EVALUATION OF FERMENTATION CHARACTERISTICS OF SIX YEAST STRAINS

by

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DEDICATION

This thesis is dedicated to my mom; my best friend, my rock and my biggest supporter. I couldn't get by without you.

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ABSTRACT

Brewer's wort contains the sugars; fructose, glucose, maltose, maltotriose and sucrose, together with dextrin and other malt solids. The objective of this study was to investigate the fermentation characteristics and sugar consumption using six yeast strains: LCC125, LCC1208, LCC1209, LCC1240, SMA and Strain A. Model wort was developed using malt extract and peptone with the addition of sugars to resemble a typical wort. Fermentation properties were determined for each strain by utilizing a spectrophotometer to measure turbidity and a digital density meter to measure wort density at specific time intervals during fermentation. Samples were taken during the fermentations and analyzed via HPLC to determine sugar profiles. From the results it was decided that flocculation significantly affects metabolism of yeast cells during fermentation. Additionally, there were significant differences among the six strains on fermentation performance, especially with LCC 1208 and 1209.

LIST OF ABBREVIATIONS AND SYMBOLS USED

ASBC	American Society of Brewing Chemists
ATP	adenosine triphosphate
DNA	deoxyribonucleic acid
FAN	free amino nitrogen
Flo1	flocculation gene 1
GAP	general amino acid permease
h	hour
HPLC	high performance liquid chromatography
LCC	Labatt culture collection
Mig1p	transcription factor involved in glucose repression
mRNA	messenger ribonucleic acid
NADH/NAD+	nicotinamide adenine dinucleotide
NewFlo	new flocculation gene
nm	nanometer
RNA	ribonucleic acid
SUC2	gene encoding for two forms of invertase (secreted and intracellular)
UDP	uridine diphosphate
YEPD	yeast extract peptone dextrose
°C	degrees Celsius
°P	degrees Plato
µm	micrometer

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CHAPTER 1 INTRODUCTION

The enjoyment of consuming alcohol is common to all civilizations and pre-dates recorded history. Some historians believe that civilizations developed from a desire to drink beer. They speculate that the transition from hunter-gatherer to farmer and the beginning to civilization was to grow crops to make beer (48).

Civilizations consuming alcoholic beverages can be found on every continent where they may have been a part of their everyday diet (5). Due to the physiological effect of alcohol and, at that time, the misunderstood fermentation process, these early civilizations often associated alcoholic beverages with religious or ritual ceremonies (22). It is thought that in certain civilizations if an offering was set before a shrine and prayed over for several days that offering would transform into an alcoholic beverage (48).

Presumably, these early fermentations were unexpected and fortunate discoveries. These early ethanol fermentations occurred where any natural source of sugar was found accompanied by yeast contamination (natural) and a supply of water (10). It was not understood that the naturally occurring yeast in soil and on plants was a critical component to creating an alcoholic fermentation. Ancient brewers and winemakers relied on these natural yeast sources to inoculate their fermentations. For most of history, fermentation was a divine mystery (48).

It is known that the preparation of some native African beers used cereals as a source of extract which involved a step where the grains where chewed by the brewer. Chewing allowed the saliva, which contains amylase, aided in the degradation of the starch content of the grain and thereby increasing the fermentability of the wort (5). Of course those early brewers could

not have made beer without yeast.

In the beginning, it was not known what exactly what occurred during fermentation. When the Bavarians created the "Reinheitsgebot" also known as the beer purity law in 1516 they made it illegal to brew beer containing anything other than water, barley malt and hops. Yeast was not added to the list of ingredients because they did not know it existed or that it was the reason for the fermentation (12).

When Pasteur started working with beer fermentation in the 1860's, many people believed yeast was not the causative agent of fermentation. Scientists at the time knew yeast was part of the mixture, but they regarded it as a by-product of the fermentation (48). They believed fermentation was caused by spontaneous generation catalyzed by air. After 15 years of experiments, Pasteur proved that alcohol is a by-product of yeast metabolizing sugars. He also theorized that the bacteria and other yeast present in beer were the cause of off-flavours (32).

The beneficial effects of fermentation extend to its bactericidal qualities in the product. Historically, beer was a useful source of dietary calories, minerals and vitamins but could also be viewed as sanitized water. Ethanol itself inhibits the growth of many microorganisms including pathogens and this inhibition is reinforced by the lowering of pH of the environment which is caused by other by-products of yeast metabolism (5).

CHAPTER 2 OBJECTIVES

This study was designed to investigate whether different yeast strains consume wort sugars at the same rate and to quantify their sugar consumption. The wort used was a model wort to ensure consistency and contain a mixture of sugars resembling a true wort. The fermentation assays were conducted under shaking, at 21°C, to negate the effects of flocculation. Sugar consumption was determined for each strain by utilizing a spectrophotometer to measure turbidity and a digital density meter to measure wort density. Turbidity measurements are an indication of the yeast in suspension of wort while changes in density measurements are an indication of carbohydrate metabolism by yeast during fermentation. These measurements were taken in triplicate at these specified time intervals during fermentation: 0, 6, 22, 26, 30, 46, 50, 54, 70, 74, 78, 122 and 194 h. Sugar consumption was quantified using sugar profiles via HPLC.

CHAPTER 3 LITERATURE REVIEW

3.1 Brewing Yeast

Yeast is the most important part of the brewing fermentation process. Yeast converts sugar to alcohol, carbon dioxide, and other compounds that influence the flavour and aroma of beer. Brewer's yeast is an eukaryote and belongs to the kingdom Fungi. By some scientific classifications, all beer-brewing strains of yeast are placed in the genus *Saccharomyces* (sugar fungus) and species *cerevisiae* (47). However, the brewing industry uses a classification which divides yeast into two types; ale-yeast (*S. cerevisiae*) and lager-yeast (*S. carlsbergensis*). The distinction is kept so as to separate yeasts used to make ales from those used to make lagers (6).

3.1.1 Description of Yeast Cell Structure

Most of the organisms in the kingdom Fungi are multicellular however yeast is a single-cell organism. A single yeast cell measure about 5-10µm in diameter and is usually spherical, cylindrical or oval in shape (5). Yeast occurs in single, pairs, chains and clusters (38). Figure 3.1 is a simplified diagram of yeast cell structure. The cell wall is a barrier that is mostly composed of carbohydrates surrounding the cell (5). It is a rigid structure which is 250 nm thick and constitutes approximately 25% of the dry weight of the cell (38). There are three cross-linked layers comprising the cell wall (Figure 3.2). The inner layer is a chitin (a long-chain polymer of a N-acetylglucosamine) layer, composed mostly of glucans; the outer layer is mostly mannoproteins while the intermediate layer is a mixture of both the inner and outer layer (48).

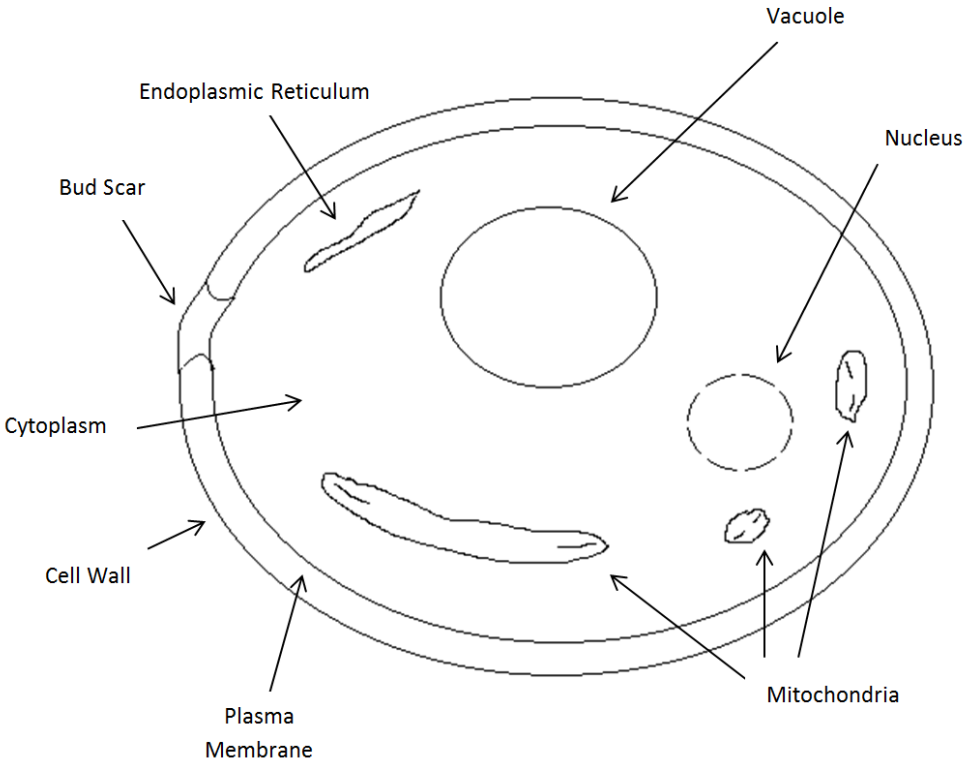

Figure 3.1 Simplified diagram of yeast cell structure (Adapted from 48).

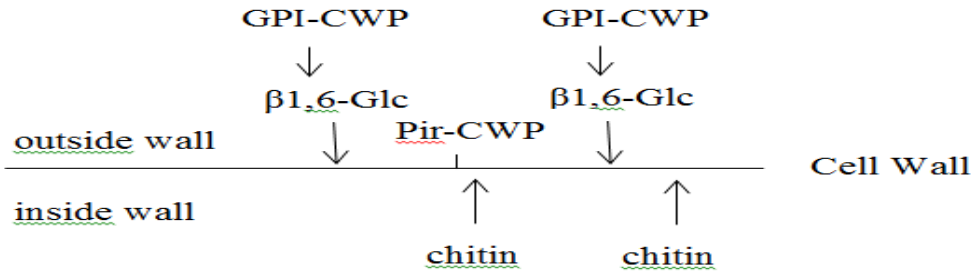

Figure 3.2 Molecular organization of the cell wall of *S. cerevisiae*. GPI-CWP are GPI-dependent cell wall proteins, Pir-CWP are pir proteins on the cell wall and β1-6-Glc are glucan molecules which are highly branched. Therefore they are water soluble which tethers GPI-CWPS to the cell wall (24).

To reproduce asexually, a yeast cell clones itself thereby creating a new daughter cell. Cell separation is achieved when the layers of the cell wall separate leaving the bud scar on the mother cell and the birth scar on the daughter cell (38). The bud scar is composed mainly of chitin. The average ale yeast cell will not bud more than 30 times over its lifetime while lager yeast will bud only 20 times before they are unable to bud further (48).

The plasma membrane is a semi-permeable lipid bilayer between the cell wall and the inside of the cell. There are several distinct roles that the plasma membrane carries out such as to provide a barrier to free diffusion of solutes, to catalyze specific exchange reactions, to store energy dissipation, to provide sites for binding specific molecules involved in metabolic signaling pathways and to provide an organized support matrix for the site of enzyme pathways involved in the biosynthesis of other cell components (18). The plasma membrane is quite fluid and flexible due to its constituents of lipids, sterols and proteins. Additionally, these constituents allow for the creation of a daughter cell.

The formation of double bonds in fatty acids controls their level of saturation. The saturation level determines the ease and extent of hydrogen bonding that can occur between fatty acids (6). Membrane fluidity is necessary for proper membrane function. Lipid bilayers are by their nature fluid and that fluidity is determined by the extent to which the lipids bind to one another (48). By controlling the level of saturation in their lipid membranes, yeast cells are able to maintain proper membrane fluidity at different temperatures which is important during fermentation. Without proper aeration yeast cells are unable to control membrane fluidity through to the end of fermentation which leads to halted fermentations and off-flavours of the final product (48).

The cytoplasm is that portion of the cell enclosed by the plasma membrane and excluding other membrane bound organelles. It is an aqueous colloidal liquid containing a multitude of metabolites (6). The cytoplasm contains intercellular fluid known as the cytosol. The cytosol contains enzymes involved in anaerobic fermentation which enable the cell to convert glucose into energy immediately after it enters the cell (48).

The mitochondrion is an organelle where aerobic respiration occurs. Mitochondria consist of a double membrane which is the location of the conversion of pyruvate (a metabolic compound) and the tricarboxylic acid cycle. The nucleus stores the cell DNA and is delimited by a lipid membrane which envelopes the nucleus and is similar to the plasma membrane. The cell uses mRNA to transfer the information out into the cytoplasm for use in protein synthesis (48).

The vacuole is a membrane bound structure that stores nutrients and is also where the cell breaks down proteins. Brewer's yeast vacuoles are large enough to be seen through light microscopy (48). The major site for proteolysis is the cell vacuole. Much of the regulation of both specific and non-specific proteolysis involved the sequestration of target proteins into vacuoles where they are exposed to proteinases (6). The endoplasmic reticulum is a network of membranes and is usually where the cell manufactures proteins, lipids and carbohydrates for membranes and secretion (48). Other microbodies are mainly made up by glycogen bodies and lipid glandules (5).

3.1.2 Comparison of Lager and Ale Yeast

The distinctions between the yeast used in ale and lager brewing are small. Traditionally, ale yeast were regarded as top fermenters which formed a frothy yeast head

on the surface of the fermenting beer and was skimmed off to be used for subsequent brews, while lager yeasts were bottom fermenters which formed little surface head and were recovered from the bottom of the fermenter (6). Today, this is a less useful distinction as many types of ale yeast now have the capacity to fall out of solution and settle at the bottom of the fermenter (1).

The optimal growth temperature of lager and ale yeast differ and this is reflected in the different temperatures used for larger fermentations 8-12˚C and for ale fermentations 12-18˚C (1). Lager and ale yeasts can also be distinguished by the ability of lager strains to ferment the disaccharide melibiose because they have α-D-galactosidase activity, which hydrolyzes melibiose to galactose and glucose while ale strains cannot. However, this is of no practical importance since the sugar does not occur in wort (6). Additionally, lager yeast strains can utilize maltotriose more rapidly than ale strains. Lager strains utilize mixtures of galactose and maltose simultaneously, whereas ale strains prefer to utilize maltose (5).

3.1.3 Flocculation

One functional definition of flocculation is that is describes the ability of yeast strains to clump together and fall out of solution. Near the end of fermentation, single cells aggregate into clumps of thousands of cells. Different strains of yeast have different flocculation characteristics. Some strains flocculate earlier during fermentation and subsequently do not attenuate (ie., finish the fermentation) normally. Flocculating too early results in a beer that is under attenuated and sweet, however, when yeast fails to flocculate entirely, it results in a beer that is cloudy with a yeasty taste (35).

Flocculation has been studied for many years and the exact mechanism is still debated. Cell wall composition is a key factor in the ability of adjacent cells to stick to each other. Yeast has a thick cell wall made up of protein and polysaccharides with a net negative surface charge due to phosphates in the cell wall (6). The extent of the negative charge depends on the yeast strain, phase of growth, oxygen availability, starvation, generation number, dehydration and cell age. Yeast cells are also hydrophobic due to exposed hydrophobic peptides and lipids (2). The primary determinant of flocculation is the yeast strain itself (36).

The minute differences in cell wall composition play a key role in flocculation behaviour and determine the degree of flocculation for a strain. Factors that influence the degree of flocculation include the original gravity of the wort, temperature of fermentation, pH of the wort, pitching rate, initial oxygen content, calcium and inorganic ion concentration, and cell age. Additionally anything that affects the health and growth rate of the yeast affects flocculation (36).

The mechanism of lectin-like cell-cell interactions has been established to explain yeast flocculation in the past two decades (36). Lectins are a structurally diverse group of proteins that are capable of binding carbohydrates while zymolectin is an anchored yeast cell wall protein that contains one or more mannose binding site (5). This mechanism proposes that specific surface proteins known as zymolectins which are present on flocculent yeast cells, bind to mannose residues of mannan molecules on neighboring cell surfaces (36). The involvement of this protein-carbohydrate interaction was suggested by Taylor and Orton (44) as flocculation can be inhibited specifically by mannose.

3.1.4 Level of Flocculence

Brewers classify yeast as high, medium and low flocculators. Ale strains can be found in all categories while lager strains are predominantly medium flocculators (Table 3.2).

Table 3.2 Differences in flocculation classification (48).

Level of Flocculence	Description
High	• Start to flocculate by day 3-5 • May need to rouse yeast • Higher levels of diacetyl and lower attenuation • Good for malty ales
Medium	• Start to flocculate by day 6-15 • Ideal for ales • Clean, balanced flavour production
Low	• Fail to begin to flocculate by day 15 • Good for Belgian beers • Makes filtering difficult

A high flocculator begins to clump in three to five days into fermentation. When the flocculated yeast clump falls to the bottom of the fermentor, it forms a solid, precipitate. In order to produce a fully attenuated beer with a high flocculating yeast strain it requires special attention such as stirring the yeast as to suspend it into the beer (48). However even then, the use of high flocculators result in unfermented sugars and/or unwanted flavor compounds such as diacetyl. Highly flocculant strains do have advantages as they can produce a brighter beer with less suspended yeast making filtration easier (50).

Medium flocculators are used in ale beer as they tend to produce “cleaner” beers with lower levels of diacetyl and esters since the yeast cells stay in suspension longer as they metabolize the carbon sources in the beer more than high flocculators and reduce diacetyl and other fermentation compounds (48). Medium flocculators will start to flocculate as sugars become less available (50). In a commercial brewery, medium flocculators are slightly more difficult to work with than high flocculators because they often require filtering for quick production. Medium flocculators additionally are used to produce hopped ale beer as their clean flavours allow for the hop aroma and flavour to come through (48).

Low flocculation yeast strains are rarely used in the brewing industry because they do not settle out of the beer creating haze and filtering problems. However, a hazy beer requires low flocculation as yeast in suspension is a wanted characteristic (48).

3.1.5 Flocculation Phenotypes

Since the 1950s, it has been recognized that flocculation is a hereditary characteristic (35). NewFlo and Flo1 are two common flocculation phenotypes found in brewer’s yeast and laboratory yeast, respectively (42). Flocculation in the NewFlo phenotype is inhibited by mannose, glucose, sucrose and maltose, while only mannose inhibits flocculation in Flo1 phenotypes (43). NewFlo is flocculent at the end of fermentation while Flo1 is heavily flocculent throughout the fermentation (43).

It has been hypothesized that these sugars inhibit flocculation by binding to lectin proteins on the yeast cell wall sites on zymolectins which project from the yeast cell wall (46). This binding prevents the zymolectins from binding to the mannose molecules present on the surface of other yeast cells (46).

The Flo1 phenotype is generally heavily flocculent throughout the fermentation since mannose is not present in wort and Flo1 zymolectins are present at a constant level (43). In contrast, NewFlo phenotype yeasts become flocculent with the decrease in fermentable sugars (37).

