)

Time (hr)	Maltotriose (g/100mL)		Maltose (g/100mL)		Glucose (g/100mL)		Fructose (g/100mL)		Ethanol (% v/v)	
0	1.072	1.125	4.588	4.829	4.234	4.463	0.319	0.307		
2	1.253	1.274	5.407	5.504	4.909	4.984	0.409	0.421		
6	1.293	1.396	5.530	5.920	4.484	4.718	0.385	0.406		
15	1.268	1.266	5.085	5.125	2.635	2.798	0.340	0.346	2.440	2.226
18	0.934	0.955	3.808	3.839	1.859	1.739	0.307	0.298	2.875	3.100
21	0.935	0.976	3.794	3.845	1.486	1.313	0.302	0.297	3.680	4.000
25	0.848	0.788	3.322	3.053	0.783	0.440	0.243	0.203	4.595	4.889
30	0.609	0.681	2.461	2.721	0.013	0.036	0.073	0.095	5.516	5.787
42	0.283	0.294	1.018	1.172					6.881	6.838
48	0.214	0.192	0.470	0.382					7.209	7.153
66	0.194	0.202	0.178	0.282					7.196	7.175

Raw sugar and ethanol data collected over time using HPLC analysis of fermenting wort for malt variety AC Metcalfe using a 4% maltose spike prior to yeast inoculation.

AC Metcalfe (4% Maltose Spike)

Time (hr)	Maltotriose (g/100mL)		Maltose (g/100mL)		Glucose (g/100mL)		Fructose (g/100mL)		Ethanol (% v/v)	
0	1.169	1.288	8.302	9.166	1.115	1.229	0.300	0.327		
2	1.218	1.376	8.620	9.660	1.162	1.276	0.361	0.399		
6	1.377	1.411	9.657	9.863	0.905	0.920	0.355	0.359		
15	1.178	1.171	8.197	8.114	0.144	0.158	0.196	0.198	2.620	2.584
18	1.024	0.872	7.294	6.290	0.100	0.052	0.183	0.138	3.146	3.328
21	0.846	0.813	6.018	5.955	0.014	0.021	0.078	0.090	3.995	3.937
25	0.687	0.701	5.055	5.436		0.009	0.038	0.065	4.710	4.205
30	0.538	0.482	4.068	3.630					5.523	5.555
42	0.241	0.239	1.264	1.157					7.157	7.176
48	0.198	0.200	0.652	0.734					7.524	7.623
66	0.190	0.191	0.275	0.221					7.544	7.501

A6. continued

Raw sugar and ethanol data collected over time using HPLC analysis of fermenting wort for malt variety AC Metcalfe using a 4% mixed sugar spike prior to yeast inoculation.

AC Metcalfe (4% Mixed Sugars Spike)										
Time (hr)	Maltotriose (g/100mL)		Maltose (g/100mL)		Glucose (g/100mL)		Fructose (g/100mL)		Ethanol (% v/v)	
0	1.723	1.944	7.459	8.293	1.691	1.833	0.388	0.431		
2	1.981	1.962	8.354	8.297	1.856	1.836	0.503	0.500		
6	2.030	1.999	8.514	8.386	1.472	1.438	0.473	0.465		
15	1.712	1.719	7.036	7.043	0.375	0.359	0.294	0.289	2.671	2.671
18	1.415	1.423	6.178	6.102	0.304	0.204	0.297	0.259	2.873	3.432
21	1.268	1.275	5.382	5.398	0.049	0.048	0.166	0.163	4.093	4.215
25	1.564	1.023	6.835	4.542			0.299	0.067	2.869	4.782
30	0.759	0.727	3.473	3.410					5.736	5.733
42	0.279	0.282	1.103	1.139					7.228	7.215
48	0.211	0.219	0.579	0.594					7.699	7.792
66	0.196	0.202	0.187	0.267					7.572	7.557

Logistic model applied to each of maltotriose, maltose, glucose, fructose, and ethanol data resulting from HPLC analysis of third round fermentations. Model parameters and corresponding ASE are summarized.

