IMPLEMENTATION OF ISA S88 BATCH CONTROL STANDARDS ON A TRADITIONAL MICROBREWERY SYSTEM

BY

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Submitted in fulfillment of the academic requirements for the degree of Master of Science (MSc) in the Discipline of Microbiology, School of Life Sciences, College of Agriculture, Engineering and Science at the University of KwaZulu-Natal (Westville Campus).

As the supervisor of the candidate, I approve this dissertation for submission

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PREFACE

The experimental work described in this dissertation was carried out in the Discipline of Microbiology, School of Life Sciences, College of Agriculture, Engineering and Science at the University of KwaZulu-Natal (Westville Campus), Durban, South Africa from July 2012 – March 2015, under the supervision of Prof B. Pillay.

These studies represent original work of the author and have not been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledged in the text.

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Publication 1:

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ABSTRACT

Beer production dates back to the Babylonian and Monks age in 1000 BC and had no standard manufacturing protocols for a very long time. It was the passing of the purity law by the Germans in 1514 that advanced beer production and handling into a more industrial approach. In the years that followed, beer production has evolved into a very technical and delicate procedure that has high quality control measures. This investigation set out to prove the hypothesis that the implementation of the ISA S88 batch control standards in a traditional microbrewery system would increase process efficiency, as well as product consistency and stability. The study commenced with modifications to an existing traditional microbrewery system that included additional stirring flaps in the mash tun, construction of a wort/water recirculation pipeline coupled with a sprinkler in the lauter tun, and additional heating belts and temperature probes as well as a removable cooling coil in the kettle. Thereafter, an experimental plan was developed to brew Premium English pale ale under consistent conditions defined by the proposed ISA S88 model where quality defining parameters included specific gravity, pH and total dissolved solids, colour, batch volumes, reducing sugars content, free amino nitrogen content, simple sugars and flavour compound concentrations. Six identical batches were brewed and apportioned for fermentation at 14, 16, and 18 °C, respectively. Racking of all fermented batches was performed at 0 °C for two weeks before bottling, conditioning and final storage of all batches of beer at 0, 4, and 18 °C, respectively. A HACH HQ 40d multimeter probe was used for all physico chemical measurements with its various probes whilst a Shimadzu UV – 1800 spectrophotometer coupled with a Shimadzu CPS temperature controller was used for all colourimetric and optical density measurements. Simple sugars and beer flavour compound concentrations were measured by means of an Agilent 7890 A gas chromatography system coupled with an Agilent GC 80 sampler and an inert mass spectrophotometry detector. In the mashing

process, the final gravity of the wort was observed to be 14.06 ± 0.18 °P, reducing sugars were found to be 89.47 ± 2.39 g/l. In the lautering stage, the three runnings resulted in 7.92 ± 0.51 °P, 3.95 ± 0.60 °P and 1.67 ± 0.15 °P gravities, corresponding to 7.67 ± 0.55 l, 7.58 ± 0.48 l and 5.45 ± 0.42 l volumes, respectively. The collective volume was 35.71 ± 0.51 l and 167.61 ± 1.71 g reducing sugars were recovered from the spent grain. In the kettle, gravity increased to 12.10 ± 0.46 °P. Upon addition of 462 ± 68.87 g maltose syrup and boiling, the final reducing sugars amount was found to be 1632.97 ± 12.64 g in 26.45 ± 1.34 l of wort. Optimum fermentation and beer storage conditions were noted to be 16 °C and 0 °C, respectively. Flavour compounds formed during this fermentation period were found to be at concentration levels of 4.52 ± 0.24 % v/v, 119.05 ± 9.66 mg/l, and 64.02 ± 7.72 mg/l for ethanol, total fusel alcohols and total esters, respectively. Beer fermented at 16 °C depleted the total simple sugars from 12.99 ± 1.25 g/l to 5.23 ± 0.24 g/l, 10.61 ± 1.61 g/l to 5.24 ± 0.29 g/l, and 8.56 ± 3.12 g/l to 4.84 ± 0.47 g/l for storage temperatures of 0 °C, 4 °C, and 18 °C, respectively. The ethanol concentrations increased during the storage period from 4.57 ± 0.39 % v/v to 5.12 ± 0.43 % v/v, 4.70 ± 0.37 % v/v to 5.24 ± 0.29 % v/v, and 4.82 ± 0.43 % v/v to 5.39 ± 0.22 % v/v for beer stored at 0 °C, 4 °C, and 18 °C, respectively. The primary fermentation temperature of 16 ºC was found to be the most ideal (r^2 = 0.9551), as it produced a very steady and predictable fermentation trend. There were no pH changes in the beer fermented at 16 ºC, implying that no mouth feel changes in the product‟s taste were significantly possible. The physical and chemical property trends, statistical analyses, and literature comparison of the produced wort and beer proved that ISA S88 batch controlling standards, even in a basic traditional microbrewery, can improve process-product quality and guarantee product quality consistency.