3.2 Assimilation of Wort Nutrients

Brewing yeast strains are heterotrophic organisms which are capable of utilizing a wide variety of nutrients to support growth and generate energy. When presented with a choice of nutrients, yeast cells will use those that are easiest to assimilate and will be selective in its uptake. Thus, assimilation of individual nutrients from wort is made complex, depending on the nutrients available (5). Of particular relevance to brewery fermentation is the assimilation of carbohydrates and nitrogenous compounds and their processes are highly regulated Not only are some components utilized in preference to others but some components are utilized in the presence of some nutrients which can inhibit the utilization of others. In consequence, uptake of carbohydrates and the various sources of nitrogen present in wort are ordered processes (5).

3.3 Sources of Carbon

Yeast can digest a wide range of organic carbon compounds. The most commonly used carbon sources are carbohydrates, including mono-, di- and trisaccharides. Brewing yeast strains cannot utilize pentose. Strains of *S. cerevisiae* metabolize a limited list of carbon sources for growth and utilization of these carbon sources are strain-specific (47).

3.3.1 Wort Carbohydrates

Yeasts can ferment the following sugars; the monosaccharides glucose and fructose, the disaccharides sucrose and maltose and the trisaccharide maltotriose (6). Typically maltose is the most abundant sugar in wort. The remainder consists mainly of glucose, fructose, sucrose, maltotriose and higher dextrins. Typical amount of sugars in a 12% wort are: Glucose + fructose, 0.9-1.2 g/100 mL; sucrose, 0.4-0.5 g/100 mL; maltose, 5.6-5.9 g/100 mL; and maltotriose, 1.4-1.7 g/100 mL; giving a sugar total of 8.3-9.3 g/100 mL (15). The unfermentable fraction of the extract (dextrins) accounts for approximately 25% of the total carbohydrates in wort (14).

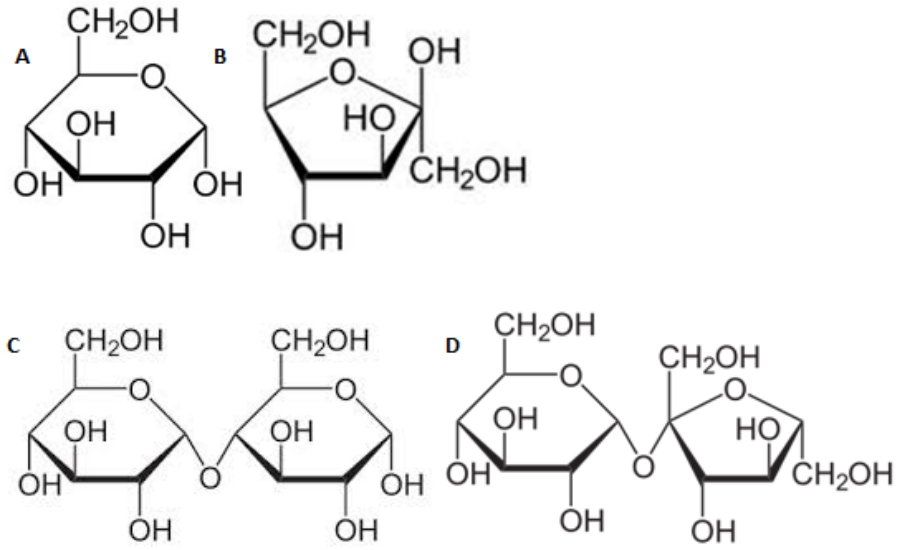

Figure 3.3 Glucose (A), fructose (B), maltose (C), sucrose D) molecules.

The monosaccharides (Figure 3.3), fructose and glucose are fermented the most rapidly, while maltotriose is fermented slowly and sometimes incompletely so traces may remain in beer. Dextrins and β-glucans which are derived from the partial degradation of

malt are not fermentable. The fermentable carbohydrates are the major energy source of the yeast while alcohol and carbon dioxide are the major metabolic products (6).

3.3.2 Uptake of Wort Sugars

Sugar uptake appears to be predominantly via active processes and against a concentration gradient (6). The uptake of sugar is complex and highly regulated. When a mixture of sugars are present yeast have mechanisms for selecting first, those which are most readily utilized. In the case of brewers' yeast in wort, the utilization of sugars is an ordered process. Ale strains *S.cerevisiae* are able to ferment glucose, sucrose, fructose, maltose, galactose, raffinose, maltotriose and occasionally trehalose. Lager strains of *S.* cerevisiae are distinguished by being able to also ferment the disaccharide melibiose (36). The patterns of uptake of sugars during an ale fermentation are that sucrose is utilized first and the resultant hydrolysis causes a short increase in the concentration of fructose (6).

Fructose and glucose are taken up simultaneously and are fully metabolized from the wort after about 24 h. Complete assimilation of glucose is followed by uptake of maltose, the major wort sugar. Maltotriose is utilized last after all assimilation of maltose. Higher polysaccharides and the dextrins are not utilized by brewing strains and those contribute to beer flavour by way of imparting fullness (6).

The sequential uptake of wort sugars reflects the genotype of the yeast and ways in which this is expressed by repression and induction and by carbon catabolite inactivation. There are specific and often multiple carriers for individual sugars. The activity of individual carriers is modulated by the spectrum and concentration of sugars

present in wort. In particular, glucose appears to be the preferred substrate and when present in the medium its presence inactivates or represses carriers for the uptake of other sugars (26).

Sucrose is assimilated via invertase which is secreted into the cell periplasm. In *S. cerevisiae* the enzyme is encoded by the SUC2 gene and it hydrolyses both sucrose and raffinose (7). A second invertase is secreted into the periplasm and this is responsible for the metabolism of extracellular sucrose. Once hydrolysed, the released fructose and glucose are taken up by facilitated diffusion. In the presence of high glucose concentrations invertase is repressed via binding of a component Mig1p to the SUC2 gene promoter (29). It has been suggested that low levels of glucose (0.1%, w/v) are actually required for maximum transcription of the USC2 gene (31).

The predominant sugar, maltose is then taken up into the cell by maltose permease enzyme (maltase) and is then split inside the cell into two glucose units by β-glucosidase (16). Maltose utilization is accomplished using the products of a multigene (MAL) family that occurs at several loci in the yeast genome and is not restricted to a single chromosome. Each locus consists of three genes: MALT which encodes for a maltose permease; MALS encoding for a maltase (α-glucosidase) and MALR which encodes for a post-transcription activator of the MALS and MALT genes (9). Both the latter two genes are induced by maltose and repressed by glucose. The maltose system is an active process requiring cellular energy. Uptake is via a proton symport system in which potassium (K^+) is exported to maintain electrochemical neutrality (34). Maltose utilization is repressed by glucose and requires the maltose for induction. When the maltose concentration falls to an undetectable level maltotriose is metabolized. Longer

chain sugars (dextrins) are not utilized by brewing yeasts (16). Figure 3.4 is a simple schematic of sugar uptake by a brewing yeast cell.

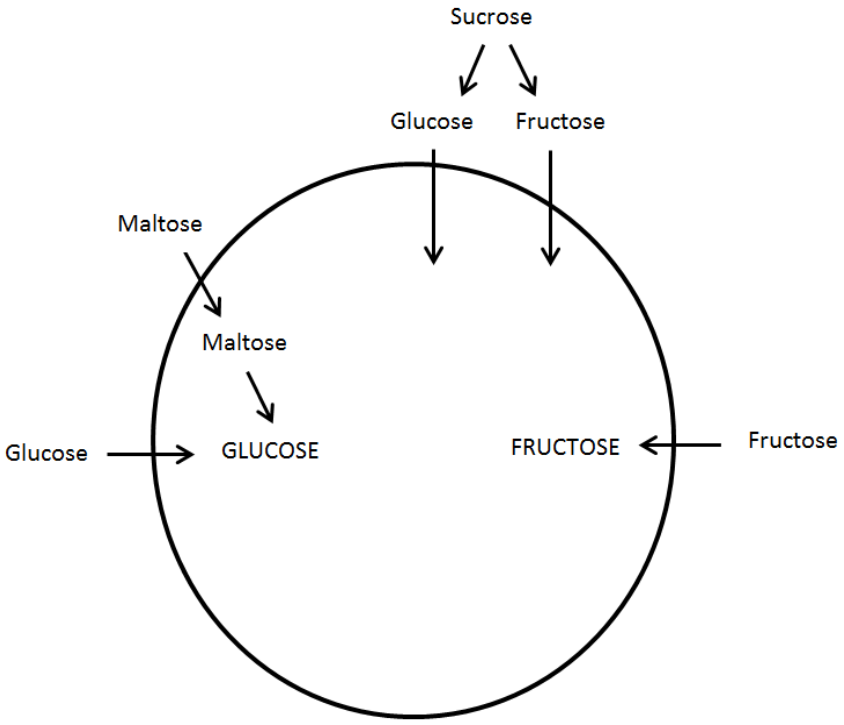

Figure 3.4 Basic diagram of sugar uptake in a yeast cell (Adapted from 16).

3.3.3 Sugar Metabolism

The catabolism of sugars provides yeast with energy and an essential activity. Several distinct pathways are involved.

One pathway is named Glycolysis or the Embden-Myerhof-Parnas pathway and is the major sugar catabolic pathway in yeast. It operates under both aerobic and anaerobic conditions. The pathway catalyzes the conversion of one molecule of glucose into two molecules of pyruvate (Figure 3.5).

The initial phosphorylation reaction, in which ATP is the phosphate donor, may be catalyzed by one of three enzymes. Hexokinases 1 and 2 show activity towards both glucose and fructose and glucokinase with glucose, alone (6). All of the glycolytic reactions are reversible with the exceptions of the initial phosphorylation of glucose, the phosphorylation of fructose-6-phosphate to yield fructose 1,6 bisphosphate and the dephosphorylation of phospho-enol-pyruvate to form pyruvate. Several of the steps are catayzed by multiple enzymes (6). Glycolysis can operate in the reverse direction as gluconeogenesis. With the aid of three additional enzymes, phospho-enol-pyruvate carboxykinase, fructose 1, 6-bisphosphatase and glucose 6-phosphate phosphatase, they catalyze the contra-flow of carbon past the irreversible steps of glycolysis (6).

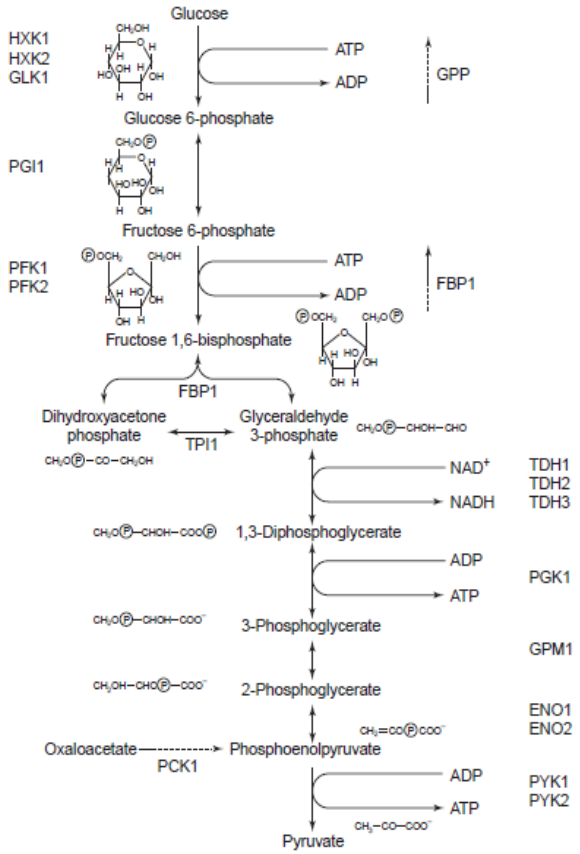

Figure 3.5 The pathway of glycolysis from Briggs et al., 2006.

Of course, there are additional sugars that feed into the glycolytic pathway as shown Figure 3.6 The utilization of these other sugars also involves reactions in which ATP is consumed and phosphorylated intermediates are formed such as glucose.

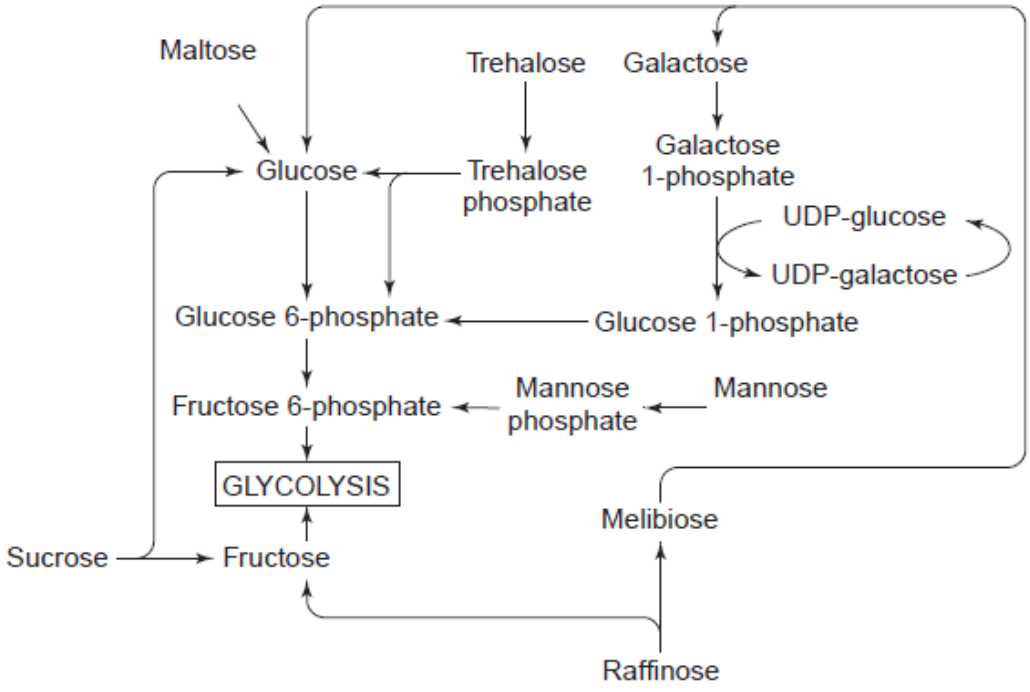

Figure 3.6 Diagram of sugars entering glycolysis from Briggs et al., 2006.

Glycolysis generates reducing power in the form of NADH. This is re-oxidized in redox balancing reactions. During the conversion of glucose to fructose 1, 6-bisphosphate, two molecules of ATP are consumed. In the later stages of glycolysis, four ATP molecules are generated in the reactions catalyzed by phosphoglycerokinase and

pyruvate kinase. Therefore, for every molecule of glucose catabolized via glycolysis there is a net gain of two molecules of ATP (6).

3.3.4 Storage Carbohydrates

S. cerevisiae accumulates two classes of storage carbohydrates, glycogen and trehalose which have roles in brewery fermentation. Glycogen serves as a true energy reserve, which may be used during periods of starvation (5). Glycogen is a polymer of α-D-glucose and has a branched structure containing chains of 10-14 residues of α -D-glucose joined by 1→4 linkages. Glycogen is synthesized from glucose, via glucose-6-phosphate and glucose-1-phosphate (Figure 3.7). The pathway uses uridine diphophate (UDP) as a carrier of glucose units. Glycogen synthase catalyzes chain elongation by successive transfer of glucosyl units from UDP-glucose to the growing α -(1-4) linked polyglucose polymer (5). A second enzyme, branching enzyme, forms the α -(1-6) glucosidic bonds which from the branch points in the growing polymer (Figure 3.8).

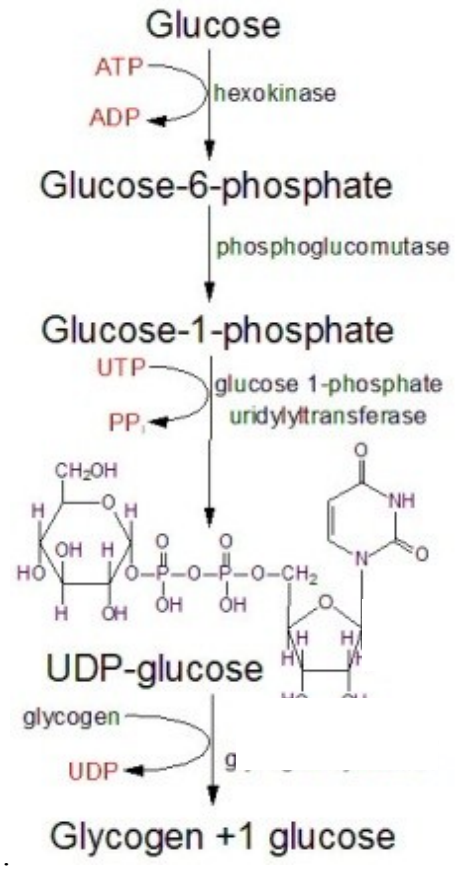

Figure 3.7 Glycogen pathway from King, 2013.

Accumulation or degradation of glycogen is controlled by yeast growth rate. The accumulation of glycogen is signaled by nutrient limitation in the presence of excess sugar . Glycogen accumulation may also occur under carbon limitation (5). When glucose falls to a concentration below that required to saturate the uptake system and growth rate is restricted then glycogen accumulation is triggered. Accumulation continues until the available sugar becomes exhausted. This glycogen storage provides an energy source for induction of the respiratory and gluconeogenic systems, which are required for utilization of ethanol (17).

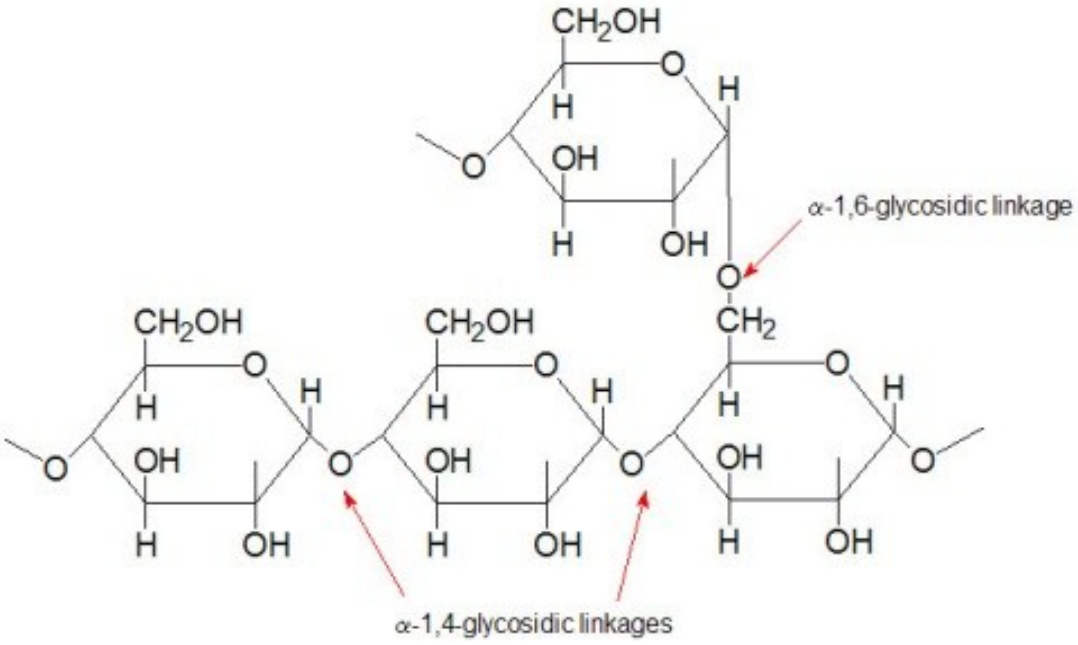

Figure 3.8 Glycogen molecule (25).

