Assessing Floor Versus Pneumatic Malting: Comparing the Effects on Malt Quality and Fermentability

by

Gerrard Kilfoil

Submitted in partial fulfilment of the requirements

of the degree of Master of Science

at

Dalhousie University

Halifax, Nova Scotia

September 2020

Dedication

To my late brother Mike. Everything has been because of you, the rest is for you.

Table of Contents

List of Tables

List of Figures

Abstract

The increase in popularity of craft beer over the past decade has resulted in a subsequent increase in the craft malting industry. Approximately 25% of craft malthouses use a labour-intensive method called floor malting for barley malt production. This research's focus was to address some main concerns around floor malting. Over two years, malting data was collected on 22 batches of floor-malted barley. Mini-fermentations of these malt samples showed that significant ($p<0.05$) correlations exist between fermentation and malting parameters. Only one floor malted sample displayed the potential for premature yeast flocculation (PYF). When compared to pneumatic malt, floor malt yielded significantly higher ($p<0.05$) extract, colour, FAN, and diastatic power as well as significantly ($p<005$) lower friability. Additionally, initial laboratory trials showed the ASBC Yeast-14 method could be shortened by one day, require fewer laboratory materials, and employ other lager yeast strains to detect PYF in malt samples.

List of Abbreviations and Symbols Used

AAC	Agriculture and Agri-food Canada
AC	Agriculture Canada
ADF	Apparent Degree of Fermentation
ASBC	American Society of Brewing Chemists
°C	Degrees Celsius
CDC	Crop Development Center
CI	Confidence Interval
cm	centimeter
CMBTC	Canadian Malting Barley Technical Centre
COA	Certificate of Analysis
CO_2	Carbon Dioxide
db	Dry Basis
DNA	Deoxyribonucleic Acid
DOE	Design of Experiment
DON	Deoxynivalenol
DP	Diastatic Power
FAN	Free Amino Nitrogen
g	grams
mL	milliliter
NSLC	Nova Scotia Liquor Convention
ppb	parts per billion
psi	pounds per square inch
PYF	Premature Yeast Flocculation
rpm	revolutions per minute
VDK	Vicinal Diketones
w/v	weight / volume
x*g*	multiplied by gravity
YEPD	Yeast Extract Peptone Dextrose
YIS	Yeast in Suspension

Acknowledgements

I would like to extend my gratitude for everyone who has helped make this possible. I have met some incredible people during the duration of this degree who have provided valuable guidance and plenty of great laughs. I am happy to extend a sincere thank you to all of you.

First off to Dr. Alex Speers, thank you so much for taking me on as a student and granting me this opportunity. I truly have learned a great deal from you, whether it was brewing science related or Monty Python references. To my supervisory committee members Dr. Allan Paulson and Dr. Tim Ells, thank you for your continued guidance during this entire process. Thank you Dr. Adel Al-Taweel for joining my committee last minute to help make this possible.

Thank you to my fellow colleagues Prachi, Lani, and Keven, I consider myself very fortunate to have become so close with all of you over the past couple years. Thank you so much for enduring my extra strong coffee and especially terrible puns. You guys have certainly helped to culture me as we have become such a close floc (that's it, no more, you can safely read on). Best of luck to each of you and your future endeavors!

Finally, I would not be where I am today without my amazing friends and family. Words cannot express how fortunate I consider myself to have all of you. You have always been there to support me and provide much needed study breaks.

Thank you all!

Chapter 1 Introduction

Beer is among the most popular beverages globally; it is also a diverse class of alcoholic drinks. Beer is typically made from a minimum of four ingredients: water, yeast, hops and malted barley. The vast selection of beers available today is a result of the wide variety of 1) it is ingredients and 2) differences in processing techniques. Consumers demand as high-quality beer, this in turn increases the demand for high-quality beer ingredients by the brewer, especially malted barley. The process of malting barley is a delicate one, as process conditions influence final malt quality. The aims of the following research are to help understand several malting process conditions and their effects on subsequent malt fermentations. In particular, this thesis aims to examine the process conditions in a floor malthouse and how these differing conditions from commercial pneumatic malting systems affect final malt quality and fermentability.

Beer consumers often overlook the process of malting. This is because malting is not as romanticized as the brewing process, since malting creates brewing precursors, whereas brewing directly makes an enjoyable consumable. However, without the malting step the resulting beer would be unrecognizable compared to the current beverage. The process of malting barley involves the controlled and limited germination of the grain ([1]). Germination is a naturally occurring biological process that the barley grains undergo in order to sprout a new plant. This process is manipulated by the maltster in order to produce a consistent, balanced enzyme- and carbohydrate-rich malt ([2]). These enzymes are later required by brewers for conversion of the grain's remaining starch reserves into fermentable sugars during brewing.

Maltsters must pay close attention to malting conditions such as time, temperature and moisture in order to produce consistently good quality malt. Aside from being a carbohydrate source, malt contributes flavours and colour to the final beer, therefore, any changes to the malting process will directly affect these two main characteristics of the beer. It is imperative to have tight controls of conditions during malting to ensure that a consistent malt gets produced to aid in the production of a consistent beer, this is a difficult feat as malting is not a sterile process. Barley is cleaned of soil, insects and debris upon arrival at the malthouse but still houses a plethora of microbes consisting of wild yeasts, bacteria, and filamentous fungi ([3,4]). These organisms will begin to proliferate under the humid and temperate conditions experienced during malting and can have detrimental effects on resulting malt quality. Without plausible commercial sterilization of barley, the only way to keep malt from microbial spoilage is to keep germination temperatures below physiological temperatures. Depending on the initial microbial load on the barley, these germination temperatures can still be high enough to support detrimental microbial proliferation.

Horton Ridge Malt and Grain Company located in Hortonville, NS became the first malthouse in Atlantic Canada in 2016. Horton Ridge uses a traditional technique for malting grains called floor malting. This technique involves the germination stage of malting being carried out on a concrete floor. This is contrary to most modern commercial systems that employ larger automated, pneumatic malting equipment. In pneumatic systems, the germination stage is carried out on large perforated beds which allows for constant airflow of humid, temperature-controlled air into

the bed of germinating grains. These beds are also constantly being turned over by automated arms which aerate and disentangles the growing rootlets from the grain kernels. As a result, pneumatic malting systems have been designed to produce uniform malt to tight specifications on a large scale. However, floor malting does not allow for as precise temperature and humidity control over the germinating grains. Thus, the germinating grains on the concrete floor may develop temperature and moisture gradients. Floor maltsters try to limit these variations by keeping the grain pile only 7-10 cm deep. As well, floor malthouses would typically open doors and windows during the cool nights to help reduce temperatures in the germination room. The aeration of germinating grains during floor malting is also different than pneumatic malting systems as a more labour-intensive approach is used. Aeration during floor malting involves a rake being pulled through the germinating grains every 4-5 hours to disperse heat and carbon dioxide as well as break up rootlets. The other stages of malting, steeping and kilning, resemble a similar but small-scale version of the commercial pneumatic malting operations.

For hundreds of years floor malting was the only method for production of malted grains. However, floor malting was always considered to be a seasonal profession. As mentioned previously, floor malthouses would open doors and windows during the nights to reduce temperatures of the germinating grains. However, during the summer months the evening temperatures could remain too hot to effectively cool down the germinating grains. This would lead to undesirable flavour development in the malt but more importantly would cause spoilage from fungal infection. Pneumatic malting systems were designed to constantly ‘push’ cool and humid air through the bed of germinating grains allowing for more effective temperature and

moisture regulation during malting than floor malthouses. During the 1940's, floor malthouses started to become obsolete in favour of pneumatic malting systems. It was during this time that materials and labourers became scarce for the malting industry. This scarcity propelled the need for automated systems that required fewer labourers ([1]). Since 1940, new malthouses began employing automated pneumatic malting systems as they required less employees, allowed for year-round malt production, and could handle larger production volumes. For these reasons floor malthouses became relatively obsolete as did corresponding research on the topic of floor malting. The few floor malthouses that remained in operation since the 1940s are mostly in Scotland and serve predominately as a tourist attraction.

Nowadays, air conditioning has helped with the growing popularity of craft malthouses employing the technique of floor malting. Ventilation ducts and air conditioning have made it possible for floor maltsters to continue malting year-round. However, there is still a need for subsequent research on the quality of floor malt being produced. Historically, floor malt was judged based on appearance, feel and taste of the subsequent beer it was able to produce ([5]). Fortunately, a more thorough analysis can now be performed on this style of malting. As it is important to see whether floor malting can effectively produce good quality malt during the hotter summer months. Even with modern refrigeration, floor malting can experience higher germination temperatures than pneumatic malting systems. This again puts summer floor malting at an increased risk of fungal proliferation during malting that can cause PYF during the production of beer as well as several other detrimental beer qualities ([3,4,6]).

Examining the occurrence of PYF in floor malt is a main focus of this thesis research. PYF is the phenomenon where yeast cells begin to aggregate and settle out of solution before all of the fermentable sugars have been consumed by the yeast ([3]). PYF usually results in slower and/or incomplete fermentations ([3]). The early removal of yeast from solution causes a variety of effects on the resulting fermentation and thus final beer quality. Less yeast in suspension leads to lower CO_2 gas evolution rates, attenuation of fermentable sugars, and alcohol content as well as higher diacetyl and sulfur dioxide (SO_2) concentrations in final beer which can result in unwanted flavour profiles ([6,7]). It has also been found that PYF can lead to higher risk of microbial infections of the beer ([7]). All of these factors can lead to major inefficiencies in the brewery as the batch of beer may require additional fermentation, blending or disposal ([7]).

As discussed earlier, floor malting has been widely discontinued over the past 80 years. Corresponding research has also been absent. A literature review of floor malting on Google Scholar resulted in many historical references but only a few research articles in the past 100 years. It is the aim of this research project to begin to fill some of the research gaps that currently exist with respect to floor malting. In doing so, this research project consisted of two sub-projects based around floor malting. The first was to examine the effects that floor malting parameters have on subsequent fermentation parameters. The second project was to examine the differences in several malt quality attributes that arise from a single barley lot that had been floor or pneumatically malted. Malt quality was judged on several aspects such as modification of endosperm, extract, enzymatic power, and β-glucan content in the respective malt.

Chapter 2 Literature Review

2.1 Barley

Barley or *Hordeum vulgare* is a part of the plant family *Gramineae*, which is diploid (2n=14). Most varieties are self-pollinating ([5,8]). The family *Gramineae* includes many other important crops that make up the agrarian economy such as wheat, oats, maize, rye, rice, sugar cane and grasses that support grazing livestock ([2,5]). Barley is a hardy and adaptable crop, with the ability to grow in many places that other grains cannot. Growing regions for barley range from sub-Arctic to sub-Tropical ([5,9,10]). Barley is also a popular crop of high altitude regions of the world such as the Andes and Himalayas ([5,11]). Along with oceanic and continental climates, barley's genetic resistance to dry heat and drought allows it to also grow in near desert climates such as North Africa ([10,11]).

Barley was one of the earliest domesticated crops by mankind ([11–15]). Evidence from archeological digs showed that the earliest barley cultivations were roughly 10,000 BCE near the Fertile Crescent ([8,13,14,16]). The Fertile Crescent, pictured in Figure 1, stretched from Western Jordan, Southeast Turkey, Northern Iraq into Western Iran ([8,13]). It is difficult to pinpoint the exact location of barley domestication as evidence shows that wild barley was present in a wider area than just the Fertile Crescent at this time ([8,13]). This reflects the versatility of even early barley varieties. As even without domestication, barley was already widely distributed and proficient at growing in a wide range of terrains ([8,13]).

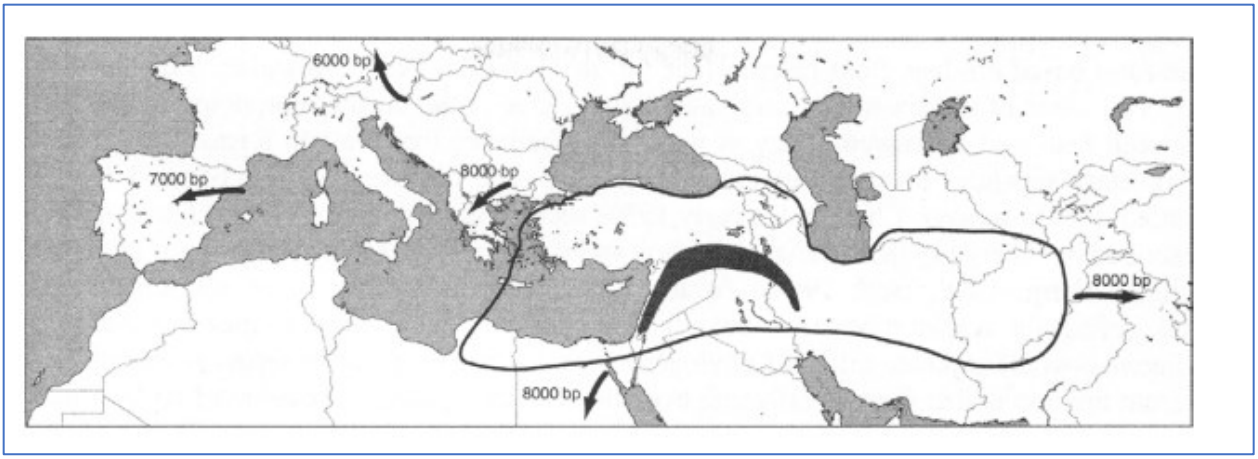

Figure 1. Map highlighting the Fertile Crescent (retrieved from von Bothmer et al. ([13])).

The timing of barley domestication coincides with the era of when human civilizations began to progress from hunters/gatherers to becoming farmers. Arguments have been made that the availability and ease of domestication of early barley cultivars played a large role with helping humans create large settlements ([13]). Human settlements became more common as sufficient amounts of food could be produced intensively on land intended to support a community. This led to the domestication of wild barley along with other plant species as well as animals at the dawn of farming communities ([13]). As early civilizations migrated, they would pack their barley seeds to sow in new areas which helps explain the vast growing regions where barley is found.

2.1.1 The Use of Barley in Brewing

Worldwide, roughly about 20-25% of the barley grown is used for malting, 2% used directly as human food, and the rest used as feed for livestock ([5,10,12]). Traditionally, malted barley was the

primary grain used to create most beer styles. Today, many different styles of beer are available that use an array of malted cereal grains. It is not uncommon to see malted barley being used along with several other grains to make up a single beer's grain bill. Malted grains such as rye, oats and wheat are other popular brewing grains that add unique characteristics to beer. However, barley remains the predominant cereal grain used for brewing beer as its specific grain features cannot be replaced by another single grain. The combination of features such as a relatively soft endosperm, intact hull, and low protein content is what makes barley the ideal grain for malting and brewing ([9,12]).

Brewing with entirely raw, unmalted barley is also possible with the incorporation of exogenous enzymes added to the steep tank during wort production ([17]). However, this process is not widely used for commercial beer production ([18]). Unmalted barley has several unfavourable characteristics compared to malted barley when it comes to brewing. Unmalted barley:

- is more dense and not as brittle, which makes it much harder to mill ([18,19])
- lacks adequate enzymes for proper conversion of stored polysaccharides into fermentable sugars ([17,19])
- generates wort with increased protein content, β-glucan content, and viscosity ([17,19]).
- affects flavour profile, resulting in wort with less higher alcohols and esters ([17]).

Brewing with entirely unmalted barley is very rare, more often is the addition of unmalted barley adjuncts to replace no more than 20% of the beer's grain bill ([10,19]). If any more than 20% of raw barley is used, then the enzymatic power from the rest of the malted grains in the beer's grain

bill will not be sufficient to produce enough wort extracts from the grains. The main benefits of using raw grains for brewing is the financial and environmental benefits it serves. In some cases, primarily craft breweries, use raw grains in the grain bill to save money. As well, the beer's carbon footprint is reduced by saved CO_2 emissions that are produced during the malting stage ([9,17,19]).

2.1.2 Barley Plant Physiology

Barley kernels are the indehiscent fruit of the monocotyledon plant ([19]). At the top of the mature plant, the arrangement of the ear is a significant identifier of various barley varieties. The ear is a firmly attached extension of the plant stem. Until flowering, the ear is contained within the sheath of the flag leaf. The axis of the ear is referred to as the rachis which is lined with nodes. Each node contains a triad, or group of three 'spikelets' which can produce a flowering plant on each side of the rachis ([5,8,20]). In barley varieties where all six spikelets are fertile and can produce a grain the ear will be vertically lined with six rows of kernels and is deemed a six-row barley variety ([2,5,8,13]). Conversely, in barley varieties where the lateral grains of each spikelets are sterile and do not produce a grain the ear will be lined with two rows of barley kernels produced from the central, fertile spikelet. These barley varieties are referred to as two-row ([2,5]). Two-row barley varieties are more commonly used for brewing as they generally have larger kernels and produce more extract with lighter colour but have less enzymatic potential then six row ([9]).

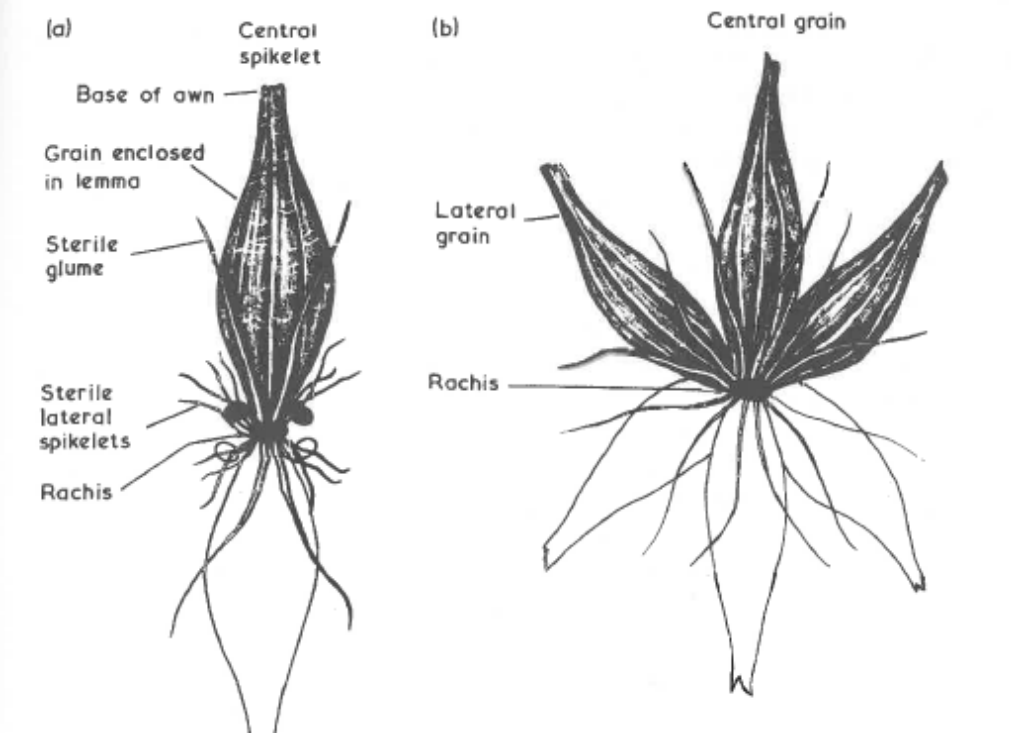

Figure 2. Diagram of barley plant showing 2-Row (left) and 6-Row (right) kernel orientations (retrieved from Malts and Malting by Briggs ([5])).

Environmental growth factors will ultimately determine the final barley kernel shape and size as well as biochemical composition ([21]). Generally, most barley kernels resemble an elongated cigar shape and contain three main components highlighted in Figure 3: the embryo, outer layers (husk, pericarp, aleurone, lemma, etc.), and the endosperm ([2,22,23]). These three components all serve important and unique roles in the kernel's development during germination.

The embryo, or germ, is the most important part of the barley kernel as it contains the acrospire that will grow to form a new plant. Housed in the embryo is limited food reserves of starch, proteins and lipids which initially are used to develop the embryo upon germination ([22]). The embryo is located at the base of the proximal end of the barley kernel. The proximal end is the

part of the kernel that is attached to the rachis located on the barley plant. The embryo is slightly angled towards the dorsal side, pointing to the eventual path of the acrospire growth upon germination. The hydration of the embryo activates a cascade of biological functions within the kernel to begin break down of the endosperm producing sugars, lipids and amino acids that the embryo consumes in order to produce a new plant ([22]).

The endosperm contains the main source of energy supplied to the embryo during respiration. The chemical composition of the endosperm is the main determinant of the overall quality of the barley ([22]). In general, the endosperm is roughly 60% of the total weight of the barley kernel and its structure consists of cells with starch granules embedded in a protein matrix ([22,24,25]). Upon hydration of the kernel, the activated enzymes in the aleurone layer begin to degrade endosperm cell wall. Endosperm walls consists of mostly of β-D-(1→3), (1→4)-glucan (75%) and arabinoxylans (20%) ([9,22]). Degradation of these endosperm wall constituents is crucial for amylolytic enzymes to access interior starch reserves. Extensive degradation of β-glucan and arabinoxylan also improves final beer quality, as increased concentration of endosperm wall polymers, mainly β-glucan, remaining in malt can result in higher viscosity wort, slower filtration times and lead to haze production in beer ([9,22,26]). The extent and pattern of endosperm modification is also a determinant of good quality malting barley ([27]). Good malting barley exhibits a rapid and uniform modification front through the endosperm during germination.

The outer layers of the barley kernel also play important roles to the survival of the barley kernel. Encasing the endosperm is the aleurone layer which is only 2-3 cell layers thick ([27]). The aleurone

is where the formation of endosperm degradation enzymes is formed. The husk serves as a protective layer against microbes and incidental threshing. However, some barley varieties are hull-less, meaning they have no outer husk. Hull-less cultivars are still used for brewing. While these varieties offer higher portion of endosperm per mass of barley, they are prone to cause stuck mashes during brewing as the husk also serves as an effective filtering material. Husk also serve as a protective coating to prevent damage to the embryo during harvest and germination ([22]).

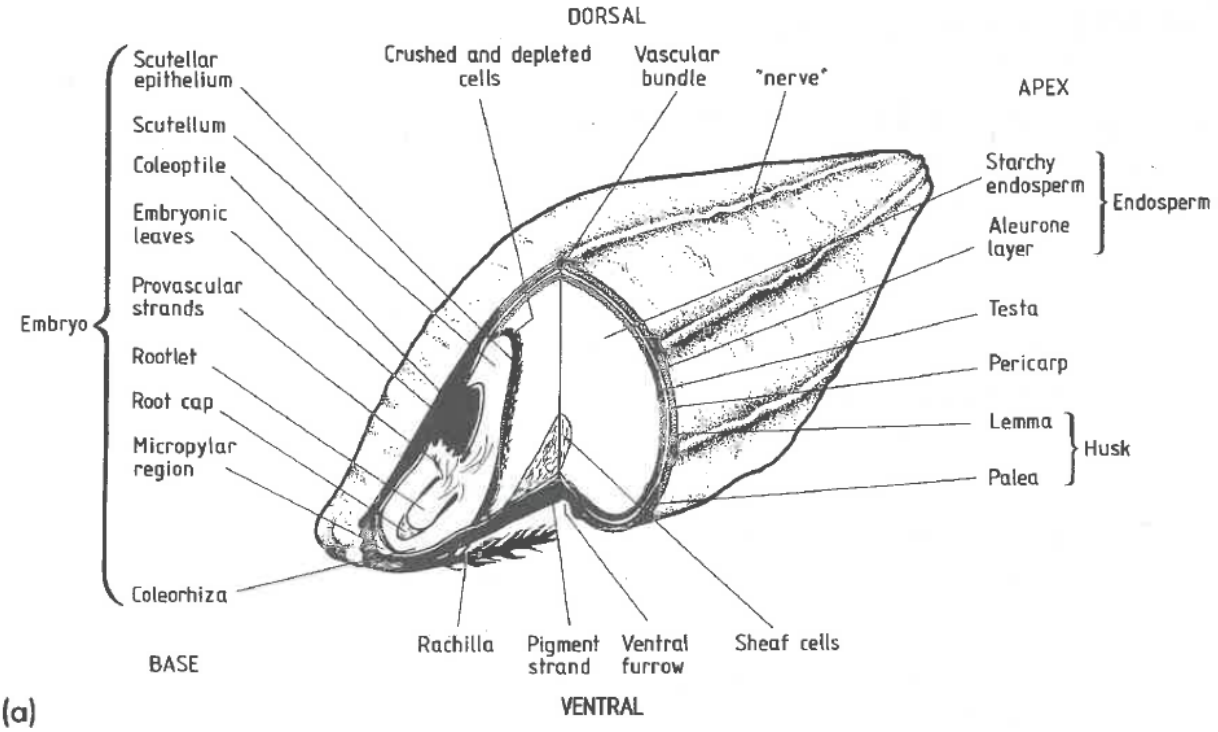

Figure 3. Labeled diagram of a cross sectional view of a barley kernel (retrieved from Malts and Malting by Briggs ([5])).

2.1.3 The Ideal Malting Barley

There is not one barley cultivar that is superior over all others to be used for malting. This is because there are certain needs of a malting barley variety from the maltster, farmer, and the brewer. Consideration must be given that specific qualities desired from the maltster may in turn be disadvantageous to the farmers, brewers and vice versa ([2]). Breeding programs work to introduce new varieties that preserve barley attributes that offer the best functionality for the entire malt production chain.

Regardless of genotype, ultimately the quality of the final barley is dependent on growing environment ([21,22]). The farmers are tasked with producing barley with consistent quality each growing season. The challenge with this is that growing conditions such as sunlight and rainfall vary between geographical locations and rarely stay consistent each year. Farmers are also at risk of economic loss if the barley produced does not meet the standards of malting barley. In North America a premium is paid by farmers for growing malting barley varieties, so farmers risk selling malting barley off at the lower cost feed barley price if malting barley standards are not met or if there is no buyer for malting barley after harvest ([5]). To help ensure consistent crops farmers desire barley attributes such as drought-, flood-, fungus-, and insect-resistance. Also, ideal barley has good post-harvest dormancy, a short growing season, and rugged stocks to withstand mechanical harvest and high yield ([2]).

Selection of barley is the first job of the maltster and arguably the most important, as all future malthouse operations are dependent on incoming barley quality. The selection of barley involves comprehensive inspection to decide if incoming barley lots meet the malthouse's standards. The acceptance of a shipment of malt at this point depends on visual inspection and certificate of analysis (COA), if available. Upon visual inspection the maltster wants barley that is free of fungal and other microbial infections, plump intact kernels, and free of debris such as dirt, rocks and rodents. Furthermore, if it is made available, the COA of the incoming barley is an important piece of information in determining the malt-ability of incoming barley. Ideally, maltsters want to see on the COA that in barley is low in nitrogen content (<12%),high in thousand kernel count weight, low beta-glucan, less than 1 ppm DON, low moisture levels, and low kernel density ([2,5,22]). Permitting sub-standard barley to enter into malt production can lower malthouse efficiency when extended production time, application of exogenous modification hormones, or final malt blending are required ([5]).

Additionally, it is beneficial to the maltster if all incoming barley is of the same variety and from similar growing conditions as this will result in similar malting aspects. Maltsters want a barley that will yield high enzyme potential and extract, as well as exhibit rapid and even water uptake and modification during germination. Barley with comparable malting patterns allow the maltster to construct a malting regime that can be used for all incoming barley ([28,29]).