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LIST OF ABBREVIATIONS

% v/v:	percentage volume per volume
% w/v:	percentage weight per volume
% w/w:	percentage weight per weight
°C:	degree Celsius
°P:	degree Plato
AATase:	alcohol acyltransferase
acyl CoA:	acetyl coenzyme A
ADP:	adenosine diphosphate
ATF:	acyltransferase
ATP:	adenosine triphosphate
BJCP:	beer judge certification program
ca.:	approximately
cfu:	colony forming units
CIP:	cleaning in place
cm:	centimetre(s)
CO_2:	carbon dioxide
DMS:	dimethyl sulphide
DMSO:	dimethyl sulphoxide
DNA:	deoxyribonucleic acid
DNS:	3, 5-dinitrosalicyclic acid
DO:	dissolved oxygen
DSC:	digital signal controller
EBC:	European brewing convention

EMP:	Embeden Meyerhof Parnas
est.:	established
Ex:	single phase motor
FAN:	free amino nitrogen
FLO:	flocculation gene
floc:	flocculum
FMCG:	fast moving consumer goods
g:	grams
g/l:	grams per litre
GC:	gas chromatography
H_2S:	hydrogen sulphide
hl:	hectolitre(s)
hr:	hour(s)
HS GC-MS:	head space gas chromatography-mass spectrometer
Hz:	hertz
ISA S88:	instrumentation, systems and automation society standards of 1988
kDa:	kilodalton(s)
kg:	kilogram(s)
kgf:	kilogram force
KW:	kilowatt(s)
l:	litre(s)
LTP:	lipid transfer protein
mA:	milliamp(s)
mg:	milligram(s)
mg/l:	Milligrams per litre

min: minute(s)

ml: millilitre(s)

Mr: relative molecular mass

MS: mass spectrometer

mtDNA: mitochondrial deoxyribonucleic acid

n.c: normally closed

n.o: normally open

NADPH: nicotinamide adenine dinucleotide phosphate

NIR: near infrared

nm: nanometre(s)

O_2: oxygen

PIH: pre-isomerised humulone

PLC: programmable logic controller

psi: pound force per square inch

Pt. 100: platinum resistance temperature detector of 100 ohms at 0 °C

PVPP: polyvinylpolypyrrolidone

QA: quality assurance

ROS: reactive oxygen specie(s)