Logistic Parameter	Maltotriose, (g/100mL)	Maltose, (g/100mL)	Glucose, (g/100mL)	Fructose, (g/100mL)	Ethanol, (% v/v)
			AC Metcalfe (4% Glucose Spike)		
P∞	0.19 (0.053)	0.19 (0.273)	-0.1 (0.306)	-0.5 (2.337)	7.26 (0.089)
Pi	1.26 (0.048)	5.51 (0.26)	4.85 (0.209)	0.38 (0.021)	-0.34 (1.139)
B	-0.1607 (0.035)	-0.1262 (0.025)	-0.2249 (0.05)	-0.1691 (0.131)	-0.1313 (0.018)
M	27.38 (1.361)	27.65 (1.509)	16.48 (0.722)	34.11 (26.27)	19.71 (2.342)
			AC Metcalfe (4% Maltose Spike)		
P∞	0.19 (0.036)	0.23 (0.315)	0.01 (0.037)	0.04 (0.041)	7.63 (0.108)
Pi	1.34 (0.044)	9.62 (0.346)	1.27 (0.055)	0.36 (0.018)	1.94 (0.216)
B	-0.167 (0.027)	-0.1316 (0.019)	-0.344 (0.058)	-0.308 (0.135)	-0.1537 (0.015)
M	23.25 (0.875)	25.78 (0.986)	8.885 (0.625)	15.73 (1.054)	26.13 (0.685)
			AC Metcalfe (4% Mixed Sugar Spike)		
P∞	0.16 (0.081)	0.05 (0.4)	0.01 (0.099)	0.17 (0.065)	7.7 (0.322)
Pi	1.99 (0.087)	8.53 (0.395)	1.93 (0.1)	0.46 (0.035)	2.36 (0.89)
B	-0.1468 (0.03)	-0.1243 (0.023)	-0.2863 (0.054)	-0.3599 (0.361)	-0.1777 (0.076)
M	25.54 (1.256)	27.29 (1.396)	10.14 (0.823)	14.94 (2.035)	27.8 (2.32)

N.B. Bracketed values denote asymptotic standard error (ASE).

A7. Two-Way ANOVA

The following are significance (p) values obtained by running multiple two-way ANOVA (Analysis of Variance) procedures on the indicated data sets, across both year and variety for each model parameter. Two-way ANOVAs were conducted by first combining all varieties and comparing the percent variation experienced between parameter values as a function of variety and growing year. Further analyses were later conducted individually between fermentation weeks, which included only those varieties fermented with a single yeast grow-up. Bolded text indicates significant difference ($p<0.05$).

Two-way ANOVA by year and variety of parameter values from the logistic function describing RE, grouped as a single data set ("All varieties") and by those varieties fermented together from a single yeast grow-up ("Grouped by week"). Reported p-values only.

RE

	Pi (p-value)			P_∞ (p-value)			B (p-value)			M (p-value)		
	All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week	
Interaction	0.7458	0.5212		**0.0139**	0.9596		0.2989	0.8330		0.8825	0.5799	
Year	**0.0072**	0.3773	Week 1	**0.0002**	0.9395	Week 1	**< 0.0001**	0.3821	Week 1	0.0980	0.5721	Week 1
Variety	**< 0.0001**	**0.0250**		**< 0.0001**	0.3007		**< 0.0001**	**< 0.0001**		**0.0059**	0.1414	
Interaction	0.7458	0.3029		**0.0139**	0.0610		0.2989	**0.0190**		0.8825	0.5178	
Year	**0.0072**	0.0661	Week 2	**0.0002**	**0.0261**	Week 2	**< 0.0001**	**0.0055**	Week 2	0.0980	0.1030	Week 2
Variety	**< 0.0001**	**0.0113**		**< 0.0001**	0.0641		**< 0.0001**	**< 0.0001**		**0.0059**	**0.0240**	
Interaction	0.7458	0.3622		**0.0139**	**0.0065**		0.2989	0.3630		0.8825	0.4603	
Year	**0.0072**	**0.0244**	Week 3	**0.0002**	**< 0.0001**	Week 3	**< 0.0001**	**< 0.0001**	Week 3	0.0980	0.1328	Week 3
Variety	**< 0.0001**	0.0796		**< 0.0001**	**< 0.0001**		**< 0.0001**	**< 0.0001**		**0.0059**	0.7981	
Interaction	0.7458	0.3770		**0.0139**	0.4413		0.2989	0.2902		0.8825	0.4153	
Year	**0.0072**	0.2871	Week 4	**0.0002**	0.1746	Week 4	**< 0.0001**	**0.0351**	Week 4	0.0980	0.9258	Week 4
Variety	**< 0.0001**	0.2530		**< 0.0001**	**0.0151**		**< 0.0001**	0.5415		**0.0059**	**0.0081**	