There will never be one barley variety that triumphs over all others for malting. This is the result of vast growing conditions where some barley varieties do better than others. Every few years

barley breeding programs release new malting barley varieties that have been under development. The top recommended malting barley varieties such as the two-row varieties AC Metcalfe and CDC Copeland are examples of barley varieties that have been developed by their respective breeding programs specifically for malting and brewing purposes ([30]). Often, brewers will blend a mix of barley varieties in order to produce a consistent brand.

Barley breeding programs work diligently to keep up with the demands of the malt production chain for high quality barley selection. As demands for desired barley traits can change over time, so does the effectiveness of bred traits such as disease resistance. Diminishing disease resistance can be a product of the improved genetic traits such as mildew resistance as this eventually leads to the spread of more noxious mildew species that are unaffected by these genes ([28]). Thus, most barley from these breeding programs have relatively short commercial life of 10-20 years until they are replaced by better coping varieties.

2.2 The Malting Process

The overall objective of malting is a controlled and limited germination of barley ([9]). Malting is a manipulation of the natural process which a barley seed undergoes. Once barley seeds are hydrated to a moisture content of roughly 45%, the barley begins respiration of the stored energy reserves in order to sprout a new plant. This process is only progressed to a certain extent before being ceased by the maltster. In doing so this allows for production of sufficient barley degradation enzymes without the depletion of the starch-rich endosperm. This is ideal for

brewers who desire a barley malt with ample carbohydrates and enough enzymatic power to break down these carbohydrates into fermentable sugars ([31]). Malting is just a portion of the barley processing chain in order to be used for brewing, as highlighted in Figure 4. The three main stages of malting are steeping, germination, and kilning ([3,32–34]). All of these malting stages are unique and have their own important role in production of malt.

2.2.1 Outline of the Malting Process

The malting process begins with the barley getting submerged in water to begin hydration of the barley kernels, this process is called steeping. Re-hydration of the barley serves two important functions. First it activates the barley's embryo to begin to produce a new plant ([5]). Secondly, it reduces barley kernel density which allows for easier transport of starch degradative as well as other important enzymes around the endosperm. Oxygen is also required by the embryo for germination, which is why water is periodically drained from the barley during steeping. This 'air rest' allows for the embryo to obtain the oxygen that it needs to continue with respiration. The cycle of water soak followed by air rest is followed until a moisture content of approximately 45% has been obtained by the barley.

Once the barley is steeped, activation of the embryo will result in a cascade of biochemical processes within the barley as germination begins. The first visible sign of germination is the chit. The chit will appear as a tiny white dot at the proximal end of the barley, from this five or six tiny rootlets will emerge. At this time the barley is drained from the steep tank and allowed to

germinate. Germination chambers vary, however, most are equipped with a method of agitating and controlling the temperature of the germinating grains. Agitation is required during germination as the barley rootlets quickly get intertwined and clump together creating insulated pockets with higher temperatures then the rest of the malt bed. Strict temperature control is required throughout germination as higher temperatures can be detrimental to final malt quality. This stage of malting naturally provides ideal conditions for microbial proliferation as grains supply ample source of food and water ([32,35,36]). To prevent spoilage, germination occurs at slightly lower temperatures around 12-16°C. Typically, germination lasts anywhere from 3-5 days. Germination length depends mostly on the malthouse conditions, barley variety and the desired malt product. The most important function here is production of degradative enzymes which will be used later during brewing. Allowing the germination to proceed longer will degrade the endosperm for energy to be used by the growing embryo and not necessarily translate into higher levels of enzymes. Therefore, modification of barley during germination is closely monitored, to obtain adequate barley modification and enzyme production without complete degradation of the endosperm. At this time the germinating barley is ready to be loaded into the kiln for the final stage of malting.

Kilning is the process of forcing hot air through the bed of barley to reduce the moisture content to below 5%. At this point germination has effectively ceased and the processed grains are now considered malt, or malted barley. The amount of heat applied during kilning is important as it ultimately determines the type of malt produced. Base malts such as Pilsner, pale ale, Vienna, Munich, etc. are kilned at temperatures between 50-70°C until the final moisture is obtained,

whereas specialty malts such as crystal, black, and coffee malts are roasted at higher temperatures which generates their characteristic colours and flavours.

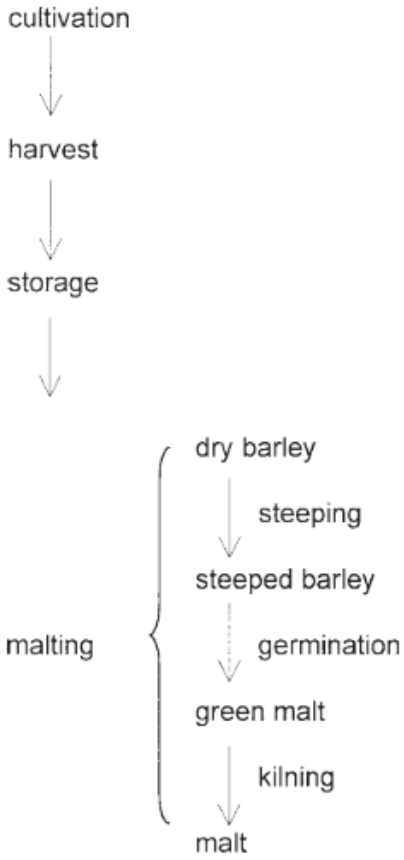

Figure 4. Overview of the stages during the malting process (retrieved from Noots et al. ([32])).

2.2.2 Malting Technology

2.2.2.1 Pneumatic Malting

Most modern malting systems use a pneumatic style of malting as it has been developed to be fully automated with high throughput. Pneumatic malting gets its name from the operation

employed during its germination stage of the malting process. In pneumatic systems, steeped barley is transferred into a stainless-steel vessel with a perforated floor that allows the constant circulation of cool, humid air through the germinating barley. This constant flow of conditioned air allows for tight control of grain bed temperatures. Incorporated in this vessel are automated arms that rotate around the vessel to provide agitation of the barley. These automated arms periodically turn the germinating grains to break up rootlets and allow for dissipation of built up heat and CO_2 that is produced during respiration of barley. With automated agitation, germination beds can be maintained of upwards of 1.2 m in depth depending on the machinery used. Most commercial malting systems have come to use large scale pneumatic systems. This was because the temperature control and agitation of barley was able to be run by computer systems requiring less hired personnel and allowing for malt to be produced on a larger scale.

2.2.2.2 Floor Malting

The term 'floor' malting is coined from its signature germination stage which involves the barley being germinated on a solid concrete floor. Without perforated floors there is no constant air flow through the grain bed to regulate temperature. To compensate for this, traditional floor malthouses would open windows of the germination room during the nighttime to allow colder external air to cool the grain bed. This method of grain temperature control is not as effective as pneumatic systems. Modern floor malthouses are equipped with air conditioners that allow for constant temperature regulation of the air in the germination room. Nevertheless, air conditioning will often keep surface grains cool but grains inside the malt pile stay insulated and

experience temperatures of up to 7℃ higher than surface grains ([5]). To combat this, floor germinations occur with relatively smaller grain bed depth of around 25 cm ([2]). Agitation of the germinating grains is more labour intensive. In order to disentangle growing rootlets a worker must walk the length of the grain bed pulling a rake with angled tongs designed to break up and flip the grains over. This also helps to dissipate heat and CO_2 from the germinating grains.

The characteristics of floor malting previously listed outline the key differences between floor and pneumatic malting systems. Between raking of floor malt the grain sits undisturbed and develops temperature, moisture and CO_2 gradients within the grain bed. Without constant airflow through the grains to regulate temperature, germination in floor malting typically experience a higher and a fluctuating pattern of temperatures throughout germination. This in turn affects the consistency and repeatability of floor malting techniques.

Historically, floor malting has been the technique used for malting for hundreds of years. One of the earliest records of widely used floor malting was given by W. Harrison in 1587 ([37]). It was not until industrialization took over that malting practices changed to become more machine dependent. Even upon the earlier years of industrialization, floor malting was still considered superior to early pneumatic malting systems such as box or drum malter. However, machine malting systems kept improving and soon floor malting was widely discontinued. The last text written concerning floor malting was published in 1908 ([38]). Industrialization resulted in new machinery that reduced the hired personnel required to operate a malthouse. More importantly the addition of forced air through the germination bed allowed for an effective method of

keeping grains consistently cool during germination. Historically, malting was a seasonal profession and was not able to operate during the summer months ([1]). During the warmer summer months, outside temperatures during the nights would not effectively cool the germinating grains. This would often lead to uncontrollable germination temperatures that would result in the entire batch being spoiled by fungal growth ([5]). Incorporating a pneumatic system to cool off grains allowed for a method of effective temperature control of germinating grains even during the hot summer months. Due to this advantage of pneumatic malting systems, the method of floor malting was largely abandoned in favour of the year-round operational systems.

2.2.3 Microflora During Malting

Barley houses a multitude of different microflora consisting of bacteria, yeasts and filamentous fungi ([39,40]). The barley microbe community is also predominately influenced by the growing conditions of barley. Wet conditions during barley growth and harvesting lead to an increase in fungi, in particularly *fusarium* spp. on the barley ([4,7,41,42]). The presence and proliferation of fungal species such as *fusarium* spp. in barley is important to keep in mind as this fungal species and its metabolites can result in several detrimental aspects of beer such as gushing ([3,7,42,43]), increased mycotoxin levels ([3,6,7,36,42,44–46]) and the occurrence of PYF ([47–51]).

A review of malting literature reported various processes of barley sterilization as a pre-step for malting. These sterilization processes have included the physical, chemical and biochemical

elimination of pathogens ([6,39]). Barley decontamination methods using processes such as heat treatment, irradiation, and use of pesticides have been used in various studies ([39,52–60]). However, all these sterilization methods are not industrially employed due to either: effectiveness, negative impact on environment, negative impact on grain quality, or the unknown effect on resulting beer. This shows that complete sterilization of incoming barley for malting is not yet practical for industry. Therefore, the presence of microbial communities present on barley during malting is unavoidable.

It is convenient to assume the malting process consists of two intertwined biochemical processes: 1) the germination of barley and 2) the lifecycles of existing microbial colonies ([34]). The objective of malting is to produce sufficient enzymes for the breakdown of barley components to supply food and nutrients for yeast during brewing. This malting objective can still be achieved even with present microbial communities. For instance, fungi can supply exogenous enzymes which aid in the degradation and modification of barley during malting ([34,61,62]). These include similar proteases, β-glucanases, and α-amylases which are produced by the barley during germination ([32,40,42,61,63–65]). Many studies have focused on the effect of fungi present during malting ([34,35,67–69,40,42,45,47,49,61,64,66]). Many of these reports have indicated that fungal activity during malting can have several positive effects on the resulting malt and beer. Malt produced with inoculated or increased fungal levels during malting have been shown to produce malt with lower friability and lower wort β-glucan content as well as higher extract, soluble nitrogen, FAN, colour, and α-amylase. In addition to these trends in malt quality, Oliveria et al. ([51]), Schwarz et al. ([61]), and Sarlin et al. ([42]) also found that barley inoculated with fungi during malting would result in higher

mycotoxin in the final malt then control barley samples ([42,51,61]). Sarlin et al. ([42]) also found that malt samples that had been inoculated with fungi during malting had a higher tendency for gushing to occur in subsequent beer made from the malt.

The different stages of malting all have various influences on microbe growth. The microbial community is dominated by increases in bacteria and yeasts during steeping. This is a result of limited oxygen and temperatures below 20°C during steeping which is ideal for these microbes ([34]). The filamentous fungi spp. *Fusarium graminearum, culmorum,* and *avenaceum* have been identified as being the most common malt spoilage fungal species ([34,51,70,71]). Growth of fungi during steeping does not proceed as quickly as other microbes as storage spores must be re-activated and mycelium must spread from kernel to kernel ([32]). The germination phase also increases microbial growth on the barley. Like the steeping phase, microbial growth is dominated by the increase in bacteria and yeast counts. Fungi loads on barley increase during germination but have been noted to fluctuate. Fungi growth tends to slow down on the second day of germination and is followed by a substantial increase on the third day ([32,51,72,73]). Kilning is essential to the microbial stability of the final malt. Overall, kilning results in final microbial load reduction of one to two orders of magnitude ([34,73,74]). Final microbial loads have been found to be substantially higher than the corresponding unmalted barley lots as remaining colonies are found to be thermally resistant microbes and are able to survive kilning by the formation of biofilms on the malt surface ([34,36,73]).

Oliveira et al. ([51]) noted that levels of the fungal species *Fusarium culmorum*, pictured in Figure 5, increased during steeping and germination but remained unchanged during kilning. Laitila et al. ([40]) found a similar pattern except for yeast levels and several bacterial species during malting, except that bacterial counts were reduced significantly during kilning. The findings of Li et al. ([73]) on fluctuations of microbial populations during malting are agreeable with the results of Oliveira et al. ([51]) and Laitila et al. ([40]) and are given in Figure 6.

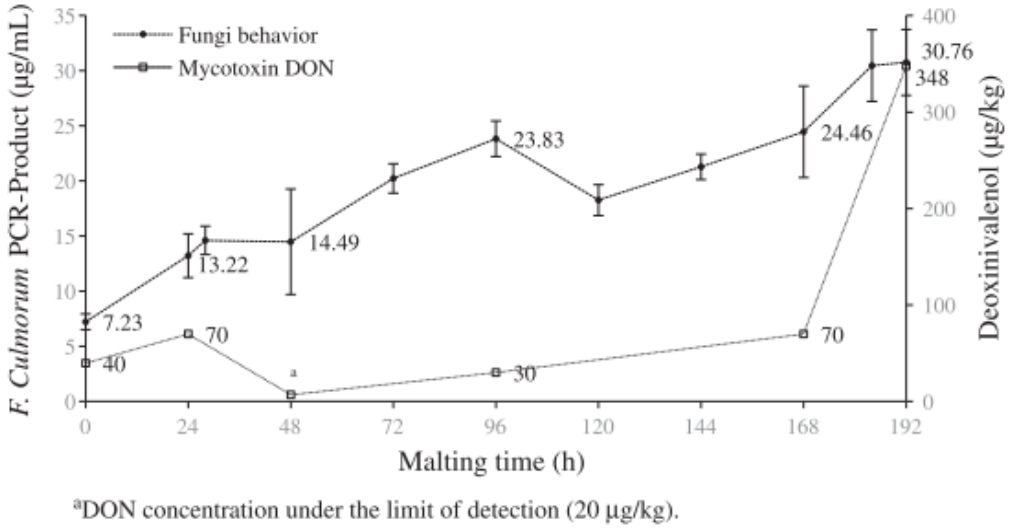

Figure 5. Diagram of *fusarium culmorum* and DON levels during malting of an artificially infected sample of barley. Steeping took place over the first 48 hours, then germination lasted from 48-168 hours and kilning lasted from 168-192 hours (retrieved from Oliveira et al. ([51])). DON levels increase significantly from the beginning to the end of germination.

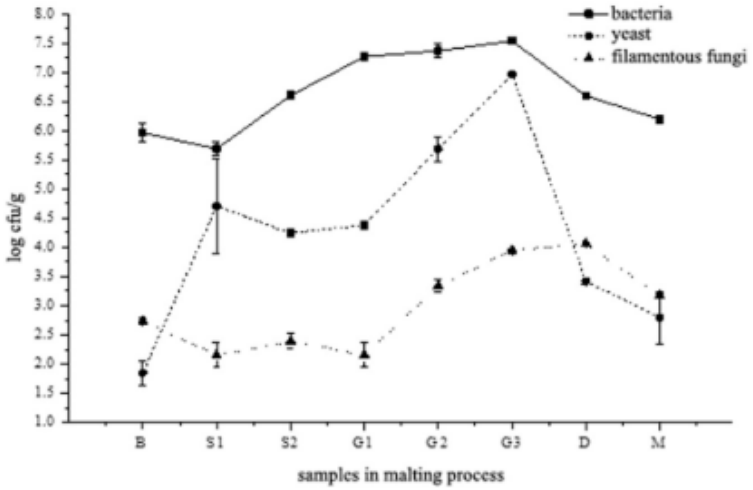

Figure 6. Growth of various microbials levels throughout the malting process. B=Barley, S1,S2,S3=Steeping, G1,G2,G3=Germination stages, D=During kilning, M=Final malt (retrieved from Li et al. ([73])). Each type of microbial's population increase during germination and are reduced during kilning.

As noted earlier, presence of microbes throughout the malting process is unavoidable. However, uncontrolled proliferation of these microbial communities would be disastrous and yield unacceptable malt. As malting is the controlled germination of barley, the limitation of microbial growth is also essential. To help limit microbial growth during malting, several general precautions are taken. During steeping, cold water (12°C) soaks limit microbial growth. The removal of steeping water also substantially decreases overall loads as well. Temperatures below typical physiological temperatures are also advised during germination to again limit the microbial reproduction. Finally, high kilning temperatures are effective at decreasing final microbial loads in malt. Appropriate heat and duration are essential during kilning at keeping final microbial loads low.

The biodiversity of the microbial communities creates an extraordinarily complex ecosystem. The steeping and germination stages of malting offer near ideal environments for microbial proliferation. Certain microbial loads can improve overall quality of malt while uncontrolled growth is damaging to resulting malt. Quantifying the exact microbial community during malting is difficult as the levels are sensitive to process conditions employed (e.g., times, temperatures and moisture content). Variability in microbial colonies also arises due to the influence of geography and harvest conditions of the incoming barley lots. All these factors add pressure on the maltster to adjust his or her process to make acceptable localized malt based on globalized standards.

2.3 Beer Brewing Process

The overall brewing process consists of three main steps: wort production, fermentation and post-fermentation processes. Beer production is different than the manufacturing of other alcoholic beverages. Beer is a non-distilled alcoholic beverage that is fermented from starch-derived mono, di and tri saccharides. Differing from wine making, beer production requires a conversion step to enzymatically degrade starches into fermentable sugars ([16]). The entire beer brewing process involves a multitude of biochemical processes at every stage which must be understood and controlled by the brewer in order to effectively brew consistent and enjoyable beer each time. Detailed outlines of all the biochemical processes occurring during brewing have filled many textbooks and consists of too much information to include in this thesis. However, a

general outline of the brewing process is given to help build the readers' understanding of the importance and influence that the malt has on overall beer production.

The first process of beer production is mashing. Wort is a general term given to the sweet sugary liquid produced from the mashed grains. As mentioned earlier, beer is produced from starch-based polysaccharides stored in the malt. In order to access the starches within the malt, the malt is first milled. By first milling the malted grains this breaks open the malt and increases the surface area of the starch-rich endosperm for easier access of degradative enzymes. Once milled, the process of mixing the malt with warm water is called mashing. This rehydrates and re-activates the stored enzymes produced during malting. Mashing durations and temperatures can vary depending on the malt being used. A malt with high enzymatic power can degrade that available starch into fermentable sugars faster than malt with low enzymatic power. Malts with high enzymatic power are often used as base malts in brewing. Some malts are heavily roasted or kilned and have little to no enzymatic power and require excess enzymatic power from the base malts to aid in starch degradation. At this time the liquid in the mash tun is sweet wort and needs to be filtered out of the spent grains. After separation of wort from spent grains the wort is then boiled. Boiling the wort has several important functions to beer production most important are wort sterilization, development of and solubilization of bittering acids from hops ([16]). Once boiling is finished, the final part of wort production is the quick cool down and aseptic transfer of wort into the fermentation vessel.

Fermentation begins once yeast is pitched into the sterile wort. Yeast metabolizes wort sugars and during anaerobic conditions will release the by-products: ethanol and carbon dioxide. The brewer designs recipes with specific wort sugar concentrations to produce a target amount of ethanol in the final beer. However, the ADF and thus final alcohol content is affected by many factors during fermentation such as viability and vitality of the yeast, temperatures, fermentation vessel, yeast nutrients and wort carbohydrate profile ([29,75–78]).

The final stage of brewing is the post-fermentation processes. These processes mainly encompass the varying methods of which breweries chose to filter, blend, carbonate, package and condition their brews ([16]). These processes are mostly influenced by the type and beer being made and the select brand that is being produced. Most times these post-fermentation processes are used to further create a unique and enjoyable beer.

2.4 Yeast

Yeast is a single cell microorganism. Reproduction of brewing yeast is usually asexual, involving the duplication of DNA then budding to produce a new “daughter” yeast cell. Yeasts are a broad and diverse category of microorganisms but only a few species are used in brewing beer.

2.4.1 Role in Brewing

There are many factors that make yeast the ideal microorganism to use for beer production. The most important function of yeast during brewing is the catalysis of conversion of wort sugars into ethanol and carbon dioxide. Yeast also plays a large influence on beer flavour. As fermentation proceeds, yeast produces desired flavours such as esters and organic acids and re-metabolizes off flavours such as VDK and acetaldehyde, as pictured in Figure 7 ([3,16,79]).

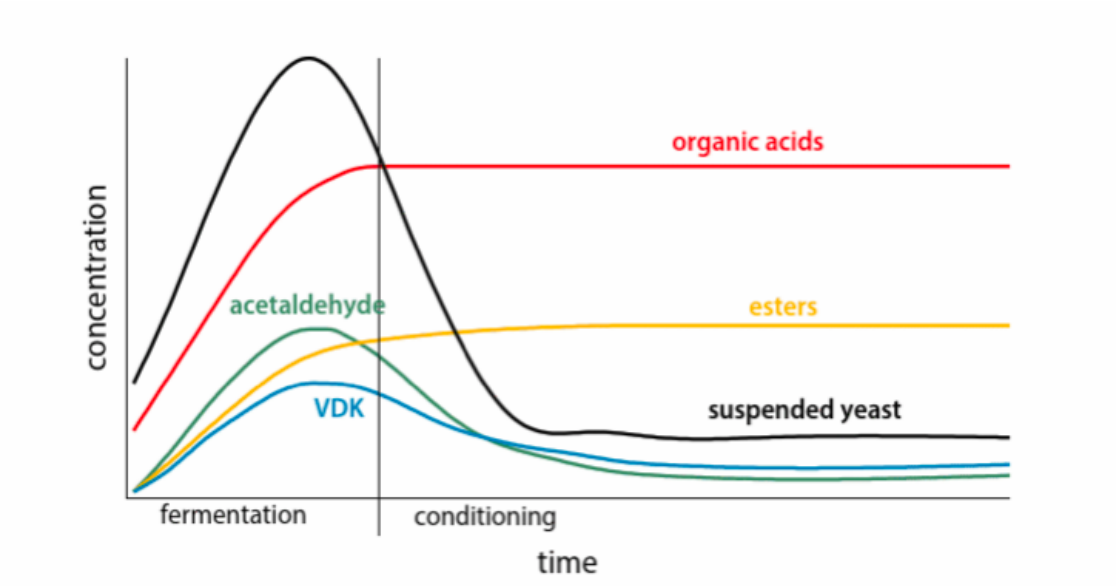

Figure 7. Diagram of relative values of yeast in suspension (black), organic acids (red), esters (yellow), acetaldehyde (green), VDK (blue) (Retrieved from Farber et al. ([16])).

The yeast strain being used during beer production plays a large role in the specific type of beer being produced. For instance, top fermenting 'ale' strains called *Saccharomyces cerevisiae* are used to produce ales, stouts and porters ([3,16,80]). These strains have cell walls that are more hydrophobic. Upon fermentation, released carbon dioxide gas within the floc remain associated with the yeast cell walls and have a buoyant effect carrying the yeast cells to the top of the

fermentation vessel. The second genus of brewing yeast are considered bottom fermenting or lager strains and are termed *Saccharomyces pastorianus* ([16]). These bottom fermenting strains are used to produce beers such as lagers and are much less diverse then the top fermenting category ([3,80]). Bottom fermenting strains have cell walls that are comparably more hydrophilic. Released carbon dioxide does not adhere as strongly to the yeast and thus the yeast cells/flocs tend to reside near the bottom of the fermentation vessel.

2.4.2 Flocculation

Yeast flocculation is an important phenomenon in the brewing industry. It serves as a natural clarifying step during production of beer and indicates the completion of fermentation. Flocced yeast can easily be removed and re-pitched if required. Yeast flocculation is described as natural tendency for yeast cells to aggregate in a non-sexual, calcium-dependent and zymolectin-mediated manner ([3,47,75,81]). This process is reversible with the aid of chelating agents or simple sugars such as glucose, sucrose and maltose ([20,49,75,81]). Flocculation is achieved by zymolectin proteins on the surface of the yeast cells binding with mannose residues on adjacent cells. This process requires calcium ([20,77,82–84]). Generally, yeast flocculation becomes favourable once fermenter conditions such as depleted fermentable sugars and shear rates are reduced ([3,77,78,81,85]). As the flocs of lager yeast become larger, they become less able to remain suspended in the ferment and begin to settle out.

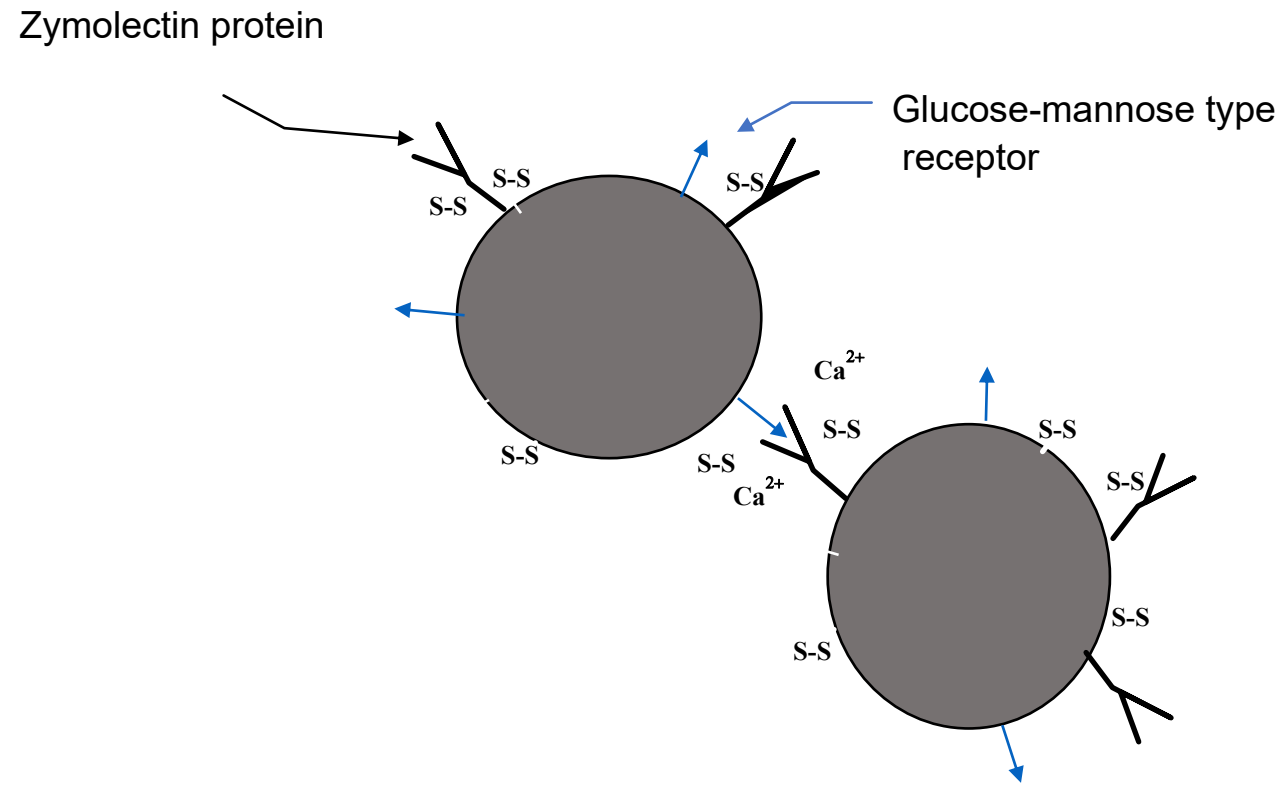

Figure 8. Diagram of zymolectin mediated flocculation of yeast (retrieved from Speers R.A. ([86])).