rpm: revolutions per minute

SAB: South African breweries

SCADA: supervisory control and data acquisition

SMM: S-methyl methionine

SO_2: sulphur dioxide

SOP: standard operating procedure

TCA: tricarboxylic acid

V AC: volts alternating current

V DC: volts direct current

VDK: vicinal diketone

VHG: very high gravity

µl: microlitre

µS: microsiemens

CHAPTER ONE: LITERATURE REVIEW

1.1 Introduction

Bibere, which means to drink, is a Latin word that beer evolved form. The Babylonian people were one of the first people to historically brew beer, dating back as far as 3000 BC. *Triticum dicoccum* was one of the first grain varieties used in a dehusked and baked form to produce flat bread. Spontaneous fermentation on the bread was done as a successive step through wild yeast action on the thick water-bread mixture (Esslinger, 2003; Atnafu and Abebaw, 2015). The Egyptians later eliminated the soaked pieces of bread and made the grain germinate thereby improving the beer. In the following years, experimental zeal grew in malting and brewing processes and around the middle ages (around 9th century) it was the Monks who started with the addition of hops to the beer, initially as a preservative to extend the shelf life of beer (Esslinger, 2009; SABMiller, 2013).

Commercial brewing began to be established in Europe between the 11th and 13th centuries, where public houses which later become pubs were opened for beer consumption and selling to the public. Hops were eventually accepted as legitimate brewing additives in the 14th century which immediately saw tree bark, bitter herbs, and berries which were previously used being replaced (Esslinger, 2003). Only the top-fermenting action of ale beer production at elevated temperatures was known amongst brewers until the sixteenth century. Later, bottom fermentation of lagers was accidentally discovered after beer was stored in cool caverns for long periods (Esslinger, 2009; SABMiller, 2013; Atnafu and Abebaw, 2015).

As brewing turned modern, malt beverages became the most popular. The natural ingredients of these basically are: treated water, malted barley, adjuncts and hops. A variety of beer styles have emerged among malt beverages, thereby defining a beer family tree over the years.

Addition of malt and hops at higher concentration levels paired with long beer aging periods became the more prominent practice of beer production.

Low-calorie, no-carbohydrate beers are made from pre-hydrolyzed wort. Fungal enzymes (glucoamylase and amylase) are used to hydrolyze the dextrin to maltose and glucose, which can be completely fermented to alcohol, the net result is a lower concentration of remaining carbohydrates (Fraizer *et al*., 1988).

Ale beer is made with top yeast and has a primary fermentation of 12.2 – 24.4 °C. This high temperature promotes rapid fermentation i.e., 5 – 7 days, and the ale produced is pale in colour, fruity-flowery aroma, and tart in taste. Weiss beer, porter and stout are ales in the sense that top yeasts are employed in their manufacture. Weiss beer is a light tart ale made chiefly from wheat. Porter and stout are dark, heavy swe*et al*es. There are also related beverages that are not necessarily malt beverages. Sonti is a rice beer or wine from India. The mould *Rhizopus sonti* and yeasts are active in the fermentation. Pulque is a Latin-American beer-like beverage containing about 6 % alcohol that results from a natural yeast fermentation of the juice of the agave, or century plant (Briggs *et al.*, 2004; SABMiller, 2013).

As the centuries went by, regulative beer production laws were passed. One such law came to be through the Bavarian dukes Wilhelm IV and Ludwig X on April 23, 1516 (Esslinger, 2003). This law was passed in the Ingolstadt parliament and was soon referred to as the purity law (Reinheitsgebot). The Law stipulated that for brewing purposes malted barley, water and hops are to be used together with yeast as the sole fermenting organism. Since its first passing in 1551 Greece, Germany and Switzerland have adhered to these very strict principles together with the greater part of Europe under what is known today as the European Brewery Convention (Angelino, 1996; Esslinger, 2009).

1.2 Barley Malting

Enzyme development paired with simultaneous degradation of higher molecular substances by the action of controlled germination, are the key objectives of malting where discrete colour and aroma characteristics can be achieved with adequate removal of unwanted off-flavour precursors e.g. S-methyl methionine (Atnafu and Abebaw, 2015). High extract yield and low malting loss are economic goals deemed necessary by brewers who seek to maintain brand integrity and standard (Esslinger, 2009). During malting, the barley starch is degraded, mainly to a mixture of polyglucose molecules that are somewhat less complex than the originals. Amylopectin tends to be degraded preferentially compared with amylose. The enzymes able to degrade the non-gelatinised starch in barley appear to be (i) phosphorylase, (ii) α-glucosidase, (iii) α-amylase, (iv) β-amylase and (v) debranching enzymes (Hough, 1991).