A7. continued

Two-way ANOVA by year and variety of parameter values from the logistic function describing Maltotriose, grouped as a single data set ("All varieties") and by those varieties fermented together from a single yeast grow-up ("Grouped by week"). Reported p-values only.

Maltotriose

	Pi (p-value)			P_∞ (p-value)			B (p-value)			M (p-value)		
	All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week	
Interaction	0.2499	0.3378		0.6747	0.9261		**0.0240**	0.1346		0.1559	0.5243	
Year	**0.0149**	0.2796	Week 1	**0.0820**	0.7169	Week 1	0.4993	0.4038	Week 1	0.1495	0.2563	Week 1
Variety	**0.0149**	0.1326		**< 0.0001**	**< 0.0001**		**0.0074**	0.9002		**< 0.0001**	**0.0151**	
Interaction	0.2499	0.2858		0.6747	0.0704		**0.0240**	**0.0169**		0.1559	0.0853	
Year	**0.0149**	**< 0.0001**	Week 2	**0.0820**	**0.0483**	Week 2	0.4993	0.3159	Week 2	0.1495	0.0572	Week 2
Variety	**0.0149**	**0.0156**		**< 0.0001**	**0.0060**		**0.0074**	**0.0036**		**< 0.0001**	**0.0211**	
Interaction	0.2499	0.3622		0.6747	**0.0065**		**0.0240**	0.5915		0.1559	0.0819	
Year	**0.0149**	**0.0244**	Week 3	**0.0820**	**< 0.0001**	Week 3	0.4993	**0.0259**	Week 3	0.1495	0.1674	Week 3
Variety	**0.0149**	0.0796		**< 0.0001**	**< 0.0001**		**0.0074**	**0.0020**		**< 0.0001**	**< 0.0001**	
Interaction	0.2499	0.2704		0.6747	0.3240		**0.0240**	0.0986		0.1559	0.0681	
Year	**0.0149**	0.7396	Week 4	**0.0820**	0.2758	Week 4	0.4993	0.5462	Week 4	0.1495	0.7106	Week 4
Variety	**0.0149**	0.2989		**< 0.0001**	**0.0167**		**0.0074**	0.6054		**< 0.0001**	0.2047	

Two-way ANOVA by year and variety of parameter values from the logistic function describing Maltose, grouped as a single data set ("All varieties") and by those varieties fermented together from a single yeast grow-up ("Grouped by week"). Reported p-values only.

Maltose

	Pi (p-value)			P_∞ (p-value)			B (p-value)			M (p-value)		
	All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week	
Interaction	0.4716	0.9848		0.7039	0.6337		0.7900	0.6392		0.5712	0.3659	
Year	0.0686	0.1908	Week 1	0.0856	0.2385	Week 1	0.0689	0.5389	Week 1	0.4270	0.5480	Week 1
Variety	0.2630	0.2705		**0.0094**	**0.0203**		**0.0018**	0.0927		0.0998	**0.0141**	
Interaction	0.4716	0.1740		0.7039	0.4944		0.7900	**0.0059**		0.5712	0.0618	
Year	0.0686	0.6872	Week 2	0.0856	0.1201	Week 2	0.0689	0.5727	Week 2	0.4270	0.1216	Week 2
Variety	0.2630	0.0762		**0.0094**	0.3861		**0.0018**	**< 0.0001**		0.0998	0.7419	
Interaction	0.4716	0.4971		0.7039	0.2997		0.7900	0.5992		0.5712	0.7531	
Year	0.0686	0.0589	Week 3	0.0856	0.9021	Week 3	0.0689	**0.0026**	Week 3	0.4270	0.7224	Week 3
Variety	0.2630	0.1705		**0.0094**	0.5821		**0.0018**	**< 0.0001**		0.0998	0.4851	
Interaction	0.4716	0.3622		0.7039	0.6453		0.7900	0.1369		0.5712	0.1585	
Year	0.0686	0.6391	Week 4	0.0856	0.4276	Week 4	0.0689	0.5621	Week 4	0.4270	0.6682	Week 4
Variety	0.2630	0.4992		**0.0094**	0.9761		**0.0018**	0.5567		0.0998	0.5310	