2.4.3 Premature Yeast Flocculation

In rare instances, a phenomenon occurs where yeast settles out of solution before the typical end of a fermentation; as mentioned earlier, this process is called premature yeast flocculation (PYF). During PYF, yeast cells aggregate and settle out of solution before all of the available sugars have been consumed ([3]). The onset of PYF is not necessarily earlier than in normal fermentation, but results in heavier flocculation then normal fermentations ([7]). Figure 9 compares typical yeast in suspension (YIS), which is represented by spectrophotometric absorbance during

fermentations of PYF and control wort. In Figure 9, flocculation during both fermentations appear to occur at the same time (20 hours) with similar YIS values. However, at 30 hours the YIS of the PYF sample is half that of the control sample as the absorbance at 600 nm is approximately 0.6 and 1.2 respectively.

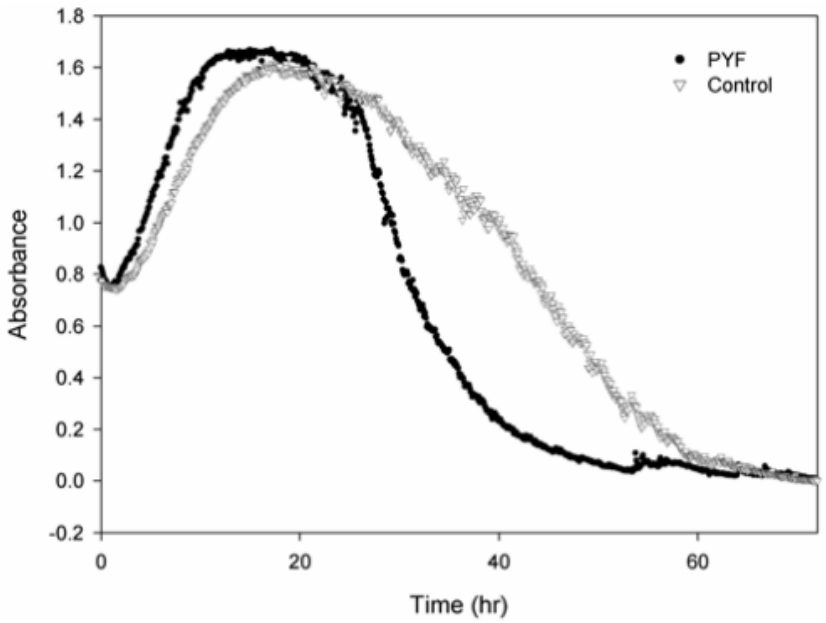

Figure 9. A comparison of YIS during a test-tube fermentation of PYF and a control malt sample. Absorbance readings at taken at 600 nm (retrieved from Lake et al. ([7])).

PYF is a result of fungal infection of the barley or malt while in the field or in the malthouse, respectively([3,34,36,45,47,49]). The exact mechanism for the creation of PYF-inducing factors is unknown, however there are two theories. PYF-inducing factors may be caused: 1) by the barley as a defense mechanism or 2) secreted by infecting fungus to aid in enzymatic degradation of the barley ([7,68,87]). Brewing scientists do not hold the former theory. There have been many reports published identifying the possible compound(s) responsible for inducing PYF, these are all

outlined in Lake et al. ([7]). More recently, Koizumi et al. ([50]) isolated a complex polysaccharide made up of a highly substituted arabinoxylan and protein component that induced PYF as concentrations as low as 30 ppb ([50]). The mechanism of PYF onset during fermentation involves the PYF-inducing factor enhancing the normal yeast flocculation process. This PYF factor has a strong affinity for yeast cell lectins and can act as a bridge between yeast cells. This creates stronger yeast flocs which in turn speeds up the flocculation process even in the presence of fermentable sugars ([88]).

PYF is not desirable by brewers as it is usually accompanied with slower or incomplete fermentations ([3]). The early separation of yeast from fermenting wort causes a variety of effects on the resulting fermentation and thus beer quality. Low end of fermentation yeast counts caused by PYF can result in lower CO_2 gas evolution rates, attenuation and alcohol content ([7]). Additionally, PYF leads to higher diacetyl and sulfur dioxide (SO_2) concentrations in final beer that lead to undesirable flavours ([6,7,47,88]). All of these can have a negative impact on the brewery financially if PYF associated beer requires any additional fermentation, blending or disposal ([7]).

Chapter 3 Effects of Germination Conditions on the Fermentation Performance of Floor Malt

3.1 Introduction

Floor malting research is scarce, and as a result this project was developed to yield further insight about previously reported issues regarding traditional floor malting. Barley generates substantial heat while germinating. For this reason, floor malting was traditionally done during the colder months of the year as maltsters could use cold external air to cool the germinating barley. During the warm summer months, outside air temperatures during the evenings were not cold enough to reduce the germination temperature of the malt. This can lead to spoilage of the malt by fungus and other microorganisms. With modern refrigeration, it is now possible to provide better air conditioning inside floor malthouses, but it is beneficial to examine if any correlation exists between external temperatures and germination temperatures during summer floor malt production. The objective of this project was to examine any influences that seasonal temperatures had on summer floor malting. This would indicate how well the current air conditioning system operated in specific floor malthouse and if it was effective enough to maintain safe germination temperatures during the summer. As a small business, Horton Ridge was not able to shut down for a prolonged period to allow researchers to employ their system to run experiments, measurements had to be taken during normal malt production. The downside of this approach was that germination parameters were not able to be set and effects of which could not be studied individually.

A mini-fermentation of each malt sample would then show if any of these recorded malting variables had significant effects on fermentation kinetics such as change in wort extract, yeast in suspension, and ADF. It was hypothesized that the elevated temperatures during summer production of floor malt would be more prone to fungal proliferation and thus result in PYF malt. If true, then identifying the specific floor malting conditions such as germination temperatures and duration that induce PYF in malt can be avoided in future floor malt production.

3.2 Materials

3.2.1 Floor Malted Barley Samples

A total of 22 floor malted barley samples were monitored, collected and examined over two years; a total of 17 in 2018 and 5 during 2019. All barley crops were organically grown. The following barley varieties were examined during this research project.

Table 1. List of barley varieties used during Chapter 1 trials.

Year:	Barley Variety:	Grown in (CAN):	Number of Samples:
2018	AC Newdale	PE	6
2018	CDC Copeland	SK	2
2018	AAC Synergy	NS	1
2018	AC Queens	PE	8
2019	CDC Copeland	SK	4
2019	AAC Synergy	NS	1

3.3 Methods

3.3.1 Floor Malting of Barley

All floor malting was done at Horton Ridge Malt and Grain Company in Hortonville, NS. Data collection and sampling were designed to not disrupt regular malt production at Horton Ridge. This allowed the maltsters to operate normally with minimal disruptions to malt production. For each malt batch the following variables were recorded: steep out moisture, average germination temperature, maximum germination temperature and germination length.

For each barley sample the malting regime was as follows:

- ***Steeping*** **-** barley samples were steeped with ground water with a series of intermediate air rests until barley moisture was >42%.
- ***Germination*** - barley was germinated on a concrete floor until acrospire length was ¾ of the kernel length. Germinating grains were raked every 4-6 hours.
- ***Kilning*** **-** took place in a forced air kiln to produce a Horton Ridge Pale Two-Row Malt. This consisted of a kilning regime of 60℃ until moisture reached 10%, then kiln temperatures increased to 70℃ until moisture was approximately 5% and then cured for 1 hour at 80℃.

3.3.2 Determination of Premature Yeast Flocculation

Each malt sample was tested for the presence of PYF using the ASBC's standard Yeast-14 method ([89]). For each malt sample collected, three 50 g malt samples were finely milled (0.2 mm) using a Laboratory disk mill (Buhler, Alzenau, DEU) Each 50 g milled sample was then put into separate mash beakers in an IEC mash bath (Thornbury, AUS) containing a stir bar and 200 mL of distilled water heated to 45°C. Once the malt was added to the mash beaker the stir bar provided agitation, and a watch glass was placed on top to prevent evaporation. The temperature was held at 45°C for 30 min and then ramped up at 1°C/min until the mash temperature reached 70°C. At this time an additional 100 mL of distilled water heated to 70°C was added to the mash beaker and a mash temperature of 70°C was maintained for 60 min. After the 60 min was complete, cold water was added to the mash bath apparatus to cool the mash bath down to room temperature. This took no longer then 15 min and no less than 10. Afterward the mash beakers are removed, and the contents were filtered through a Whatman #4 fluted filter paper. The first 100 mL of filtrate was returned to the filter. Once the wort was filtered, it was autoclaved for 20 min at 121°C then cooled to room temperature and refrigerated at 4°C overnight. Following the cold break, the wort was centrifuged in 50 mL centrifuge tubes at 3300 *xg* for 15 min to remove the trub. If necessary, wort was diluted to 12.6°P with distilled water to 410 mL of wort at 12.6°P, dextrose (Sigma Aldrich, Oakville, ON) at 4% w/v to produce a wort with a starting gravity of 16.1°P.

Wort was pitched with a *saccharomyces pastorianus* strain called SMA that was obtained from White Laboratories via CMBTC, Winnipeg, MB. Yeast was prepared from an agar slant, a 1-μL sterile loop was used to transfer the yeast aseptically into a 125 mL Erlenmeyer flask containing 50 mL of YEPD. A foam bung was used as a stopper for the flask which was then incubated on an orbital shaker at 100 rpm and 30°C for 24 hours. Next, the yeast slurry was centrifuged in a 50 mL Falcon tube at 3000*xg* for three minutes. The supernatant solution was poured off and the yeast pellet was resuspended in distilled water. The centrifugation and resuspension process were performed two more times. A yeast count was then performed on a haemocytometer and the yeast slurry was pitched at a rate of 1.5 x. 10^7 cells/mL into two 250 mL flasks containing 100 mL of YEPD each. The 100 mL flasks were stoppered with foam bungs and incubated on the orbital shaker at 100 rpm at 30 °C for 24 hours. Following incubation, the yeast was centrifuged again following the same procedure as before and resuspended into a single tube where another yeast count was performed.

The wort was pitched with the prepared yeast at a rate of 1.5 x 10^7 cells/mL. This was defined as the start of the fermentation and the apparent extract was recorded. Afterward, the wort was dispensed into 30 sterile test tubes, containing a boiling chip, that held 15 mL of wort each. These test tube fermenters were stoppered with sterile foam bungs and placed in a water bath set to 21.0°C.

Test tube samples were analyzed at fermentation times of 0, 1, 5, 22, 26, 30, 46, 50, 54, 70, and 78 hours or as close to these times as practical. At time 0-hour, one density reading was taken

directly from the wort pitched with yeast. At time 1 hour, one test tube was sampled (destructive sampling) recording the density and yeast in suspension. At time 5 hours, two test tubes were sampled (destructive sampling) with YIS and density being recorded. At all future sampling times, three test tubes were destructively sampled to get triplicate measurements of YIS and density.

Detection of the presence of PYF was done using YIS control charts generated by Armstrong et al. ([88]) shown in Figure 10. Armstrong et al. ([88]) created the control chart and determined the upper and lower bounds of normal YIS values during fermentations of non-PYF malt using the ASBC's mini-fermentation method ([89]). The upper and lower bounds of this control chart are placed two standard deviations away from the mean absorbance values throughout the fermentation. This mean value was obtained from pooled data of mini-fermentations of 77 malt samples. A positive PYF result is signaled when a YIS data point drops below the lower control limit outlined by Armstrong et al. ([88]). This signals a significant variation from normal YIS values during fermentation.

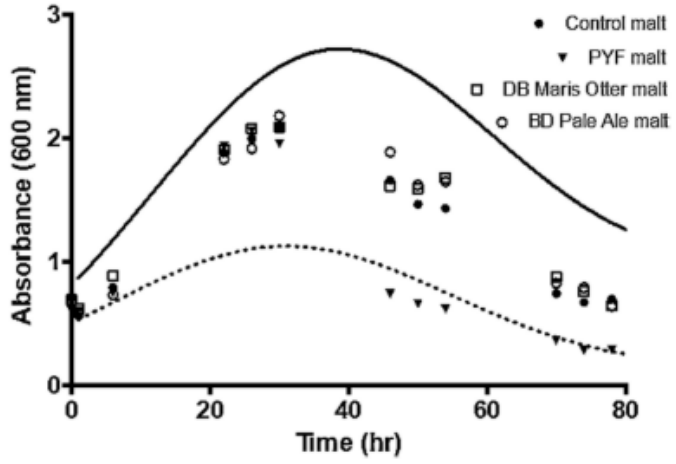

Figure 10. Detection of the presence of PYF was done using a YIS control chart generated by Armstrong et al. ([88]). Armstrong et al. ([88]) created a control chart outlining the upper and lower bounds of normal YIS values during fermentations of non-PYF malt using the ASBC's mini-fermentation method ([89]).

3.3.3 Moisture Testing

Determination of malt moisture was done following the ASBC's Malt-3 method ([90]). Malt samples of 5.00 g (+/- 0.01 g) were placed into a drying oven set to 103°C for three hours. After three hours the samples were removed and set in a desiccator to cool to room temperature before the mass of the sample was taken. The moisture content of each sample was then determined. Samples were returned to the oven for an additional hour after which samples were again cooled and moisture determined until a steady reading was obtained.

3.3.4 Measuring Density of Wort/Beer

Density was recorded following ASBC's Beer-2 method using a handheld digital density meter (Anton Paar, DMA 35A, Graz, AUT).

3.3.5 Recording Germination Temperature

During the germination of each malt sample, 3-6 thermocouples (Omega Environmental, Montreal, QC) were placed in different locations and heights within the malt bed. A GL240 data logger (GraphTec, Irvine, CA) was used to record germination temperatures.

3.3.6 Daily External Temperature Information

Daily temperature data was used from the government weather station in Kentville, NS located 17 kilometers away from Horton Ridge Malthouse ([91]). Average and maximum daily temperatures were determined for each sample of malt over the days that the malt was being germinated.

3.3.7 Measurement of Acrospire Growth

Measurement of barley acrospires during germination were performed as stated in the ASBC's Malt-2 Physical Tests ([92]). All samples were germinated until the acrospires were ¾ of the length of the barley kernel.

3.3.8 Statistical Analysis

Each malt sample's change in apparent extract (AE) and YIS during mini-fermentation was mathematically modelled using ASBC's Yeast-14 method ([89]). The mathematical equations representing these two curves are given in Figures 11 and 12. Correlation coefficients between floor malting parameters and change in the Apparent Extract (AE) variables as well as floor malting parameters and YIS variables were determined using Excel (Microsoft Corp, Redmond, WA).

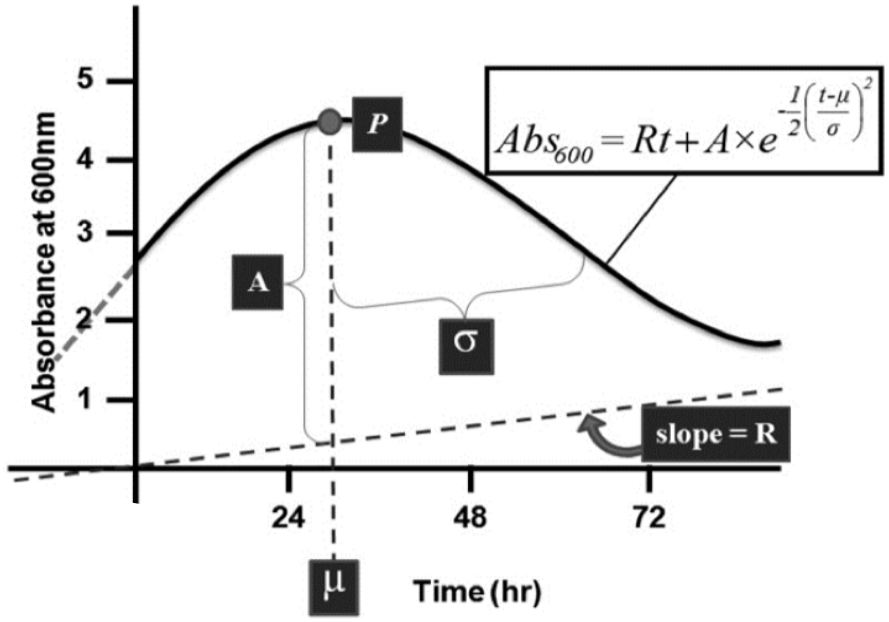

Figure 11. Tilted Gaussian fit of Absorbance for YIS (taken from ASBC Yeast-14 (85)). Abs600 = Absorbance at any time t, A = Absolute Amplitude, R = Slope Term, σ = width factor, μ = the midpoint, P = peak absorbance reading of YIS

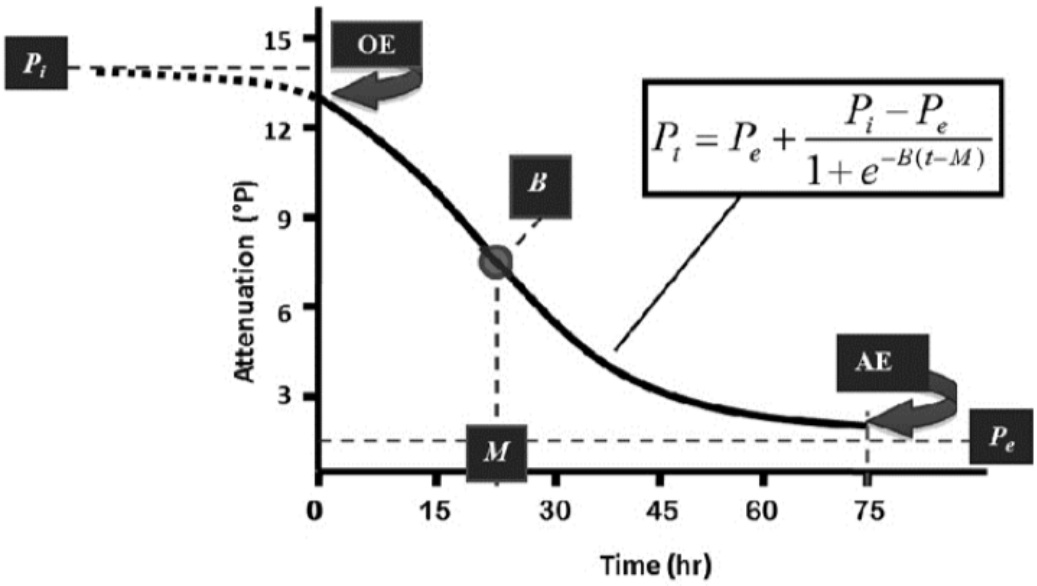

Figure 12. Nonlinear regression fit of change in apparent extract during mini-fermentation (taken from ASBC Yeast-14 (85)). Pt= Extract Value at time t, Pe= Final asymptotic value, Pi= Initial asymptotic value, B= A function of the slope at the inflection point, M= The time to reach the inflection point, OE = original extract (°P) reading taken at t=0.

3.3.9 Experimental Design

This experiment was designed to assess several aspects of floor malting simultaneously. Each barley sample was malted to produce a typical Horton Ridge Pale Two-Row Malt. During the malting of each sample the following variables were recorded: steep out moisture, average germination temperature, maximum germination temperature, and germination length.

Resulting malt samples were collected and analyzed at Dalhousie University (Halifax, NS). A mini-fermentation was performed on each malt sample to gather the information on extract, ADF and YIS during fermentation. The malting and fermentation variables of each floor malt sample were compared in groups to find the influence of malting variables on corresponding fermentation kinetics. Data was grouped according to variety; typical malting varieties were grouped together and compared against feed barley varieties.

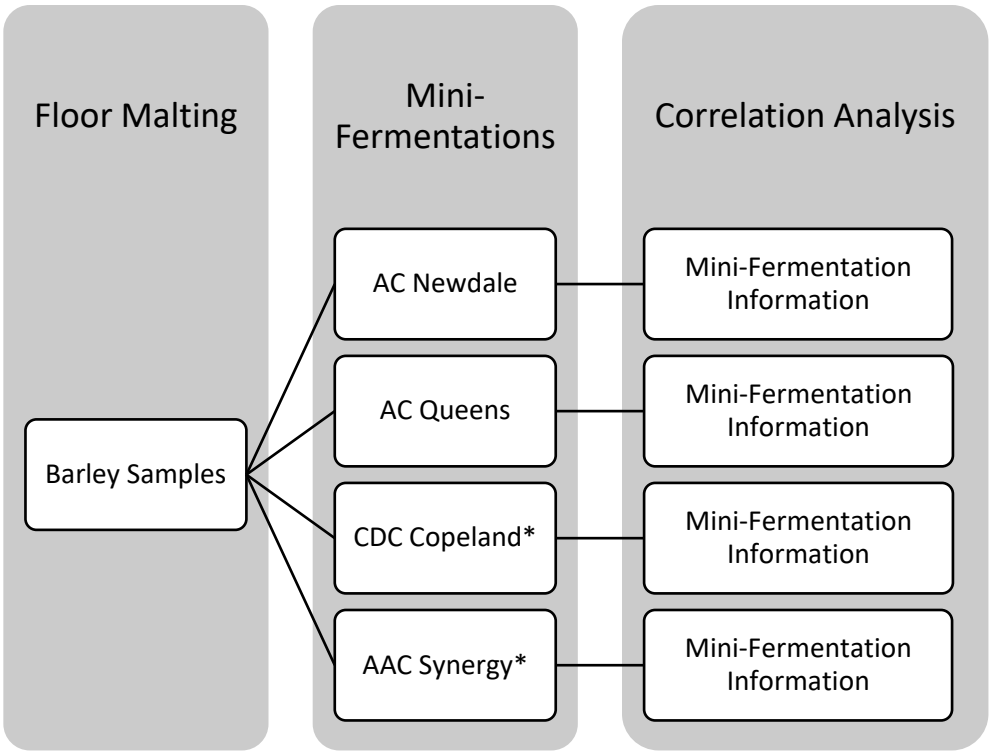

Figure 13. Diagram of the experimental design for testing of summer floor malt for PYF. *= barley varieties sampled in both year one and two

3.4 Results and Discussion.

3.4.1 Influence of External Temperatures on Germination Temperatures in a Floor Malthouse.

The germination temperatures of the malt samples during 2018 and the corresponding external daily temperature information were compiled in Table 2. Of the 17 floor malted samples in 2018, 14 had the germination time and temperature data recorded for the entire germination. However, for the three samples #225, #226, and #239 the data logger was not available to record temperature information. The average temperatures for these three samples were calculated

from the malthouse temperature log, where malthouse workers recorded germination temperatures using a handheld temperature probe and took reading before each raking.

Table 2. Correlation of daily and malt germination temperatures during 2018 data collection.

	External Avg. Temperature	**External Max. Temperature**	**Avg. Germination Temperature**	**Max. Germination Temperature**
External Avg. Temperature	1			
External Max. Temperature	0.918*	1		
Avg. Germination Temperature	0.521*	0.449	1	
Max. Germination Temperature	0.248	0.273	0.444	1

* = significant at $p<0.05$, $r>0.482$ ($n=17$)

The highest average germination temperature was recorded to be 23.6°C for sample #243 which began germinating on the floor September 2nd, 2018. The lowest average germination temperature was 18.7°C for the sample #251 which began on September 25th, 2018. The highest maximum germination temperature experienced was 31.5°C by samples #245 and #246 which began malting September 8th and September 12th, 2018 respectively. Daily temperature data showed that the highest temperature during the sampling time was 33.4°C during the malting of sample #227. Highest average daily temperature was 24.0°C during the floor malting of sample #232.

Comparisons of external temperatures with the malthouse germination temperatures states one significant correlation according to Table 1. The average external temperature and average germination temperatures were found to be significantly correlated ($p<0.05$) with floor malt samples recorded between June 26th, 2018 and October 18th, 2018. Other comparisons of interest such as the effect of maximum external temperatures on germination temperatures were non-significant ($p>0.05$).

3.4.2 Effects of Floor Malting on the Change in AE during Fermentation of Malting versus Feed Barley Varieties.

Subsequent wort production and fermentation of each floor malted sample yielded several notable correlations as shown in Table 3. It was found that the average wort extract (% db) yield between malting (CDC Copeland, AC Newdale, and AAC Synergy) and feed (AC Queens) barley varieties was not significantly different ($p>0.05$) after being floor malted. However, the fermentation performance was found to be different between malting and feed barley varieties. All malting barley varieties yielded an average ADF of greater than 83%. The average ADF for the feed barley AC Queens barley was significantly ($p<0.05$) lower at 76.2%.

The average germination length was significantly shorter ($p<0.05$) for the malting barley varieties compared to AC Queens barley. Germination of AC Queens took at least another full day for required modification to be reach during floor malt production. Average germination

temperatures varied between barley varieties. Average germination temperatures were highest for AC Newdale and AC Queens varieties with 21.1°C and 22.4°C respectively.

Table 3. Malting and fermentation data on the three malting and one feed barley varieties that were floor malted.

Barley	Malt (M) or Feed (F) Barley	Extract (% db)	Apparent Degree of Fermentation (ADF %)	Germination Length (Days)	Average Germination Temperature (°C)
AC Newdale (n = 6)	M	85.9± 2.7[b]	84.7± 3.0[c]	4.1± 0.6[d]	21.1± 0.9[f,g]
CDC Copeland (n = 6)	M	87.6± 2.1[b]	89.0± 1.4[c]	4.3± 0.7[d]	18.2± 1.8[e]
AAC Synergy[a] (n = 2)	M	86.7± 6.1[b]	83.2± 7.0[c]	4.3± 0.7[d]	16.8± 3.7[e,g]
AC Queens (n = 8)	F	82.9± 3.4[b]	76.2± 1.7	5.4± 0.3	22.4± 0.6[f]

a: calculations based on only two samples.
b,c,d,e,f,g: Values located in the same column and denoted with the same superscript are not significantly different ($p>0.05$).

A summary the logistical model of AE variables can be found in Table 4 for each barley variety. Logistical model variables were not significantly different between CDC Copeland and AC Newdale and so the data for each were pooled for further correlation analysis. AC Queens barley variety yielded a set of logistical model variables that were unique from other varieties so the data could not be pooled.

Correlation analysis was carried out on the pooled data of CDC Copeland and AC Newdale barley varieties, as shown in Table 5. Between recorded malted parameters and Logistical Model

variables there were two significant correlations found. Steep out moisture was significantly correlated with resulting wort extract ($p<0.05$, $r = -0.727$). The maximum germination temperature was also significantly correlated with P_i and ADF ($r = -0.622$ and $r = -0.606$, $p<0.05$), respectively.