Barley firstly goes through the sieving, screening and magnetic metal removal stages after which it is dried to a moisture content of 12 % by weight, which allows storage without damaging the embryo. The steeping process follows where by the cleaned grain is added into the steeping tank and moisture levels are allowed to increase to levels of 38 – 42 %. This steeping process is achieved by a series of interchanging wet-dry stands which may amount to 6 in total, depending on barley variety, maturity, plump size, water sensitivity, etc. Once water and oxygen (through aeration) are added, germination of the grain starts and the embryo develops rootles and acrospires. Partial nutrient consumption and endosperm modification occur during germination where the aim of this controlled process is to activate and produce high enzymic activity and cell wall degradation, but not allowing the new plant to develop fully. Parameters that affect germination are moisture, temperature, ratio of air to carbon dioxide and time. A temperature range favourable to uniform germination is set

between 14 – 18 °C, whilst an air supply sufficient enough to guarantee normal respiration and adequate CO_2 removal is supplied (Fix, 1999; Esslinger, 2009).

Steeping provides definite moisture content appropriate to the physiological characteristics of the barley. A 15 hour steeping stage raises the moisture content in the tanks from 14 – 30 %, followed by a subsequent dry steeping phase (16 – 24 hours) with moisture set at 30 %, where barley water sensitivity is noted to decline (Esslinger, 2003). By allowing the moisture content to rise up to 38 % in the second steeping stage, the kernels germinate evenly in an anticipated duration of 14 – 20 hours, evident by the formation of tiny chits (barley shoots). Adequate spraying in the germination box ensures the rise in moisture content to its final value of approximately 45 %. For problematic barley, moisture levels may go as high as 46 – 47 % in order to reduce malting time, however, high moisture steeps do not produce high-quality malts (Fix, 1999; Esslinger, 2009).

Malting is performed to promote chemical transformations, the main purpose being the induction and increase of hydrolytic enzymes. The most important groups of these are the cytolytic enzymes, proteolytic enzymes, amylases, and phosphatases. Cytolytic enzymes (β-glucanases and cytase) break down the hemicelluloses to low molecular mass materials making them responsible for the degradation of cell-wall structures, which is crucial in enabling hydrolytic enzymes to access the protected endosperm (Esslinger, 2003). Proteolytic enzyme action for a significant amount of protein hydrolysis is necessary for successful cell-wall degradation. Low germination temperatures, high kernel moisture and optimized process durations are some of the most favourable conditions for efficient proteolysis. If germination is allowed to continue for elongated periods, the consumption of low-molecular protein material becomes imminent where these are used for acrospire and rootlet development. (Steiner *et al.*, 2012). Germinating barley contains α-amylase, β-amylase, limit dextrinase, and α-glucosidase, whose combined action can theoretically degrade amylose and

amylopectin to glucose (Gupta *et al.*, 2010). Malting is important for synthesis and activation of these activities, but only a small amount (≈12 %) of the starch is actually degraded during the production of pale malts. Excessive starch degradation results in a loss of both malt yield and malt extract (Ullrich, 2011).

Proteolytic enzymes are believed to mediate the release of bound β-amylase during germination and this process is accompanied by the appearance of additional β-amylase isoforms. These are probably generated by limited proteolysis of the polypeptides found in protein matrices that have starch granule cells embedded in them (Lewis and Young, 2001; Ullrich, 2011). Little to no inactivation of the endoproteases enzymes during kilning was reported by B. L. Jones, (2005) as one of the main factors responsible for the release of the amylases enzymes. Unlike α-amylase, β-amylase is synthesized during grain development and is stored in the mature endosperm ready for digestion of the starch reserves during germination. Most lines contain about 1mg of β-amylase per gram dry weight (≈1 % of the total seed protein). β-amylase also acts as a storage protein in that the amount increases under conditions of high nitrogen availability (Ullrich, 2011).