3.3.5 Alcohol Production

The products of fermentation are alcohol and carbon dioxide. The following equation describes yeasts conversion of sugar to ethanol:

$$\text{Glucose} + 2\ \text{ADP} + 2\ \text{phosphate} \rightarrow 2\ \text{ethanol} + 2\ CO_2 + 2\ \text{ATP}$$

The equation can be split into two main parts: glucose to pyruvate, then pyruvate to ethanol.

Glucose to pyruvate occurs in the cytosol. Enzymes in the cytosol catalyze this reaction and the other metabolic reactions. However, not all pyruvate ends up as ethanol. Pyruvate conversion has two possible paths. The first possible path for pyruvate to enter a mitochondrion and get separated into to carbon dioxide and water, this known as aerobic

respiration. The second possible path is for pyruvate to remain in the cytosol, where the yeast cell convert it to acetaldehyde and then to ethanol (48).

Yeast obtains more energy from converting pyruvate into carbon dioxide and water in the presence of oxygen. Yeast only produce ethanol when under conditions of high sugar levels and low oxygen levels, known as anaerobic conditions.

Yeast cells rely on the co-enzyme nicotinaminde adenine dinucleotide (NAD+ and NADH) in enzymatic reduction-oxidation reactions, including in the conversion of acetaldehyde to ethanol by the alcohol dehydrogenase enzyme. Yeast use NAD+ in the initial breakdown of glucose. If there is oxygen present, pryuvate from this step goes to the mitochondira where it enters the Krebs cycle (a.k.a. tricarboxylic or citric acid cycle) to yield an energy-rich compound known as adenosine triphosphate (ATP). ATP is important to a yeast cell as it provides the cell energy for protein synthesis and DNA replication necessary for budding. If the yeast is without oxygen, pyruvate from that step does not go through the Krebs cycle (48). This leads to a buildup of pyruvate and in turn leaves the cell without ATP, and NAD+. In order to create NAD+, which is necessary for the generation of ATP, the yeast cell breaks down pyruvate to lactic acid which is catalyzed by the enzyme lactic dehydrogenase and now the cells are able to generate the NAD+ they need (48).

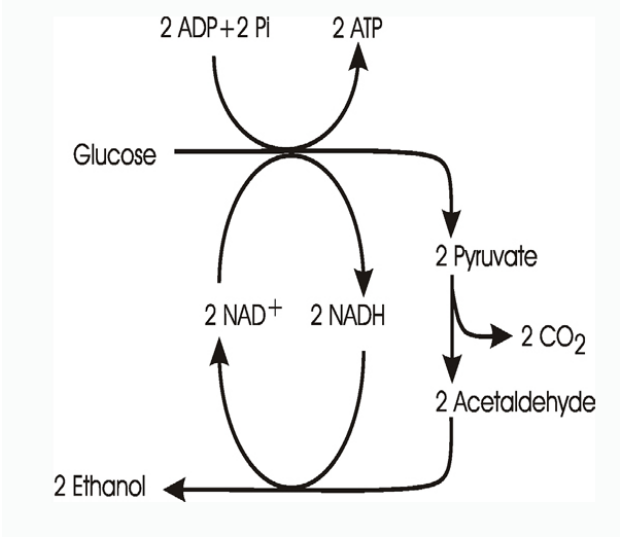

Figure 3.9 Simplified diagram of the pathway for glucose to ethanol during fermentation (25).

There is another way yeast will ferment anaerobically and still produce ethanol known as the Crabtree effect. This is very important to brewing. If there is a high enough glucose concentration, even in the presence of oxygen, yeast will still produce ethanol. Brewers wort always contain more than the 0.4% glucose required to cause the Crabtree effect so that fermentation always results in alcohol (5).

3.4 Nitrogenous Components

Nitrogenous components of wort account for 4-5% of the total dissolved solids. The bulk (85-95%) of the total is in the form of amino acids, small peptides and proteins (28). The relative proportions of each of these groups of nitrogenous components depend on the composition of the barley and the conditions of wort production (6).

Worts can contain more than 1000 mg/L total nitrogen, depending on the materials used and the process conditions during malting and fermentation, although 700-800 mg/L is more typical (5). The fraction of soluble nitrogen is reported as 20% protein,

22% polypeptides and 58% peptides and free amino acids (8). Free amino acid concentrations are within the range 150-250 mg/L in a wort of 10.5°P (5). Recommended free amino nitrogen concentrations of wort is of the order of 150-200 mg/L where oxygen is the limiting substrate. A third to a half of the amino acids in wort arise from the action of preoteases (mainly carboxypeptidases) during mashing while the remainder being derived directly from malt and formed during malting (5). Malt carboxypeptidases have maximal activity at temperatures between 40 and 60°C and are inactivated at 70°C therefore the temperature at which mashing is conducted has a crucial impact on the FAN (Free Amino Nitrogen) content of worts. All free amino acids can be utilized by yeast during fermentation under appropriate conditions other than proline, which requires oxygen. Some 40% of the small peptides are also utilized (5).

3.4.1 Uptake of Wort Nitrogenous Compounds

Nitrogenous components in wort are heterogenous. In a Canadian lager wort, nitrogenous compounds were roughly distributed as 20% protein, 30-40% polypeptides, 30-40% amino acids, 10% nucleotides (23). Amino acids hold the most significance for fermentation performance and beer quality (Table 3.3). The uptake of wort amino acids uses a number of permeases, some specific for individual amino acids and a general amino acid permease (GAP) which has a broad substrate specificity (5). It has been recognized that there are 16 different amino acid transport systems in yeast (21). Out of these 16 different transport systems, 12 are constitutive and the remaining 4 are subject to regulation by the nitrogen sources present in the growth medium. The presence of an outside supply of certain nitrogenous nutrients renders the utilization of other nitrogenous compound by repressing the enzymes responsible for their uptake (20). Amino acid

uptake is an active process which requires energy. The patterns of uptake are complex and there are several regulatory mechanisms being evident (5). Therefore, the spectrum of permeases present, their specificity, competition for binding to individual permeases and feedback inhibition of specific permeases by amino acids in the intracellular pool and feedback inhibition of specific permeases by amino acids in the intracellular pool and other nitrogenous components are all influential (5).

The GAP permease is a high-affinity type, which is one of the group subjects to nitrogen catabolite repression. That means the maximum activity of this carrier is only expressed when nitrogen is limiting. It was concluded that the specific permeases were likely to be involved in uptake of amino acid for anabolic pathways, and protein synthesis, whereas GAP permease and other others, which show nitrogen catabolite repression, had catabolic roles. This provides an explanation as to why some amino acids are used in preference to ammonia when it is supplied as a mixture. Thus although nitrogen gas is a preferred nitrogen source for catabolic reactions, certain amino acids may be used first for direct incorporation into proteins (5).

Table 3.3 Classes of wort amino acids in order of assimilation during fermentation (Adapted from Boulton & Quain, 2001).

Class A	Class B	Class C	Class D
Arginine	Histidine	Alanine	Proline
Asparagine	Isoleucine	Ammonia	
Aspartate	Leucine	Glycine	
Glutamate	Methionine	Phenylalanine	
Glutamine	Valine	Tyrosine	
Lysine		Tryptophan	
Serine			
Threonine			

3.5 Small Scale Fermentations

It is necessary to have appropriate laboratory scale fermentations systems in order to study the biochemistry that occurs during brewery fermentation, to assess the properties of individual yeast strains, to screen and select new yeast strains and to develop novel processes. There is a quick miniature fermentation assay approved by the ASBC that has successfully been used to test malt fermentability and to distinguish between normal fermenting wort and wort that causes premature yeast flocculation. This assay use less wort and requires only 78 h for completion This fermentation assay involves warm fermentation temperatures of 21°C, test tube as fermentation vessels and 4% (wt/v) glucose supplemented wort (27). The fermentation profiles that this assay produces were found to be similar to those of tall tube fermentations (previous fermentation assay) and require fewer materials and time (27).

3.6 HPLC Analysis of Sugars

High performance liquid chromatography (HPLC) is an efficient and reproducible method of carbohydrate analysis. The column choice also plays a large role in carbohydrate analyses. The speed of analysis as well as the overall separation is based on column factors such as length, phase type and loading. In order to separate beer carbohydrates an ion exchange column is used (13).

Ion exchange chromatography, as the name suggests separates molecules by taking advantage of a charge differential. There are two approaches to ion exchange separations; cation and anion stationary exchange. The stationary phase is predominantly polymer based and functionalized with acidic groups to produce cation materials or basic groups to produce anion exchanges (30). Cation exchange media are generally of carboxylic acid or sulfonic acid functionality to promote weak and strong cation exchangers, respectively. Conversely, the weak and strong ion exchange media are typically functionalized as tertiary or quaternary amines. Ion exchange chromatography is generally carried out in aqueous environment where the charged components of a mixture are desorbed from the stationary phase by either changing the pH of the eluent or by adding a stronger counter-ion and effectively displacing the analyte (13).

A column developed specifically for the separation of those saccharides found in beer and corn syrup that can be used to monitor starch hydrolysis is the BP-100 Carbohydrate Ag+ from Benson Polymeric (Figure 3.9). This column provides rapid oligosaccharides separations through sulfonated highly cross-linked styrene-divinylbenzene copolymer resin in the silver form is stable and can resolve saccharides as large as DP-7 (4). This column requires an isocratic system and therefore uses de-ionized

water as its mobile phase. This water needs to be pre-filtered and degassed for greater performance and column life. Additionally all samples must be filtered before injected must be filtered to provide maximum protection for the analytical column (4).

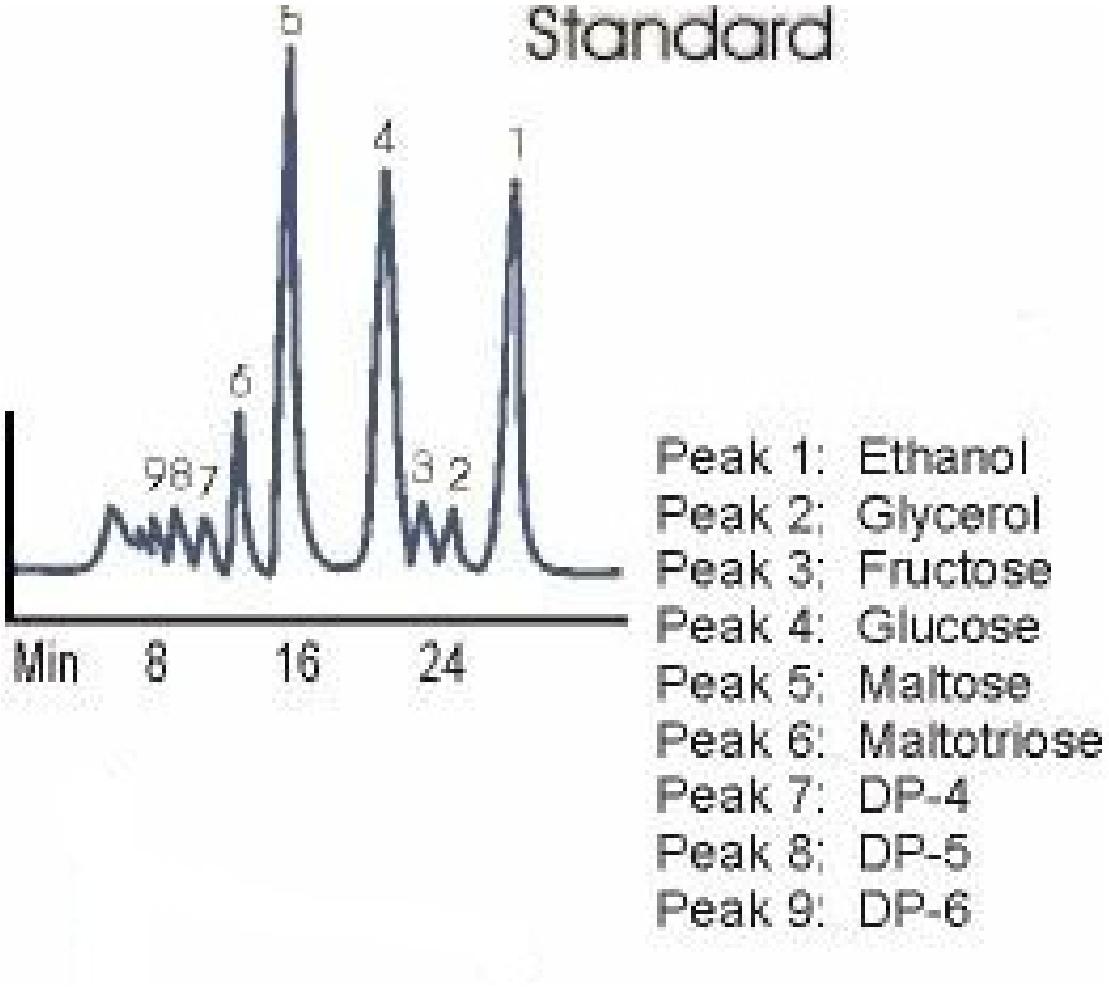

Figure 3.10 Chromatograph of a standard domestic beer from Benson Polymeric.

CHAPTER 4 MATERIALS AND METHODS

Figure 4.1 shows a schematic of the experimental design for this research. This research began with the idea of two experiments; fermenting with only yeast strain SMA and fermenting with yeast strain SMA and five other strains. When fermenting with SMA only, the experiments were broken down into a fermentation using a model wort with one sugar (either fructose, glucose, maltose or sucrose), and a fermentation using a model wort containing maltose only. These fermentations were then fermented in either a static or dynamic environment (or both) and absorbance and Plato values were measured then analyzed.

When comparing fermentations of 6 different yeast strains, the experiments consisted of using a model wort and a mixture of sugars (to resemble a true wort) then the fermentation was carried out in an dynamic environment where the samples were then measured for absorbance and Plato values and sugar concentrations at the beginning and end of fermentation.

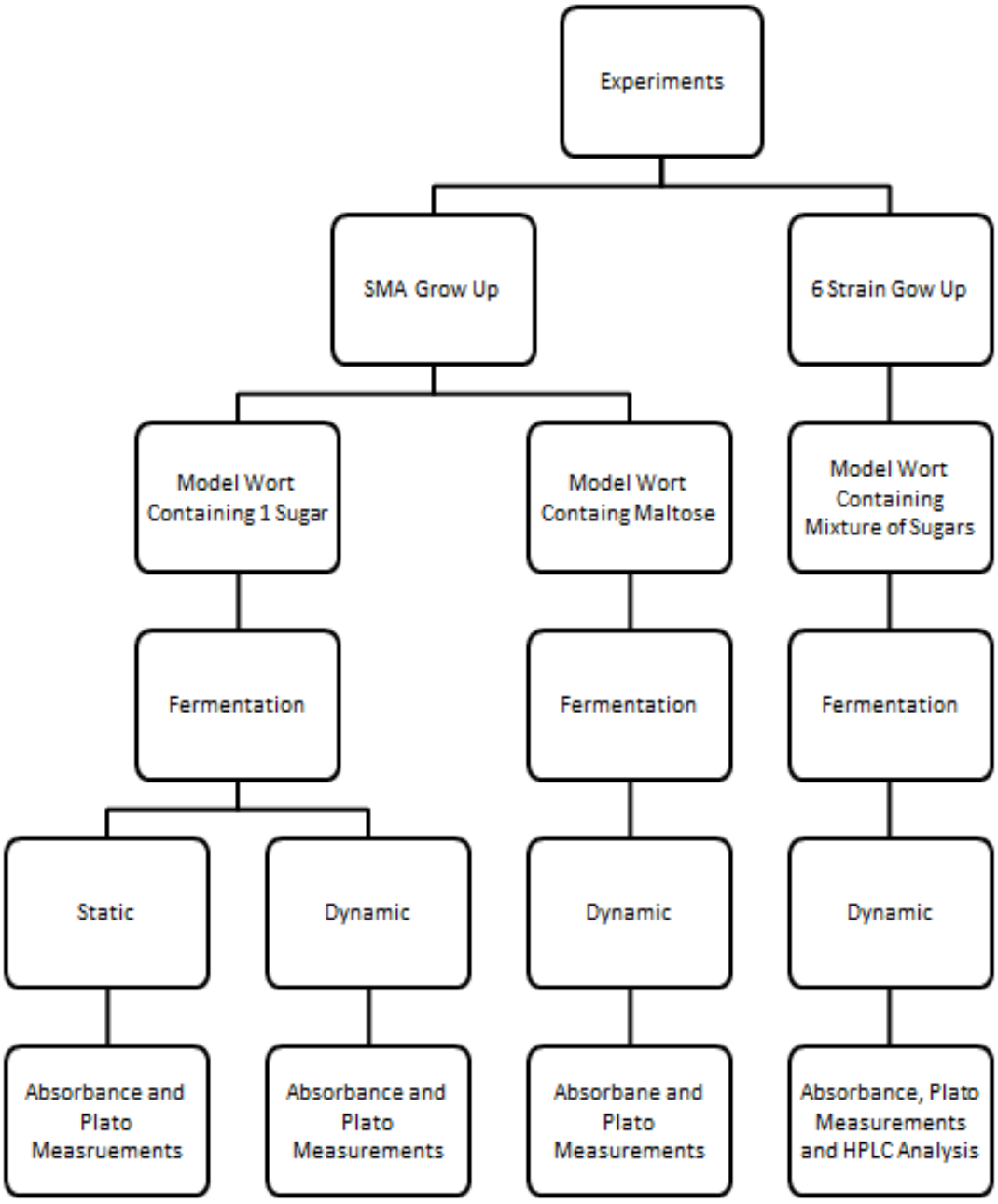

Figure 4.1 Schematic of experimental design.

4.1 Yeast Strains

Six yeast strains were chosen in order to examine their ability to ferment the same model wort (Table 3.1). Four strains were from the Labatt Culture Collection (LCC): 125, 1208, 1209, and 1240. One of the remaining two strains was SMA from Wyeast Laboratories while the other, referred to as Strain A was a proprietary industrial Australian strain.

Table 4.1 Description of the six yeast strains examined (33).