Table 4. Logistical Model variables for each barley variety of floor malted samples.

Barley	P_i (95% CI Range)	P_e (95% CI Range)	B (95% CI Range)	M (95% CI Range)
AC Newdale (n=6)	15.07[a] (14.28-15.86)	2.25[b] (1.87-2.62)	-0.12[c] (-0.15- (-0.10))	32.17[d,e] (29.69-34.66)
CDC Copeland (n=6)	15.93[a] (15.59-16.26)	1.72[b] (1.49-1.94)	-0.11[c] (-0.12-(-0.10))	33.98[d] (32.79-35.17)
AAC Synergy (n=2)	15.62[a] (14.99-16.25)	2.57[b] (1.64-3.5)	-0.12[c] (-0.14-(-0.10))	31.98[e] (31.82-32.15)
AC Queens (n=8)	16.21[a] (15.85-16.56)	3.69 (3.98-3.40)	-0.09 (-0.09-(-0.08))	32.87[e] (31.46-34.29)

a,b,c,d: Values located in the same column and denoted with the same superscript are not significantly different ($p>0.05$).
NOTE: P_i is not equal to OE but is equal to the asymptotic value that apparent extract function approaches at OE. However, P_e is equal to AE, which is the exact value at the end of fermentation.

The significant correlations of logistical model of AE with malting variables of feed barley variety AC Queens are in Table 6. Only two significant correlations were detected, both involving average germination temperature. Average germination temperature had significant correlations ($p<0.05$) of 0.728 with both the logistical variable "M" and the wort extract. Other correlations were present in Tables 5 and 6 then what has been discussed, however, only correlations between malting parameters (A-E) with Logistical model variables (F-K) were of interest in this project.

Table 5. Correlation table of Logistical model variables on pooled data of CDC Copeland and AC Newdale. Bold print indicates significant correlation ($p<0.05$).

	A)	*B)*	*C)*	*D)*	*E)*	*F)*	*G)*	*H)*	*I)*	*J)*	*K)*
A) Steep out moisture (%)	1.000										
B) Max. Germination Temp.(°C)	0.347	1.000									
C) Avg. Germination Temp. (°C)	-0.013	**0.587**	1.000								
D) Germination Length (days)	-0.263	-0.250	-0.514	1.000							
E) Malt Moisture (%)	0.211	**0.618**	0.423	-0.256	1.000						
F) Pi	-0.309	**-0.622**	-0.195	0.165	-0.274	1.000					
G) Pe	0.190	0.532	0.306	0.257	0.488	-0.391	1.000				
H) B	-0.012	-0.483	-0.180	-0.063	-0.041	**0.808**	**-0.577**	1.000			
I) M	0.066	-0.336	-0.178	-0.308	0.051	0.521	**-0.617**	**0.858**	1.000		
J) Extract (%db)	**-0.727**	-0.440	-0.151	0.252	-0.261	0.395	-0.237	0.124	-0.028	1.000	
K) ADF (%)	-0.214	**-0.606**	-0.308	-0.203	-0.471	0.563	**-0.979**	**0.707**	**0.683**	0.275	1.000

Significance at $p<0.05$, $r>0.575$, $N=12$.

Table 6. Correlation table of Logistical model and malting variables of AC Queens samples. Bold print indicates significant correlation ($p<0.05$).

	A)	*B)*	*C)*	*D)*	*E)*	*F)*	*G)*	*H)*	*I)*	*J)*	*K)*
A) Steep out moisture (%)	1.000										
B) Max. Germination Temp.(°C)	**-0.738**	1.000									
C) Avg. Germination Temp. (°C)	-0.472	0.581	1.000								
D) Germination Length (days)	0.423	-0.103	-0.457	1.000							
E) Malt Moisture (%)	0.006	0.302	-0.180	0.442	1.000						
F) Pi	-0.074	0.029	0.285	0.388	0.362	1.000					
G) Pe	-0.011	-0.424	0.127	-0.075	-0.237	0.589	1.000				
H) B	-0.210	0.215	0.356	0.371	0.440	**0.964**	0.559	1.000			
I) M	0.118	0.145	**0.728**	0.035	-0.034	0.588	0.187	0.524	1.000		
J) Extract (%db)	0.118	0.145	**0.728**	0.035	-0.034	0.588	0.187	0.524	1.000	1.000	
K) ADF (%)	0.020	0.472	-0.037	0.177	0.327	-0.403	**-0.975**	-0.386	-0.008	-0.008	1.000

Significance at $p<0.05$, $r>0.706$, $N=8$.

3.4.3 Effects of Floor Malting on the Change in YIS during Fermentation of Malting versus Feed Barley Varieties.

Each of the malting barley varieties (AC Newdale, CDC Copeland, and AAC Synergy) yield tilted Gaussian variables that were not significantly ($p>0.05$) different from each other as highlighted in Table 7. Therefore, YIS data were pooled for the three malting barley varieties: AC Newdale, CDC Copeland, and AAC Synergy. AC Queens yielded a set of tilted Gaussian variables that were unique from the malting barley varieties.

Table 7. Tilted Gaussian model variables representing YIS during fermentation of malt and feed barley varieties. Mean +/- 95 CI range was included for each variable.

Barley	R (95% CI Range)	A (95% CI Range)	Mu (95% CI Range)	Sigma (95% CI Range)
AC Newdale	0.003[a] (0.000-0.007)	1.867[b] (1.756-1.977)	39.027[c,d] (34.455-43.599)	19.852[e] (18.641-21.064)
CDC Copeland	0.004[a] (0.001-0.007)	1.901[b] (1.718-2.084)	43.210[c] (42.488-43.932)	20.572[e] (19.718-21.427)
AAC Synergy	0.003[a] (0.002-0.003)	1.933[b] (1.882-1.984)	42.238[c,d] (37.301-47.174)	21.426[e] (19.733-23.118)
AC Queens	0.013 (0.010-0.016)	1.441 (1.329-1.553)	38.298[d] (36.831-39.764)	21.377[e] (20.117-22.636)

a,b,c,d: Values located in the same column and denoted with the same superscript are not significantly different ($p>0.05$).

Pooling the data from the malting barley varieties gives a sample size of 14. Table 8 contains the correlations of the recorded malting parameters and the tilted Gaussian YIS variables of the pooled malting barley fermentation data. Only two significant correlations existed between recorded malting parameters and Gaussian variables. These were that germination length was

found have a significant correlation of 0.844 and -0.724 on the Gaussian variables: “R” and “A”, respectively.

Table 8. Correlation table of tilted Gaussian model for YIS with malting parameters of malting barley varieties. Bold print indicates significant correlation (p<0.05).

	A)	*B)*	*C)*	*D)*	*E)*	*F)*	*G)*	*H)*	*I)*
A) Steep out moisture (%)	1.000								
B) Max. Germination Temp.(°C)	0.423	1.000							
C) Avg. Germination Temp. (°C)	0.217	**0.653**	1.000						
D) Germination Length (days)	-0.317	-0.262	-0.517	1.000					
E) Malt Moisture (%)	0.199	**0.620**	0.405	-0.236	1.000				
F) R	-0.214	-0.242	-0.452	**0.844**	-0.217	1.000			
G) A	0.110	-0.012	0.261	**-0.724**	-0.117	**-0.863**	1.000		
H) μ	-0.054	-0.436	-0.334	-0.226	-0.029	-0.292	0.379	1.000	
I) σ	-0.218	-0.250	-0.374	-0.190	0.180	-0.232	0.106	**0.727**	1.000

Significance at p<0.05, r>0.532, N=14.

A similar correlation analysis between recorded malting parameters with Gaussian variables for AC Queens was shown in Table 9. However, no significant correlations were found between recorded malting parameters and Gaussian YIS variables. Other correlations were present in Tables 8 and 9, however, only correlations between malting parameters (A-E) with Gaussian model variables (F-I) were of interest in this project.

Table 9. Correlation table of tilted Gaussian model for YIS with malting parameters of AC Queens. Bold print indicates significant correlation (p<0.05).

	A)	*B)*	*C)*	*D)*	*E)*	*F)*	*G)*	*H)*	*I)*
A) Steep out moisture (%)	1.000								
B) Max. Germination Temp.(°C)	**-0.738**	1.000							
C) Avg. Germination Temp. (°C)	-0.472	0.581	1.000						
D) Germination Length (days)	0.423	-0.103	-0.457	1.000					
E) Malt Moisture (%)	0.006	0.302	-0.180	0.442	1.000				
F) R	-0.660	0.704	0.503	0.201	0.123	1.000			
G) A	0.670	-0.671	-0.580	-0.162	-0.065	**-0.962**	1.000		
H) μ	0.577	-0.409	0.165	-0.322	-0.010	-0.682	0.595	1.000	
I) σ	0.003	-0.327	0.028	-0.443	-0.114	-0.092	0.216	0.190	1.000

Significance at p<0.05, r>0.706, N=8.

Of the 22 floor malted samples, only one displayed a positive tendency for PYF as per the PYF control chart developed by Armstrong et al. ([88]). YIS plots for the other 21 floor malted samples can be found in the Appendix. The PYF positive sample was #226 and Figure 14 shows its respective YIS during the mini-fermentation. Figure 14 shows that at 49 hours one of the triplicate measures of YIS drops below the lower limit of the control chart generated by Armstrong et al. ([88]) which was indicative of PYF.

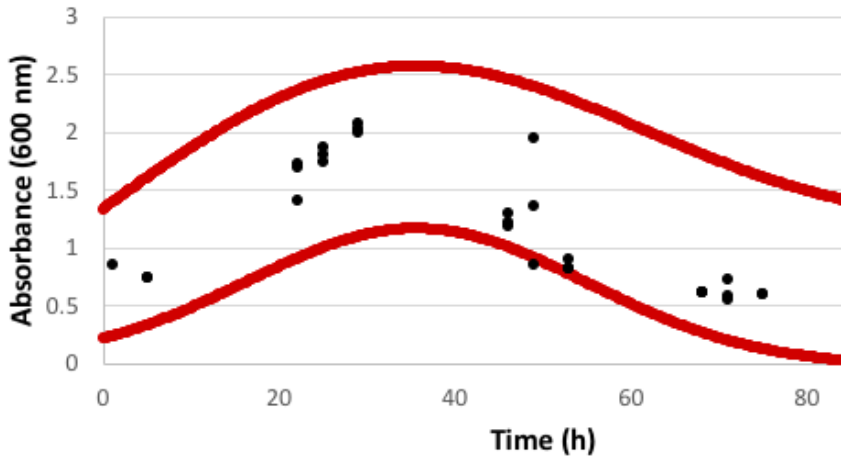

Figure 14. Yeast in suspension during a test tube fermentation of floor malted sample #226. The red lines indicate the upper and lower bounds for detecting PYF created by Armstrong et al. ([88]).

3.4.4 Discussion

The correlation results from Table 2 infer that there was a significant ($p<0.05$), but weak, correlation between external temperatures on germination temperatures, confirming that higher sustained temperatures outside the malthouse resulted in higher temperatures during

germination. The results gave evidence that the cooling system operating in the germination room were not enough to maintain desired germination temperatures during 2018 sampling. Looking at the germination temperature data in Table 13 in the appendix, it appears the highest average and maximum germination temperatures experienced were during summer months. Germination information on samples #251 and #256 which were malted in late September and October, respectively, showed the lowest average and maximum germination temperatures.

It appears that during 2018 Horton Ridge's germination temperatures were influenced by external temperatures but in a different way than traditional floor malthouses. Hotter days impact the ability of the heat exchangers installed by Horton Ridge to maintain cool germination temperatures. The heat exchangers that provide air conditioning to the germination room must draw air from outside, cool it down, and then circulate it into the germination room. When the temperature of the air outside was hotter, it required more work by the heat exchangers to cool the air down before getting circulated into the germination room. At Horton Ridge during the hot summer months in 2018 the heat produced from the germinating barley exceeded the refrigeration capacity of the cooling system located in the germination room. When the heat produced from the germinating barley was greater than the capacity of the heat exchangers to supply cold air it led to an overall increase in germination temperatures. Once summer ended and outside air was not as hot, then the heat exchangers could provide cold air more effectively and at this point keep average germination temperatures below 20°C.

Possible solutions to assist summer floor malting would be to invest in another heat exchanger that would only operate during the summer months when the extra cooling capacity was required. Another solution would be to cut back malting production volumes. This would reduce the amount of germinating barley and thus the amount of heat produced by the germinating barley. This would then also require less work from the existing heat exchangers and help to maintain safe, cool germination temperatures all year round.

As anticipated, the malting barley varieties performed superior to the feed barley variety for malting purposes. Even though AC Queens yielded an average wort extract equal to that of the malting barleys, it was at the expense of a significantly longer malting process and lower ADF.

It could be argued that the differences in ADF between the malting varieties and the AC Queens barley could have been a result of the longer germination period. To rule this out trials could have been done on four-day germinations of feed barley to be comparable to the malting barley varieties, as well as vice versa. However, the samples of floor malt were collected during regular production at Horton Ridge. The study did not allow control of the malting procedure at Horton Ridge. Any changes to malting procedures could interfere with established demand which could affect branding and result in a financial loss for the malthouse. Therefore, only a hands-off approach was followed in monitoring the malting process and recording data.

However, it was still deemed valid to conclude that modification occurs more rapidly in the malting barley varieties then the feed barley in this study. Horton Ridge maintains consistent

germinations among batches by ceasing germination when the average acrospire length in the malt batch gets to be ¾ of the length of the grain seed. Thus, the extra day required during germination of AC Queens barley was indicative of a natural slower modification process that was an intrinsic property of that barley variety. Toffoli et al. ([93]) studied the effects of several germination temperature regimes on malting and feed barley varieties. In every case the malting variety had greater friability then feed barley varieties and extract after a 113 hour germination ([93]). This supports our findings as it exemplifies that within the window of normal germination temperatures, feed barley varieties require longer germination time to obtain similar modification and extract as typical malting varieties.

The factor(s) responsible for the experimental differences in ADF are more difficult to conclude between the malting and feed barley varieties. Even though initial wort extract was not found to be significantly different among final malt samples, the fermentability was significantly and substantially lower for AC Queens. Although PYF can affect final ADF, the variations in ADF between feed and malting barley varieties can be postulated to be a result of differing carbohydrate profiles and not fungal contamination. Gunkel et al. ([94]) studied the carbohydrate profiles after malting of several known good and poor malting barley varieties. These researchers found that poor quality malting barley varieties exhibited lower ADF because their carbohydrate profiles consisted of relatively higher non fermentable carbohydrates concentrations ([94]). Although it was outside the scope of this research project, the exact profile of AC Queens malt carbohydrates could explain differences in fermentation. The finding from Gunkel et al. ([94]) can be used to explain the difference in ADF between the malting and feed barley varieties in Table

3, as AC Queens barley samples could of resulted in more non-fermentable carbohydrates being produced during malting. This would result in a typical extract yield for AC Queens barley but reduce the fermentability or ADF of the subsequent wort.

For the correlation analysis in Table 5, the data from CDC Copeland and AC Newdale was pooled to represent malting barley varieties. This was deemed valid as both barley groups yielded a complete series of logistical variables that were not significantly different from each other as shown in Table 4. By pooling this data, it allowed for a more robust analysis of the correlation of malting variables on the function representing change in AE during fermentation. However, AAC Synergy could not be pooled since its data yielded a set of logistic model variables that were unique from the other malting barley varieties. In Table 5, the correlation of highest significance was between steep out moisture and wort extract ($r = -0.727$). This correlation insinuates that for these barley samples, higher extract can be obtained when lower steep out moisture content was used during malting. This effect was contradictory to knowledge about typical malting regimes as barley is usually steeped until the moisture content is above 45%. All 2018 samples had steep moistures that were in the range of typical final moisture contents after steeping, except for sample #243 having a moisture of 41.8% which would be considered low. The data suggests that there exists an optimum moisture content when germinating CDC Copeland and AC Newdale in order to maximize extract produced, as CDC Copeland and AC Newdale samples with steep out moistures <45% yielded higher extracts. This effect would certainly have diminishing returns as adequate moisture content would still be required during germination.

However, if these barley varieties can be effectively germinated at lower-then-typical moisture contents then this would save water, time, and energy at the malthouse.

The other significant findings in Table 5 were that of the influence that higher peak germination temperature would decrease the P_i and ADF of the resulting malt fermentation of AC Newdale and CDC Copeland. P_i represents the asymptotic value that OE approaches in the logistical function, and initial extract has been found to be reduced at higher germination since starch reserves are more quickly depleted to support respiration. This agrees with other studies such as Toffoli et al. ([93]) and Agu ([95]) on germination temperatures effects on extract. Other studies such as Cole and Mitchell ([96]) have reported similar findings on increased germination temperatures diminishing effect on ADF. The main cause for the reduction of ADF is the increased acrospire growth at higher temperatures during germination. The increased acrospire growth leads to higher consumption of sugars that would have remained in the malt to be consumed by the yeast thus reducing initial extract and ADF ([96]).

A similar correlation analysis on AC Queens barley, in Table 6, exhibited a significant ($p<0.05$) correlation of increased germination temperature with an increase in the logistical model variable “M”. The variable “M” corresponds to the inflection point of the change in AE curve or in simpler terms, the time at which sugar consumption during fermentation was quickest. This correlation suggests the change in germination temperature affects the carbohydrate profile of the wort which in turn affects the speed at which the yeast consumes these sugars.

The data in Table 6 also showed that increases in the average germination temperatures of AC Queens barley will correlate to subsequent increases in wort extracts. This information can help to develop an optimum malting regime for feed barley varieties, specifically AC Queens. Many studies have shown that higher germination temperature increases enzyme activity during malting. The increased enzyme concentration as a result of higher germination temperatures may be beneficial to the modification during germination of AC Queens. Feed barley varieties are known to have more β-glucans and protein content. From the literature review, it was noted that in order to access the starch reserves in the endosperm, the β-glucan and protein matrix that contain the starch must first be degraded. It then seems intuitive for higher β-glucan and protein barley varieties such as feed barleys that modification could be improved from increased enzymes associated with higher germination temperatures.

When assessing malting effects on YIS in Table 7 the data from CDC Copeland, AC Newdale, and AAC Synergy were pooled. This was deemed valid as each malting barley variety yielded a series of Gaussian variables that were not significantly different as shown in Table 7. Pooling this data allowed for a more robust correlation analysis to generalize a trend among malting barley varieties. Without pooling, the data from AAC Synergy would have been excluded from correlation analysis as it only had a sample size of two.

Correlations in Table 8 found that germination time influenced the tilted Gaussian model variable "A" for the pooled data of malting barley varieties. The Gaussian variable "A" mathematically represents the peak amount of YIS during fermentation. A correlation (r = -0.724) between these

two variables suggests that as germination time was allowed to proceed longer, the peak YIS would diminish. Conceptually this make sense, as theoretically if germination time was extended then more sugars are consumed by the growing acrospire. Therefore, there would be less sugars available to supply food for yeast growth and reproduction, in turn lowering peak YIS during fermentation. This gives insight for optimal malting of the varieties AC Newdale and CDC Copeland. If the brewer desires a higher peak YIS during fermentation of these barley varieties than a shorter germination time needs to be communicated to the maltster.

However, there appeared to be no correlation of malting parameters on yeast performance during fermentation for feed barley variety AC Queens in Table 9. This may be in part to small sample size being available as well as external effects during malting that were not able to be recorded.

Only one of the samples displayed a PYF tendency, that was sample #226. Pictured in Figure 14, sample #226's yeast in suspension appears to be 'normal' until readings are taken after the onset of flocculation. Upon flocculation, yeast drops out of solution faster and results in one YIS data point below the lower control limit, while the other two data points of the triplicate remain within the PYF control chart. Sample #226 was indicative of the variability of PYF in malt samples as the YIS readings during mini-fermentation are known to be heteroscedastic, meaning the natural variation in the sample YIS readings is larger during the middle of the mini-fermentation (between 40-65 h) than at the beginning or end. This makes it more difficult to definitively conclude a PYF malt sample with confidence, as a positive PYF test can be falsely concluded as a

result of the increased variation in sample YIS readings during this time during the mini-fermentation. Assessing the germination conditions of #226 found in Table 14, it is evident that sample #226 did not undergo any unique malting conditions. The PYF positive sample was not germinated for the longest or at the highest temperature which further attests to the variability of PYF occurrence and the difficulty by researchers to pinpoint the most significant factors which cause PYF.

With potentially one of 22 floor malted samples exhibiting PYF during this experiment. It remains unclear what set of malting conditions was responsible for causing PYF, as the positive PYF sample did not experience any unique malting conditions that other floor malted samples did not at least partly experience as well. However, this could indicate that there exists a very specific set of malting conditions that will induce PYF, as it was not simply high germination temperatures that cause PYF but perhaps a certain duration at a specific germination temperature range in which fungal activity may favour the formation of PYF inducing factors. Alternatively, it could also be another set of malting parameters that were not recorded that were responsible for inducing PYF. One such factor could be the individual fungal load that was present in this particular batch of PYF malt. As previously discussed in the literature review, different microbes proliferate better at certain temperatures during malting. Since each malt sample were not malted simultaneously, each batch experienced a unique temperature profiles during malting. It would be expected that for each malt sample, the microbial dynamic would be unique during malting as well. Other barley-malt handling processes could also be responsible for the PYF positive results. Alternatively, since no specific malting parameters could be pinpointed as causing PYF in this

experiment it could be possible that the PYF-inducing factor was created during storage or handling of the barley/malt. For instance, this one PYF positive malt sample could have been exposed to more humid storage or shipping conditions (e.g., collected/transported on a rainy day).

Chapter 4 Comparing Floor versus Pneumatic Malting on Quality of Resulting Malt and Beer.

4.1 Introduction

There were three main reasons for the industrialization of the malting process to become a fully automated pneumatic system: 1) less workers, 2) less manual labour during production, and 3) more control of malting conditions. While floor malting may be at a technological disadvantage, it does offer a less costly malt production method, particularly for small-scale operations. Since floor malting has less control over malting process variables than pneumatic systems, more manual labour was required by maltsters in order to produce adequate malt.

There has been a substantial increase in the craft beer market in North America occurring over the past few years. A look at the brief history of craft beer in Nova Scotia is an example of this of this pattern. The 2018-2019 year-end report for Nova Scotia Liquor Corporation (NSLC) stated $16.7 million in craft beer sales, an increase of 27.3% from the year before ([97]). Additionally, the NSLC now carries products from 29 craft breweries which is up from only six craft breweries just five years previous ([97]). But what may have gone unnoticed was the subsequent increase in the craft malting industry as well. New craft malt houses are opening and employing traditional methods such as floor malting, to produce malted grains.

As mentioned in the literature review, floor malthouses have been relatively obsolete over the past 80 years in favour of pneumatic malting systems. However, floor malting appeals to craft

maltsters as a less costly malting operation for small scale production. According to the Craft Maltsters Guild at the time of writing, in North America there are roughly 100 craft malthouses and 25 of which are floor malthouses ([98]). The revamped popularity of the floor malt production assures that there needs to be more subsequent research done involving this malting method, as future research can provide quality assurance and confidence to owners who then contribute to the craft beer industry and the economy.

The objective for this research project was to explore the differences that may arise from malt that has been produced pneumatically and from floor malting. This in turn will benefit the malting industry as it will provide the unique characteristics, if any, that exist between the malt production techniques. This malt comparison information would be useful for the malthouses for either quality assurance, or advertising purposes. Either way, the rising popularity of floor malting has produced a research opportunity in the malting industry that has been relatively untouched for the past 80 years.

4.2 Materials

4.2.1 Barley

A single sourced 2017 harvest CDC Copeland malting barley variety was used exclusively for this trial. The CDC Copeland barley was divided with a sample being pneumatically malted at CMBTC in Winnipeg, MB and a sample being floor malted at Horton Ridge Malt and Grain Co. in

Hortonville, NS. Malted barley samples were malted and packaged at their respective malthouses and delivered to Dalhousie University (Halifax, NS) where they were stored away from sunlight at room temperature until further analysis could take place.

4.3 Methods

4.3.1 Malting Regime

The following malting regime was used:

Steeping: Immersion in 12°C water for 10 hours, followed by an 8-hour air rest, then 10-hour water soak, 8-hour air rest, 4-hour soak then final drain and begin germination.

Germination: The germination stage for each malting technique was different from each other. The germination stage of the pneumatically malted barley at CMBTC occurred in a HDP Micromalting system (MacDonald Steel HDP, Cambridge, ON) at a consistent temperature of 14°C for 96 hours, whereas germination for the floor malted barley took place on a concrete floor in an air-conditioned room with temperature ranging from 12.3°C – 23.0°C.

Kilning- Forced-air style kilns were used on both malts as follows: 50°C until malt moisture was below 10%, then 60°C for 3 hours, 70°C for 1 hour, then 80°C until malt moisture reached 5%.

4.3.2 Malt Quality Analysis

Malt quality analysis was carried out by Hartwick College Center for Craft Food & Beverage (Hartwick, New York, USA). All malt analysis procedures were performed according to the following ASBC standard method of analysis:

4.3.2.1 Moisture Testing: Determination of malt moisture was done following the ASBC's Malt-3 method ([90]).

4.3.2.2 Friability: Friability was determined using ASBC's Malt-12 ([99]).

4.3.2.3 Extract: Extract was determined using ASBC's Malt- 4 ([100])

4.3.2.4 Color: Wort color was determined according to ASBC's Beer-10 ([101]).

4.3.2.5 Wort β-glucan: Wort β-glucan was determined using ASBC's Wort-18B-Segmented Flow Analysis ([102]).

4.3.2.6 Soluble Protein: ASBC's Wort-17 ([103])

4.3.2.7 Total Protein: ASBC's Malt-8B ([104])

4.3.2.8 FAN: ASBC's Wort-12B ([105])

4.3.2.9 Diastatic Power: ASBC's Malt-6C ([106])

4.3.2.10 α- Amylase: ASBC's Malt-7C ([107])

4.3.2.11 pH: ASBC's Wort-8 ([108])

4.3.3 Experimental Design

The difference between pneumatic and floor malting styles was analyzed by assessing the resulting quality of malt generated by each malthouse on a single sourced malting barley lot. As highlighted in Figure 15, samples of CDC Copeland barley were sent to a pneumatic and floor malthouse at CMBTC and Horton Ridge Malthouse, respectively. Each malthouse was instructed to make a pale ale base malt. During malting, both the pneumatic and floor malthouse followed identical steeping and kilning regimes. This would allow any differences in resulting malt quality to be a product of the germination conditions experienced at the different malthouses.