There is a surprising array of proteases present in germinating barley. At least five are endopeptidases i.e., enzymes able to cleave randomly at any peptide linkage chain of the amino acids making up the protein. Their activity increases about 20-fold during germination. Other endopeptidases are metallo-enzymes whose activity can be seriously impaired by chelating the metal present in the molecule (Hough, 1991).

Maillard reactions contribute a huge role in the formation of flavour profiles, colour, caramelization of sugars, and degradation of phenolics and lipids in malt. Kilning is the most important stage for flavour and colour development and for removal of unwanted green malt flavours (Briggs, 2002). The Maillard reaction involves interaction of amino acids and reducing sugars to form glycosylamines. Glycosylamines are unstable and undergo

rearrangement to form ketosamines. The ketosamines can further react to form reductones, short-chain hydrolytic fission products, or brown nitrogenous polymers (melanoidins). The reactions are favoured by high temperature and low moisture levels (Ullrich, 2011).

Maillard derived heterocyclic compounds are responsible for flavours and aroma in malt. Oxygen heterocyclic compounds such as furans contribute toffee-caramel flavours, while coffee-nutty and roasted flavours are known to be contributed by nitrogen heterocyclics, such as pyrazines. Strecker aldehydes are derived from amino acids when heated with diketones or reductones. Isovaleraldehyde, derived from leucine, has a strong malty flavour. Flavour substances derived from fatty acids include aldehydes, alcohols and lactones. Trans-2-hexenol and trans-2cis-6-nonodienal are responsible for the green or grassy aroma of green malts (Seaton, 1993; Ullrich, 2011). Other important components are phosphates-ca. 0.3 %, minerals 2.5 – 3.5 %, vitamins-ca. $0.5 * 10^{-3}$ %, and phenolic substances-ca. 0.2 % (Esslinger, 2009).

Low molecular weight products of the Maillard reaction are flavourants contributing mostly to the flavour of dark speciality malts. Polymeric high molecular weight melanoidins are synthesized as end-products that are flavour inactive, coinciding with vicinal diketone and antioxidant scavenging radical level declines due to the effect of the Maillard reaction. (Coghe *et al.*, 2004).

Also present in the germinating corn are peptidases which cleave amino acids or simple peptides from the proteins. The most important are the carboxypeptidases which liberate amino acids. They are named in this manner due to their action of attacking the chain at the end where there is a free carboxyl group. Among the wide range of amino acids liberated is proline which can only be utilized by yeast under aerobic conditions and therefore after a brewery fermentation, the beer is rich in proline compared with other amino acids (Hough, 1991).

The kilning process which comes after barley germination has the importance of stopping the chemical and biological transformations in the barley, drying the grain for storage purposes and also the driving off of grass-like flavours dominated mostly by vegetative and green malt flavours. Malt colour and quantification of volatile intermediates can be used to determine the extent and rate of the Maillard reaction during kilning. The curing temperatures combined with duration of the malt‟s exposure to such conditions also have a direct impact on the Maillard reaction extent (Esslinger, 2003; Briggs *et al.*, 2004; Coghe *et al.*, 2004).

Moisture content, colour and extract are the main characters observed for dark speciality malts. These characteristics however, are inadequate for brewing performance predictions, dark malt flavour profiles and beer stability (Coghe *et al.*, 2004; Esslinger, 2009).