Strain	Ale/Lager	Level of Flocculence	Flocculation Phenotype	Genotype	Source
LCC 125	Ale	High	NewFlo	Not specified	40
LCC 1208	Unknown	Unknown	Unknown	α mating type, MAL, POF	NA
LCC 1209	Ale	Flocculent (level not specified)	Flo1	α mating type, MAL, POF	39, 41
LCC 1240	Ale	low	Flo1	Unknown	33
SMA	Lager	Medium	NewFlo	Not specified	49, 50
A	Lager	Not specified	NewFlo	Not specified	19

4.2 Yeast Cultures and Handling

The strains of yeast, Labatt Culture Collections 125, 1208, 1209 and 1240, SMA and strain A were originally received in both slanted and slurry form. The slurry was transferred to yeast extract peptone dextrose (YEPD) agar slants consisting of 20 g/L dextrose (BioShop, Burlington, ON), 20 g/L peptone (Bioshop, Burlington, ON), 10 g/L yeast extract (BioShop, Burlington, ON) and between 25-30 g/L agar (BD, Sparks, MD). The slants were incubated (New Brunswick Scientific Co., Edison, NJ), at 30 °C for 48 h. After incubation, the slants were stored at 4 °C and re-slanted every three to four months.

4.3 Yeast Grow-Up and Cell Count

The yeast grow-up and fermentation method followed the method from The American Society of Brewing Chemists (4). Aseptically, one loopful of yeast from a YEPD agar slant was inoculated into a 125 mL Erlenmeyer flask containing 50 mL of YEPD broth. The flask was covered using a foam bung. Two 125 mL flasks were used per sample. The flaks were then shaken aerobically. After 24 hours of shaking at 30°C and 100 rpm, the yeast was then washed by centrifuging the slurry (3000 x g for 3 min) in 50 mL centrifuge tubes and then discarding the supernatant. The yeast pellet was resuspended in deionized water and then centrifuged, again. This process was done three times to ensure a clean yeast slurry. The washed yeast slurry was resuspended then a diluted with 0.1N sodium acetate buffer (Yeast 4). Cells were counted using a haemocytometer (Hausser Scientific Partnership, Horsham, PA), and the following equation was used to determine the amount of yeast slurry needed to pitch into 250 mL YEPD in Erlenmeyer flasks:

$$vi = \frac{r * V0}{n} \quad (4.1)$$

Where vi is the volume of yeast slurry (mL), r is the standard pitching rate (1.5 x 10^7 cells/mL), v_0 is the wort volume and n is the cell count of the slurry (cells/mL)

After the vi volume of yeast slurry was calculated, that volume was inoculated into four 250 mL Erlenmeyer flasks that each contained 100 mL of YEPD broth per sample. These flasks were then incubated in the same manner as the 125 mL Erlenmeyer flasks.

4.4 Model Wort

The wort contained 12 g/L malt extract (BD, Sparks, MD) and 13.5 g/L peptone (BioShop, Burlington, ON) to ensure excess nitrogen. Depending on the experiment, 77.88 g (14.9% w/v) of sugar fructose (BioShop, Burlington, ON), glucose (BioShop, Burlington, ON), maltose (Fisher Scientific, Fair Lawn, NJ) or sucrose (Fisher Scientific, Fair Lawn, NJ)) or 16 g/L fructose (BioShop, Burlington, ON) and glucose (BioShop, Burlington, ON), 78.7 g/L maltose (Fisher Scientific, Fair Lawn, NJ) and 6.7 g/L sucrose (Fisher Scientific, Fair Lawn, NJ). The model wort was autoclaved (Soma Technology, Inc., Bloomfield, CT) for 20 min at 121 °C then stored at 4 °C for 24 h.

4.5 Fermentation

4.5.1 Adjustment and Aeration

Depending on the experiment, 18 g (4%) of lab grade D-glucose (BioShop, Burlington, ON) was added to 450 mL of the cooled wort. The wort was then aerated to saturation by using a medical grade oxygen tank (Vital Aire, Mississauga, ON) and bubbling oxygen into the wort for 5 minutes.

4.5.2 Pitching

After the second 24 h of shaking at 21°C and 100 rpm, the yeast was then washed in the same manner as described in Section 4.2. All identical yeast strains were then combined in a single tube and spun down one final time. The washed yeast was resuspended in sterile distilled water, pitched and counted in the same manner as described in Section 4.2, and added into 450 mL of aerated wort to the rate of $1.5x10^7$ cells/mL.

4.5.3 Fermentation Conditions

The pitched wort was placed in 30-39, 20 mL sterile test tubes at 15 mL aliquots. Each test tube contained 1 large, PTFE, sterile boiling stone. The test tubes were sealed with a sterile foam bung and fermented for 143-194 hours at 21 °C in either a static water bath or an orbital shaker (New Brunswick Co., Edison, NJ).

4.5.4 Experimental Measurements

Triplicate turbidity and density readings were taken at 0, 6, 22, 26, 30, 46, 50, 54, 70, 74, 78, 122, 194 hours or as close to these times as possible. Three 15 mL samples were measured at each sampling time. The turbidity of the samples was measured at 600nm (Thermo Scientific, Mississauga, ON). An aliquot of 3.5 mL of the sample was taken from the top of each of the three test tubes using a pipette. Once the sample has been transferred into the cuvette, the cuvette was then tapped before measurement to ensure any bubbles attached to the walls were dislodged. Additionally, the absorbance samples are measured against a blank of deionized water. The fermenting samples were filtered through 20-25 μm filter paper (Whatman Ltd., Maidstone, GBR) to prevent interference from gas bubbles, into a clean test tube until filtrate was at a depth of 2cm.

The density of the filtrate was measured in degrees Plato (°P) with a DMA 35N density meter (Anton Parr, Graz, AUT).

4.6 Non-Linear Modeling

Turbidity measurements were analyzed using the following Gaussian equation:

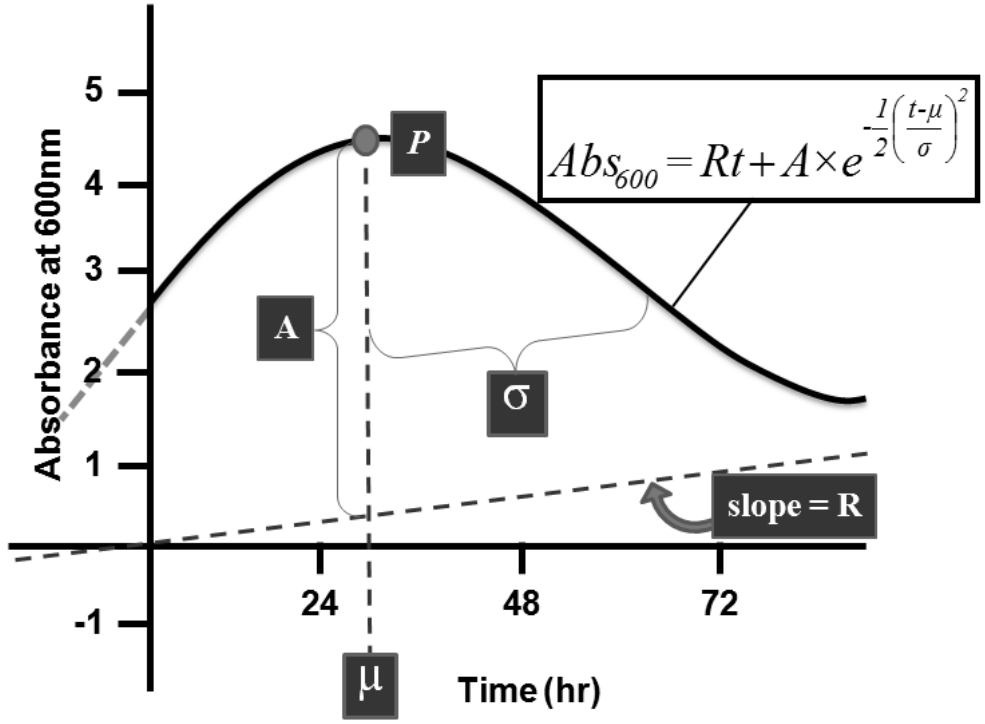

Figure 4.2 Absorbance Curve- Non-linear tilted-Gaussian fit (3).

where the mean μ variance, σ^2 A the amplitude and R the slope were determined using Prism (Ver. 5.0b, Prism Software Corporation, Irvine, CA). The parameters μ and σ2 for each sample were analyzed using a one-way ANOVA with $\alpha = 0.05$ and comparisons were completed to determine differences between the samples.

Density measurements were analyzed using the Logistic equation described in Figure 4.3.

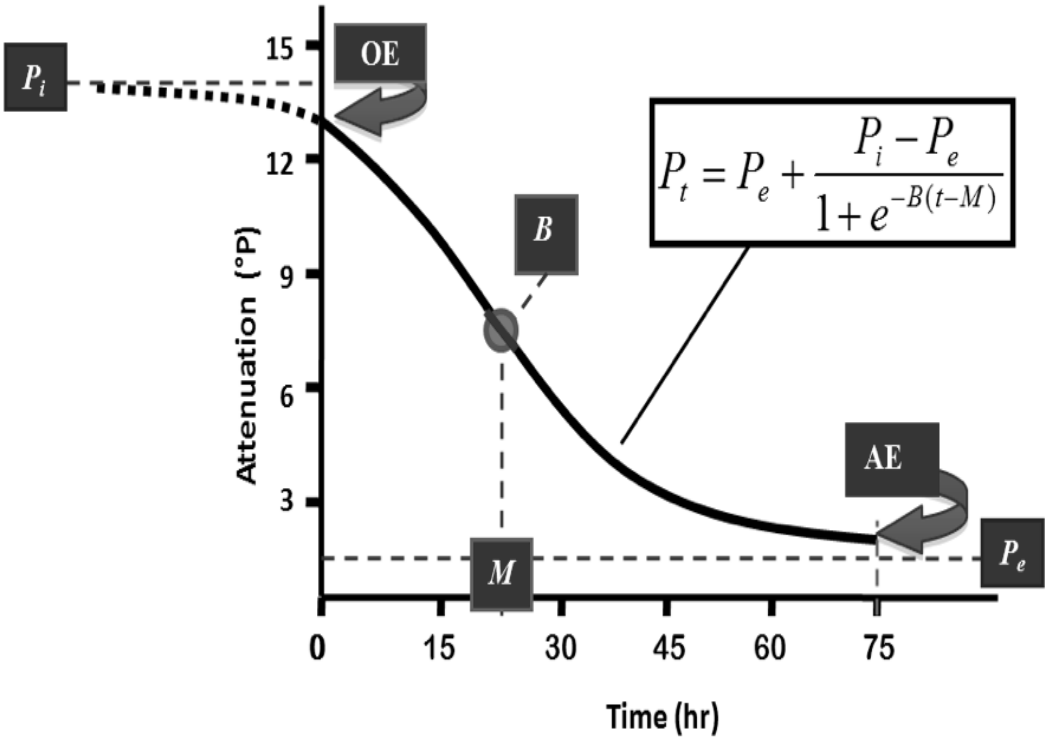

Figure 4.3 Apparent Extract Curve- Non-linear sigmoidal fit of decline in density (3).

where Pt is the extract value (°P) at time t, Pe is the final asymptotic extract value (°P), Pi is the initial asymptotic extract value (°P), B (°P/h) is the rate of fermentation which is proportional to the slope at the inflection point and M is the time to reach the inflection point (h) using Prism. The parameters Pe, Pi, B and M, for each sample were analyzed using a one-way ANOVA with $\alpha = 0.05$ and comparisons were completed to determine differences between the samples.

4.7 Fermentation Part 1: SMA Yeast, One Sugar

SMA yeast was grown-up as described in Section 4.2. Eight 500mL of model wort was made containing 12 g/L malt extract, 13.5 g/L peptone and 74.88g (14.9%) of fructose, glucose, maltose or sucrose and autoclaved for 20 min at 121°C. The autoclaved

wort was cooled for 24 h at 4 °C. After 24 h, the cooled wort was measured to 450 mL then aerated for 5 min, as described in Section 4.4.1. The yeast that had been growing for 48 h was washed and pitched into 450 mL of aerated wort at the rate of $1.5x10^7$ cells/mL. The pitched wort was dispensed into in 30, 20 mL test tubes at 15 mL aliquots, each containing one large sterile boiling stone. The test tubes were sealed with a foam bung and fermented for 143 hours at 21 °C. Four samples of the pitched wort containing one type of sugar were fermented in either a static water bath while the other four samples were fermented in an orbital shaker. Triplicate turbidity and density measurements were taken at 0, 6, 22, 26, 30, 46, 50, 54, 70, 74, 78, 120, 143 hours. These measurements were analyzed using the Gaussian and Logistic equation.

4.8 Fermentation Part 2: SMA Yeast, Four Different Slants

Four slants of SMA yeast were grown-up as described in Section 4.2. Four 500mL of model wort was made containing 12 g/L malt extract, 13.5 g/L peptone and 77.88 g (14.9%) maltose then autoclaved for 20 min at 121 °C. The autoclaved wort was cooled for 24 h at 4 °C. After 24 h, the cooled wort was measured to 450 mL then aerated for 5 min, as described in Section 4.4.1. The yeast that had been growing for 48 h was washed and pitched into 450 mL of aerated wort to the rate of $1.5x10^7$ cells/mL. The pitched wort was placed in 30, 20 mL test tubes at 15 mL aliquots, each containing one large sterile boiling stone. The test tubes were sealed with a foam bung and fermented for 144 hours at 21 °C, 175 rpm in an orbital shaker. Triplicate turbidity and density measurements were taken at 0, 6, 22, 26, 30, 46, 50, 54, 70, 74, 78, 120, 144 hours. These measurements were analyzed using the Gaussian and Logistic equation.

4.9 Fermentation Part 3: Six Yeast Strains, Mixture of Sugars

Labatt Culture Collections 125, 1208, 1209 and 1240, SMA and strain A were grown-up as described in Section 4.2. Each yeast strain was fermented in triplicate. 500 mL/strain of model wort was made containing 12 g/L malt extract, 13.5g/L peptone and 16 g/L fructose and glucose, 78.7 g/L and 6.7 g/L sucrose then autoclaved for 20min at 121°C. The autoclaved wort was cooled for 24 h at 4 °C. After 24 h, the cooled wort was measured to 450 mL then aerated for 5 min, as described in Section 4.4.1. The yeast that had been growing for 48 h was washed and pitched at into 450 mL of aerated wort to the rate of $1.5x10^7$ cells/mL. The pitched wort was placed in 30, 20 mL test tubes at 15 mL aliquots, containing 1 large sterile boiling stone. The test tubes were sealed with a foam bung and fermented for up to 194 hours at 21°C at 175 rpm in an orbital shaker. Triplicate turbidity and density measurements were taken at 0, 6, 22, 26, 30, 46, 50, 54, 70, 74, 78, 122, 194 hours. These measurements were analyzed using the Gaussian and Logistic equation. After density readings at the beginning and end of fermentation, 1.5mL of the sample was placed in an eppendorf tube and stored at -35°C for sugar and alcohol analysis.

4.10 Sugar, Glycogen and Alcohol Analysis

The frozen eppendorf samples were thawed at room temperature. Once thawed, the samples taken at the beginning of fermentation were diluted 1:10 while samples taken at the end of fermentation were not diluted. Using a 10mL syringe each sample was transferred to a HPLC vial, after being filtered (0.45 μm) prior to analysis. Once all the samples were filtered and transferred to HPLC (Waters, Milford, MA) vials they were put through the HPLC for sugar and alcohol analysis. The column used for carbohydrate and

alcohol detection was BP-100 Ag+, Silver form for Carbohydrate Analysis column (Benson Polymeric, Reno, NV) and the solvent used was deionized, degased water. The HPLC produced peaks and calculated their areas. These areas were then calculated into concentrations, taking into account the dilution factor and the concentration of the standards used.

4.11 Yeast Viability

4.11.1 Reagents

Yeast viability was determined using a slide-culture technique (Yeast 6) from The American Society of Brewing Chemists (4). To prepare the medium needed 0.3 g malt extract, 0.3 g yeast extract, 1.0 g glucose, 0.5 g peptone, 6.0 g maltose, 1.5g agar, 1.5 g zinc sulfate dissolved in 100 mL distilled water then transfer 1 mL of this solution and transfer to 100mL of distilled water.

4.11.2 Methods

Once homogeneous the mixture was then autoclaved for 20 min at 121 °C. After sterilization, 1mL of molten MYGP medium was spread into six Petri dishes over a glass slide. Once the agar solidified, two drops of a yeast in suspension (1×10^6 cells/mL) was placed onto the surface of the agar at each end of the slide. The Petri dish was covered and incubated at 25 °C for 12-16 h. After incubation the slide cultures were examined at 200-250x magnification.

4.11.3 Calculations

Cells that gave rise to microcolonies were counted as viable while single cells that had not giving rise to microcolonies were considered dead. These counts were then used for the following yeast viability calculation:

$$\%\ Viable\ Cells = \frac{Number\ of\ Microcolonies}{Total\ Number\ of\ Cells\ and\ Microcolonies}\ x\ 100 \quad (4.2)$$

4.12 Free Amino Nitrogen

4.12.1 Reagents

The quantity of free amino nitrogen in wort available to yeast during fermentation was determined using a method from The American Society of Brewing Chemists (method Wort 12) (4). This method measures amino acids, ammonia, and, to some extent, end-group α-amino nitrogen in peptides and proteins. The reagents used were ninhydrin colour reagent consisting of 3.964 g disodium hydrogen phosphate (Fisher, Fair Law, NJ), 2.378 g monosodium phosphate (Fisher, Fair Law, NJ), 0.198 g ninhydrin (Sigma-Aldrich, Oakville, ON), 0.119 g fructose (BioShop, Burlington, ON) diluted to 100 mL distilled water; dilution solution consisting of 2 g potassium iodate (Fisher, Fair Lawn, NJ) in 600 mL distilled water and 400 mL 95% ethanol; glycine standard stock solution consisting of 0.1072 g glycine (BioShop, Burlington, ON) in 100 mL distilled water and glycine standard solution consisting of 1 mL glycine standard stock solution in 100 mL distilled water. This glycine standard solution contains 2 mg/L of amino nitrogen.

4.12.2 Methods

After the reagents were prepared 2 mL glycine standard solution was transferred to test tubes in triplicate and model wort was prepared as described in Section 4.2. One mL of model wort was transferred into 100 mL distilled water then 2 mL of diluted wort was transferred into test tubes in triplicate. The blank was then prepared using 2 mL of distilled water and placed into test tubes in triplicate. One mL of ninhydrin colour reagent

was added to all 9 test tubes. The tubes were covered and heated for 16 min in boiling water then cooled for 20 min at 20 °C. An aliquot of 5 mL of dilution solution was added to all 9 test tubes after cooling. The test tubes were mixed thoroughly using a vortex (Scientific Industries, Bohemia, NY) and absorbance measured at 570 nm against distilled water. After readings were obtained, average absorbance measurements for the triplicate samples were calculated.

4.12.3 Calculations

The average absorbance for the glycine standard was subtracted from the average absorbance of the blank, while the average absorbance for the diluted wort samples were also subtracted from the average absorbance of the blank. From these subtractions the free amino nitrogen concentration was calculated using the following equation:

$$\text{Free Amino Nitrogen } \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{net absorbance of wort}}{\text{net absorbance of glycine standard}} \text{ x 2 x 100} \qquad (4.3)$$

In the above equation, 2 represents the concentration of amino nitrogen in the glycine standard solution while 100 represents the dilution factor. The reported Free Amino Nitrogen is reported in mg/L and is rounded off to whole numbers.