A7. continued

Two-way ANOVA by year and variety of parameter values from the logistic function describing Glucose, grouped as a single data set ("All varieties") and by those varieties fermented together from a single yeast grow-up ("Grouped by week"). Reported p-values only.

Glucose

	Pi (p-value)			P_∞ (p-value)			B (p-value)			M (p-value)		
	All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week	
Interaction	0.1027	**< 0.0001**		0.9976	0.8280		0.1628	0.1737		0.1781	0.0793	
Year	0.6807	0.4792	Week 1	0.8622	0.9368	Week 1	**0.0360**	0.5472	Week 1	**0.0152**	0.7856	Week 1
Variety	**< 0.0001**	**< 0.0001**		0.7339	0.5968		**< 0.0001**	**0.0019**		**< 0.0001**	**0.0012**	
Interaction	0.1027	0.2504		0.9976	0.9112		0.1628	0.4763		0.1781	0.2296	
Year	0.6807	0.5655	Week 2	0.8622	0.7654	Week 2	**0.0360**	0.7848	Week 2	**0.0152**	0.3188	Week 2
Variety	**< 0.0001**	**0.0089**		0.7339	0.5725		**< 0.0001**	**< 0.0001**		**< 0.0001**	0.0560	
Interaction	0.1027	**0.0053**		0.9976	0.9721		0.1628	0.3843		0.1781	0.3560	
Year	0.6807	0.1249	Week 3	0.8622	0.7561	Week 3	**0.0360**	**< 0.0001**	Week 3	**0.0152**	**< 0.0001**	Week 3
Variety	**< 0.0001**	**< 0.0001**		0.7339	0.7022		**< 0.0001**	**< 0.0001**		**< 0.0001**	0.2911	
Interaction	0.1027	0.8094		0.9976	0.4594		0.1628	0.8583		0.1781	0.3387	
Year	0.6807	0.3250	Week 4	0.8622	0.8093	Week 4	**0.0360**	**0.0340**	Week 4	**0.0152**	0.5530	Week 4
Variety	**< 0.0001**	0.5332		0.7339	0.6961		**< 0.0001**	**< 0.0001**		**< 0.0001**	**0.0009**	

Two-way ANOVA by year and variety of parameter values from the logistic function describing Fructose, grouped as a single data set ("All varieties") and by those varieties fermented together from a single yeast grow-up ("Grouped by week"). Reported p-values only.

Fructose

	Pi (p-value)			P_∞ (p-value)			B (p-value)			M (p-value)		
	All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week	
Interaction	**< 0.0001**	0.6406		0.7215	0.2220		**0.0240**	0.3285		**0.0133**	0.4438	
Year	**< 0.0001**	**0.0460**	Week 1	0.2814	0.2523	Week 1	0.2409	0.6272	Week 1	0.1526	0.2898	Week 1
Variety	**< 0.0001**	**< 0.0001**		**0.0280**	**0.0121**		**< 0.0001**	**0.0224**		**< 0.0001**	**0.0100**	
Interaction	**< 0.0001**	**0.0010**		0.7215	0.6575		**0.0240**	0.1483		**0.0133**	0.2940	
Year	**< 0.0001**	**< 0.0001**	Week 2	0.2814	0.5885	Week 2	0.2409	0.8533	Week 2	0.1526	0.8802	Week 2
Variety	**< 0.0001**	**< 0.0001**		**0.0280**	0.5204		**< 0.0001**	**0.0066**		**< 0.0001**	**0.0365**	
Interaction	**< 0.0001**	**< 0.0001**		0.7215	0.3425		**0.0240**	0.3428		**0.0133**	**0.0002**	
Year	**< 0.0001**	**< 0.0001**	Week 3	0.2814	0.4200	Week 3	0.2409	0.5400	Week 3	0.1526	**0.0029**	Week 3
Variety	**< 0.0001**	**< 0.0001**		**0.0280**	0.2100		**< 0.0001**	0.6698		**< 0.0001**	**< 0.0001**	
Interaction	**< 0.0001**	**0.0004**		0.7215	0.8471		**0.0240**	**0.0007**		**0.0133**	0.5119	
Year	**< 0.0001**	**< 0.0001**	Week 4	0.2814	0.5022	Week 4	0.2409	0.3175	Week 4	0.1526	0.0599	Week 4
Variety	**< 0.0001**	**< 0.0001**		**0.0280**	0.9254		**< 0.0001**	**0.0088**		**< 0.0001**	**< 0.0001**	