The moisture content, which is especially relevant to the storage quality of freshly harvested barley, may range from 12 to 20 %. A 12 – 14 % range is the normal result mostly measured by the Near Infrared Spectroscopy (NIR) technique (Angelino, 1996). Higher moisture levels for storage purposes promote the formation of slack malt, which is lower in carbohydrate content, flavour aroma, and extraction yield due to its milling difficulty. For brew house usage, a malt moisture content of about 4.5 % is required, as this promotes dry milling, friability and efficient cracking of the malt without experiencing over-ground powdery grain (Sileoni *et al.*, 2010; SABMiller, 2013).

1.3 Mashing

The mashing process constitutes ground malt and other prepared grist material mixed carefully with treated liquor at a specific temperature. Sometimes the mash may vary with respect to compositions i.e., 100 % malt, unmalted cereal and malt mixture with exogenous enzymes, etc. Strength, extract (solubles suspended in solution), and the volume of liquid in which the solids are dispersed, partly characterize wort in this stage (Briggs *et al.*, 2004). Solid particles are rendered soluble in brewing liquor during mashing by heat and enzymatic actions (Esslinger, 2003). Sweet wort is dense, sweet, coloured and sticky. It has a highly complex matrix where substances present include simple sugars, dextrins, β-glucans, pentosans, trace-residual starch, phosphates, lipids, proteins, peptides and amino acids, phenolic substances, dissolved inorganic ions, yeast growth factor vitamins, organic acids, bases and nucleic breakdown products (Briggs *et al.*, 2004). Sweet wort typically contains solids consisting of about 90 – 92 % carbohydrates, 4 – 5 % nitrogen-containing substances and 1.5 – 2 % ash (MacWilliam, 1968). During the primary fermentation of wort, the simple sugars are converted by means of numerous yeast metabolic pathways, to ethyl alcohol, fusel alcohols, esters, vicinal diketones and organic sulphur compounds (Landaud *et al.*, 2001).

Before 1945 the English infused liquor and malt to form a thick mash with homogeneously ground malt(s) combined with 5 – 15 % of rice or maize adjunct. Mixing of the grist with hot liquor (water) at a temperature chosen to give a particular „first heat" was a standard initiating protocol for brews. 30 min (minutes) later, after observing negative iodine starch test results, hot water was introduced from the vessel bottom (underlet) to raise the mash temperature and after 2 – 3 hours, wort collection, clarification by recirculation and sparging would begin. Typically on mashing in, the liquor/grist ratio would be 2.15 – 2.42 hl/100 kg grist and the temperature would be 63.4 – 67.2 ºC. After underletting, with additions of hot water, the temperature of the mash would be 66.6 – 68.8 ºC. The final wort was collected separately

whilst the solid remains were sparged with 3.76 – 4.30 hl/100 kg of liquor at 75 – 77 ºC. Thus the whole process from mashing to collecting the wort would take at least six hours (Briggs *et al.*, 2004). The driving factors to these optimized industrial practices were reported by C. W. Bamforth, (2000) as cost saving, quality improvement, safety adherence, and promoted sales opportunities. These are similar performance areas of interest to those of the South African Breweries (SAB), where all procedures in the brew house are governed by cost, quality, deliverables, safety, and morale (SABMiller, 2013).

The choice of good quality malt minimizes the odds of brewing a poor mash and allows the stands to be shortened to 1 – 1.5 hours. Addition of some hydrolytic enzymes in the mash shortens and accelerates wort separation time. A direct lauter tun mashing regime gives an opportunity for fine grist use and fast separations. By running faster sparges paired with short mash stand times a higher performance delivery rate can be achieved in the brewery at the expense of extract loss and short lauter runs (faster grain bed compaction). The total volumes of liquor used in the old England breweries were typically 6.98 – 7.52 hl/100 kg grist (Briggs *et al.*, 2004).