CHAPTER 5 RESULTS AND DISCUSSION

5.1 Fermentation Part 1: SMA Yeast, One Sugar

5.1.1 Absorbance and Apparent Extract Static Environment

Absorbance measured at 600 nm is an indication of yeast in suspension as it provides the relative amount of suspended solids present during fermentation at a specific time. A measure of the degree of flocculation is made by observing the change in turbidity over as time as the yeast flocs aggregate and fall out of suspension. Absorbance curves describe the characteristic growth phase of yeast cell in a nutrient media. The start of the curve denotes suspended cells present in a given volume of wort after pitching. Yeast cells then start consuming sugars and nutrients present in wort and produce alcohol and CO_2. The log phases involve growth and cell division which increases yeast cell numbers causes an increase in absorbance. At the point where nutrient depletion occurs, the cells reach stationary phase where the growth and division stop. When yeast cells stop dividing they begin to flocculate and sediment at the bottom of the fermentation tubes. This marks the decline in turbidity and absorbance will begin to approach zero as yeast continues to floc and fall out of solution. Unfortunately, there has been no accepted theory which provides a quantitative relationship between turbidity measurements and the degree of flocculation (11).

Figure 5.1 shows the changes in absorbance of four fermenting model worts containing either fructose, glucose, maltose or sucrose, as a carbon source for the SMA pitched yeast in a static water bath. It can be observed (with the aid of Table 5.1), that glucose yielded the highest amount of yeast in suspension at an absorbance of 1.769 ±

0.136, with fructose at an absorbance of 1.715 ± 0.127, followed by sucrose 1.690 ± 0.097 than maltose with an absorbance of 1.549 ± 0.082. It can also be observed that glucose reaches its average amount of yeast in suspension sooner than the other sugars at 35 h ± 2.1 into fermentation, with fructose at an average of 36 h ± 2.3, followed by sucrose 38 h ± 1.6 than maltose with an average amount of yeast in suspension of 47 h ± 2.6. There is quite a bit of noise in Figure 5.1 this is due to the nature of the experiment, fermenting in test tubes. The advantage of fitting these curves is that it clarifies the random variation of the fermentations. According to Table 5.1 the best fit fermentation to the Gaussian equation is sucrose, then maltose, then fructose followed by glucose. A global F-test showed that all four curves in Figure 5.1 to be significantly different (α=0.05, p=0.0003).

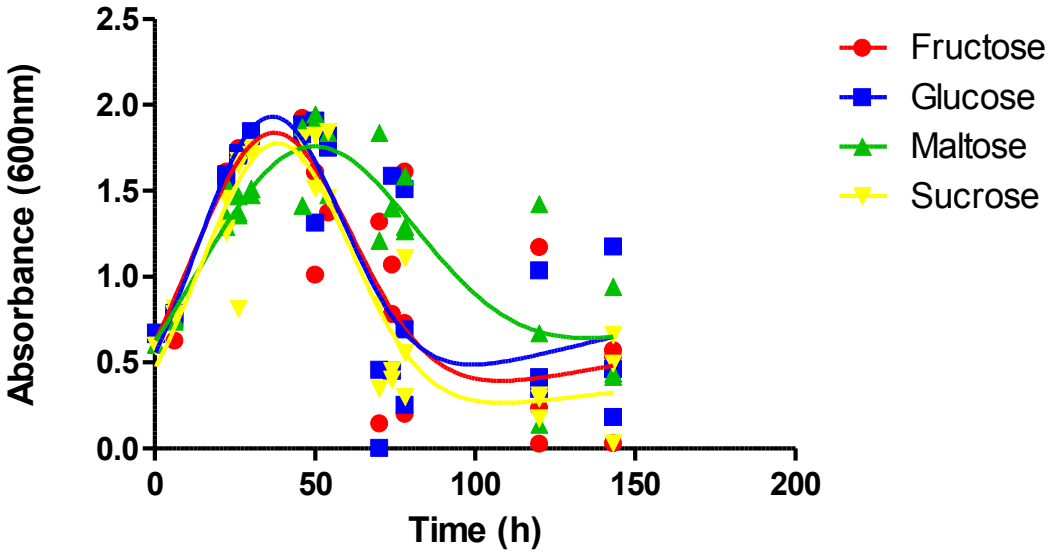

Figure 5.1 Comparison of decline in absorbance at 600 nm during fermentation of wort using one sugar in a static water bath at 21°C for 143h. The curves titled Gaussian fits of the absorbance data collected equation.

Table 5.1 Comparison of the statistical analysis of the absorbance during fermentation of wort using one sugar in a static water bath 21°C for 143 h.

	Fructose	**Glucose**	**Maltose**	**Sucrose**
Amplitude	1.715 ± 0.127	1.769 ± 0.136	1.549 ± 0.082	1.690 ± 0.097
Mean (hours)	36 ± 2.3	35 ± 2.1	47 ± 2.6	38 ± 1.6
SD	25.31 ± 2.49	23.06 ± 2.28	34.43 ± 3.22	23.46 ± 1.68
r	0.003 ± 0.001	0.005 ± 0.001	0.004 ± 0.001	0.002 ± 0.001
Coefficient of Determination	0.7006	0.6852	0.7816	0.8251
Absolute Sum of Squares	3.541	3.832	1.639	1.872
Number of Points Analyzed	33	33	33	33

Note ± values denote the asymptotic standard error.

Density (gravity) represents the relative amount of fermentable sugars (extract) present in the wort at the given time. Density or apparent extract is measured in degree Plato (°P) at specific time intervals after the beginning of a fermentation run. The highest density of the wort, which had been adjusted to 14.8 °P, was recorded at the start of a run. Once, the wort was pitched with yeast, the fermentable sugars were utilized by the yeast cells and converted to alcohol and carbon-dioxide. The utilization of sugars begins the decline of apparent extract with respect to time.

It is reasonable that the fermentation with glucose and fructose have higher amounts of yeast in suspension compared to the other sugars. Glucose and fructose molecules are taken up into the cell by facilitated diffusion (29). While maltose is taken up into the cell by an enzyme which splits maltose into two glucose units (16) and sucrose is metabolized and split into a fructose and glucose unit (29). Glucose and fructose are more easily taken up into the yeast cell compared to maltose and sucrose. Since fructose and glucose are more easily taken up, the fermentation's contained only those sugars have more CO_2 being produced resulting in a vigorous and movement, resulting in greater yeast in suspension than maltose and sucrose. This also explains why glucose reached its average amount of yeast in suspension sooner than fermentations with other sugars.

Figure 5.2 shows the changes in apparent extract of four fermenting model worts containing either fructose, glucose, maltose or sucrose, as a carbon source for the SMA pitched yeast in a static water bath. It can be observed (with the aid of Table 5.2), that fructose has the highest final asymptotic extract value of 2.073°P ± 0.433, then glucose at -0.6003°P ± 0.813, then maltose at -0.6116°P ± 0.496 then sucrose had a final asymptotic

value of -0.7304°P ± 0.869. These negative apparent extract values signify that the density was less than water which means that all the sugars were consumed and there was a high concentration of ethanol, since the density of ethanol is less than that of water. The fermentation with the highest initial asymptotic extract value is maltose with 23.59°P ± 3.36 then sucrose at 22.03°P ± 5.00, then glucose at 21.99°P ± 5.80, then fructose had the lowest final asymptotic value of 16.66°P ± 1.34. The fermentation with the steepest slope at the inflection point which is proportional to the rate of fermentation is fructose with 0.08425°P ± 0.01869, then glucose with 0.04423°P ± 0.01271, then sucrose with 0.04013 ± 0.01075 and the slowest fermentation is maltose with a slope of 0.03275°P/h ± 0.004841. The fermentation which was the fastest time to reach the inflection point was glucose at 22.29 h ± 12.43, then maltose at 24.22 h ± 8.48, then sucrose at 27.17 h ± 11.66, and the slowest fermentation to reach the inflection point is fructose at 28.34 h ± 2.97. A global F-test showed that all four curves in Figure 5.2 to be significantly different (α=0.05,p=0.0011).

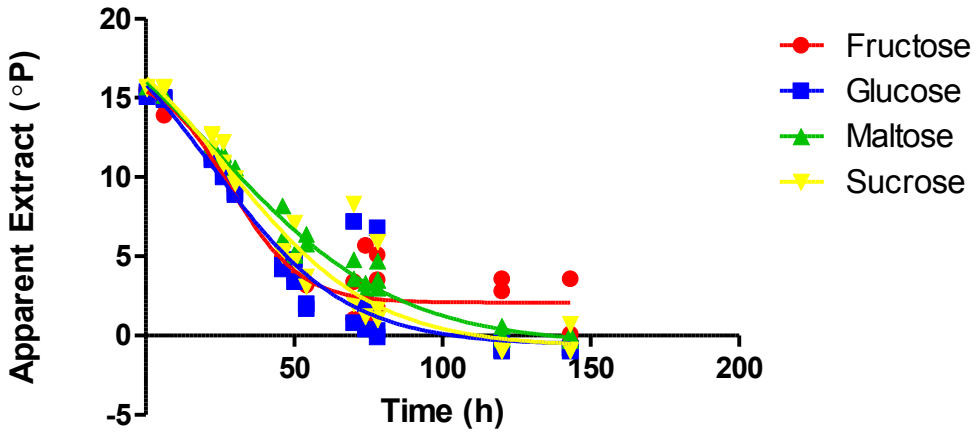

Figure 5.2 Comparison of decline in apparent extract during fermentation of wort using one sugar in a static water bath at 21°C for 143h. The curves represent logistic equation of the apparent extract data collected.

Table 5.2 Comparison of the statistical analysis of the apparent extract during fermentation of wort using one sugar in a static water bath at 21°C for 143h.

	Fructose	**Glucose**	**Maltose**	**Sucrose**
Pe	2.073 ± 0.433	-0.6003 ± 0.813	-0.6116 ± 0.496	-0.7304 ± 0.869
P0	16.66 ± 1.34	21.99 ± 5.80	23.59 ± 3.36	22.03 ± 5.00
B	0.08425 ± 0.01869	0.04423 ± 0.01271	0.03275 ± 0.004841	0.04013 ± 0.01075
M	28.34 ± 2.97	22.29 ± 12.43	24.22 ± 8.48	27.17 ± 11.66
Coefficient of Determination	0.9424	0.9285	0.9862	0.9384
Absolute Sum of Squares	50.4	80.04	13.33	71.39
Number of Points	33	33	33	33

Analyzed

Maltose had the highest value for apparent extract. This is reasonable as maltose is metabolized slower and therefore its fermentation has a higher value of extract than the other fermentations. It is also reasonable that fructose and glucose have lower initial apparent extracts as they are metabolized more quickly and therefore would have less extract compared to the other fermentation. This is also why glucose and fructose had steeper "B values" which is the slope and the inflection point and is proportional to the rate of fermentation; the steeper the slope, the faster the rate of fermentation.

5.1.2 Absorbance and Apparent Extract Dynamic Environment

Figure 5.3 shows the changes in absorbance of four fermenting model worts containing either fructose, glucose, maltose or sucrose, as a carbon source for the SMA pitched yeast in a dynamic environment. It can be observed (with the aid of Table 5.3), that all four fermentations are in significantly the same (α=0.05, p=0.0736). They all have the same highest level of yeast in suspension with an absorbance of 1.975 ± 0.058 and all four fermentations have an average yeast in suspension occur at the same point of 47h ± 1.2.

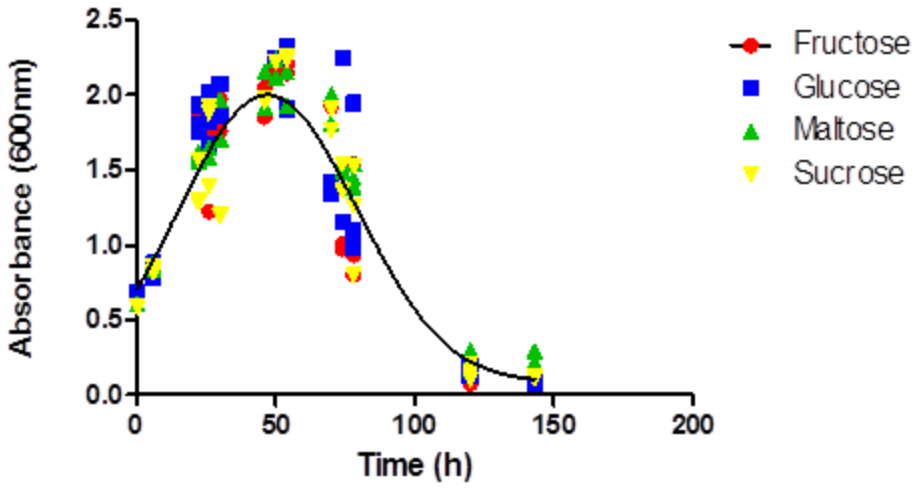

Figure 5.3 Comparison of decline in absorbance at 600 nm during fermentation by SMA yeast of wort using one sugar in a static water bath at 21°C for 143h. The curves represent tilted Gaussian fits of the absorbance data collected.

Table 5.3 Comparison of the statistical analysis of the absorbance during fermentation of wort using one sugar in an orbital shaker at 21°C for 143h.

	Global Curve
Amplitude	1.975 ± 0.058
Mean (h)	47 ± 1.2
SD	32.48 ± 1.533
r	0.001 ± 0.001
Coefficient of Determination	0.8901
Absolute Sum of Squares	1.88
Number of Points Analyzed	33

Figure 5.4 shows the changes in apparent extract of four fermenting model worts containing either fructose, glucose, maltose or sucrose, as a carbon source for the SMA pitched yeast in an dynamic environment. It can be observed (with the aid of Table 5.4), that all four fermentations are in significantly the same (α=0.05, p=0.2102). They all have the same final asymptotic extract value of -1.104 ± 0.253°P ± 0.4, the same initial asymptotic extract value of 17.82 ± 0.795, the same slope at the inflection point which is proportional to the rate of fermentation of 0.060 ± 0.005 and the same time to reach the inflection point at 33.32h ± 1.78.

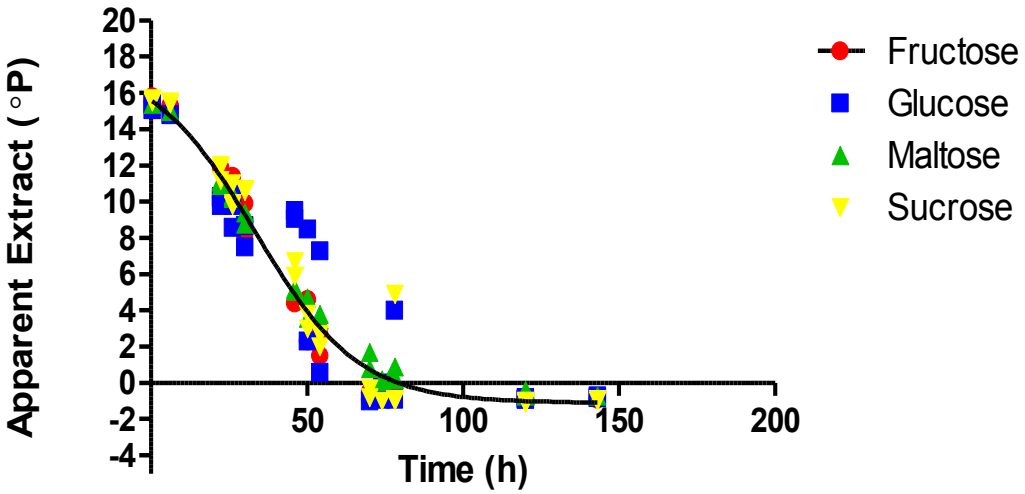

Figure 5.4 Comparison of decline in apparent extract during fermentation by the yeast of wort using one sugar in an orbital shaker at 21°C for 143h. The curves represent logistic equation of the apparent extract data collected.

Table 5.4 Comparison of the statistical analysis of the apparent extract during fermentation of wort using one sugar in a at 21°C for 143h.

	Global Curve
Pe	-1.104 ± 0.253
P0	17.82 ± 0.795
B	0.060 ± 0.005
M	33.32 ± 1.78
Coefficient of Determination	0.9838
Absolute Sum of Squares	21.73
Number of Points Analyzed	33

As the results show a vigorous fermentation has no effect on yeast in suspension and apparent extract therefore metabolism. It didn't matter that glucose and fructose are more easily metabolized. The vigorous fermentation counter acts the effects of flocculation. Since the yeast cells are not able to clump together, they remain suspended and have access to all nutrients. When yeast cells have flocculated together many yeast cells do not have access to nutrients and therefore fermentation slows. The vigorous fermentation of shaking and high levels of yeast in suspension create a high CO_2 production resulting in an even more vigorous fermentation.

As these four figures (5.1, 5.2, 5.3, and 5.4) depict, metabolism is not affected by flocculation when the fermentation is very vigorous. This means that in order to compare metabolism between yeast strains the effects of flocculation need to be removed by conduction a dynamic fermentation.

5.2 Fermentation Part 2: SMA Yeast, Four Different Slants

5.2.1 Absorbance

Figure 5.5 shows the absorbance values of four fermentations in model wort carried out by with four different yeast slants of SMA yeast containing maltose as the sugar source while fermenting in an orbital shaker. It can be observed (with the aid of Table 5.5), that the fermentation started on July 5 with slant 2 has the highest amount of yeast in suspension at an absorbance of 2.329 ± 0.048, with July 9 and slant 4 at an absorbance of 2.236 ± 0.061, followed by July 3 with slant 1 at 2.224 ± 0.048 then July 7 and slant 3 with an absorbance of 2.189 ± 0.028. It can also be observed that July 7 reaches its average amount of yeast in suspension sooner than the other sugars at 44 h ± 0.6 into fermentation, with July 9 at an average of 48 h ± 1.5, followed by July 5 at 50 h ± 1.11 than July 3 with an average amount of yeast in suspension at 53 h ± 1.4. According to Table 5.5 the best fit fermentation to the Gaussian equation is July 7, then July 5, then July 3 followed by July 9. A global F-test showed that all four curves in Figure 5.5 to be significantly different ($\alpha=0.05$, $p<0.0001$).