A7. continued

Two-way ANOVA by year and variety of parameter values from the logistic function describing AE, grouped as a single data set ("All varieties") and by those varieties fermented together from a single yeast grow-up ("Grouped by week"). Reported p-values only.

AE

	Pi (p-value)			P_∞ (p-value)			B (p-value)			M (p-value)		
	All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week		All varieties	Grouped by week	
Interaction	0.5764	0.4713		**0.0139**	0.9404		**0.0244**	0.8645		0.7050	0.4747	
Year	**0.0314**	0.3171	Week 1	**< 0.0001**	0.8176	Week 1	**< 0.0001**	0.2306	Week 1	0.2264	0.4784	Week 1
Variety	**< 0.0001**	**0.0169**		**< 0.0001**	0.2376		**< 0.0001**	**< 0.0001**		**0.0030**	0.0926	
Interaction	0.5764	0.1097		**0.0139**	**0.0398**		**0.0244**	**0.0036**		0.7050	0.1742	
Year	**0.0314**	0.2963	Week 2	**< 0.0001**	**0.0030**	Week 2	**< 0.0001**	**0.0271**	Week 2	0.2264	0.6673	Week 2
Variety	**< 0.0001**	**0.0205**		**< 0.0001**	**0.0164**		**< 0.0001**	**< 0.0001**		**0.0030**	0.0976	
Interaction	0.5764	0.3463		**0.0139**	**0.0059**		**0.0244**	0.3548		0.7050	0.4380	
Year	**0.0314**	**0.0227**	Week 3	**< 0.0001**	**< 0.0001**	Week 3	**< 0.0001**	**< 0.0001**	Week 3	0.2264	0.1270	Week 3
Variety	**< 0.0001**	0.0743		**< 0.0001**	**< 0.0001**		**< 0.0001**	**< 0.0001**		**0.0030**	0.8008	
Interaction	0.5764	0.1590		**0.0139**	0.6821		**0.0244**	0.0599		0.7050	0.2297	
Year	**0.0314**	0.6438	Week 4	**< 0.0001**	0.1260	Week 4	**< 0.0001**	0.0964	Week 4	0.2264	0.6131	Week 4
Variety	**< 0.0001**	0.7097		**< 0.0001**	**0.0121**		**< 0.0001**	0.1798		**0.0030**	0.0928	

A8. Raw Attenuation Data

The following summarizes all attenuation data (AE) collected from all varieties throughout second round fermentations (measured in ^{o}P) using a digital density meter:

Time (Hr)	CDC Bold (2007)	AC Metcalfe (2007)	Harrington (2007)	CDC Dolly (2007)	TR251 (2007)	CDC Copeland (2007)	TR 306 (2007)	CDC Kendall (2007)	McLeod (2007)
0									
1	14.6	14.7	14.6	14.7	14.6	14.8	14.7	14.7	14.7
1	14.6	14.7	14.7	14.7	14.6	14.8	14.7	14.7	14.7
1	14.6	14.7	14.7	14.7	14.6	14.8	14.7	14.7	14.7
6	14	14.1	14	14	14	14.1	14.1	14.2	14.3
6	13.9	14.1	14	13.9	14	14.2	14.1	14.1	14.2
6	13.9	14.1	14.1	13.9	14	14.2	14.1	14.1	14.2
22			6.7			7	6.9	7.1	8.3
22			7.2			7.2	6.8	7.2	8.6
22			6.9			7.2	7.2	7.3	8.6
23		6.9			6.9				
23		7.1			7.2				
23		7.3			7.1				
26	5.4	6.2	4.9	7	5.3	5.2	5.3	5.3	7
26	5.7	6.6	4.6	7	5.6	5.3	5.4	5.6	6.8
26	5.2	5.6	5.1	7	5.4	5.3	5.3	5.4	6.8
30	4.2	4.3	3.4	6.4	4.4	4.2	4.2	4.2	5.7
30	4.5	4.1	3.6	6.2	4.4	4.1	4.2	4.2	5.8
30	4.2	4.1	3.5	6.3	4.5	4.2	4.2	4.4	5.7
46		2.2	1.9		2.4	2.1	2.1	2	3.3
46		2.2	1.9		2.5	2.6	2.1	2.2	3.2
46		2.2	1.9		2.5	2	2.2	2.2	3.1
46.5	2.1			3.5					
46.5	2			3.8					
46.5	2			3.8					
50	2.1	2.1	1.8	3.4	2.4	1.9	2.1	1.8	2.9
50	2.7	2.1	1.8	3.3	2.2	1.9	2.3	1.9	4.5
50	3.5	2	1.8	3.5	2.5	2	2.2	2	3.1
54	2	2	1.8	3.2	2.1	1.9	2.2	1.9	2.9
54	1.9	2	1.8	3	2.3	1.9	3	1.8	2.9
54	3.2	1.9	1.7	3	2.2	1.8	2	1.6	3.1
70	1.8	1.9	1.7	2.4	2.1	1.8	1.9	1.7	2.8
70	1.9	1.9	1.8	2.5	2.1	1.8	1.9	1.8	2.7

(continued from pg. 129)

Time (Hr)	CDC Bold (2007)	AC Metcalfe (2007)	Harrington (2007)	CDC Dolly (2007)	TR251 (2007)	CDC Copeland (2007)	TR 306 (2007)	CDC Kendall (2007)	McLeod (2007)
70	1.9	1.9	1.7	2.1	2.1	1.7	1.9	1.8	2.6
74	1.8	1.9	1.7	2.4	2	3	2	1.8	3.5
74	2.3	1.9	1.7	2.3	2	1.8	1.9	1.8	2.6
74	1.9	1.8	1.8	2.4	2.4	1.7	2.7	1.8	2.7
78	1.8	1.9	1.8	2.4	2	1.8	1.9	1.8	2.6
78	1.9	1.8	1.8	2.3	2	1.8	2	1.7	2.7
78	1.8	1.9	1.7	2.4	2	1.8	3	1.8	3

Time (Hr)	CDC Helgason (2007)	BM9752D-17 (2007)	CDC Bold (2008)	AC Metcalfe (2008)	Harrington (2008)	CDC Dolly (2008)	TR251 (2008)	CDC Copeland (2008)	TR 306 (2008)
0									
1	14.6	14.5	14.9	14.7	14.7	14.8	14.7	14.7	14.8
1	14.7	14.6	14.9	14.7	14.7	15	14.7	14.7	14.8
1	14.7	14.6	14.9	14.7	14.7	14.8	14.7	14.7	
6	14.2	13.6	14.1	14.1	14.1	14.2	14.1	14.1	14.1
6	14.2	13.9	14.1	14	14	14.2	14.1	14	14.1
6	14.1	14	14.1	13.7	14	14.2	14.2	14	
22	8.1				7.6			7.5	7.4
22	8.1				7.9			7.4	7.7
22	7.9				7.6			7.5	7.4
23		7.6		7.1			7.3		
23		7.5		7.3			7.4		
23		7.6		7.2			7.4		
26	6	6.2	5.9	6.1	6.1	7.5	6.2	6.2	6
26	6	6.2	5.7	6.2	6	7.6	6.2	6	6.1
26	6.1	6.2	5.8	6.1	6.1	7.6	6.2	6.1	5.8
30	5.9	5	4.7	4.7	5.3	6.8	5.2	5	4.8
30	5	5.6	4.7	4.7	5.1	6.8	5.3	6.5	4.7
30	5.3	5	5.2	4.5	5	6.8	5.2	5	4.8
46	2.5	2.9		2.5	2.8		2.9	2.6	2.5
46	2.3	2.8		2.5	2.8		3.1	2.6	2.6
46	2.6	2.6		2.5	2.8		2.9	2.5	2.7
46.5			3.4			4.4			
46.5			2.3			6			
46.5			2.2			4.1			
50	2.2	2.6	2.2	2.4	2.5	3.9	2.6	2.3	2.3