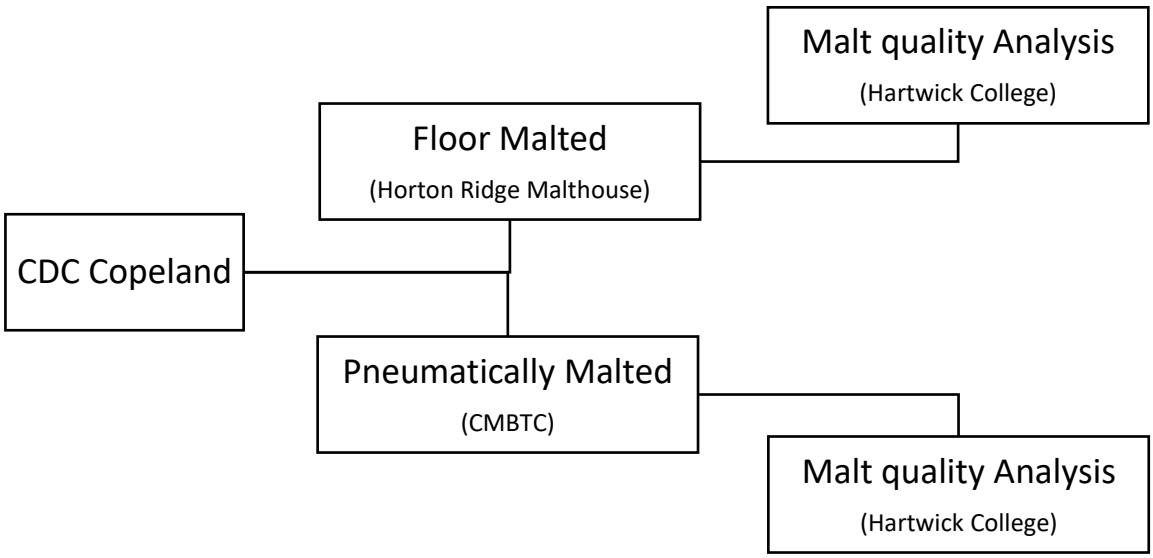

Figure 15. Flow chart outlining the experimental design for examining the effects of floor versus pneumatic malting on final malt quality.

4.3.4 Data Analysis

Graphing YIS data on Armstrong et al. ([88]) PYF control charts were performed on Excel (Microsoft Office). Error bar calculation was performed on Prism 8.4.2 (GraphPad, San Diego, CA).

4.4 Results and Discussion

4.4.1 Effects of Floor versus Pneumatic Malting on Malt Quality

The germination conditions during floor and pneumatic malting varied slightly although attempts were made to duplicate the malting conditions. Germination temperatures for both floor and pneumatic processes are presented in Table 10. The data in Table 10 shows that floor malting exhibited higher average and more variable germination temperatures. Floor malting had an average germination temperature of 19.6℃ and a maximum germination temperature of 23.0℃, Whereas pneumatic malting temperatures remained consistent at 14℃ throughout germination.

There were several notable differences in malt quality between floor and pneumatically malted barley, which are presented in Table 11. The moisture, extract, colour, FAN and diastatic power were all found to be significantly ($p<0.05$) higher in the floor malt. Friability was significantly higher in pneumatic malt. Notably, both floor and pneumatic malting produced malt that was

not significantly different in terms of wort soluble nitrogen, α-amylase content, and wort β-glucan content. Overall, the malt specifications list in Table 11 for floor and pneumatic malt are both considered suitable for further brewing use.

Table 10. Germination Temperatures experienced during the floor and pneumatic malting trials of CDC Copeland.

Malt:	Avg. Temp. (℃)	Max. Temp. (℃)
Floor	19.6	23.0
Pneumatic	14	14

When designing this experiment, it was hypothesized that differences in the final malt would correspond to differing germination temperatures between the two malting techniques. The germination temperatures were notably higher and more variable during floor malting compared to the pneumatic malting procedure. With this information, it was expected that resulting malt quality would be in accordance with other studies done of varying temperatures during germination. Previous research has showed that higher germination temperatures lead to final malt with generally lower FAN levels ([93,95,96]), extract ([93,95,96,109]), ADF ([96]), and β-glucanase activity ([93]), while increasing friability ([93]). Comparing the malt quality attributes presented in Table 11, it appears the difference in malt quality does not correspond with previous research undertaken at increased germination temperatures. This suggests that germination temperatures are were not the most influential factor when anticipating the differences in quality of floor versus pneumatic malt.

Table 11. Comparison of malt quality attributes from CDC Copeland barley after being Floor and Pneumatically malted.

Malt Quality Attribute	Floor Malted	Pneumatically Malted	SD (95%)
Moisture (%)	5.6	4.5	0.04*
Friability (%)	87.3	91	0.75*
Extract (% db)	80.1	79.6	0.18*
Color (SRM)	1.69	1.57	0.05*
β-glucans (mg/L)	90	104	7.6
Soluble Protein (%)	5.14	5.07	0.04
Total Protein (%)	12.8	13.0	0.12
FAN (mg/L)	195	191	1.5*
Diastatic Power (°L)	175	164	2.5*
α-Amylase (D.U.)	70.7	69.3	1.8
Filtration	Normal	Normal	/
Clarity	Clear	Clear	/
pH	5.96	5.91	/

**= indicates a statistical difference ($p<0.05$).*

/= indicates no SD given.

SD is based on eight successive analysis (on differing days) of the same malt sample([98])

4.4.2 Effects of Malting Technique on Fermentation Parameters

Assessing both floor and pneumatic malt's YIS values during fermentation yielded no indication of PYF. Neither sample's YIS values fell below the lower control limit determined by Armstrong et al. PYF control chart pictured in Figure 16 ([88]). Figure 17 shows the average YIS throughout fermentation of floor and pneumatic malt samples. In Figure 17, YIS values only indicated one significant difference which occurred before onset of flocculation at 25 hours. At all other sampling times, YIS was not significantly different between fermentations of floor and pneumatic malt. This was important to note as it represents identical yeast flocculation patterns for each fermentation.

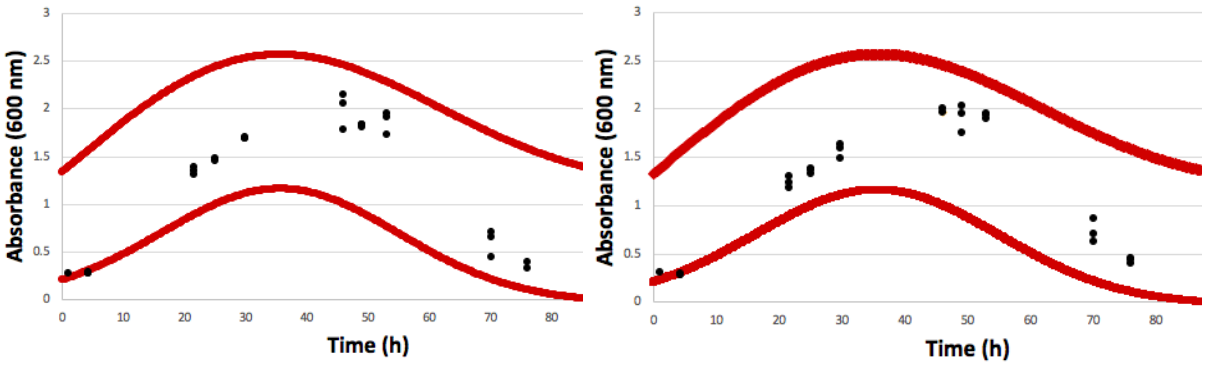

Figure 16. (Left) Mini-fermentation triplicate measure of yeast in suspension values at each sampling time for pneumatically malted sample. Graphed with PYF control chart created by Armstrong et al. ([88]). (Right) Mini-fermentation yeast in suspension values for Floor malted sample along with Armstrong et al. ([88])'s PYF control chart.

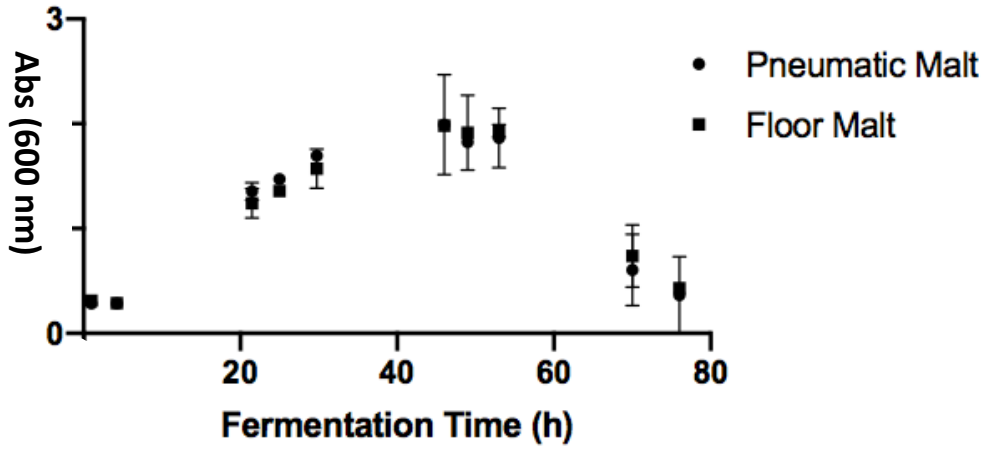

Figure 17. Average YIS values with 95% CI error bars during a test tube fermentation of floor and pneumatically malted CDC Copeland. Other than time= 25 hours, error bars of each sample overlap. This indicates no significant difference between the average YIS values between pneumatic and floor during mini-fermentation at these times.

Using the Logistical model for apparent extract during fermentation, the calculated ADF values for the floor and pneumatic malt were 88% and 90%, respectively. The ADF was calculated using the change in apparent extract as listed in Equation 1. ADF was calculated using the apparent extract data from fermentations, where OE represents apparent extract (°P) of solution immediately after pitching yeast, and AE represents apparent extract of solution (°P) 75 hours after yeast pitching.

Equation 1: $$ADF = \frac{OE-AE}{OE} = \frac{P_0-P_{75}}{P_{75}}$$

Figure 18 shows the average apparent extract for the fermentation of each malt sample during the mini-fermentation. As shown in Figure 18, the apparent extract at each sampling time was not significantly different between floor and pneumatic malt samples.

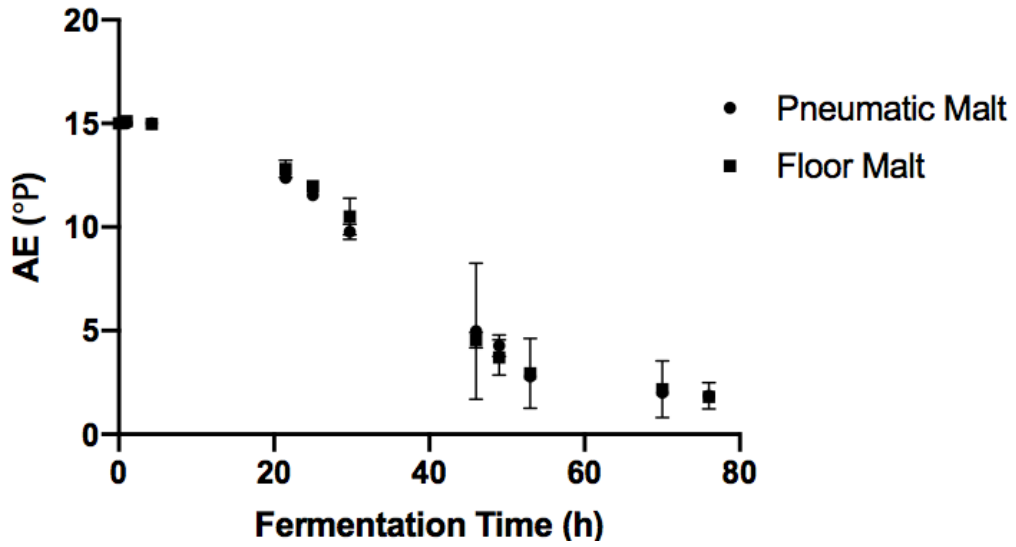

Figure 18. Average values of apparent extract (°P = plato reading) with 95% CI error bars during a test tube fermentation of floor and pneumatically malted CDC Copeland. Error bars of the pneumatic and floor malt samples overlap at each sampling time, indicating no significance difference ($p<0.05$) in AE at these times.

4.4.3 Discussion

When designing this experiment, it was hypothesized that differences in the final malt would correspond to differing germination temperatures between the two malting techniques. The germination temperatures were notably higher and more variable during floor malting compared to the pneumatic malting procedure. With this information, it was expected that resulting malt quality would be in accordance with other studies done of varying temperatures during germination. Previous research has showed that higher germination temperatures lead to final malt with generally lower FAN levels ([93,95,96]), extract ([93,95,96,109]), ADF ([96]), and β-glucanase activity ([93]), while increasing friability ([93]). Comparing the malt quality attributes presented in Table 11, it appears the difference in malt quality does not agree with previous research done on increased

germination temperatures. This means germination temperatures were not the most influential factor when anticipating the differences in quality of floor versus pneumatic malt in this experiment.

To rationalize the differing results in malt quality found in Table 11 other aspects of each malting techniques were analyzed, such as the influence that indigenous microbial populations had on final malt quality. Explaining the difference in malt quality between floor and pneumatic malt in Table 11 by considering relative microbial populations had more success than considering germination temperatures alone. It has been found that higher fungal activity during malting results in elevated levels of several important degradation enzymes such as β-glucanase, xylanase ([40,63]), proteases([45,61]), and α-amylases ([40,61,63]). This increase in enzymatic power was reportedly due to the additional exogeneous fungal enzymes present which aid in the modification process during malting.

Fungal activity was not monitored during the malting trials in Chapter 4. However, presuming that floor malting had increased fungal levels during malting would more accurately correlate to the discrepancy in malt quality between floor and pneumatic malt. Previous studies such as Laitila et al. (2007) reported similar findings in malt quality of lower friability and wort β-glucan as well as higher extract, soluble nitrogen, FAN, colour and α-amylase in malt made with higher fungal activity during germination ([40]). The findings from Laitila et al. (2007) correlate exactly with the presented results in Table 11 if it is assumed that pneumatic malting is a reduced microbe environment when compared to floor malting. This assumption can be supported by several

comparatives between pneumatic and floor malting environment's promotion of microbials which has already been discussed in the literature review. Other studies have also found similar results regarding elevated microbial levels and lower wort β-glucan ([4,42,68]), degree of modification ([42]) as well as higher extract ([62,68]), soluble nitrogen ([42,62,68]), FAN ([42,68]) α-amylase ([42,62]), diastatic power ([62]) and darker wort ([42]). As such, it seems logical that the discrepancy in malt quality between the floor and pneumatic malt was a result of the local make up and relative population of microbial communities in each malthouse.

PYF evaluations are usually done after the yeast begins to settle out of the fermenting beer. Before this point, YIS values for PYF positive malt can resemble PYF negative malt which makes these readings trivial and not conclusive for PYF detection but necessary for fitting the Tilted Gaussian Model of Absorbance as mentioned in the ASBC's Yeast-14 method ([89]). The discrepancy of YIS at fermentation time 25 hours in Figure 17 may be a result of wort composition differences such as carbohydrate profile, oxygenation, and/or yeast nutrients available. However, the exact wort composition was not thoroughly studied as it was not a focus of the research project; the focus was on the flocculation patterns of malt produced from the two styles of malting.

Overall, floor and pneumatic malt exhibited similar fermentability as both have nearly identical YIS and change in AE throughout fermentation. Additionally, both malts yield adequate fermentability to be used for brewing (i.e., PYF absent, low end of fermentation yeast counts and high ADF).

Chapter 5 Accelerating the ASBC's Yeast-14 PYF Detection Technique and Determining the PYF Sensitivity Among Lager Yeast Strains.

5.1 Introduction

PYF has been a difficult issue for brewing researchers to assess. An incomplete or slow fermentation may be blamed on PYF malt. Other possible factors could be responsible for poor fermentations such as low oxygenation, low pitched yeast rate, poor yeast vitality and viability ([7]). PYF has been known to be sporadic, and without specific knowledge of the PYF-inducing compound, such as the conformation and synthesis pathway, it is difficult to predict PYF in malt samples. Detection of PYF malt can only be performed once its subsequent wort fermentation has begun. Without pre-emptive measures in place for detecting PYF, by the time PYF malt has been confirmed in a commercial fermentation it may already be detrimental to the beer. To salvage affected fermentations, further processing may be required, whether this involves re-pitching or blending ([7]).

PYF, although rare, has increasingly become a concern for breweries, even more so for commercial breweries as they stand to suffer increased production losses due to a PYF affected fermentation. The brewing industry requires a rapid and robust method for detecting PYF malts before these malts are used in the brewhouse. The current standard method recognized by the ASBC for detecting PYF involves a miniature fermentation carried out in test tubes ([89]). This method is advantageous compared to industrial fermentations as it only requires less then 450 mL of prepared wort. Even still, the ASBC method requires sample preparation and requires six

days to complete the entire fermentation procedure. In addition, only a single *saccharomyces pastorianus* strain, SMA (VLB-Berlin, Berlin, DEU), has been tested to effectively detect PYF during the mini-fermentation. It remains unknown if other lager yeast strains can be employed to give definitive PYF results using the ASBC Yeast-14 method.

The objective of this research project was to assess the current ASBC's Yeast-14 method to further accelerate and simplify the procedure to obtain a definitive PYF result in less time than what the current method states. In order to accomplish this, there were several variables of the Yeast-14 method assessed. Firstly, to simplify the Yeast-14 procedure the elimination of the oxygenation of wort step was assessed. Anecdotal reports have suggested that this step may not be required due to ample oxygen provided to the yeast during propagation as well as introduction of oxygen into the wort during sample preparation. Secondly, to decrease the time required to detect PYF in the ASBC's Yeast-14 method the use of a one-day yeast propagation was compared to the two-day propagation currently employed. To further accelerate the Yeast-14 method, YIS values of fermentations of PYF-positive and PYF-negative malts were compared on the third day of fermentation to determine if PYF-positive malts could be statistically distinguished by this time. The current method calls for fermentation to progress for four days for PYF analysis. Additionally, this project was designed to further test the robustness of the Yeast-14 method to determine whether other commercial lager yeast strains could be employed, as this would clarify if the Yeast-14 method was only valid if used with the SMA yeast strain or if other lager strains that are readily accessible in the brewing industry could be used as well.

5.2 Materials

5.2.1 Yeast Strains

Two yeast strains were used during this experiment. SMA yeast (VLB-Berlin, Berlin DEU) was used as well as an industrial strain identified as Lager “A” (Molson-Coors, ON).

5.2.2 Malt

Both the PYF negative and positive control malt samples for the experiment were obtained directly from Canada Malting Co. (Calgary, AB).

5.2.3 Oxygen

Medical grade oxygen (Air Liquide Co., Dartmouth, NS) was used for oxygenation of wort samples.

5.3 Methods

5.3.1 Mini-fermentation

Mini-fermentations were carried out using the standard method: ASBC Yeast-14 ([89]). Differences from the ASBC method occurred during the yeast propagation and oxygenation steps; these changes are outlined in Chapter 5.3.2 and 5.3.3, respectively.

5.3.2 Propagation of Yeast

Two different yeast propagation procedures were employed that involved propagation of yeast over one- or two-day time intervals. The first method was the two-day yeast grow up procedure outlined in the ASBC's Yeast-14 method ([89]). The one-day yeast propagation method consisted of aseptically transferring 1 μL of yeast from a slant into a 250 mL Erlenmeyer flask containing 100 mL of sterile YEPD. The YEPD was made as in the ASBC's Yeast-14 method ([89]). After pitching the yeast, the 250 mL Erlenmeyer flask was stoppered with a foam bung and incubated on a rotary shaker at 100 rpm and 30°C for 24 hours. After 24 hours the yeast slurry was washed, centrifuged and counted as in ASBC's Yeast-14 method ([89]).

5.3.3 Oxygenation Method

Prepared wort was oxygenated using medical grade oxygen (Air Liquide Co., Dartmouth, NS). Oxygen was dispensed into the wort using a 2.0 μm stainless steel diffusion stone (Noble Grape, Halifax, NS) at 13.8 kPa for 5 minutes.

5.3.4 Data Analysis

Graphing, error bar calculation and difference testing were performed on Prism 8.4.2 (GraphPad, San Diego, CA) according to ASBC Yeast-14 ([85]).

5.3.5 Experimental Design

In order to determine the significance of the multiple variables being assessed simultaneously a factorial DOE was employed, the layout of which is given in Table 12. Each set of fermentations used a PYF-positive and a PYF-negative (control) malt to directly compare the effects of yeast strain, oxygenation levels and yeast propagation techniques.

Table 12. Factorial DOE employed for testing significance of oxygenation, yeast propagations days and yeast strain on detecting PYF.

Malt Sample #1 (Control)					Malt Sample #2 (PYF)			
Trial #:	Fermentation:	Yeast # (1/2)	Oxygenation (Yes/No)	Yeast Grow up days (1/2)	Trial #:	Yeast # (1/2)	Oxygenation (Yes/No)	Yeast Grow up days (1/2)
1	1	1	Yes	1	1	1	Yes	1
2	1	1	Yes	2	2	1	Yes	2
3	2	1	No	1	3	1	No	1
4	2	1	No	2	4	1	No	2
5	3	2	Yes	1	5	2	Yes	1
6	3	2	Yes	2	6	2	Yes	2
7	4	2	No	1	7	2	No	1
8	4	2	No	2	8	2	No	2

Yeast identification #:

1) SMA 2019
2) Lager “A”

Oxygenation:

Yes = oxygenated with medical grade oxygen for 5 minutes, as stated in the ASBC’s Yeast-14.
No = no additional oxygenation performed.

Yeast grow up days:

2 = two-day yeast propagation method
1 = one-day yeast propagation method

YIS data from each sample after mini-fermentation was analyzed on Prism 8.4.2 (GraphPad, San Diego, CA) to generate a set of Tilted Gaussian model of absorbance as outlined in ASBC's Yeast-14 method ([89]). Pairwise comparison of fits was performed to determine if one set of Gaussian variables of YIS could be fitted to the two sets of data at the significance level α = 0.05. For this statistical analysis the null hypothesis was: one curve can be fitted to both sets of data.

If one set of Gaussian variables can be fitted to both data sets, then the experimental YIS data from each data set was not significantly different at significance level α = 0.05. Pairwise comparisons of fits were performed on data to identify any significant differences between yeast propagation days and oxygenation method of each yeast strain.

5.4 Results and Discussion

5.4.1 Effect of Oxygenation during ASBC's Yeast-14 Method on the Sensitivity of Detecting PYF.

SMA Yeast:

Both oxygenated and non-oxygenated trials could distinguish between control and PYF malt by monitoring YIS during a mini-fermentation. Oxygenated fermentations corresponding to the one- and two-day yeast propagations are pictured in Figures 19 and 20, respectively. In both oxygenated wort trials, a significant difference ($p<0.05$) could be determined between PYF and

control malt using respective YIS during fermentation. The time at which PYF and control samples could be statistical differentiated varied slightly depending on the yeast propagation method and will be discussed in more detail in the next chapter.

YIS data from the non-oxygenated fermentation trials are displayed in Figures 21 and 22. In both trials, a significant difference ($p<0.05$) in YIS between PYF and Control malts was detected at 51.5 hours of fermentation.

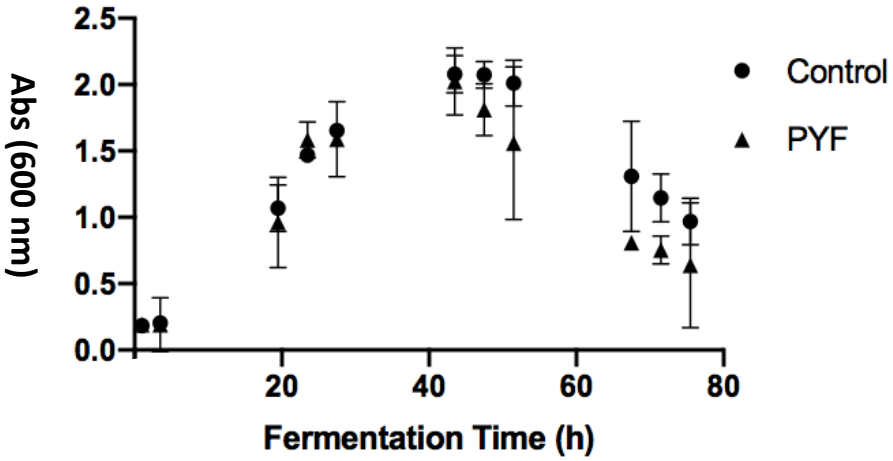

Figure 19. YIS during a mini-fermentation of oxygenated wort pitched with SMA yeast from a one-day propagation method. Readings are an average of triplicate measurements with error bars corresponding to the 95% CI.

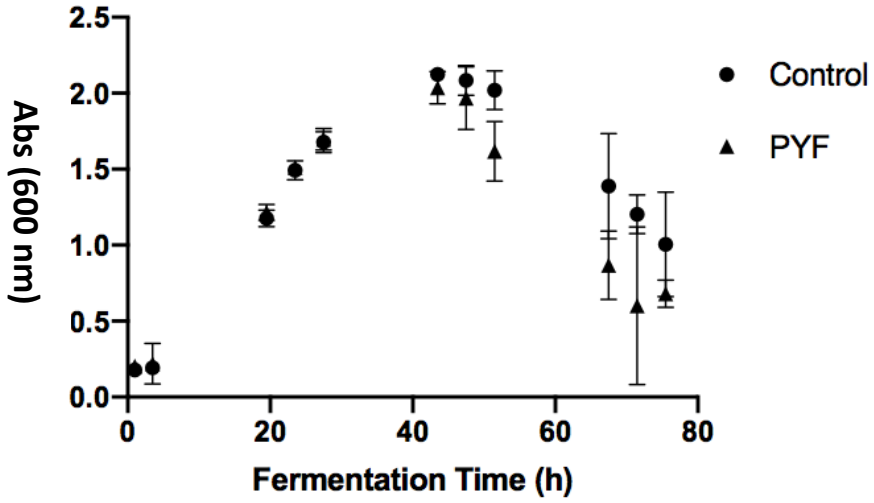

Figure 20. YIS during a mini-fermentation of oxygenated wort pitched with SMA yeast from a two-day propagation method. Readings are an average of triplicate measurements with error bars corresponding to the 95% CI.

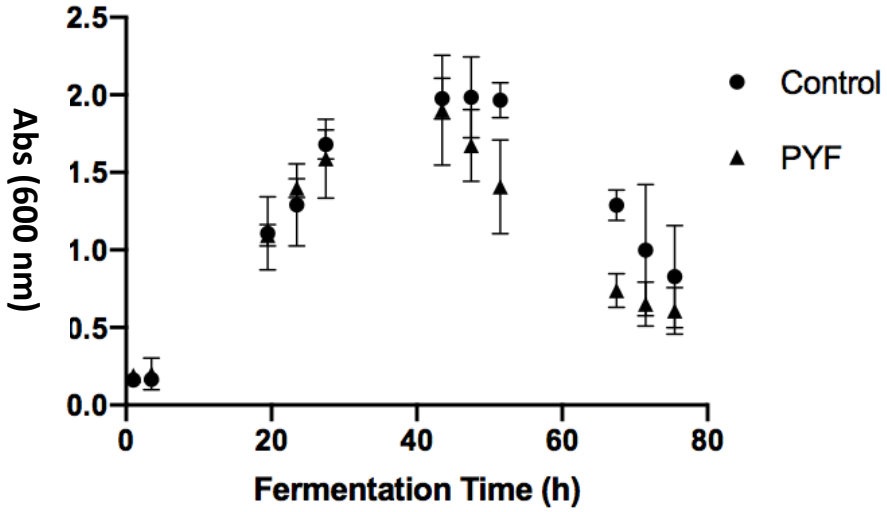

Figure 21. YIS during a mini-fermentation of non-oxygenated wort pitched with SMA yeast from a one-day propagation method. Readings are an average of triplicate measurements with error bars corresponding to the 95% CI.