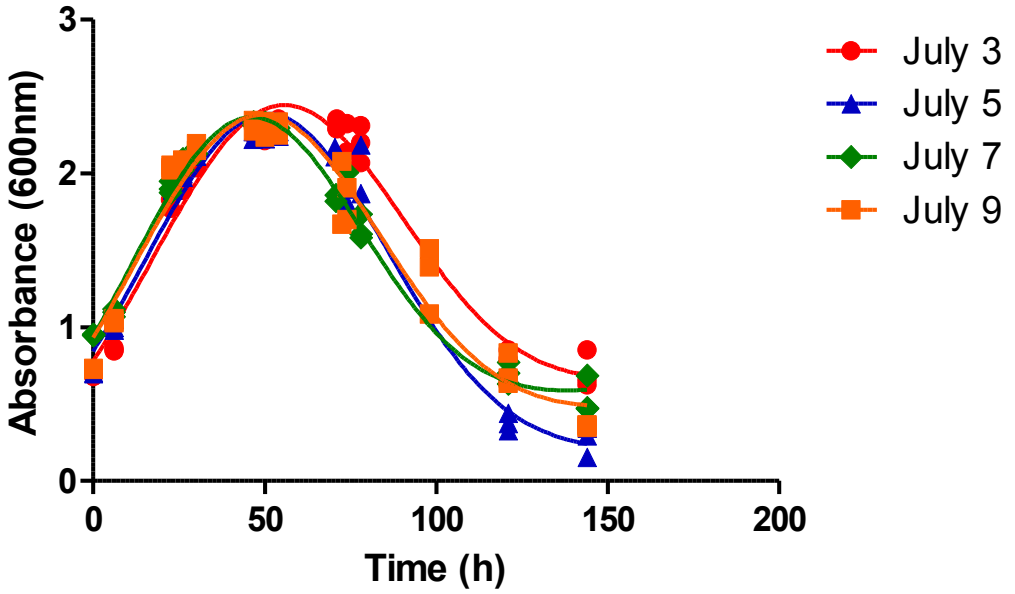

Figure 5.5 Comparison of decline in absorbance (600nm) during fermentation of four different yeast slants using maltose as a carbon source in an orbital shaker at 21°C for 144 h. The curves represent titled Gaussian fits of the absorbance data collected.

Table 5.5 Comparison of the statistical analysis of the absorbance during fermentation of four different yeast slants using maltose as a carbon source in an orbital shaker at 21°C for 144 h.

	July 3	**July 5**	**July 7**	**July 9**
Amplitude	2.224 ± 0.048	2.329 ± 0.048	2.189 ± 0.028	2.236 ± 0.061
Mean (h)	53 ± 1.4	50 ± 1.1	44 ± 0.6	48 ± 1.5
SD	36.79 ± 1.50	35.24 ± 1.31	34.03 ± 0.74	35.88± 1.61
r	0.004 ± 0.001	0.001 ± 0.001	0.004 ± 0.001	0.003 ± 0.001
Coefficient of Determination	0.9631	0.9703	0.9851	0.9432
Absolute Sum of Squares	0.5465	0.5264	0.1885	0.8841
Number of Points Analyzed	33	32	33	33

5.2.2 Apparent Extract

Figure 5.6 shows the apparent extract values of four fermentations of using a model wort with four different yeast slants of SMA yeast containing maltose as the sugar source while fermenting in an orbital shaker. It can be observed (with the aid of Table 5.6), that July 7 has the highest final asymptotic extract value of -0.555°P ± 0.103, then July 9 at -0.682°P ± 0.123, then July 5 at -0.717°P ± 0.096 then July 3 had a final asymptotic value of -0.810°P ± 0.129. Again, these negative apparent extract values signify that the density was less than water. The fermentation with the highest initial asymptotic extract value is July 9 with 20.61°P ± 1.02 then July 5 at 20.16°P ± 0.62, then July 3 at 19.56°P ± 0.63, then July 7 had the lowest final asymptotic value of 18.75 ± 0.51. The fermentation with the

steepest slope at the inflection point which is proportional to the rate of fermentation is July 7 with 0.064 ± 0.003, then July 9 with 0.056 ± 0.003, then July 5 with 0.054 ± 0.002 and the slowest fermentation is July 3 with a slope of 0.046 ± 0.002. The fermentation which was the fastest time to reach the inflection point was July 9 at 20.27 ± 1.93, then July 5 at 22.48 ± 1.23, then July 7 at 24.74 ± 0.99, and the slowest fermentation to reach the inflection point is July 3 at 29.04 ± 1.52. A global F-test showed that all four curves in Figure 5.6 are significantly different (α=0.05, $p<0.0001$).

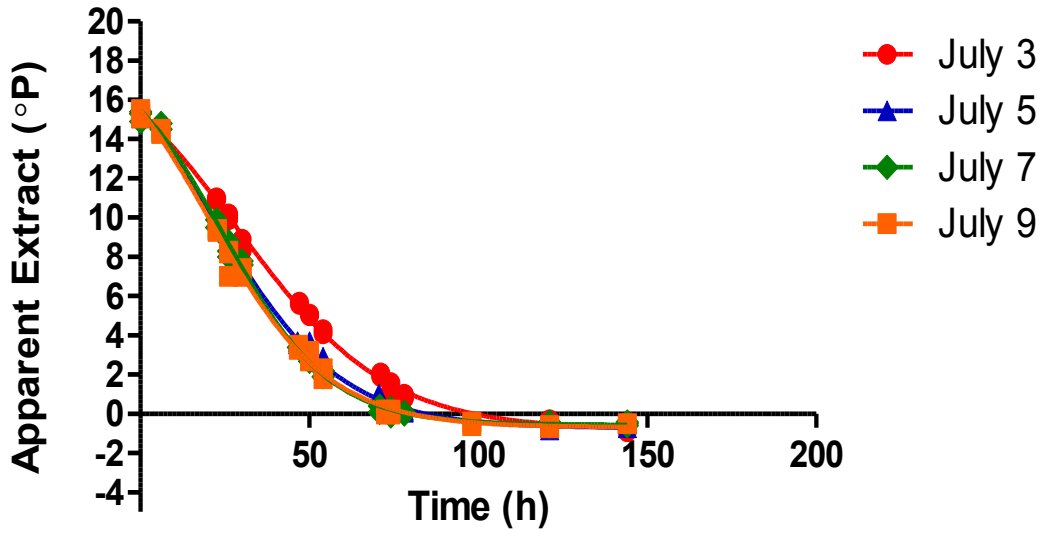

Figure 5.6 Comparison of the decline in apparent extract during fermentation of four different yeast slants using maltose as a carbon source in an orbital shaker at 21°C for 144 h. The curves represent logistic equation of the apparent extract data collected.

Table 5.6 Comparison of the statistical analysis of the apparent extract during fermentation of four different yeast slants using maltose as a carbon source in an orbital shaker at 21°C for 144 h.

	July 3	**July 5**	**July 7**	**July 9**
Pe	-0.810 ± 0.129	-0.717 ± 0.096	-0.555 ± 0.103	-0.682 ± 0.123
P0	19.56 ± 0.63	20.16 ± 0.62	18.75 ± 0.51	20.61 ± 1.02
B	0.0456 ± 0.002	0.0538 ± 0.002	0.0642 ± 0.003	0.0568 ± 0.003
M	29.04 ± 1.52	22.48 ± 1.23	24.74 ± 0.985	20.27 ± 1.93
Coefficient of Determination	0.998	0.9985	0.998	0.997
Absolute Sum of Squares	2.063	1.526	2.203	3.122
Number of Points Analyzed	33	33	33	32

The results indicate that conduction of the same experiments four times in triplicate will produce statistically different fermentations. However, this is to be expected with the use of biological materials as in biology an experiment which is repeated under the same conditions will give a comparable but not the same result (45). This is what has occurred here, the same yeast strain was used and was fermented under the same conditions however lead to similar but not exactly the same (statistically) results. This proves that for future experiments, fermentations need to be done many times in order to observe a true average of measured values during fermentations.

5.3 Fermentation Part 3: Six Yeast Strains, Mixture of Sugars

5.3.1 Absorbance

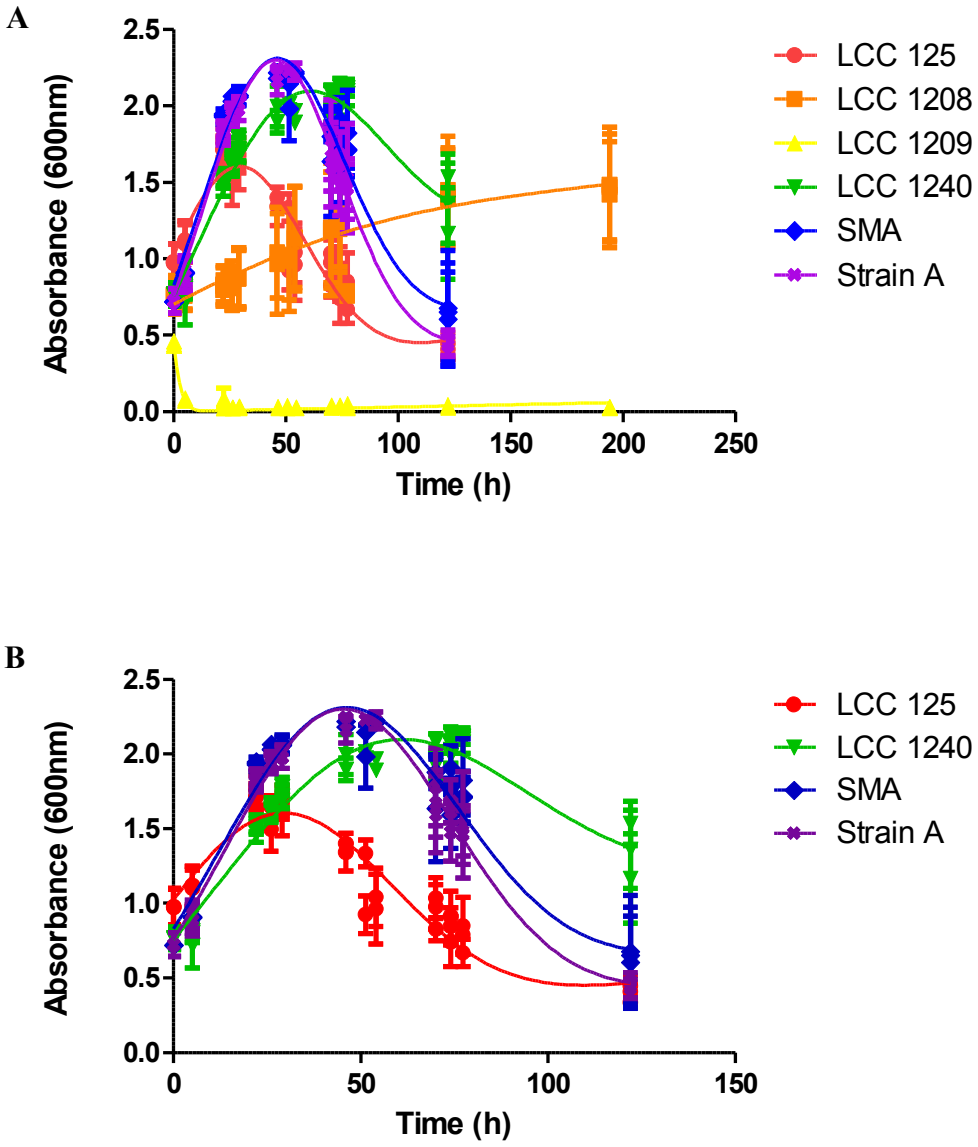

Figure 5.7 Comparison of decline in absorbance (600 nm) during fermentation by six different yeast strains in model wort containing a mixture of sugars as a carbon source in an orbital shaker at 21°C for 122-194 h. The curves represent tilted Gaussian fits. (A) Same curve as A without the abnormal curves of LCC 1208 and LCC 1209 (B).

Figure 5.7 shows the absorbance values of six different yeast strain fermentations using a model wort containing a mixture of sugars in an orbital shaker. It can be seen that all six curves are quite different from one another and two curves are abnormal; LCC 1208 and LCC 1209 do not follow the standard Gaussian shape indicating abnormal levels of yeast in suspension during fermentation. Yeast stain, LCC 1208 had a steady rise in yeast in suspension levels throughout fermentation and LCC 1209 had very little yeast in suspension throughout fermentation. Fermentations using SMA and Strain A had similar yeast in suspension levels throughout fermentation and at their highest values around the same point during fermentation. However, after this point SMA seemed to have more yeast in suspension at the end of fermentation. Yeast strain, LCC 125 had a normal yeast in suspension curve, however, it had lower values than SMA and Strain A, and reached a higher yeast in suspension value before the two. LCC 1240 also exhibited a normal yeast in suspension curve however it reached its highest level of yeast in suspension value after LCC 125, SMA and Strain A, and also ended with a higher yeast in suspension level at the end of fermentation.

Table 5.7 Comparison of the statistical analysis of the absorbance during fermentation of four different yeast strains using a mixture of sugars as a carbon source in an orbital shaker at 21°C for 122-194 h.

	LCC 125	LCC 1240	SMA	Strain A
Amplitude	1.499	1.654	2.097	2.158
Mean (h)	27	52	44	44
SD	31.01	42.01	32.22	30.43
r	0.003724	0.007821	0.004774	0.003193
	Std. Error			
Amplitude	0.040	0.057	0.058	0.045
Mean	1.4	2.4	1.1	0.7
SD	1.51	2.27	1.34	0.88
Coefficient of Determination	0.7593	0.8729	0.8215	0.8991
Absolute Sum of Squares	4.53	2.97	6.361	3.666
Number of points Analyzed	99	99	99	99

5.3.2 Apparent Extract

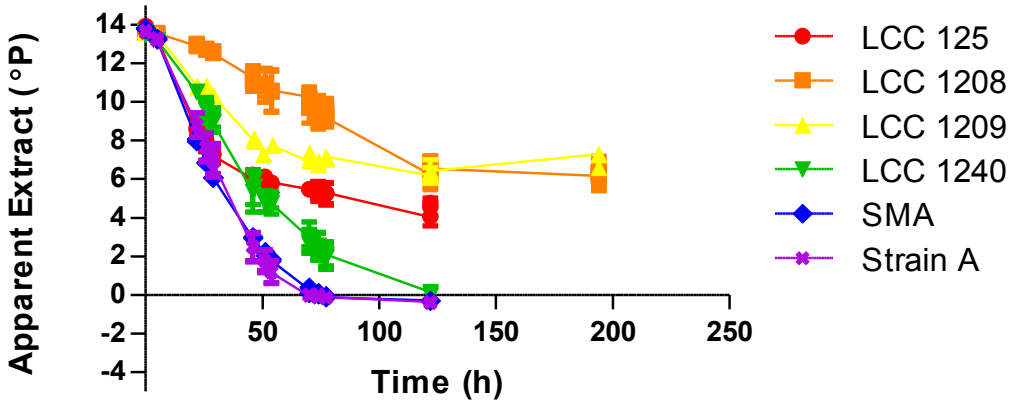

Figure 5.8 Comparison of the decline in apparent extract during fermentation of six different yeast strains using a mixture of sugars as a carbon source in an orbital shaker at 21°C for 122-194 h. The curves represent the fit of the logistic equation to the apparent extract data (n=9).

Figure 5.7 shows the apparent extract values of six different yeast strain fermentations using a model wort containing a mixture of sugars in an orbital shaker. It can be seen that LCC 1208 had the highest Plato values throughout fermentation and therefore did not ferment the wort to completion. LCC 1209 also had high Plato values throughout fermentation, however not as high as LCC 1208 but the two yeast strains had similar Plato values after 100 h into fermentation. LCC 125 had the third highest Plato values at the end of fermentation of around 5°P which indicates that fermentation was not completed. The other three strains, LCC 1240, SMA and Strain A did have end Plato values below 2°P, indicating that they completed fermentation. However, LCC 1240 was slower to complete while SMA and Strain A had similar Plato values throughout fermentation.

Table 5.8 Comparison of the statistical analysis of the apparent extract during fermentation of four different yeast strains using a mixture of sugars as a carbon source in an orbital shaker at 21°C for 122-194 h.

	LCC 125	**LCC 1240**	**SMA**	**Strain A**
Pe	5.157	-0.224	-0.509	-0.449
P0	18.98	17.52	19.61	15.66
B	0.077	0.042	0.057	0.077
M	8.22	31.16	16.94	26.43
		Std. Error		
Pe	0.133	0.319	0.120	0.165
P0	3.07	1.02	0.93	0.53
B	0.013	0.004	0.003	0.006
M	6.35	2.81	1.72	1.04
Coefficient of Determination	0.9553	0.9779	0.9936	0.9828
Absolute Sum of Squares	43.24	44.85	15.79	46.21
Number of points Analyzed	99	99	99	99

5.3.3 Carbohydrate and Alcohol Analysis

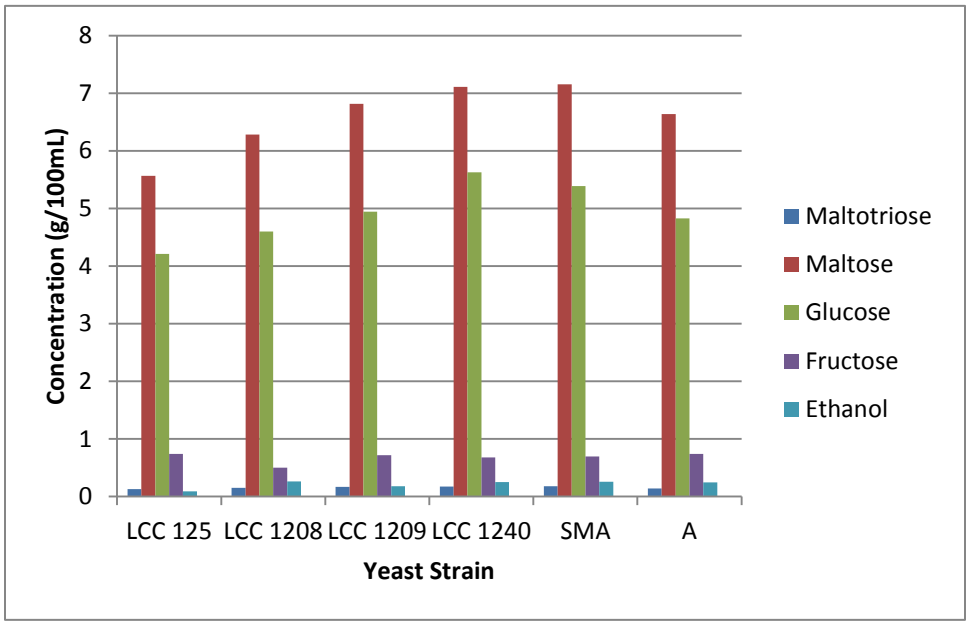

Figure 5.9 Initial sugar consumption by six different yeast strains after fermentation of model wort using a mixture of sugars as a carbon source in an orbital shaker at 21°C at t=0.25 h into fermentation.

Figure 5.8 shows the sugar consumption of each yeast strain at 0.25 h into fermentation. All fermentations began with the same amount of each sugar. After 0.25 h into fermentation it can be seen that LCC 215 utilized maltotriose, maltose, and glucose the fastest compared to other yeast strains and LCC 1208 utilized fructose the fastest. SMA was the slowest of the strains to utilize maltotriose and maltose, LCC 1240 was the slowest to use glucose and LCC 125 was the slowest to use fructose. LCC 1208 was the fastest at producing ethanol while LCC 125 was the slowest at producing ethanol. This may be an indication that utilizing fructose the fastest produces ethanol faster.