(continued from pg. 130)

Time (Hr)	CDC Helgason (2007)	BM9752D-17 (2007)	CDC Bold (2008)	AC Metcalfe (2008)	Harrington (2008)	CDC Dolly (2008)	TR251 (2008)	CDC Copeland (2008)	TR 306 (2008)
50	2.3	3.1	2.2	2.4	2.6	3.9	2.7	2.4	2.3
50	2.2	3.7	2	2.3	2.5	3.9	2.6	2.3	2.3
54	2.7	2.6	2.1	2.3	3.2	3.6	2.5	2.2	3.5
54	2.7	2.5	2.1	2.3	2.3	3.4	2.4	2.2	2.5
54	3.8	2.2	2.1	2.3	2.4	3.4	2.4	2.1	2.3
70	1.8	2.1	1.9	2.1	3.9	2.7	2.2	2	2.2
70	1.9	2.1	2.1	2.2	2.2	2.7	2.2	2	2.2
70	1.8	2	1.9	2.2	2.2	2.6	2.2	2	2.8
74	1.9	2	2	2.2	2.2	2.6	2.2	2	2.2
74	1.8	2	2.1	2.2	2.2	2.6	2.3	3.2	2.2
74	1.8	2.1	2.4	2.2	2.2	2.6	2.2	2	2.2
78	1.8	2	2	2.2	2	2.7	2.1	2	2.2
78	1.9	2	3.1	2.2	2.2	2.6	2.2	2	2.2
78	1.8	2	2	2.2	3.7	2.6	2.2	2	2.2

Time (Hr)	CDC Kendall (2008)	McLeod (2008)	CDC Helgason (2008)	BM9752D-17 (2008)
0				
1	14.7	14.6	14.5	14.7
1	14.7	14.6	14.5	14.7
1	14.7	14.6	14.5	14.7
6	14.1	14.1	14	14.1
6	14.1	14.1	14.1	14.1
6	14.1	14.1	14	14.1
22	7.4	9	8.3	
22	7.4	9.1	8.8	
22	7.4	9	8.4	
23				7.5
23				7.7
23				7.3
26	5.8	8.1	6.5	6.1
26	5.7	7.8	6.4	6.6
26	5.8	7.8	6.6	6.4
30	5.2	6.8	5	5.2
30	5.1	6.8	5.8	5.2
30	4.7	6.8	5.3	5.3

(continued from pg. 131)

Time (Hr)	CDC Kendall (2008)	McLeod (2008)	CDC Helgason (2008)	BM9752D-17 (2008)
46	2.4	4.6	2.6	3
46	2.5	4.6	2.5	2.7
46	2.4	4.5	2.6	2.6
46.5				
46.5				
46.5				
50	2	4.2	2.4	2.5
50	2.1	4.2	2.3	2.6
50	2.3	4.2	2.4	2.6
54	2	3.9	2.3	2.4
54	2	4.7	2.4	2.2
54	2	4	2.2	2.5
70	1.9	3.4	2	2.1
70	2	2.2	2	2.2
70	1.9	3.2	1.9	2.1
74	1.9	3	1.9	2.1
74	1.9	3.1	2	2.1
74	1.9	4.5	2.1	2.1
78	1.9	3.3	1.7	2.1
78	1.9	2.9	2.3	2.1
78	1.9	3	1.9	2.1