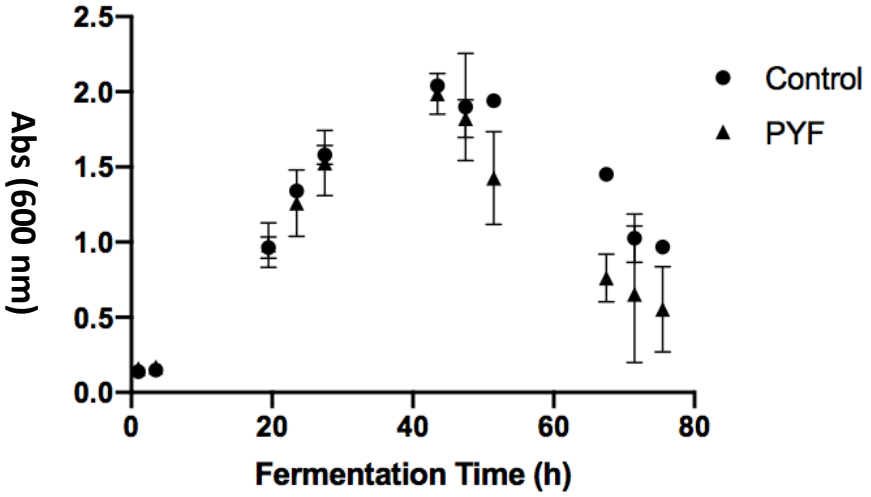

Figure 22. YIS during a mini-fermentation of non-oxygenated wort pitched with SMA yeast from a two-day propagation method. Readings are an average of triplicate measurements with error bars corresponding to the 95% CI.

Lager "A" Yeast:

The oxygenation of wort had a significant impact on the ability to detect PYF using fermentations with Lager "A" yeast. Only oxygenated wort resulted in an experimental trial that was able to distinguish between PYF and control malt samples. YIS during the oxygenated fermentations are pictured in Figure 23 and 24. From Figure 21, PYF and control malt samples were significantly distinguished at 47.5 hours. This was the earliest successful distinction between PYF and control malt samples of any of the experimental fermentations.

In both experimental fermentations of non-oxygenated wort samples pictured in Figure 25 and 26, there was no significant difference in YIS between the PYF and control sample at any time during the fermentation.

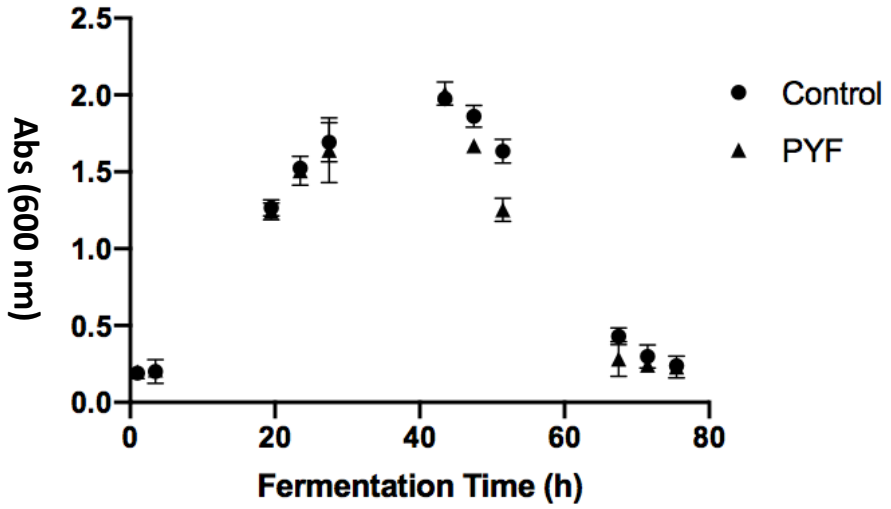

Figure 23. YIS during a mini-fermentation of oxygenated wort pitched with Lager "A" yeast from a one-day propagation method. Readings are an average of triplicate measurements with error bars corresponding to the 95% CI.

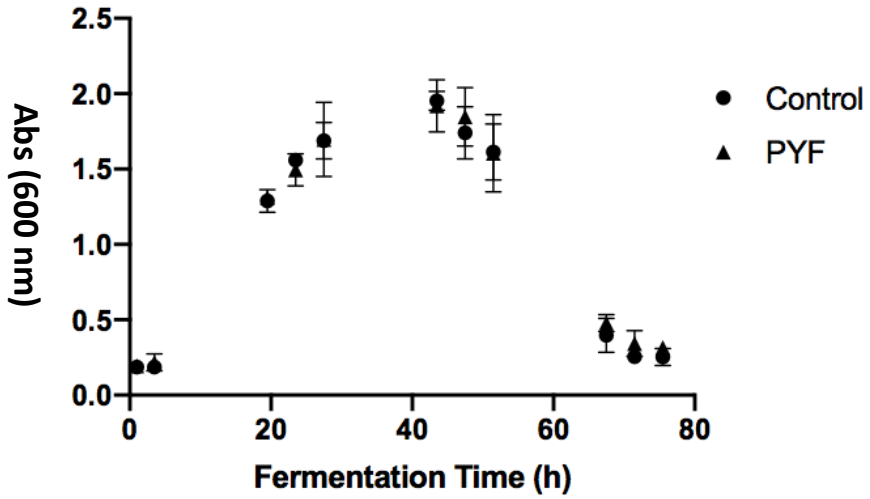

Figure 24. YIS during a mini-fermentation of oxygenated wort pitched with Lager "A" yeast from a two-day propagation method. Readings are an average of triplicate measurements with error bars corresponding to the 95% CI.

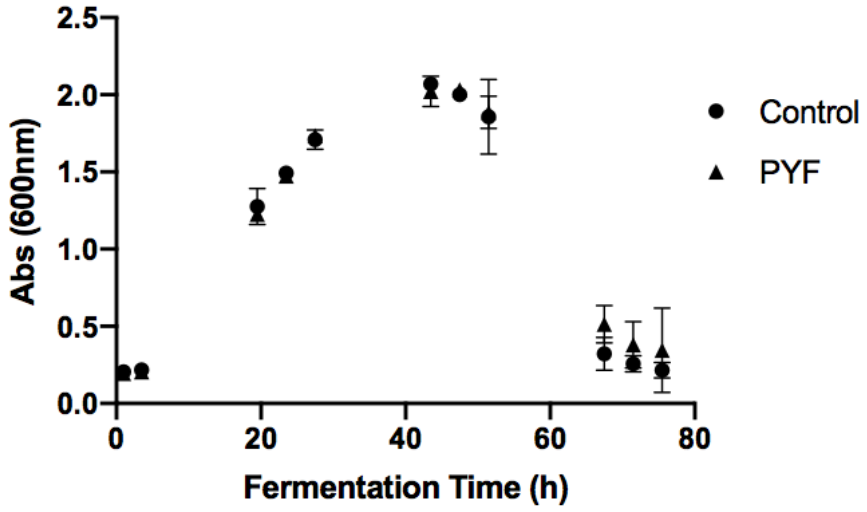

Figure 25. YIS during a mini-fermentation of non-oxygenated wort pitched with Lager "A" yeast from a one-day propagation method. Readings are an average of triplicate measurements measure with error bars corresponding to the 95% CI.

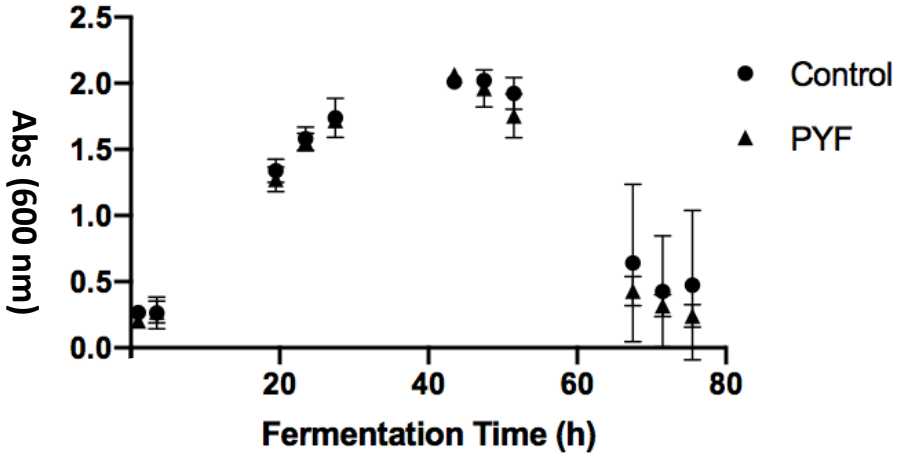

Figure 26. YIS during a mini-fermentation of non-oxygenated wort pitched with Lager "A" yeast from a two-day propagation method. Readings are an average of triplicate measurements with error bars corresponding to the 95% CI.

Pair wise comparisons of Tilted Gaussian model for YIS variables of each mini-fermentation also confirmed different YIS patterns. A full summary of the statistical analysis performed on Prism

8.4.2 (GraphPad. San Diego, CA) on the comparison of fits can be found in the Appendix. Only six fermentation trials yielded a set of YIS model variables that were significantly ($p<0.05$) different between PYF and control sample. The fermentation conditions of these six trials were:

- SMA Yeast, Oxygenated Wort, One Day Yeast Propagation (Table 15)
- SMA Yeast, Oxygenated Wort, Two Day Yeast Propagation (Table 16)
- SMA Yeast, Non-Oxygenated Wort, One Day Yeast Propagation (Table 17)
- SMA Yeast, Non-Oxygenated Wort, Two Day Yeast Propagation (Table 18)
- Lager "A" Yeast, Oxygenated Wort, One Day Yeast Propagation (Table 19)
- Lager "A" Yeast, Non-Oxygenated Wort, Two Day Yeast Propagation (Table 22)

5.4.2 Effects of Shortening the Yeast Propagation Method during the ASBC's Yeast-14 Method on the Sensitivity of Detecting PYF.

SMA Yeast:

For SMA lager yeast, the propagation time had a significant effect on the overall procedure's ability to distinguish between PYF and control malts. The oxygenated wort fermentation using a two-day yeast propagation method displayed in Figure 20, was able to detect a significant difference ($p<0.05$) in YIS between PYF and control malt samples at 51 hours, whereas, oxygenated wort pitched with the one-day yeast propagation method displayed in Figure 19, showed a significant difference ($p<0.05$) in YIS during fermentation between PYF and control after 67 hours.

Yeast propagation time did not influence the procedure's ability to distinguish between non-oxygenated wort fermentations of PYF and control malts. The non-oxygenated wort fermentations in Figure 21 and 22 were both able to distinguish ($p<0.05$) between PYF and control malts after 51 hours.

Lager "A" Yeast:

The yeast propagation time had a significant effect on the overall procedure's ability to distinguish between PYF and control malts. According to Figure 23 and 24, fermentations with Lager "A" were only able to distinguish between PYF and control malt under one condition: Wort must be oxygenated and pitched with Lager "A" from the one-day yeast propagation. Oxygenated wort pitched with Lager "A" yeast that underwent the two-day yeast propagation did not yield YIS values that were significantly different between PYF and control.

There was no significant difference ($p<0.05$) between YIS of PYF and control malt samples in non-oxygenated wort samples regardless of yeast propagation days.

5.4.3 Discussion

A statistical difference ($p<0.05$) between PYF and control malt samples could be found using both oxygenated and non-oxygenated worts using the Yeast-14 method. Both the SMA and Lager "A"

yeasts strains exhibited scenarios where the respective fermentations could distinguish between PYF and control malt samples. Using each yeast sample, there existed trial fermentations conditions that were able to successfully distinguish between PYF and control malt samples after 51 hours.

The impact of wort oxygenation on the ASBC's Yeast-14 method to detect PYF shows promising results for the objective of simplifying the method. The data suggests that eliminating the extra oxygenation step will still result in accurate PYF distinction from control samples. As well, it was possible to identify PYF from control malt using another commercial lager yeast strain (Lager "A") for the mini-fermentation method. These proposed changes to the Yeast-14 method offer a substantial reduction in time, sample preparation, and money required for successful PYF identification.

The SMA yeast strain was the more successful of the lager yeast strains employed for detecting PYF when assessing the yeast propagation method. The data suggest that regardless of the yeast propagation method, SMA could detect PYF in mini-fermentations after 51 hours. The same was not true for the Lager "A" yeast strain, which was only able to distinguish PYF from control malt under one fermentation condition: the wort was oxygenated and pitched with a one-day yeast propagation. The conditional PYF detection using Lager "A" yeast shows that there must be an influence of the available oxygen in wort on the flocculation of the yeast. Even though Lager "A" yeast was not as universal in terms of fermentation conditions in detecting PYF, it did allow for the earliest successful detection. Using the previously mentioned conditions for detecting PYF

with Lager “A” yeast, this trial was able to statistically differentiate between PYF and control malt samples after 47 hours of fermentation. This was four hours earlier than any successful trial using SMA lager yeast.

These results have several implications when attempting to improve the current ASBC Yeast-14 method. Firstly, PYF can successfully be detected after only 47.5 or 51.5 hours, depending on whether the fermentation was performed with Lager “A” or SMA, respectively. Still, these results show that sample testing does not need to proceed for the entire 78 hours as recommended by the current method. Secondly, the Yeast-14 method could be shortened by another 24 hours when using SMA or Lager “A” yeast for PYF detection, as a one-day propagation of both these yeast strains went on to successfully distinguished PYF from control. However, if using Lager “A” yeast then oxygenation of wort is required. This research shows that the current ASBC Yeast-14 method would not be universally employable to use with all lager yeast strains. The influence of oxygenation affects fermentations of different yeast strains differently. This could be due to the natural oxygen requirements of that yeast strain for regular physiological functions.

In general, SMA yeast showed more versatility to fermentation conditions while remaining able to distinguish between PYF and control malt using a mini-fermentation. This research showed evidence that when using SMA yeast the ASBC Yeast-14 can be shortened to include only a one-day yeast grow up. This would reduce the overall ASBC Yeast-14 method by 24 hours and still have the power to statistically ($p<0.05$) distinguish between PYF and control malt samples.

Chapter 6 Conclusion

6.1 Chapter 3: Effects of Germination Conditions on the Fermentation Performance of Floor Malt Conclusion

As discussed in the literature review, floor malting was historically known to be a seasonal profession. The aim of this research project was to assess improvements of floor malting with modern air conditioning to be able to produce malt year-round. After showing an influence of external temperatures on germination temperatures in the summer of 2018, Horton Ridge implemented better cooling systems for the next summer to have better control over germination temperatures. Of the 22 malt samples tested only one tested positive for PYF. The exact malting conditions that induced PYF are still unknown as other batches under similar conditions did not show any PYF which further attests to the natural variability of PYF.

The malting performance of feed barley variety AC Queens was inferior to that of typical malting varieties CDC Copeland, AAC Synergy, and AC Newdale. AC Queens required longer germination time for adequate modification compared to typical malting varieties. As well, this research showed that feed barley can be malted to generate adequate malt specifications such as extract but still will not perform as well in the brewhouse (lower ADF). This shows the importance of the feedback loop from brewer to maltster, as barley that produces adequate malting specifications may still lack in brewing performance.

These malting trials also gave insight into developing optimal floor malting regimes specific to each barley varieties. It was found that in order to increase extract and ADF for AC Newdale and CDC Copeland, lower germination temperatures are needed. Alternatively, for feed barley AC Queens, the wort extract can be increased if higher germination temperatures are employed.

6.1.1 Future Work

Future research objectives would be to perform similar trials again except in a more controlled setting. This would allow control over each germination parameter to better assess the influence of each variable. This was not possible during trials at Horton Ridge as data collection could not impede the daily production of floor malt. Further testing would be to monitor CO_2 levels within the germinating grains to assess the possible influences this may have on resulting quality and fermentability of floor malt.

6.2 Chapter 4: Comparing Floor versus Pneumatic Malting on Quality of Resulting Malt and Beer Conclusion

Initially, the influence of temperature during germination was expected to be the prominent factor on determining final malt quality. However, final quality of floor and pneumatic malts did not appear to correlate with published reports on influence of germination temperature on final malt quality. Instead, the discrepancies in final malt quality between floor and pneumatic malt were more accurately represented by published reports that studied influence of microbial loads, in particular fungal levels, during germination on final malt quality. However, microbial

proliferation is influenced by temperature, so the original hypothesis cannot be completely discarded.

Previous reports have shown that fungal communities have a substantial effect on quality aspects of the final malt ([34,35,67–69,40,42,45,47,49,61,64,66]). Studies that were performed with lab-infected barley during malting resulted in final malt that yielded satisfactory malt specifications. However, it is not enough to approve incoming malt based on its COA, as the actual fermentability of the malt is equally important. This results from this project suggest that floor malt can yield adequate malt in terms of both malt quality and fermentability.

The several differences in malt quality attributes discovered between floor and pneumatic malt helps to enlighten the research community about modern floor malt comparison to pneumatic malt, with differences in local microbial communities being a predominant factor in the differences in malt quality produced between floor and pneumatic malthouses. This offers several interesting implications about future malting. Firstly, since the local microbial communities in malthouses can influence resulting malt, this can add a further uniqueness to craft malthouses. This uniqueness can be advertised as a "terroir" of the specific malthouse. Secondly, microbials are unavoidable during malting which opens the door to the possibility of inoculating a starter culture during malting to help control the dynamic of the malthouse microbial community. The known influences of specific microbial levels during malting can lead to the possibility of malthouses using an inoculation of a starter microbial culture to aid in the production of malt. A starter culture that can improve malthouse efficiency by speeding up

modification and degradation of endosperm components while competitively inhibiting the growth of detrimental microbial pathogens would be beneficial to malthouses. This could increase malting volumes, decrease malting times and improve overall malthouse efficiency.

This research assists in filling the prolonged absence that exists regarding floor malting research, in particularly, how floor malting influences final malt quality in comparison to commercial pneumatic systems. This research offers valuable information because of the inclusion of a modern operational floor malthouse for experimental trials and not simulated malting trials in a lab setting.

6.2.1 Future Work

In future research, it would be beneficial to collect swabs throughout both pneumatic and floor malting settings to identify and quantify respective microbial communities, as identified differences in malt quality between floor and pneumatic malt did not correlate well with literature findings on differences in germination temperature. It could be that the differences in malt quality was a direct result of differing quantities and variety of microbials present in the respective malthouses. Identification of microbial communities within each malthouse also offers several other benefits, as suggested by several researchers about the potential benefits of using a start microbial culture during malting ([4,32,34,36,110]). Malthouses using a starter culture could have more control over the dynamic of the microbial community present during malting which could then aid in modification or deter other more detrimental microbial communities.

6.3 Chapter 5: Chapter 5 Accelerating the ASBC's Yeast-14 PYF Detection Technique and Determining the PYF Sensitivity Among Lager Yeast Strains Conclusion

Of the two lager yeast strains evaluated, the SMA yeast strain proved to be the most robust for PYF detection using a mini-fermentation. SMA yeast was able to detect PYF in a test tube fermentation regardless of the oxygenation and yeast propagation method employed in this experiment. In comparison, Lager "A" yeast was still able to successfully detect PYF but only under one specific set of conditions. This may rule out Lager "A" yeast from being the most desirable yeast to use in universal PYF detection but does not mean it would be impractical to use. On the contrary, the results from this research project show that other yeasts can be successfully used to detect PYF rather than SMA yeast which the current method requires. Further investigations into the possibility of other commercially available lager yeast's ability to detect PYF would be beneficial to make the method even more universal.

The results from this experiment offer plausible changes to the ASBC's Yeast-14 method for detecting PYF. Evidence suggests that the entire ASBC Yeast-14 method can be shortened from six days down to four days for successful PYF detection in malt. This would involve shortening the yeast propagation from two days down to one as well as reducing fermentation time to at least 51 hours. This prospective method can be further simplified as this research concluded that the oxygenation step stated by the current method was not required to distinguish PYF.

Overall, these proposed changes offer an improvement over the current method in terms of detecting PYF. One drawback was that the new suggested method still involves a parallel

fermentation with a known non-PYF malt (control). However, future works would be to develop a new YIS chart used for PYF prediction similar to Armstrong et al. ([88]) that would be specific to a new Yeast-14 method if the proposed changes were implemented.

6.3.1 Future Work

A suggestion for future works would be to expand testing to employ other lager yeast strains. This will further determine the robustness of the method to detect PYF. It appears individual yeast characteristics have an influence on the subsequent flocculation of that yeast, specifically the wort oxygen sensitivity. For this reason, it would be beneficial to seek out lager yeast strains that are commercially accessible and are not oxygen sensitive to employ for further testing.

References

(1) Marshall, F. G. Recent developments in the malting industry. *J. Inst. Brew.* **1953**, *59* (1), 10–15.

(2) Briggs, D. E.; Hough, J. S.; Stevens, R.; Young, T. W. *Malting and Brewing Science*, 2nd ed.; Kluwer Academic/Plenum Publishers: New York, USA. **1981.**

(3) Bokulich, N. A.; Bamforth, C. W. The microbiology of malting and brewing. *Microbiol. Mol. Biol. Rev.* **2013**, *77* (2), 157–172.

(4) Nierop, S. N. E. Van; Rautenbach, M.; Axcell, B. C.; Cantrell, I. C. The impact of microorganisms on barley and malt quality—A review. *J. Am. Soc. Brew. Chem.* **2006**, *64* (2), 69–78.

(5) Briggs, D. E. *Malts and Malting*, 1st ed.; Blackie Academic and Professional: London, GBR. **1998**.

(6) Wolf-Hall, C. E. Mold and mycotoxin problems encountered during malting and brewing. *Int. J. Food Microbiol.* **2007**, *119*, 89–94.

(7) Lake, J. C.; Speers, R. A. A discussion of malt-induced premature yeast flocculation. *Master Brew. Assoc. Am. Tec. Quarterly* **2008**, *45* (3), 253–262.

(8) Zohary, D.; Hopf, M. *Domestication of Plants in the Old World: The Origin and Spread of Cultivated Plants in West Asia, Europe and the Nile Valley*, 3rd ed.; Oxford University Press: Oxford, GBR. **2000**.

(9) Gupta, M.; Abu-Ghannam, N.; Gallaghar, E. Barley for brewing: characteristic changes during malting, brewing and applications of its by-products. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9* (3), 318–328.

(10) *Genetics and Improvement of Barley Malt Quality*; Zhang, G., Li, C., Eds.; Zhejiang University Press: Hangzhou, CHN. **2009**.

(11) Bothmer, R. von; Sato, K.; Knüpffer, H.; Hintum, T. van. Barley Diversity-an Introduction. In *Diversity in Barley (Hordeum Vulgare)*; Elsevier Science B.V. Amsterdam, NLD, **2003**, 3–8.

(12) Baik, B.-K.; Ullrich, S. E. Barley for food: characteristics, improvement, and renewed interest. *J. Cereal Sci.* **2008**, *48*, 233–242.

(13) Bothmer, R. von; Sato, K.; Komatsuda, T.; Yasuda, S.; Fischbeck, G. The Domestication of Cultivated Barley. In *Diversity in Barley (Hordeum Vulgare)*; Elsevier B.V.: Amsterdam, NLD, **2003**, 9–27.

((14) Hancock, J. F. Cereal Grains. In *Plant evolution and the Origin of Crop Species*. MPG Books Group Ltd.: Cornwall, GBR. **2012**, 132–147.

(15) Reed, C. A. Origins of Agriculture. In *Plant Evolution and the Origin of Crop Species*. MPG Books Group Ltd.: Cornwall, GBR. **2012**, 99–113.

(16) Farber, M.; Barth, R. *Mastering Brewing Science: Quality and Production*; John Wiley & Sons Inc.: Hoboken, NJ, **2019**.

(17) Zhuang, S.; Shetty, R.; Hansen, M.; Fromberg, A.; Hansen, P. B.; Hobley, T. J. Brewing with 100 % unmalted grains: barley, wheat, oat and rye. *Eur. Food Res. Technol.* **2017**, *243* (3), 447–454.

(18) Bamforth, C. W. *Scientific Principles of Malting and Brewing*; American Society of Brewing Chemists: St. Paul, MN. **2006.**

(19) Kok, Y. J.; Ye, L.; Muller, J.; Ow, D. S. W.; Bi, X. Brewing with malted barley or raw barley: what makes the difference in the processes? *Appl. Microbiol. Biotechnol.* **2019**, *103* (3), 1059–1067.

(20) Vallejo, J. A.; Sánchez-Pérez, A.; Martínez, J. P.; Villa, T. G. Cell aggregations in yeasts and their applications. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 2305–2318.

(21) Coventry, S. J.; Barr, A. R.; Eglinton, J. K.; McDonald, G. K. The determinants and genome locations influencing grain weight and size in barley (*Hordeum Vulgare L.*). *Aust. J. Agric. Res.* **2003**, *54*, 1103–1115.

(22) Fox, G. P. Chemical Composition in Barley Grains and Malt Quality. In *Genetics and Improvement of Barley Malt Quality*. Zhejiang University Press: Hangzhou, CHN. **2009**, 37–50.

(23) Palmer, G. H. Cereal science and malting technology - The future. *J. Am. Soc. Brew. Chem.* **1992**, *50* (4), 121–132.

(24) Brennan, C. S.; Harris, N.; Smith, D.; Shewry, P. R. Structural differences in the mature endosperms of good and poor malting barley cultivars. *J. Cereal Sci.* **1996**, *24* (2), 171–177.

(25) Schwartz, P.; Li, Y. Malting and Brewing Uses of Barley. In *Barley: Production, Improvement, and Uses*. Blackwell Publishing Ltd.: Oxford, GBR. **2011**, 478–521.

(26) Sadosky, P.; Schwarz, P. B.; Horsley, R. D. Effect of arabinoxylans, β-glucans, and dextrins on the viscosity and membrane filterability of a beer model solution. *J. Am. Soc. Brew. Chem.* **2002**, *60* (4), 153–162.

(27) Brennan, C. S.; Amor, M. A.; Harris, N.; Smith, D.; Cantrell, I.; Griggs, D.; Shewry, P. R. Cultivar differences in modification patterns of protein and carbohydrate reserves during malting of barley. *J. Cereal Sci.* **1997**, *26*, 83–93.

(28) Swanston, J. S.; Taylor, K. A comparison of some rapid tests to assess extent of modification during a laboratory scale malting procedure. *J. Inst. Brew.* **1988**, *94* (5), 311–314.

(29) Legge, W. G.; Edney, M. J.; Eglinton, J. K.; Rossnagel, B. G.; Barr, A. R.; Collins, H. M. Importance of endosperm modification for malt wort fermentability. *J. Inst. Brew.* **2012**, *113* (2), 228–238.

(30) CMBTC 2020-2021 Recommended Malting Barley Varieties. Canadian Malting Barley Technical Centre. Winnipeg, MN. **2020**.

(31) Petters, H. I.; Flannigan, B.; Austin, B. Quantitative and qualitative studies of the microflora of barley malt production. *J. Appl. Bacteriol.* **1988**, *65*, 279–297.