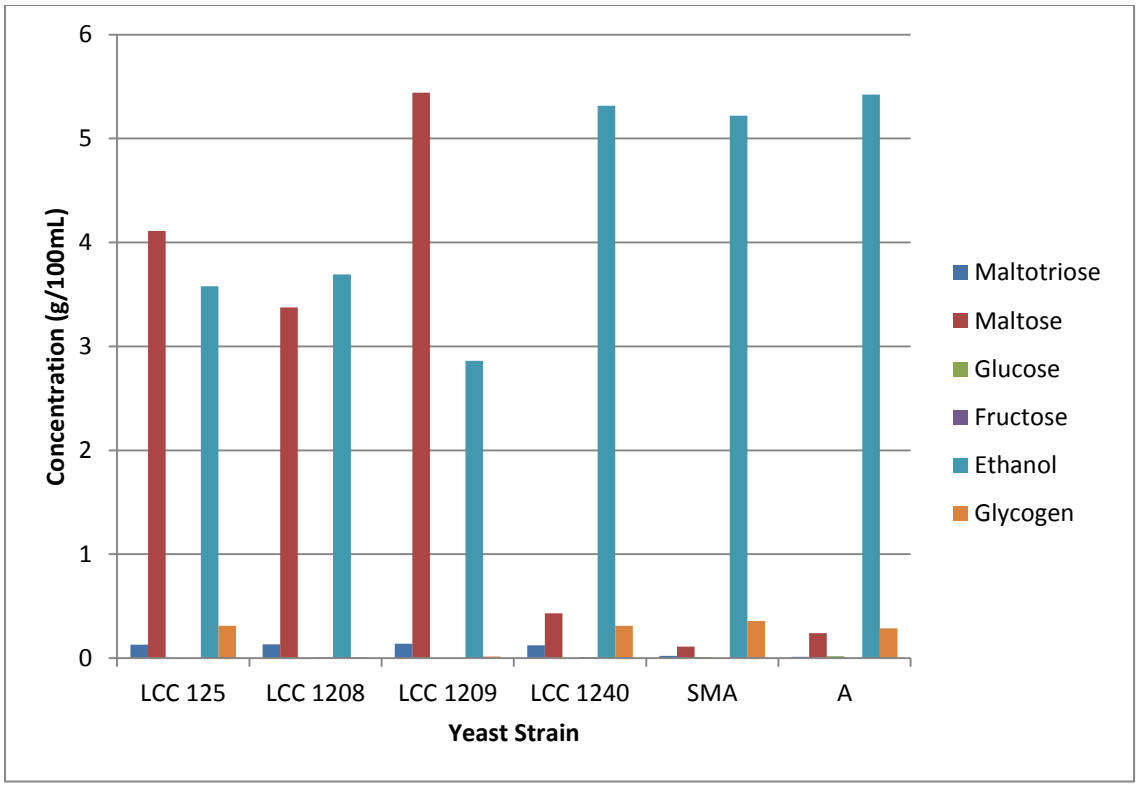

Figure 5.10 Final sugar consumption by six different yeast strains after the fermentation of model wort with a mixture of sugars as a carbon source in an orbital shaker at 21°C at t=122 and/or 194 h into fermentation.

Figure 5.9 shows the sugar consumption of each yeast strain at the end of fermentation (122 h or 194 h). At the end of fermentation it can be seen (Table 5.9) that Strain A utilized the most maltotriose while LCC 1209 utilized the least, SMA utilized the most maltose while LCC 1209 utilized the least; LCC 1209 and LCC 1240 utilized the most glucose while Strain A utilized the least and LCC 125, LCC 1240 and SMA utilized the most fructose while LCC 1240 utilized the least. Strain A produced the most ethanol and LCC 1209 produced the least amount. Glycogen is produced as a storage carbohydrate which is synthesized by glucose (5). SMA produced the most glycogen while LCC 1208 produced the least. This means that SMA consumed glucose the fastest

while LCC 1208 consumed glucose the slowest since when glucose levels fall below the necessary levels for growth, glycogen accumulation is triggered. Table 5.9 contains the numerical concentrations of the carbohydrates and alcohol analysis from the HPLC.

Table 5.9 Sugar and ethanol concentrations of the fermentation of six different yeast strains using a mixture of sugars as a carbon source in an orbital shaker at 21°C for 122-194h.

	Maltotriose (g/100mL)	**Maltose (g/100ml)**	**Glucose (g/100mL)**	**Fructose (g/100mL)**	**Ethanol (g/100mL)**	**Glycogen (g/100mL)**
Wort	0.0804 ± 0.0286	0.3299 ± 0.0645	0.4605 ± 0.0202	0.0188 ± 0.1421		0
Wort Plus	0.1779 ± 0.0295	7.1842 ± 0.0635	6.2582 ± 0.0147	0.7631 ± 0.2842	0.0068 ± 0.3452	0

Fermentation at t=0.25 h						
LCC 125	0.1306 ± 0.0347	5.5648 ± 1.3720	4.2082 ± 1.0303	0.7416 ± 0.3700	0.0894 ± 0.1265	0
LCC 1208	0.1527 ± 0.0206	6.2811 ± 0.8085	4.5987 ± 0.6092	0.5012 ± 0.3590	0.2628 ± 0.0155	0
LCC 1209	0.1687 ± 0.0166	6.8174 ± 0.6093	4.9418 ± 0.4418	0.7168 ± 0.0709	0.1817 ± 0.0704	0
LCC 1240	0.1749 ± 0.0313	7.1101 ± 1.1957	5.6272 ± 0.7532	0.6811 ± 0.1487	0.2498 ± 0.0166	0
SMA	0.1770 ± 0.0168	7.1529 ± 0.6379	5.3880 ± 0.5405	0.6948 ± 0.0964	0.2583± 0.0351	0
Strain A	0.1401 ± 0.0320	6.6381 ± 0.5654	4.8295 ± 0.3953	0.7397 ± 0.0836	0.2438 ± 0.0300	0
Fermentation at t=122 h						
LCC 125	0.1296 ± 0.0205	4.1124 ± 0.5080	0.0012 ± 0.0033	0	3.5792 ± 0.2916	0.3109 ± 0.0200
LCC 1208	0.1515 ± 0.0755	4.2240 ± 2.0495	0.0062 ± 0.0070	0.0192 ± 0.0222	3.6502 ± 1.0701	0.0062 ± 0.007
LCC 1209	0.1619 ± 0.0070	6.6893 ± 0.2100	0	0	2.4034 ± 0.0661	0.0155± 0.0070
LCC 1240	0.1247 ± 0.0155	0.4316 ± 0.2944	0	0.0096 ± 0.0031	5.3138 ± 0.1692	0.3121 ± 0.0142
SMA	0.0211 ± 0.0027	0.1119 ± 0.0131	0.0100 ± 0.0036	0	5.2196 ± 0.4605	0.3572 ± 0.0220
Strain A	0.0142 ± 0.0127	0.2412 ± 0.1110	0.0181 ± 0.0009	0.0011 ± 0.0031	5.4224 ± 0.1006	0.2862 ± 0.0117

	Fermentation at t=194 h					
LCC 1208	0.1320 ± 0.0100	3.3744 ± 0.3480	0.0039 ± 0.0562	0.0053 ± 0.0815	3.8919 ± 0.1656	0.0074 ± 0.0027
LCC 1209	0.1400 ± 0.0137	5.4403 ± 0.4928	0	0	2.5625 ± 0.1510	0.0170 ± 0.0013

Note that 'Wort" is wort without any added sugar while "Wort Plus" is wort with added sugar

As mentioned by Boulton & Quain in 2001 (5), lager yeast strains tend to assimilate maltotriose more rapidly than ales while ale yeast strains assimilate maltose more rapidly than lager strains. According to Figure 5.8, 5.9 and Table 5.9 the two known lager strains consumed maltotriose more rapidly than the ales which is in accordance to the present knowledge, however the ales used during this study did not follow the present knowledge. The known ales; LCC 125, 1209 and 1240 had a high concentration of maltose reaming after fermentation compared to the lagers. It is unsure why these particular yeast strains performed in a way opposite to what is known. Perhaps they did not assimilate maltose as they should because there was enough glucose present. The genes which encodes for maltose permease which splits maltose into two glucose units are repressed when there is a high concentration of glucose available (16). When the maltose concentration falls to an undetectable level maltotriose is metabolized (34). This is why maltotriose was not consumed during fermentation for LCC 125, 1209 and 1240.

LCC 125 is known to be a high flocculator (40). .LCC 125 begins to flocculate earlier during fermentation relative to the other strains, indicating a high flocculating yeast strain. LCC 1209 is a flocculent strain but the level is not known (39, 41). LCC 1209 falls out of solution very early during fermentation indicating that it is a very high flocculating

yeast strain. LCC 1240 is a low flocculator (Sobczak, J., personal communication, July 29, 1998). LCC 1240 is the last yeast strain out the six to flocculate and has the highest amount of yeast in suspension of all the strains at the end of fermentation. SMA is classified as a medium flocculator (19). SMA begins to flocculate later than LCC 125 but before LCC 1240 indicating it is a medium flocculator and also has a final yeast in suspension value between that of LCC 125 and LCC 1240. Strain A's flocculation level is unknown however it begins to flocculate around the same time as SMA and therefore Strain A could also be a medium flocculator.

LCC 125 has a NewFlo genotype which means that the yeast strain is flocculatant at the end of fermentation while the Flo1 genotype yields a yeast strain that is heavily flocculent throughout the fermentation (43). LCC 125 was observed to be flocculent at the end of fermentation as it had one of the lowest yeast in suspension values at the end of fermentation. LCC 1209 and 1240 both have Flo1 phenotypes. LCC 1209 is very flocculation throughout fermentation as the yeast flocculate near the beginning of fermentation and remain flocculated (even as fermentation is shaking). LCC 1240 also does flocculated throughout fermentation. LCC 1240 is that last to flocculate and has the highest yeast in suspension level compared to the other strains studied. Mannose inhibits flocculation in Flo1 phenotypes (43). Perhaps yeast strain, LCC 1240 may have been inhibited by a structure present in the model wort similar to mannose. It is known that fructose and glucose are assimilated rapidly in all strains while maltotriose is assimilated slowly while usually traces of maltotriose remain as observed for all yeast strains used in this study.

5.4 Yeast Viability and Free Amino Nitrogen Analysis

Table 5.10 Percentage of viable yeast cells during the fermentation of all experiments.

Yeast	% Viable Cells
LCC 125	97
LCC 1208	90
LCC 1209	99
LCC 1240	95
SMA	100
Strain A	99

Table 5.10 shows the percentage of viable yeast cells for yeast strain used. SMA had the most viable cells at 100% viable while LCC 1240 had the least at 95% viable. It is important that all yeast cells are healthy in this study as to not affect the outcome of the results. Table 5.10 shows that the yeast strains were healthy and therefore their health does not affect the results of this study.

The concentration of free amino nitrogen in the wort used for all experiments was 390 mg/L ± 3. It is important that the model wort contained enough FAN for healthy growth and an optimal fermentation and a FAN concentration in a wort of 14°P of 390 mg/L ± 3 of FAN is more than enough for adequate yeast growth. Free amino acid concentrations are within the range 150-250 mg/L in a wort of 10.5°P (5)

CHAPTER 6 CONCLUSION

It was found that flocculation significantly affects metabolism of yeast cells during fermentation of beer. When a fermentation is vigorous or when the fermenter is shaken, yeast does not have a chance to flocculate thereby allowing the yeast to have complete access to all available nutrient. It was discovered that when examining metabolism in yeast cells, the fermentation must take place in a dynamic environment to ensure that flocculation does not occur.

The vigorous shaking was an addition to the already, approved, miniature fermentation assay and it was vital to understand how reliable the results where once a new component was added to the method. It was discovered that the use of the shaking, along with the fermentation assay that the results were not statistically reproducible. Therefore it was decided that for future experiments, each fermentation would need to be completed in triplicates (in addition to the triplicates measured during each fermentation) to obtain an average and ensure comparability of future results.

With the six yeast strains used it was observed that if the yeast in suspension fell below the normal amount then it corresponded to a poor fermentation where the amount of carbohydrates metabolized were low, the rate of fermentation slow and the fermentation never actually reached completion to 2°P. Due to the poor fermentation, a high concentration of maltose and low ethanol concentration remained at the end of fermentation was determined by HPLC analysis. Such were noted for strain LCC125, LCC1208 and LCC 1209. It is important to note that yeast strains LCC 125 and LCC 1209 had low amounts of yeast in suspension during fermentation resulting in a poor fermentation, however, fermentation with LCC 1208 had a relatively high amount of

yeast in suspension during fermentation but still resulted in a poor fermentation. The cause of LCC 1208 having a poor fermentation despite the high amount of yeast in suspension is unknown (infected yeast does not seem to be a factor). The opposite results were found if the levels of yeast in suspension was high throughout the fermentation, that it would correspond to a good fermentation where the amount of carbohydrates metabolized were high, the rate of fermentation was fast and the fermentation reached completion to a final apparent extract to around 0°P. Due to the speedy fermentation, low concentrations of maltose and high ethanol concentrations were obtained at the end of fermentation. This fermentation pattern was observed in strains LCC 1240, SMA and Strain A. These results may be due to the strain being an ale or larger, the strains level of flocculence and/or the strains flocculation phenotype. These results were validated as the yeast strains were tested viable from a viability test and there was enough nitrogen in the wort for proper yeast growth from a FAN analysis.

These results could be used by any brewing company that is interested in using a different yeast strain than their usual. For example, if a brewery was to require a new yeast strain for their brew, a good recommendation would be yeast strain LCC 1240. Yeast strain LCC 1240 was the fastest fermenting strain in the present study. This characteristic would be attractive to a large company as the quicker the product can leave the facility, the lower the cost of production and the greater the profit. However, yeast strain LCC is a highly flocculent yeast strain, therefore fermentation with this strain would require attention in the form of stirring and rousing the yeast. For optimal results using this yeast, the fermentation should be monitored and stirred.

Additionally, these results show that this method of shaking and the mini-fermentation assay can be used for further experiments on other yeast strains to determine their sugar metabolism and their performance during fermentation.

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APPENDIX A: RAW DATA

Table A.1 Fermentation Part 1: SMA yeast, one sugar, and static environment- absorbance data.

Time (h)	Fructose	Glucose	Maltose	Sucrose
0.25	0.673	0.673	0.606	0.596
0.25	0.674	0.669	0.608	0.6
0.25	0.671	0.666	0.609	0.595
6	0.628	0.788	0.751	0.778
6	0.792	0.777	0.761	0.782
6	0.795	0.754	0.74	0.815
22.25	1.592	1.597	1.353	1.454
22.25	1.55	1.588	1.544	1.262
22.25	1.611	1.554	1.292	1.28
26	1.747	1.719	1.359	1.675
26	1.716	1.718	1.47	1.65
26	1.715	1.704	1.373	0.814
30	1.822	1.819	1.477	1.751
30	1.795	1.846	1.512	1.711
46	1.924	1.829	1.872	1.709
46	1.849	1.887	1.415	1.808
50	1.609	1.316	1.947	1.821
50	1.011	1.909	1.908	1.513
54	1.373	1.751	1.838	1.844
54	1.809	1.822	1.476	1.462
70	0.144	0.003	1.211	0.35
70	1.32	0.457	1.838	0.836
74	0.783	0.454	1.398	0.401
74	1.069	1.587	1.405	0.454
78	1.612	0.693	1.293	0.302
78	0.2	1.51	1.267	0.558
78	0.731	0.256	1.587	1.109
120	0.028	0.346	0.672	0.176
120	1.174	0.414	0.141	0.29
120	0.237	1.037	1.425	0.309
143	0.032	1.176	0.419	0.032
143	0.574	0.462	0.438	0.495
143	0.536	0.182	0.942	0.661

Table A.2 Fermentation Part 1: SMA yeast, one sugar, and dynamic environment-absorbance data.

Time (h)	Fructose	Glucose	Maltose	Sucrose
0.25	0.681	0.685	0.611	0.583
0.25	0.678	0.687	0.607	0.583
0.25	0.679	0.681	0.607	0.59
6	0.826	0.783	0.891	0.814
6	0.836	0.802	0.86	0.848
6	0.847	0.88	0.838	0.862
22.25	1.889	1.751	1.6	1.567
22.25	1.88	1.807	1.563	1.282
22.25	1.851	1.937	1.618	1.304
26	1.682	1.821	1.652	1.39
26	1.782	1.667	1.588	1.88
26	1.227	2.015	1.586	1.929
30	1.976	1.859	1.967	1.195
30	1.763	2.073	1.709	1.2
46	2.051	0.484	2.16	1.982
46	1.858	0.406	1.917	1.953
50	2.121	2.247	2.242	
50	2.176	0.699	2.116	2.223
54	2.21	2.329	2.155	2.23
54	2.144	1.91	1.93	2.256
70	1.924	1.423	2.007	1.766
70	1.378	1.342	1.814	1.905
74	0.976	1.154	1.547	1.538
74	1.009	2.248	1.495	1.361
78	0.934	0.988	1.391	0.798
78	1.543	1.1	1.444	1.259
78	0.801	1.947	1.545	1.529
120	0.121	0.199	0.243	0.105
120	0.099	0.127	0.176	0.206
120	0.077	0.144	0.294	0.146
143	0.145	0.131	0.219	0.114
143	0.105	0.123	0.292	0.11
143	0.08	0.075	0.203	0.123

Table A.3 Fermentation Part 1: SMA yeast, one sugar and static environment- apparent extract data.

Time (h)	Fructose	Glucose	Maltose	Sucrose
0.25	15.6	15.1	15.7	15.7
0.25	15.6	15.4	15.7	15.7
0.25	15.5	15.4	15.7	15.6
6	13.9	15	15.2	15.7
6	14.7	14.9	15.2	15.6
6	14.9	15	15.2	15.6
22.25	11.2	11.2	12.1	11.8
22.25	11.2	11.1	12	12.6
22.25	11.1	11.1	12.1	12.7
26	10.1	10.1	11.2	10.9
26	10.1	10.1	11.4	10.8
26	10.2	10	11.5	12.2
30	9.1	8.9	10.6	9.6
30	9.2	9	10.6	9.9
46	4.9	4.2	6.1	5.3
46	4.6	4.4	8.2	5.1
50	3.6	4.8	5.2	7.1
50	3.5	3.4	5.1	4.7
54	3.3	2	5.8	3.7
54	3.2	1.7	6.4	3.1
70	3.4	0.8	3.6	8.3
70	1	7.2	4.8	2
74	0.8	0.4	2.8	1.2
74	5.7	2	3.3	1.2
78	5.1	0.3	3.5	5.9
78	3.5	-0.1	3	1.7
78	1.8	6.8	4.7	1
120	3.6	-0.9	0	-1
120	2.8	-1	0.6	-0.9
120	-0.6	-0.4	-0.2	-0.8
143	3.6	-0.8	-0.2	0.7
143	0.1	-1	-0.3	-0.9
143	-0.4	-0.8	0.1	-1

Table A.4 Fermentation Part 1: SMA yeast, one sugar, and dynamic environment- apparent extract data.