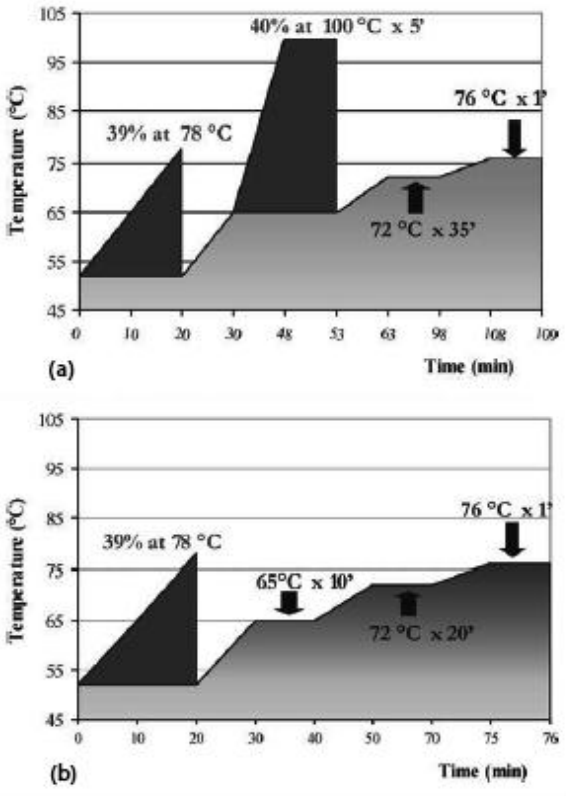


Figure 1.1. A comparison of brew house mashing profiles between (a) double decoction method and (b) single decoction method (Montanari *et al.*, 2005).

Figure 1.1 shows a comparison between two traditional decoction methods performed in the mashing stage. The critical control parameters at this stage are temperature and time as illustrated above. In a three step classical decoction mash, a grist/liquor ratio of 4.8 – 5.4 hl/100 kg grist is used to make light beers, whilst a thicker mash of 3 – 4 hl/100 kg is used for stronger brews (Briggs *et al.*, 2004). These decoction profiles are most notable by their varying boiling times. The grist may be mashed in with cold water and the temperature is raised to 35 – 40 ºC by underletting hot liquor whilst stirring. Usually the mash is kept at a low saccharification temperature range for about 2 hours so as to enable the effects of maltase, phytase, β-glucanase, and proteases enzymes to complete. The pH of the mash may fall, partly due to the activities of lactic acid bacteria. After about one hour into this period a third of the mash is transferred to the decoction vessel and is heated to boiling, often with a

rest at 65 – 70 ºC to allow α-amylase to liquefy the starch. After 15 min for pale beers and 45 min for dark beers, at 100 ºC, the hot partition of the mash is added back to the mash tun, thus increasing the temperature to 50 – 53 ºC. During the next rest the surviving enzymes begin to attack the gelatinized and liquefied starch. A second decoction increases the temperature of the main mash to about 65 – 70 ºC. A third decoction brings the temperature to 76 ºC after which the entire batch is transferred to the lauter tun vessel for mash separation. Well modified malt use proves this tedious process to be unnecessary and time consuming, hence the added advantage of such malts. Many faster and more economical double- and single-decoction procedures have been implanted in many breweries for lager production (Briggs *et al.*, 2004; Montanari *et al.*, 2005).

1.3.1. Biochemistry of malting and mashing

As the main starch source in beer brewing, barley has a number of advantages over other grain. There are two forms of starch present in the grain, amylose and amylopectin. The former is a glucose polymer comprising some 1000 – 4000 units of glucose; it therefore has a molecular weight of about 200 000 – 800 000 units. Each glucose is linked to its neighbour by what is termed an α-1, 4 bond. This linkage means that the reducing group of glucose at the number 1 position is no longer effective (Hough, 1991).