(32) Noots, I.; Delcour, J. A.; Michiels, C. W. From field to barley to malt: detection and specification of microbial activity for quality aspects. *Crit. Rev. Microbiol.* **1998**, *25* (2), 121–153.

(33) Nischwitz, R.; Cole, N. W.; MacLeod, L. Malting for brewhouse performance. *J. Inst. Brew.* **1999**, *105* (4), 219–227.

(34) Justé, A.; Malfliet, S.; Lenaerts, M.; De Cooman, L.; Aerts, G.; Willems, K. A.; Lievens, B. Microflora during malting of barley: overview and impact on malt quality. *BrewingScience* **2011**, *64*, 22-31.

(35) Prentice, N.; Sloey, W. Studies on barley microflora of possible importance to malting and brewing quality. I. The treatment of barley during malting with selected microorganisms. *Proceedings. Annu. Meet. - Am. Soc. Brew. Chem.* **1960**, *18* (1), 28–33.

(36) Raulio, M.; Wilhelmson, A.; Salkinoja-Salonen, M.; Laitila, A. Ultrastructure of biofilms formed on barley kernels during malting with and without starter culture. *Food Microbiol.* **2009**, *26* (4), 437–443.

(37) Harrison, W. *The Description of England*, Edelen, G. ed.; Cornell University Press: New York, USA **1587** (Reprinted in 1968).

(38) Lancaster, H. Practical Floor Malting. In *The Brewing Trade Review*; London, GBR, **1908**.

(39) Los, A.; Ziuzina, D.; Bourke, P. Current and future technologies for microbiological decontamination of cereal grains. *J. Food Sci.* **2018**, *83* (6), 1484-1493.

(40) Laitila, A.; Kotaviita, E.; Peltola, P.; Home, S.; Wilhelmson, A. Indigenous microbial community of barley greatly influences grain germination and malt quality. *J. Inst. Brew.* **2007**, *113* (1), 9-20.

(41) Kostelanska, M.; Hajslova, J.; Zachariasova, M.; Malachova, A.; Kalachova, K.; Poustka, J.; Fiala, J.; Scott, P. M.; Berthiller, F.; Krska, R. Occurrence of deoxynivalenol and its major conjugate, deoxynivalenol-3- glucoside, in beer and some brewing intermediates. *J. Agric. Food Chem.* **2009**, *57,* 3187-3194.

(42) Sarlin, T.; Laitila, A.; Pekkarinen, A.; Haikara, A. Effects of three *fusarium* species on the quality of barley and malt. *J. Am. Soc. Brew. Chem.* **2005**, *62* (2), 43–49.

(43) Postulkova, M.; Riveros-Galan, D.; Cordova-Agiular, K.; Zitkova, K.; Verachtert, H.; Derdelinckx, G.; Dostalek, P.; Ruzicka, M. C.; Branyik, T. Technological possibilities to prevent and suppress primary gushing of beer. *Trends in Food Science and Technology*. **2016**, *49,* (2016), 64-73.

(44) Piacentini, K. C.; Savi, G. D.; Pereira, M. E. V; Scussel, V. M. Fungi and the natural occurrence of deoxynivalenol and fumonisins in malting barley (*Hordeum Vulgare L.*). *Food Chem.* **2015**, *187*, 204-209.

(45) Oliveira, P. M.; Waters, D. M.; Arendt, E. K. The impact of *fusarium* culmorum infection on the protein fractions of raw barley and malted grains. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 2053-2065.

(46) Spanic, V.; Marcek, T.; Abicic, I.; Sarkanj, B. Effects of *fusarium* head blight on wheat grain and malt infected by *fusarium culmorum*. *Toxins.* **2018**, *10* (1), 1–12.

(47) Van Nierop, S. N. E.; Cameron-Clarke, A.; Axcell, B. C. Enzymatic generation of factors from malt responsible for premature yeast flocculation. *J. Am. Soc. Brew. Chem.* **2004**, *62* (3), 108–116.

(48) Blechova, P.; Havlova, P.; Havel, J. The study of premature yeast flocculation and its relationship with gushing of beer. *Monatsschift fuer Brauwiss.* **2005**, *58* (9–10), 64–78.

(49) Kaur, M.; Bowman, J. P.; Stewart, D. C.; Sheehy, M.; Janusz, A.; Speers, R. A.; Koutoulis, A.; Evans, D. E. TRFLP analysis reveals that fungi rather than bacteria are associated with premature yeast flocculation in brewing. *J. Ind. Microbiol. Biotechnol.* **2012**. *39*, 1821-1832.

(50) Koizumi, H.; Kato, Y.; Ogawa, T. Structural features of barley malt polysaccharides inducing premature yeast flocculation. *J. Am. Soc. Brew. Chem.* **2009**, *67* (3), 129–134.

(51) Oliveira, P. M.; Mauch, A.; Jacob, F.; Waters, D. M.; Arendt, E. K. Fundamental study on the influence of *fusarium* infection on quality and ultrastructure of barley malt. *Int. J. Food Microbiol.* **2012**, *156*, 32–43.

(52) Ma, Z.; Zhang, L.; Liu, J.; Dong, J.; Yin, H.; Yu, J.; Huang, S.; Hu, S.; Lin, H. Effect of hydrogen peroxide and ozone treatment on improving the malting quality. *J. Cereal Sci.* **2020**, *91* (2020), 1-8.

(53) Evrendilek, G. A.; Karatas, B.; Uzuner, S.; Tanasov, I. Design and effectiveness of pulsed electric fields towards seed disinfection. *J. Sci. Food Agric.* **2019**, *99* (7), 3475–3480.

(54) Wang, R.; Chen, B.; Wang, T.; Li, P.; Ding, F. Effects of chlorine dioxide on the germination, oxidative metabolism and growth of barley seedlings (*hordeum vulgare L.*). *Sci. Rep.* **2019**, *9* (1), 1–8.

(55) Chen, D.; Chen, P.; Cheng, Y.; Peng, P.; Liu, J.; Ma, Y.; Liu, Y.; Ruan, R. Deoxynivalenol decontamination in raw and germinating barley treated by plasma-activated water and intense pulsed light. *Food Bioprocess Technol.* **2019**, *12* (2), 246–254.

(56) Szőke, C.; Nagy, Z.; Gierczik, K.; Székely, A.; Spitkól, T.; Zsuboril, Z. T.; Galiba, G.; Marton, C. L.; Kutasi, K. Effect of the afterglows of low pressure Ar/N2-O2 surface-wave microwave discharges on barley and maize seeds. *Plasma Process. Polym.* **2018**, *15* (2), 1–12.

(57) Rood, L.; Koutoulis, A.; Bowman, J. P.; Evans, D. E.; Stanley, R. A.; Kaur, M. Control of microbes on barley grains using peroxyacetic acid and electrolysed water as antimicrobial agents. *Food Microbiol.* **2018**, *76*, 103–109.

(58) Spanoghe, M.; Allard, O.; Delvoye, S.; Marique, T.; Van Koninckxloo, M. Industrial-scale malting barley (*hordeum vulgare L.*) seed disinfection by fog of ozonated water Application. *Ozone Sci. Eng.* **2016**, *38* (2), 115–123.

(59) Sauer, D. B.; Burroughs, R. Disinfection of seed surfaces with sodium hypochloite. *Phytpathology* **1986**, *76* (7), 745–749.

(60) Kottapalli, B.; Wolf-Hall, C. E.; Schwarz, P. Effect of electron-beam irradiation on the safety and quality of *fusarium*-infected malting barley. *Int. J. Food Microbiol.* **2006**, *110* (3), 224–231.

(61) Schwarz, P. B.; Jones, B. L.; Steffenson, B. J. Enzymes associated with *fusarium* infection of barley. *J. Am. Soc. Brew. Chem.* **2002**, *60* (3), 130–134.

(62) Sloey, W.; Prentice, N. Effects of *Fusarium* isolates applied during malting on properties of malt. *Proceedings. Annu. Meet. - Am. Soc. Brew. Chem.* **1962**, *20* (1), 24–29.

(63) Hoy, J. L.; Macauley, B. J.; Fincher, G. B. Cellulases of plant and microbial origin in germinating barley. *J. Inst. Brew.* **1981**, *87*, 77–80.

(64) Yin, X. S.; MacGregor, A. W.; Clear, R. M. Field fungi and β-glucan solubilase in barley kernels. *J. Inst. Brew.* **1989**, *95*, 195–198.

(65) Kanauchi, M.; Bamforth, C. W. Growth of trichoderma viride on crude cell wall preparations from barley. *J. Agric. Food Chem.* **2001**, *49* (2), 883–887.

(66) Doran, P. J.; Briggs, D. E. Microbes and grain germination. *J. Inst. Brew.* **1993**, *99*, 165–170.

(67) Speers, R. A.; MacIntosh, A. J. Assessing the effect of fungal infection of barley and malt on premature yeast flocculation. *J. Am. Soc. Brew. Chem.* **2014**, *72* (1), 66–72.

(68) Laitila, A.; Manninen, J.; Priha, O.; Smart, K.; Tsitko, I.; James, S. Characterisation of barley-associated bacteria and their impact on wort separation performance. *J. Inst. Brew.* **2018**, *124* (4), 314–324.

(69) Kelly, L.; Briggs, D. E. The influence of the grain microflora on the germinative physiology of barley. *J. Inst. Brew.* **1992**, *98*, 395–400.

(70) Wegulo, S. N.; Baenziger, P. S.; Hernandez Nopsa, J.; Bockus, W. W.; Hallen-Adams, H. Management of *fusarium* head blight of wheat and barley. *Crop Prot.* **2015**, *73*, 100–107.

(71) Geißinger, C.; Whitehead, I.; Hofer, K.; Heß, M.; Habler, K.; Becker, T.; Gastl, M. Influence of *fusarium avenaceum* infections on barley malt: Monitoring changes in the albumin fraction of barley during the malting process. *Int. J. Food Microbiol.* **2019**, *293*, 7–16.

(72) Schwarz, P. B.; Casper, H. H.; Beattie, S. Fate and development of naturally ocurring *fusarium* mycotoxins during malting and brewing. *J. Am. Soc. Brew. Chem.* **1995**, *53* (3), 121–127.

(73) Li, X.; Cai, G.; Wu, D.; Zhang, M.; Lin, C.; Lu, J. Microbial community dynamics of dan'er barley grain during the industrial malting process. *Food Microbiol.* **2018**, *76*, 110–116.

(74) Douglas, B. P. E.; Flannigan, B. A microbiological evaluation of barley malt production. *J. Inst. Brew.* **1988**, *94*, 85–88.

(75) Sampermans, S.; Mortier, J.; Soares, E. V. Flocculation onset in *saccharomyces cerevisiae*: The role of nutrients. *J. Appl. Microbiol.* **2005**, *98*, 525-531.

(76) Jin, Y. L.; Alex Speers, R. Flocculation of *saccharomyces cerevisiae*. *Food Res. Int.* **1998**. *31* (6-7), 421-440.

(77) Speers, R. A.; Wan, Y. Q.; Jin, Y. L.; Stewart, R. J. Effects of fermentation parameters and cell wall properties on yeast flocculation. *J. Inst. Brew.* **2006**, *112* (3), 246–254.

(78) Lake, J. C.; Speers, R. A.; Porter, A. V; Gill, T. A. Miniaturizing the fermentation assay: Effects of fermentor size and fermentation kinetics on detection of premature yeast flocculation. *J. Am. Soc. Brew. Chem.* **2008**, *66* (2), 94–102.

(79) Boulton, C.; Box, W. Formation and Disappearance of Diacetyl During Lager Fermentation. In *Brewing Yeast Fermentation Performance*; Smart, K., Ed.; Balckwell Science Ltd.: Oxford, GBR. **2003**, 183–196.

(80) Karabín, M.; Jelínek, L.; Kotrba, P.; Cejnar, R.; Dostálek, P. Enhancing the performance of brewing yeasts. *Biotechnol. Adv.* **2018**, *36*, 691–706.

(81) Stratford, M. Lectin-mediated aggregation of yeasts - yeast flocculation. *Biotechnol. Genet. Eng. Rev.* **1992**, *10* (1), 283-342.

(82) Verstrepen, K. J.; Derdelinckx, G.; Verachtert, H.; Delvaux, F. R. Yeast flocculation: What brewers should know. *Appl. Microbiol. Biotechnol.* **2003**, *61*, 197–205.

(83) Dengis, P. B.; Nelissen, L. R.; Rouxhet, P. G. Mechanisms of yeast flocculation: comparison of top- and bottom-fermenting strains. *Appl. Environ. Microbiol.* **1995**, *61* (2), 718–728.

(84) Speers, R. A.; Smart, K.; Stewart, R.; Jin, Y.-L. Zymolectins in *saccharomyces cerevisiae*. *Lett. J. Inst. Brew.* **1998**, 104-298.

(85) Jin, Y.-L.; Speers, R. A. Effect of environmental conditions on the flocculation of *saccharomyces cerevisiae*. *J. Am. Soc. Brew. Chem.* **2000**, *58* (3), 108–116.

(86) Speers, R. A. Brewing Science: Advances and Challenges (Invited Presentation). Halifax, NS **2005**.

(87) Vidgren, V.; Londesborough, J. 125th anniversary review: Yeast flocculation and sedimentation in brewing. *J. Inst. Brew.* **2011**, *117*, 475–487.

(88) Armstrong, M.; MacIntosh, A. J.; Josey, M.; Speers, R. A. Examination of premature yeast flocculation in U.K. Malts. *Master Brew. Assoc. Am. Tec. Quarterly*. **2018**, *55* (3), 54–60.

(89) ASBC Methods of Analysis, online. Yeast 14. Miniature fermentation assay. Approved **2011**. *American Society of Brewing Chemists.* St. Paul, MN.

(90) ASBC Methods of Analysis, online. Malt 3. Moisture. Approved **1958**, rev. 2010. *American Society of Brewing Chemists.* St. Paul, MN.

(91) Daily temperature data report for Kentville, NS. Government of Canada. Website: https://climate.weather.gc.ca/climate_data/daily_data_e.html?timeframe=2&Year=2018&Month=7&Day=3&hlyRange=1999-01-12%7C2020-02-03&dlyRange=1996-07-01%7C2020-0202&mlyRange=1996-07-01%7C2007-07-01&StationID=27141&Prov=NS&urlExtension=_e.html&searchType=stn (Accessed: February 11, 2020)

(92) ASBC Methods of Analysis, online. Malt 2. Physical tests: Length of acrospire. Approved **1958**, rev. 2012. *American Society of Brewing Chemists.* St. Paul, MN.

(93) Toffoli, F.; Gianinetti, A.; Cavallero, A.; Finocchiaro, F.; Stanca, A. M. Effects of pulses of higher temperature on the development of enzyme activity during malting. *J. Inst. Brew.* **2003**, *109* (4), 337–341.

(94) Gunkel, J.; Voetz, M.; Rath, F. Effect of the malting barley variety (*hordeum vulgare L*.) on fermentability. *J. Inst. Brew.* **2002**, *108* (3), 355–361.

(95) Agu, R. C.; Palmer, G. H. The effect of temperature on the modification of sorghum and barley during malting. *Process Biochem.* **1997**, *32* (6), 501–507.

(96) Cole, N.; Mitchell, K. Some Effects of steeping and germination parameters on grain hydration and respiration and consequent malt quality. *Master Brew. Assoc. Am. Tec. Quarterly* **1998**, *35* (2), 8–11.

(97) Nova Scotia Liquor Corporation. NSLC Annual Report 2018-2019. Halifax, NS. **2019**, 1–52.

(98) MacLeod, A. Personal communication. **2020**.

(99) ASBC Methods of Analysis, online. Malt 12. Malt modification by friability (international method). Approved **1988**, rev. 2009. *American Society of Brewing Chemists.* St. Paul, MN.

(100) ASBC Methods of Analysis, online. Malt 4. Extract. Approved **1958**, rev. 2011. *American Society of Brewing Chemists.* St. Paul, MN.

(101) ASBC Methods of Analysis, online. Beer 10. Color . Approved **1958**, rev. 2015. *American Society of Brewing Chemists.* St. Paul, MN.

(102) ASBC Methods of Analysis, online. Wort 18. β-glucan in congress wort: Segmented flow analysis. Approved **2011**. *American Society of Brewing Chemists.* St. Paul, MN.

(103) ASBC Methods of Analysis, online. Wort 17. Protein in unhopped wort by spectrophotometry . Approved **1990**, rev. 2010. *American Society of Brewing Chemists.* St. Paul, MN.

(104) ASBC Methods of Analysis, online. Malt 8. Protein content by combustion. Approved **2011**. *American Society of Brewing Chemists.* St. Paul, MN.

(105) ASBC Methods of Analysis, online Wort 12. Free amino nitrogen (international method): Segmented flow analysis. Approved **2011**. *American Society of Brewing Chemists.* St. Paul, MN.

(106) ASBC Methods of Analysis, online. Malt 6. Diastatic power: Automated flow analysis. Approved **1997**, rev. 2010. *American Society of Brewing Chemists.* St. Paul, MN.

(107) ASBC Methods of Analysis, online. Malt 7. Alpha-amylase: Automated flow analysis method. Approved **2007**, rev. 2010. *American Society of Brewing Chemists.* St. Paul, MN.

(108) ASBC Methods of Analysis, online. Wort 8. pH (hydrogen ion concentration). Approved **1958**, rev. 2010. *American Society of Brewing Chemists*. St. Paul, MN.

(109) Baxter, E.; O'Farrell, D. Effects of raised temperatures during steeping and germination on proteolysis during malting. *J. Inst. Brew.* **1980**, *86*, 291–295.

(110) Linko, M.; Haikara, A.; Ritala, A.; Penttilä, M. Recent advances in the malting and brewing industry. *J. Biotechnol.* **1998**, *65*, 85–98.

Appendix A Chapter 3 Yeast in Suspension Curves

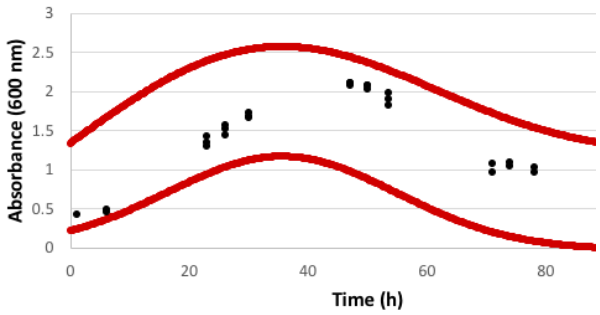

Figure 27. Yeast in suspension during a test tube fermentation of floor malted sample #225. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

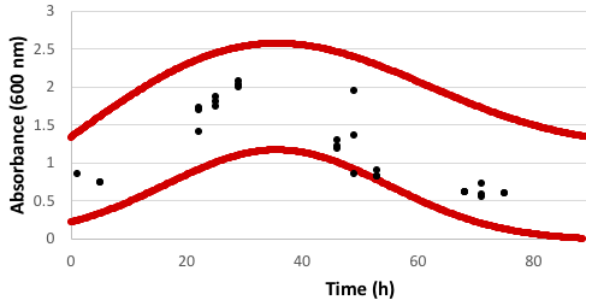

Figure 28. Yeast in suspension during a test tube fermentation of floor malted sample #226. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

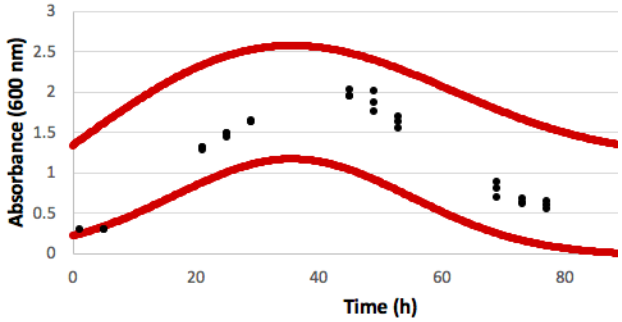

Figure 29. Yeast in suspension during a test tube fermentation of floor malted sample #228. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

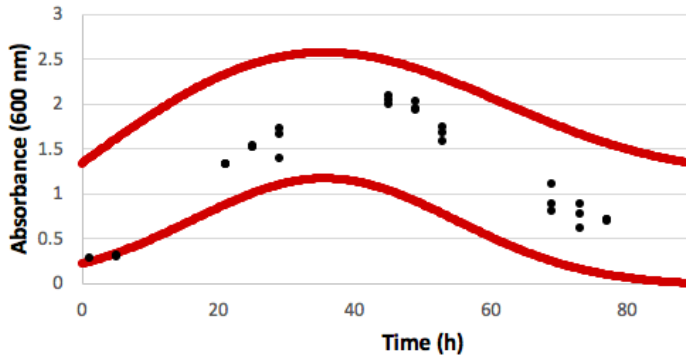

Figure 30. Yeast in suspension during a test tube fermentation of floor malted sample #229. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

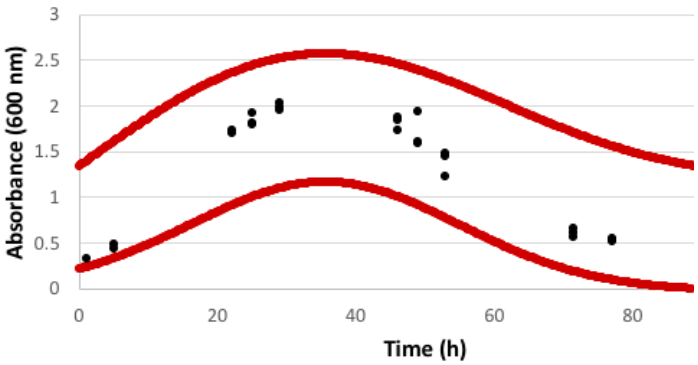

Figure 31. Yeast in suspension during a test tube fermentation of floor malted sample #230. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

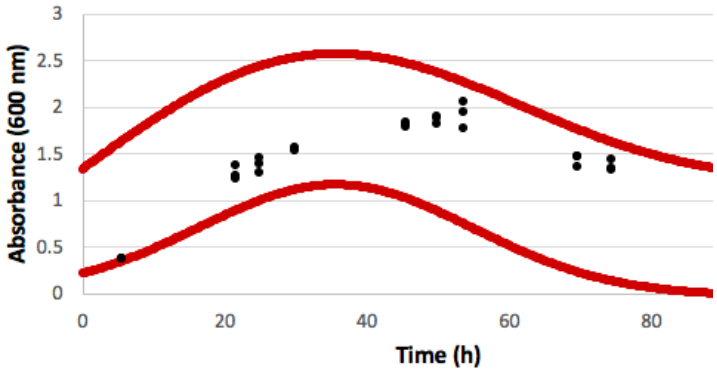

Figure 32. Yeast in suspension during a test tube fermentation of floor malted sample #232. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

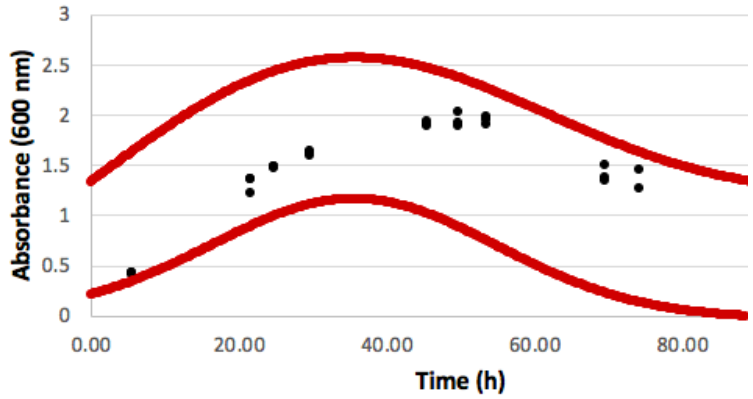

Figure 33. Yeast in suspension during a test tube fermentation of floor malted sample #234. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

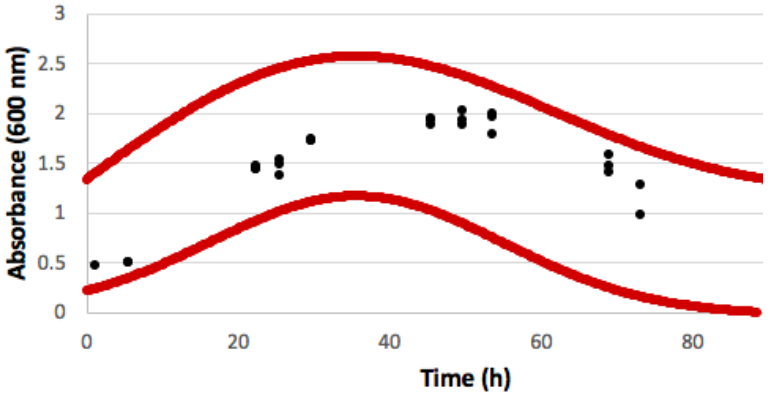

Figure 34. Yeast in suspension during a test tube fermentation of floor malted sample #236. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

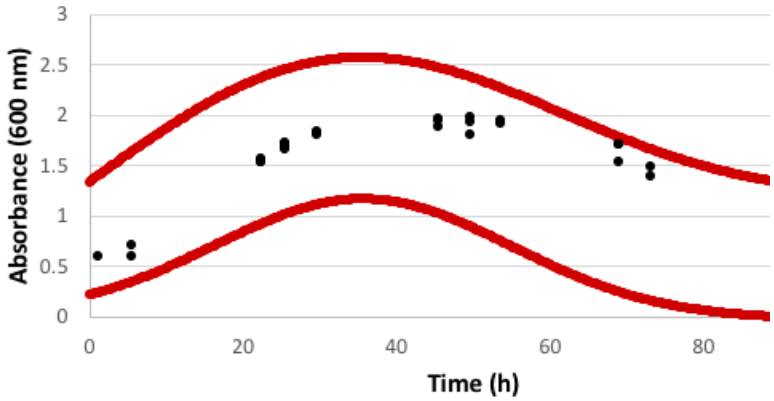

Figure 35. Yeast in suspension during a test tube fermentation of floor malted sample #239. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

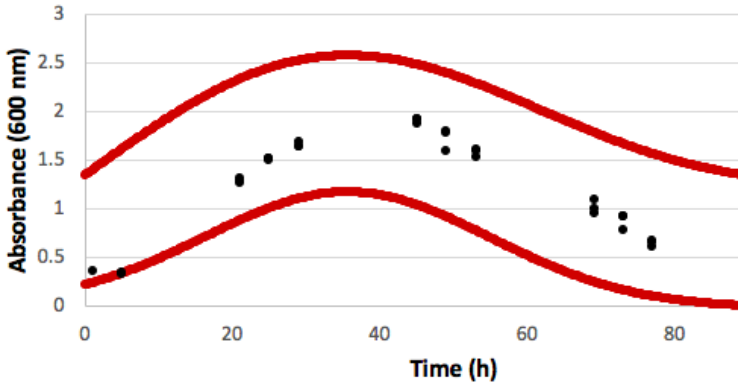

Figure 36. Yeast in suspension during a test tube fermentation of floor malted sample #240. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