Time (h)	Fructose	Glucose	Maltose	Sucrose
0.25	15.6	15.1	15.4	15.6
0.25	15.8	15.4	15.4	15.7
0.25	15.8	15.4	15.4	15.5
6	15.3	14.8	15	15.5
6	15	14.8	15	15.4
6	15.3	14.8	15	15.5
22.25	10.3	10.2	10.9	11.2
22.25	10.3	10.3	11.1	11.9
22.25	11.9	9.8	11.2	12
26	10.7	9.8	10.2	11
26	10.2	10.4	10.3	9.8
26	11.4	8.6	10.4	9.9
30	8.2	8.7	8.8	10.6
30	9.9	7.5	9.4	10.7
46	4.4	9.1	5.2	5.9
46	4.4	9.5	5.1	6.7
50	4.6	2.3	4.7	3
50	2.3	8.5	3.6	3.8
54	1.5	0.6	3.8	2.7
54	2.7	7.3	3.5	2
70	-0.5	-1	1.7	-0.8
70	-0.9	-1	0.8	-0.3
74	-0.8	-0.9	0.2	-1
74	-0.9	0	0.1	-1
78	-0.8	-0.9	0.3	4.9
78	-0.4	-0.9	0.2	-0.9
78	-0.6	4	0.9	-1
120	-0.8	-0.9	-0.7	-1.1
120	-0.8	-0.8	-0.6	-1
120	-0.8	-0.9	-0.4	-1.1
143	-0.8	-0.8	-0.7	-1
143	-0.8	-0.7	-0.7	-1
143	-0.9	-0.8	-0.7	-0.9

Table A.5 Fermentation Part 2: SMA yeast, four different slants, and static environment-absorbance data.

Time (h)	July 3	July 5	July 7	July 9
0	0.685	0.704	0.952	0.731
0	0.681	0.707	0.956	0.73
0	0.678	0.699	0.949	0.723
6	0.865	1.055	1.122	1.057
6	0.851	1.001	1.101	1.038
6	0.847	0.983	1.071	1.029
22.5	1.878		1.951	
22.5	1.828		1.875	
22.5	1.774		1.902	
22.75				2.024
22.75				2.054
22.75				2.035
23		1.897		
23		1.936		
23		1.894		
26	1.98	2.001	2.021	2.087
26	1.891	1.978	2.03	2.078
26	1.929	1.977	2.096	2.073
30	2.037	2.114	2.167	2.152
30	2.042		2.132	2.193
46.5		2.274		
46.5		2.224		
46.75			2.336	2.346
46.75			2.315	2.276
47	2.251			
47	2.245			
50	2.214	2.274	2.333	2.236
50	2.228	2.228	2.333	2.34
54	2.276	2.277	2.275	2.333
54	2.354	2.245	2.298	2.251
70.5		2.172		
70.5		2.111		
70.75			1.82	
70.75			1.861	
71	2.353			
71	2.291			
72.5				1.672
72.5				2.08
74	2.323	1.807	1.693	1.702
74	2.14	1.902	2.01	1.906
78	2.311	2.186	1.736	
78	2.07	1.775	1.584	

78	2.202	1.871	1.608	
98				1.511
98				1.086
98				1.392
121	0.793	0.376	0.634	0.637
121	0.85	0.328	0.701	0.669
121	0.851	0.441	0.775	0.831
144	0.851	0.157	0.685	0.358
144	0.626	0.294	0.471	0.365
144	0.653	0.347	0.476	0.347

Table A.6 Fermentation Part 2: SMA yeast, four different slants, and static environment-apparent extract data.

Time (h)	July 3	July 5	July 7	July 9
0	15.1	15.1	14.9	15.1
0	15.1	15.1	15.3	15.5
0	15.1	15.2	15.4	15.3
6	14.5	14.4	14.5	14.3
6	14.5	14.4	14.8	14.3
6	14.7	14.4	14.8	14.5
22.5	10.9		9.5	
22.5	11		9.9	
22.5	11		9.9	
22.75				9.3
22.75				9.3
22.75				9.4
23		9.4		
23		9.6		
23		9.6		
26	9.9	8.6	8.3	8.3
26	10.2	8.9	8	8.2
26	10.1	8.6	8.8	7
30	8.9	7.3	7.6	7
30	8.3	7.6	7.8	7.4
46.5		3.6		
46.5		3.7		
46.75			3.4	3.5
46.75			3.4	3.3
47	5.7			
47	5.6			
50	5	3.1	2.7	2.7
50	5.1	3.7	2.7	3.2
54	4.1	2.5	2.2	2.3
54	4.3	2.9	1.9	1.8
70.5		1		
70.5		0.9		
70.75			0.1	
70.75			0.4	
71	2.1			
71	1.9			
72.5				0.2
72.5				0
74	1.6	0.3	0.3	0

74	1.2	0.4	-0.1	0.2
78	0.8	0.1	0	
78	1	0.1	0.1	
78	0.9	0.2	0.1	
98				-0.4
98				-0.4
98				-0.6
121	-0.6	-0.7	-0.5	-0.6
121	-0.3	-0.8	-0.6	-0.6
121	-0.6	-0.6	-0.4	-0.7
144	-0.9	-0.7	-0.5	-0.5
144	-0.5	-0.5	-0.4	-0.5
144	-0.5	-0.5	-0.6	-0.5

Table A.7 Fermentation Part 3: six yeast strains, mixture of sugars, and static environment-absorbance data.

Time (h)	LCC 125			LCC 1208			LCC 1209		
0	1.2	0.948	0.787	0.996	0.578	0.721	0.465	0.475	0.445
0	1.201	0.948	0.772	0.971	0.577	0.72	0.466	0.427	0.402
0	1.198	0.943	0.774	1.002	0.577	0.72	0.472	0.42	0.439
5	1.335	1.132	0.892	0.955	0.63	0.695	0.085	0.083	0.093
5	1.321	1.089	0.895	0.994	0.64	0.701	0.059	0.08	0.087
5	1.348	1.112	0.88	0.998	0.63	0.708	0.064	0.06	0.098
22	1.854	1.738	1.543	0.764	0.649	0.978			
22	1.859	1.621	1.669	0.723	0.659	1.086			
22	1.942	1.61	1.719	0.756	0.682	1.051			
22.25							0.023	0.022	0.024
22.25							0.023	0.026	0.028
22.25							0.22	0.017	0.022
26	1.707	1.505	1.73	0.754	0.638	1.03			
26	1.748	1.604	1.647	0.669	0.656	1.148			
26	1.58	1.21	1.697	0.692	0.643	1.093			
26.25							0.026	0.024	0.023
26.25							0.03	0.025	0.03
26.25							0.028	0.026	0.024
29	1.762	1.309	1.72	0.684	0.657	1.272			
29	1.851	1.579	1.708	0.736	0.644	1.231			
29	1.52	1.623	1.718	0.724	0.644	1.247			
29.25							0.023	0.03	0.024
29.25							0.024	0.033	0.031
29.25							0.05	0.021	0.032
46	1.372	1.393	1.437	0.636	0.635	1.628			
46	1.092	1.468	1.469	0.841	0.659	1.622			
46.5							0.028	0.028	0.024
46.5							0.028	0.033	0.029
50.5							0.03	0.028	0.019
50.5							0.053	0.031	0.025
51.25	1.211	1.283	1.512	0.847	0.628	1.606			
51.25	0.682	0.974	1.116	0.666	0.646	1.648			
54	0.498	1.24	1.148	1.053	0.619	1.748			
54	0.689	1.361	1.07	0.982	0.629	1.784			
54.5							0.025	0.025	0.024
54.5							0.034	0.023	0.026
70	0.853	0.685	0.946	0.814	0.707	2.055			
70	0.898	0.895	1.313	0.932	0.76	1.925			
70	1.196	0.692	1.039	0.89	0.733	1.938			

70.25							0.045	0.026	0.027
70.25							0.034	0.024	0.026
70.25							0.066	0.024	0.03
74	0.772	0.443	1.016	1.062	0.779	1.962	0.041	0.028	0.03
74	0.672	0.778	1.086	0.815	0.74	2.027	0.029	0.061	0.029
74	0.785	0.711	1.243	1.179	0.762	1.991	0.041	0.033	0.032
77.25	0.847	0.549	0.612		0.76		0.028	0.024	0.023
77.25	1.189	0.532	0.826		0.778		0.031	0.043	0.025
77.25	0.725	0.766	0.876		0.788		0.08	0.043	0.024
122	0.31	0.473	0.554	1.77	0.749	1.655	0.027	0.044	0.034
122	0.394	0.306	0.309	1.752	0.747	1.848	0.044	0.056	0.026
122	0.438	0.503	0.279	1.56	0.784	1.867	0.03	0.025	0.03
194				1.795	0.779	1.834	0.025	0.028	0.026
194				1.846	0.732	1.877	0.033	0.026	0.026
194				1.659	0.733	1.865	0.029	0.03	0.03

Table A.8 Fermentation Part 3: six yeast strains, mixture of sugars, and static environment-absorbance data.

Time (h)	LCC 1240			SMA			Strain A		
0	0.626	0.864	0.821	0.786	0.69	0.685	0.875	0.606	0.696
0	0.623	0.856	0.821	0.783	0.692	0.689	0.878	0.603	0.698
0	0.62	0.853	0.812	0.783	0.687	0.69	0.875	0.601	0.692
5	0.419	0.901	0.835	0.942	0.937	0.835	1.061	0.739	0.869
5	0.689	0.923	0.8	0.934	0.933	0.868	1.069	0.708	0.877
5	0.699	0.946	0.799	0.944	0.905	0.865	1.097	0.771	0.911
22	1.376	1.587	1.448	1.995	1.831	1.965	1.895	1.73	1.762
22	1.463	1.647	1.493	1.995	1.895	1.954	1.943	1.732	1.819
22	1.415	1.589	1.534	1.952	1.887	1.976	1.949	1.775	1.826
26	1.497	1.667	1.651	2.062	1.964	2.066	2.054	1.801	1.973
26	1.467	1.752	1.655	2.095	1.965	2.04	2.053	1.855	1.887
26	1.534	1.678	1.691	2.110	2.000	2.081	2.032	1.856	1.947
29	1.563	1.789	1.799	2.122	1.948	2.134	2.067	1.938	1.923
29	1.565	1.784	1.726	2.067	1.954	2.148	2.123	1.882	1.931
29	1.706	1.9	1.741	2.114	1.974	2.119	2.102	1.93	2.005
46	1.897	2.028	1.768	2.244	2.13	2.174	2.321	2.151	2.015
46	1.864	1.863	2.264	2.272	2.156	2.224	2.278	2.187	2.279
51.25	2.024	2.033	1.993	2.196	2.185	2.06	2.154	2.259	2.266
51.25	1.945	2.048	1.931	1.565	2.141	2.239	2.234	2.222	2.242
54	1.814	1.918	1.945	2.208	2.17	2.301	2.244	2.212	2.294
54	1.96	2.046	1.9	2.238	2.191	2.206	2.111	2.283	2.283

70	1.977	1.751	2.04	2.098	1.737	1.8	1.126	2.011	1.654
70	1.809	2.222	2.014	1.511	2.082	1.813	1.277	2.055	1.67
70	2.106	1.97	2.162	2.047	0.922	1.938	1.322	2.219	1.796
74	1.99	2.085	1.899	1.585	1.207	1.988	1.467	1.671	1.702
74	2.054	2.167	1.965	1.701	1.885	1.759	1.096	1.727	1.602
74	2.059	2.241	2.067	1.794	2.201	1.73	1.398	1.79	1.865
77.25	2.094	2.175	2.037	1.798	1.958	1.374	0.949	2.178	1.452
77.25	2.099	2.202	1.937	1.582	1.602	1.97	1.075	1.738	1.56
77.25	2.011	2.18	2.168	1.266	2.08	2.127	1.165	1.632	1.609
122	1.742	0.861	1.485	0.368	1.295	0.29	0.306	0.481	0.504
122	1.35	1.425	1.831	0.304	1.429	0.295	0.289	0.585	0.47
122	1.309	0.59	1.597	0.308	1.222	0.285	0.403	0.557	0.483

Table A.9 Fermentation Part 3: six yeast strains, mixture of sugars, and static environment-apparent extract data.

Time (h)	LCC 125			LCC 1208			LCC 1209		
0	14.3	13.3	13.8	13.2	13.8	13.8	13.5	13.6	13.7
0	14.1	13.6	13.9	13.2	13.8	13.9	13.6	13.8	13.8
0	14.4	13.5	13.9	13.1	13.9	13.9	13.7	13.7	13.7
5	13.8	13.1	13.5	13	13.8	13.7	13.3	13.4	13.5
5	13.5	13.1	13.5	13	13.8	13.8	13.3	13.5	13.6
5	13.6	12.9	13.5	13.1	13.8	13.8	13.3	13.5	13.7
22	7.8	8.2	9.8	12.8	12.6	13.3			
22	7.9	8.5	9.3	12.8	12.9	13.3			
22	7.9	8.6	9.5	12.8	12.9	13.3			
22.25							10.8	10.8	10.8
22.25							10.8	10.8	10.7
22.25							10.9	10.8	10.7
26	7.1	7.7	8.4	12.7	12.3	13.1			
26	7.3	7.6	8.6	12.7	12.5	13			
26	7	8.1	8.9	12.8	12.4	13.1			
26.25							10.8	11	10.8
26.25							10.9	10.6	10.6
26.25							10.7	10.5	10.7
29	6.6	7.1	8.2	12.6	12.5	12.8			
29	6.6	7.1	8.3	12.7	12.4	12.8			
29	6.6	7.1	8	12.6	12.3	12.7			
29.25							10.2	10.2	10.5
29.25							10	10.3	10.6
29.25							10.2	10.5	10.3
46	5.4	6.4	6.2	12.2	11.5	10.1			
46	5.5	6.5	6.7	12.1	11	10.2			
46.5							8.2	7.7	8
46.5							8	8.3	8.1
50.5							6.8	7.6	7.5
50.5							6.8	7.9	7.2
51.25	5.2	6.2	6.4	11.9	11.6	9.1			
51.25	5.4	6.4	6.6	12	11.1	9.7			
54	5.1	5.9	6.2	12.1	11	8.5			
54	5.2	5.9	6.4	12	11.3	8.5			
54.5							7.8	7.9	7.6
54.5							8	7.5	7.7
70	4.9	5.6	5.9	11.3	9.8	9.7			
70	4.8	5.7	6	11.3	8.8	9			
70	4.6	5.7	6.2	11.1	9.5	9.8			

70.25							6.9	6.7	7.2
70.25							6.7	6.8	7.6
70.25							7.4	7.2	7.6
74	4.5	5.4	6	10.7	8.2	9.1	7	6.5	6.7
74	4.7	5.5	6.2	10.9	8.2	9.6	6.8	6.9	7.1
74	4.6	5.7	6	10.6	8.5	9.6	6.5	7.1	7.1
77.25	4.2	5.3	6.2	10.7	9.1	9.2	6.9	7.1	7.1
77.25	4.3	5.4	6.1	10	8.5	9.4	6.6	7.1	7.5
77.25	4.6	5.4	5.9	10.1	8.4	9.1	7.2	7.1	7.2
122	3.4	3.8	5	4.8	7.6	6.4	5.9	7	5.7
122	4.1	4.8	5.4	5	7.6	6.5	6.5	6.9	5.6
122	3.8	4.5	5.4	5.4	7.7	6.6	7.1	6.9	5.3
194				4.8	7.4	6.3	7.3	7.3	7.3
194				4.9	7.6	6.7	7.3	7.1	7.4
194				5	7.3	6.6	6.1	6.7	7

Table A.10 Fermentation Part 3: six yeast strains, mixture of sugars, and static environment-apparent extract data.

Time (h)	LCC 1240			SMA			Strain A		
0	13.6	13.4	13.4	13.4	13.9	14	13.5	13.9	13.7
0	13.7	13.7	13.4	13.5	13.9	14	13.1	14	13.7
0	13.8	13.6	13.5	13.6	14	14	13.4	14	13.6
5	13.2	13.2	13	13.1	13.4	13.4	12.9	13.9	13.2
5	13.3	13.1	13	13.1	13.4	13.4	12.9	13.5	13.2
5	13.5	13.1	13	13	13.3	13.4	12.9	13.5	13.1
22	11.2	9.9	10.5	7.6	8.6	8.1	7.6	9.7	9.1
22	11.1	10	10.5	7.5	8.5	8.2	7.6	9.6	9.2
22	11	10.2	10.4	7.6	8.5	7.8	7.6	9.7	9.2
26	10.4	9	9.5	6.3	7.4	6.9	6.3	8.8	7.7
26	10.7	8.9	9.5	6.2	7.3	7	6.3	8.6	8.2
26	10.6	8.4	9.6	6.2	7.4	6.9	6.5	8.6	8
29	10	8.3	8.6	5.5	6.6	6.2	5.6	7.7	7.6
29	10.1	8.4	9.1	5.5	6.6	6.1	5.5	8	7.7
29	9.4	8.3	9.1	5.5	6.6	6.2	5.4	8	7.2
46	6.4	4.3		2.5	3.5	2.8	1.1	3.5	3
46	6.5	4.7		2.4	3.7	3	1	3.4	3
46.5			6						
46.5			5.6						
51.25	5.6	3.9	4.9	1.5	2.6	2.1	0.8	2.6	2
51.25	5.8	3.9	4.9	2	2.7	2.1	0.6	2.6	2.1

54	5.6	4.2	5	1.4	2.5	1.7	0	2	1.6
54	5.4	3.7	5.1	1.2	2.4	1.7	0.2	1.9	1.4
70	3.8	2.3	2.8	-0.1	0.5	0.5	-0.4	0.1	0.2
70	4.2	1.6	3.3	-0.2	0.4	0.2	-0.4	0.6	0
70	3.5	1.8	2.6	0.1	0.8	0.3	-0.5	0.5	-0.1
74	3.4	1.1	3.1	-0.1	0.5	-0.1	-0.4	0.3	-0.1
74	3.1	1.3	3.3	-0.2	0.3	0	-0.4	0.4	0.2
74	2.9	1.4	2.7	0.1	0.1	0.2	-0.4	0.2	-0.2
77.25	2.8	0.8	2.2	-0.1	0.1	-0.3	-0.5	0.5	-0.5
77.25	3	0.7	2.5	-0.2	0.3	-0.2	-0.6	0.2	-0.1
77.25	2.9	1	2.4	-0.1	-0.2	-0.1	-0.4	0.3	-0.2
122	0.4	0.1	0	-0.2	-0.4	-0.2	-0.5	-0.2	-0.3
122	0	0.2	0	-0.3	-0.1	-0.4	-0.4	-0.2	-0.6
122	-0.2	-0.2	0.3	-0.3	-0.2	-0.4	-0.5	-0.2	-0.3