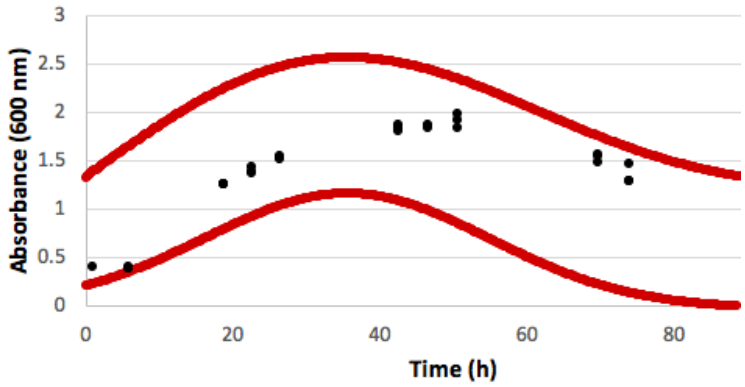

Figure 37. Yeast in suspension during a test tube fermentation of floor malted sample #243. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

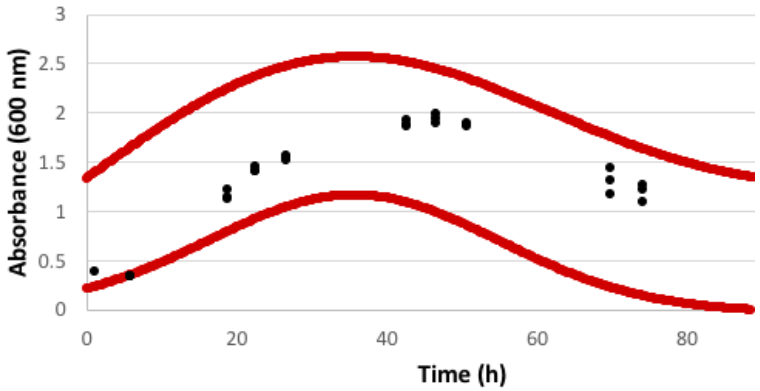

Figure 38. Yeast in suspension during a test tube fermentation of floor malted sample #244. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

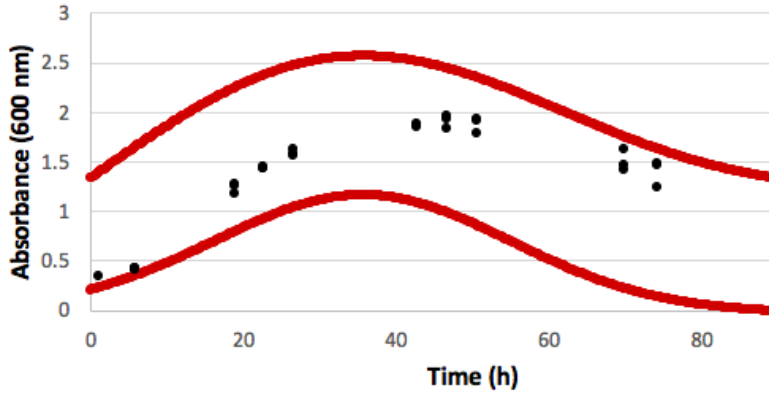

Figure 39. Yeast in suspension during a test tube fermentation of floor malted sample #245. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

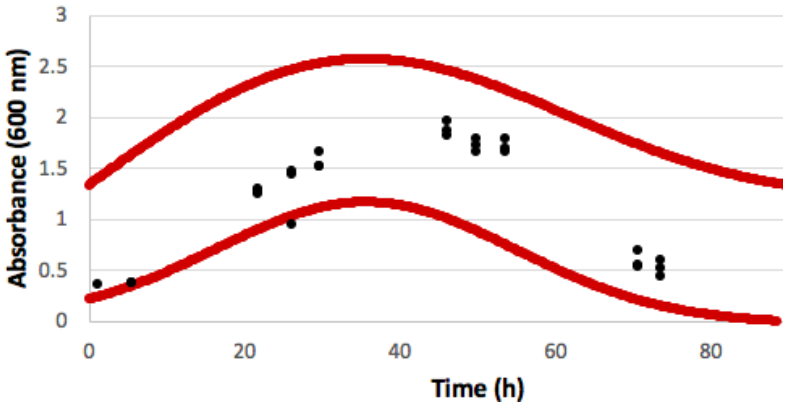

Figure 40. Yeast in suspension during a test tube fermentation of floor malted sample #246. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

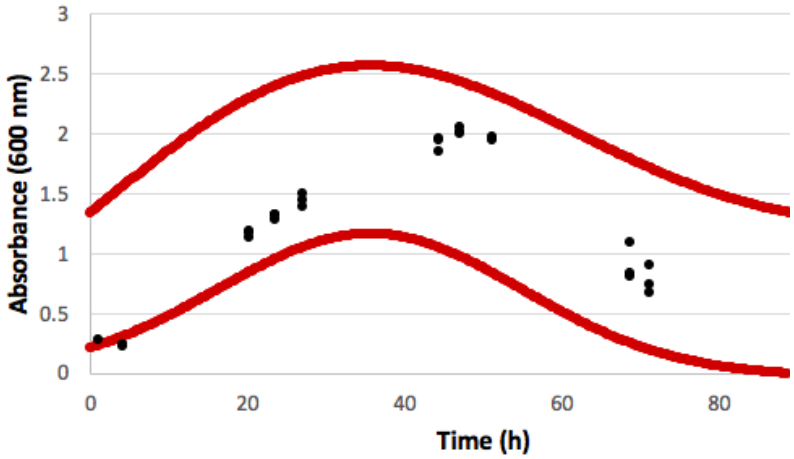

Figure 41. Yeast in suspension during a test tube fermentation of floor malted sample #247. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

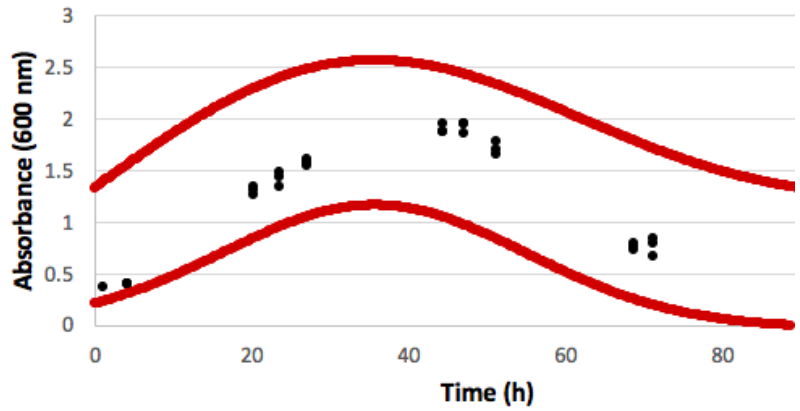

Figure 42. Yeast in suspension during a test tube fermentation of floor malted sample #251. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

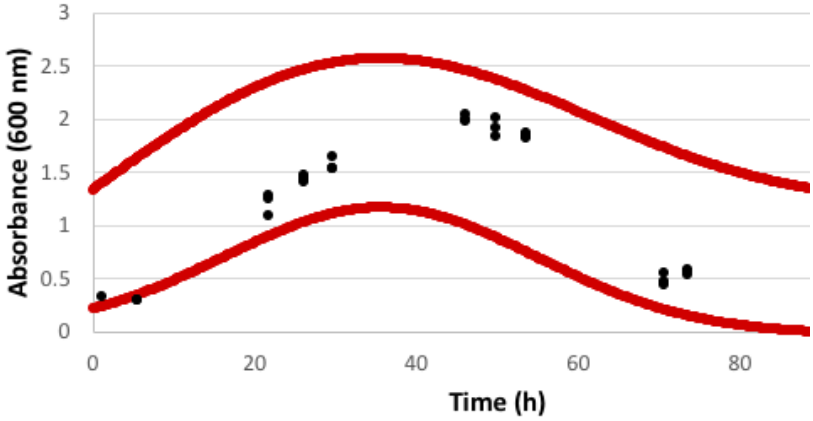

Figure 43. Yeast in suspension during a test tube fermentation of floor malted sample #256. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

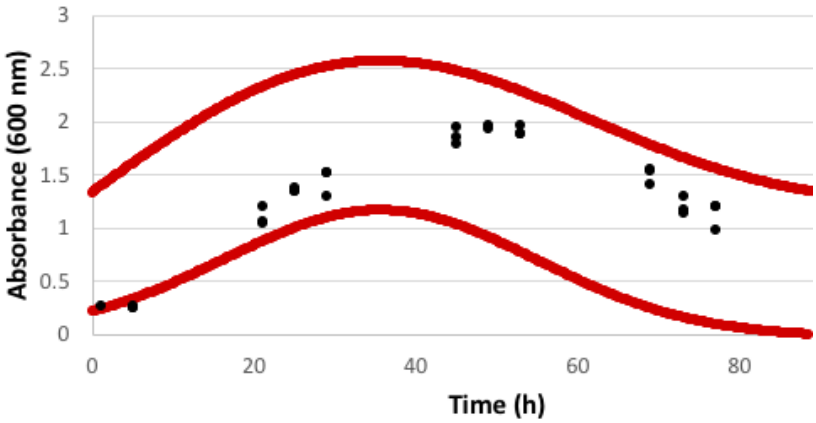

Figure 44. Yeast in suspension during a test tube fermentation of floor malted sample #353. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

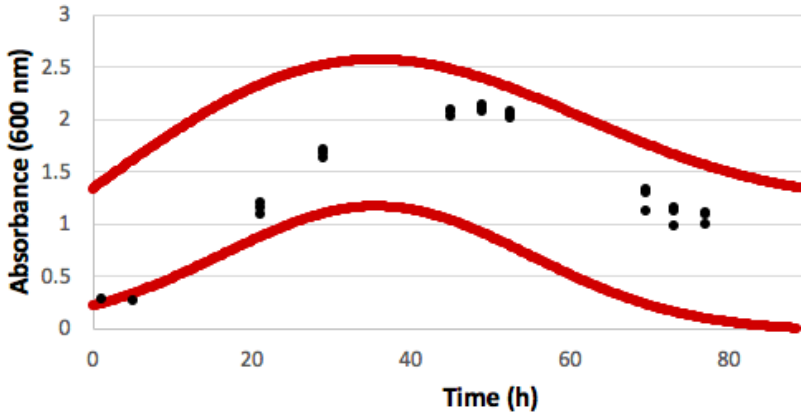

Figure 45. Yeast in suspension during a test tube fermentation of floor malted sample #360. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

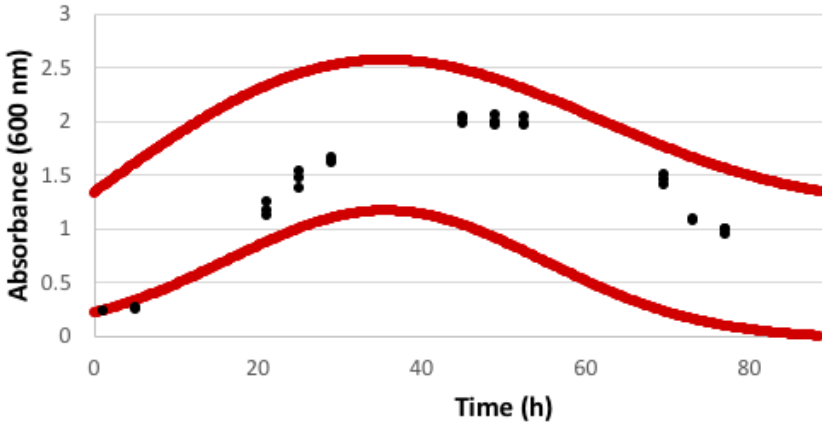

Figure 46. Yeast in suspension during a test tube fermentation of floor malted sample #362. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

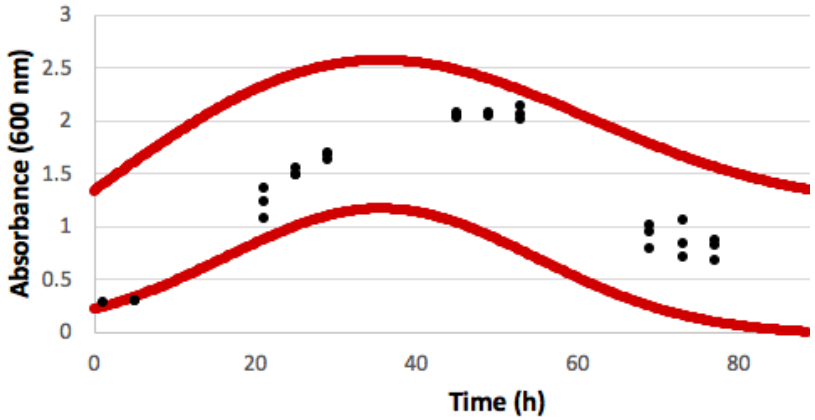

Figure 47. Yeast in suspension during a test tube fermentation of floor malted sample #372. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88]).

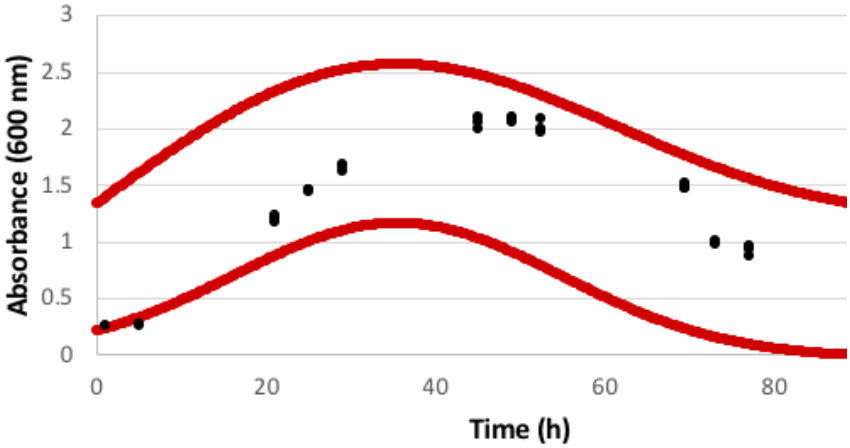

Figure 48. Yeast in suspension during a test tube fermentation of floor malted sample #389. Upper and lower bounds (red) for detecting PYF created by Armstrong et al. ([88])

Appendix B Chapter 3 Data Collected from all Floor Malted Samples

Table 13. Summer 2018 daily temperature data and germination temperature information. Average malt temperature for batch #225 & #226 was calculated using a digital handheld probe instead of the data logger.

Batch #	Day on Floor (2018)	Germination Length (days)	External Avg. Temp. (°C)	External Max. Temp. (°C)	Avg. Germination Temp. (°C)	Max. Germination Temp. (°C)
225	26-Jun	3.79	18.0	27.0	19.6	26.6
226	30-Jun	4.77	22.3	31.7	20.7	29.4
227	04-Jul	4.73	21.5	33.4	20.8	24.3
228	08-Jul	4.69	19.8	29.5	20.1	25.1
230	17-Jul	3.85	20.6	30.3	22.8	24.7
232	24-Jul	5.69	24.0	30.4	22.9	26.5
234	01-Aug	5.71	23.2	30.5	23.0	26.5
236	08-Aug	4.75	21.7	28.7	22.5	25.7
239	19-Aug	5.65	19.0	26.1	21.6	25.6
240	22-Aug	5.71	11.9	22.8	20.6	23.5
243	02-Sep	4.67	20.0	28.4	23.6	28.6
244	05-Sep	5.67	15.2	28.4	22.5	27.9
245	08-Sep	5.63	14.3	24.5	22.4	31.5
246	12-Sep	2.75	17.6	26.7	21.5	31.5
247	16-Sep	3.04	19.2	26.5	22.2	25.1
251	25-Sep	3.96	13.3	22.8	18.7	22.7
256	18-Oct	3.85	6.6	16.6	19.6	23.0

Table 14. Malting information on all 22 barley samples used in Chapter 3.

Batch #	Year	Barley Type	PYF presence	Steep Out Moisture (%)	Malt Moisture (%)	Max. Germination Temp. (°C)	Avg. Germination Temp. (°C)	Days on Floor (Days)
225	2018	AC Newdale	negative	44.1	5.59	26.6	19.6	3.79
226	2018	AC Newdale	positive	43.4	4.82	29.4	20.7	4.77
228	2018	AC Newdale	negative	44.5	5.80	25.1	20.1	4.69
229	2018	AC Newdale	negative	44.8	4.55	29.0	21.8	4.81
230	2018	AC Newdale	negative	43.4	5.63	24.7	22.8	3.85
232	2018	AC Queens	negative	43.9	4.80	26.5	22.9	5.69
234	2018	AC Queens	negative	44.4	4.80	26.5	23.0	5.71
236	2018	AC Queens	negative	44.2	4.70	25.7	22.5	4.75
239	2018	AC Queens	negative	43.4	4.48	25.6	21.6	5.65
240	2018	AC Queens	negative	44.7	4.60	23.5	20.6	5.71
243	2018	AC Queens	negative	41.8	3.77	28.6	23.6	4.67
244	2018	AC Queens	negative	44.4	4.17	27.9	22.5	5.67
245	2018	AC Queens	negative	42.2	5.62	31.5	22.4	5.63
246	2018	AC Newdale	negative	46.5	6.67	31.5	21.5	2.75
247	2018	CDC Copeland	negative	42.0	4.66	25.1	22.2	3.04
251	2018	AAC Synergy	negative	44.2	4.17	22.7	18.7	3.96
256	2018	CDC Copeland	negative	43.9	3.32	23.0	19.6	3.85
353	2019	CDC Copeland	negative	44.5	4.52	22.7	16.5	4.98
360	2019	CDC Copeland	negative	44.0	4.60	24.3	16.1	4.98
362	2019	CDC Copeland	negative	41.2	4.93	24.0	17.7	5.03
372	2019	CDC Copeland	negative	45.3	3.65	22.3	17.1	3.77
389	2019	AAC Synergy	negative	41.5	4.65	21.6	14.9	4.69

Appendix C Chapter 5 Non-linear Logistic Fitting.

Table 15. Results summary on the comparison of fits between the YIS data from mini-fermentation of PYF and control sample. Fermentation conditions were: SMA yeast, Oxygenated wort, One-day yeast propagation. Taken from Prism 8 (GraphPad, San Diego, CA) data analysis output comparing difference between each data set at significance level α = 0.05.

Table of results	SMA ABS Control O2 D=1	SMA ABS PYF O2 D=1	Global (shared)
	Y	Y	Y
Comparison of Fits			
Null hypothesis			One curve for all data sets
Alternative hypothesis			Different curve for each data set
P value			<0.0001
Conclusion (alpha = 0.05)			Reject null hypothesis
Preferred model			Different curve for each data set
F (DFn, DFd)			22.57 (4, 52)
Different curve for each data set			
Best-fit values			
Amplitude	1.771	1.795	
Mean	40.86	37.30	
SD	19.11	15.49	
r	0.008917	0.007819	
95% CI (profile likelihood)			
Amplitude	1.669 to 1.907	1.668 to 1.936	
Mean	39.29 to 42.86	36.09 to 38.94	
SD	17.33 to 21.09	13.43 to 18.06	
r	0.005326 to 0.01158	0.004899 to 0.009906	

Table 16. Results summary on the comparison of fits between the YIS data from mini-fermentation of PYF and Control sample. Fermentation conditions were: SMA yeast, Oxygenated wort, Two-day yeast propagation. Taken from Prism 8 (GraphPad, San Diego, CA) data analysis output comparing difference between each data set at significance level α = 0.05.

Table of results	SMA ABS Control O2 D=2	SMA ABS PYF O2 D=2	Global (shared)
	Y	Y	Y
Comparison of Fits			
Null hypothesis			One curve for all data sets
Alternative hypothesis			Different curve for each data set
P value			<0.0001
Conclusion (alpha = 0.05)			Reject null hypothesis
Preferred model			Different curve for each data set
F (DFn, DFd)			40.76 (4, 52)
Different curve for each data set			
Best-fit values			
Amplitude	1.768	1.891	
Mean	40.54	37.61	
SD	19.43	16.84	
r	0.009591	0.006318	
95% CI (profile likelihood)			
Amplitude	1.667 to 1.900	1.807 to 1.976	
Mean	38.97 to 42.53	36.64 to 38.79	
SD	17.67 to 21.37	15.36 to 18.52	
r	0.006089 to 0.01222	0.004106 to 0.008076	

Table 17. Results summary on the comparison of fits between the YIS data from mini-fermentation of PYF and Control sample. Fermentation conditions were: SMA yeast, Non-oxygenated wort, One-day yeast propagation. Taken from Prism 8 (GraphPad, San Diego, CA) data analysis output comparing difference between each data set at significance level α = 0.05.

Table of results	SMA ABS Control non-O2 1d	SMA ABS PYF non-O2 D1	Global (shared)
	Y	Y	Y
Comparison of Fits			
Null hypothesis			One curve for all data sets
Alternative hypothesis			Different curve for each data set
P value			<0.0001
Conclusion (alpha = 0.05)			Reject null hypothesis
Preferred model			Different curve for each data set
F (DFn, DFd)			33.00 (4, 52)
Different curve for each data set			
Best-fit values			
Amplitude	1.814	1.705	
Mean	41.83	36.55	
SD	19.91	15.72	
r	0.005934	0.007074	
95% CI (profile likelihood)			
Amplitude	1.658 to 2.058	1.632 to 1.781	
Mean	39.52 to 44.95	35.83 to 37.38	
SD	17.49 to 22.64	14.45 to 17.15	
r	7.709e-005 to 0.009888	0.005666 to 0.008260	

Table 18. Results summary on the comparison of fits between the YIS data from mini-fermentation of PYF and Control sample. Fermentation conditions were: SMA yeast, Non-oxygenated wort, Two-day yeast propagation. Taken from Prism 8 (GraphPad, San Diego, CA) data analysis output comparing difference between each data set at significance level α = 0.05.

Table of results	SMA ABS Control non-O2 2d	SMA ABS PYF non-O2 D2	Global (shared)
	Y	Y	Y
Comparison of Fits			
Null hypothesis			One curve for all data sets
Alternative hypothesis			Different curve for each data set
P value			<0.0001
Conclusion (alpha = 0.05)			Reject null hypothesis
Preferred model			Different curve for each data set
F (DFn, DFd)			34.06 (4, 52)
Different curve for each data set			
Best-fit values			
Amplitude	1.683	1.762	
Mean	41.91	38.02	
SD	19.46	15.04	
r	0.008328	0.006786	
95% CI (profile likelihood)			
Amplitude	1.539 to 1.912	1.668 to 1.866	
Mean	39.59 to 45.05	37.10 to 39.21	
SD	16.99 to 22.24	13.46 to 16.95	
r	0.002794 to 0.01202	0.004712 to 0.008357	

Table 19. Results summary on the comparison of fits between the YIS data from mini-fermentation of PYF and Control sample. Fermentation conditions were: Lager "A" yeast, Oxygenated wort, One-day yeast propagation. Taken from Prism 8 (GraphPad, San Diego, CA) data analysis output comparing difference between each data set at significance level α = 0.05.

Table of results	L-A ABS Control O2 D=1	L-A ABS PYF O2 D=1	Global (shared)
	Y	Y	Y
Comparison of Fits			
Null hypothesis			One curve for all data sets
Alternative hypothesis			Different curve for each data set
P value			<0.0001
Conclusion (alpha = 0.05)			Reject null hypothesis
Preferred model			Different curve for each data set
F (DFn, DFd)			10.04 (4, 52)
Different curve for each data set			
Best-fit values			
Amplitude	2.137	2.066	
Mean	38.15	36.34	
SD	17.34	15.31	
r	-0.0002455	0.001272	
95% CI (profile likelihood)			
Amplitude	2.080 to 2.194	1.982 to 2.156	
Mean	37.51 to 38.86	35.72 to 37.01	
SD	16.45 to 18.30	14.22 to 16.53	
r	-0.001751 to 0.001064	-0.0001152 to 0.002492	

Table 20. Results summary on the comparison of fits between the YIS data from mini-fermentation of PYF and Control sample. Fermentation conditions were: Lager "A" yeast, Oxygenated wort, Two-day yeast propagation. Taken from Prism 8 (GraphPad, San Diego, CA) data analysis output comparing difference between each data set at significance level α = 0.05.

Table of results	L-A ABS Control O2 D=2	L-A ABS PYF O2 D=2	Global (shared)
	Y	Y	Y
Comparison of Fits			
Null hypothesis			One curve for all data sets
Alternative hypothesis			Different curve for each data set
P value			0.3705
Conclusion (alpha = 0.05)			Do not reject null hypothesis
Preferred model			One curve for all data sets
F (DFn, DFd)			1.092 (4, 52)
Different curve for each data set			
Best-fit values			
Amplitude	2.102	2.060	
Mean	37.50	37.76	
SD	17.15	17.54	
r	7.790e-005	0.0007227	
95% CI (profile likelihood)			
Amplitude	2.029 to 2.175	1.986 to 2.135	
Mean	36.72 to 38.40	36.91 to 38.75	
SD	16.03 to 18.38	16.34 to 18.85	
r	-0.001753 to 0.001627	-0.001272 to 0.002383	

Table 21. Results summary on the comparison of fits between the YIS data from mini-fermentation of PYF and Control sample. Fermentation conditions were: Lager "A" yeast, Non-oxygenation wort, One-day yeast propagation. Taken from Prism 8 (GraphPad, San Diego, CA) data analysis output comparing difference between each data set at significance level α = 0.05.

Table of results	L-A ABS Control non-O2 D=1	L-A ABS PYF non-O2 D=1	Global (shared)
	Y	Y	Y
Comparison of Fits			
Null hypothesis			One curve for all data sets
Alternative hypothesis			Different curve for each data set
P value			0.1083
Conclusion (alpha = 0.05)			Do not reject null hypothesis
Preferred model			One curve for all data sets
F (DFn, DFd)			2.002 (4, 51)
Different curve for each data set			
Best-fit values			
Amplitude	2.322	2.237	
Mean	39.40	39.73	
SD	17.55	17.92	
r	-0.002281	-0.0008525	
95% CI (profile likelihood)			
Amplitude	2.221 to 2.430	2.143 to 2.344	
Mean	38.34 to 40.69	38.67 to 41.02	
SD	16.15 to 19.12	16.60 to 19.39	
r	-0.005305 to 0.0001044	-0.003844 to 0.001541	

Table 22. Results summary on the comparison of fits between the YIS data from mini-fermentation of PYF and Control sample. Fermentation conditions were: Lager "A" yeast, Non-oxygenated wort, Two-day yeast propagation. Taken from Prism 8 (GraphPad, San Diego, CA) data analysis output comparing difference between each data set at significance level α = 0.05.

Table of results	L-A ABS Control non-O2 D=2	L-A ABS PYF non-O2 D=2	Global (shared)
	Y	Y	Y
Comparison of Fits			
Null hypothesis			One curve for all data sets
Alternative hypothesis			Different curve for each data set
P value			0.0102
Conclusion (alpha = 0.05)			Reject null hypothesis
Preferred model			Different curve for each data set
F (DFn, DFd)			3.686 (4, 52)
Different curve for each data set			
Best-fit values			
Amplitude	2.178	2.244	
Mean	39.22	38.85	
SD	18.77	17.78	
r	0.0003464	-0.001220	
95% CI (profile likelihood)			
Amplitude	2.034 to 2.355	2.177 to 2.315	
Mean	37.53 to 41.49	38.08 to 39.72	
SD	16.66 to 21.25	16.75 to 18.89	
r	-0.004588 to 0.003814	-0.003183 to 0.0004422	