Superior taste of beer, control of the germination process, and brewing technology available are some of the advantages barley has in the brewing industry. Storing conditions of barley are vital and determine what kind of malt will be produce for brewing (Esslinger, 2009). On a biochemical level, malting and mashing can be viewed largely as a controlled process of endosperm mobilization, where the primary objective of malting is to modify the endosperm (i.e., overall physical/biochemical changes that occur in the barley endosperm). The

endosperm of well-modified malt is friable and easily crushed, while that of poorly modified malt is hard and steely. This physical change results from the degradation of cell walls and protein within the endosperm (Ullrich, 2011).

The α-glucans (amylose and amylopectin of the starch) are the most important carbohydrates in barley (Esslinger, 2003). An amylose molecule, due to the presence of only one functional reducing group at the molecule ends, has no more reducing power than a single molecule of glucose. When iodine dissolved in potassium iodide solution is used for amylose treatment, the iodine finds a position in the coils of the structure and the amylose-iodine complex has a blue-black colour (Hough, 1991). Amylopectin is also a polymer of glucose but is bigger and branched in nature; with a molecular weight in excess of 500 000 units. Most of the units of glucose are linked by α-1, 4 bonds but there are occasional instances of another bond, α-1, 6. The effect of this is to make the molecule branched but, like amylose, there is only one functional reducing group in the molecule. Iodine stains amylopectin but produces a reddish colour (Hough, 1991; Lewis and Young, 2001).

Other carbohydrate components include β-glucans (cellulose, hemicellulose and gums), pentosans, as well as minute portions of low molecular mass sugars (Esslinger, 2003). Arabinoxylans and β-glucans are present in barley in soluble and insoluble forms, and processes of both solubilization and degradation occur during malting and mashing. The β-glucans are primary endosperm cell wall polysaccharides in barley, and their importance in brewing can mainly be attributed to the higher molecular weight fractions and their impact on viscosity. Failure to adequately degrade β-glucans can result in reduced malt extraction, lautering and filtration difficulties (Ullrich, 2011).

The most important enzymes in malting and brewing are the α and β amylase. They are so-called because they yield a carbohydrate product with a carbon bearing its hydroxyl group in the α position or the β position, respectively. The α amylase is a metallo-endo-enzyme and attacks randomly, hydrolyzing any α-1-4 linkage except those close to a branching point and those close to the end of the molecule. Thus in the case of amylose, the enzyme yields straight-chained molecules of differing lengths, and with amylopectin, mixed products of branched and unbranched molecules and for this reason α amylase does not produce significant amounts of fermentable sugars (glucose, maltose , etc.). The cleavage lowers the size of the original starch molecule and reduces viscosity of the starch significantly thus α-amylase being known as a liquefying enzyme. Heat is known to render solubility to starch, but it has been acknowledged that heating in the presence of active α-amylase facilitates a more efficient hot-water-extraction during mashing (Hough, 1991; Lewis and Young, 2001). β-amylase attacks α-1-4-links from the non-reducing ends of amylose and amylopectin molecules to release the disaccharide maltose. The enzyme can almost completely hydrolyze amylose to maltose, but is unable to bypass the 1, 6-branch points in amylopectin (Gupta *et al.*, 2010). The main consequence of the cleaving action of β-amylase is to provide maltose sugar, a readily diffusible carbohydrate that can be used by the barley embryo. To the brewer, it is an easily fermented sugar, the main constituent in the wort (Hough, 1991).

During mashing, α-amylase catalyzes the hydrolysis of 1, 4-α-linkages in amylose and amylopectin in an endo-manner to yield linear and branched (1, 6: 1, 4-α-linked) dextrins. This activity is important as it greatly reduces the molecular weight of the starch and in turn mash viscosity. It also provides additional substrate of 1, 4- α-linkages from the non-reducing end, to release maltose. β-amylase hydrolyzes amylose to maltose and a small amount of maltotriose to maltose, but cannot bypass the 1, 6-branch points in amylopectin. Limit

dextrinase catalyzes the hydrolysis of 1, 6-α-linkages in branched dextrins, yielding linear dextrins (Ullrich, 2011).

Starch breakdown is most important during mashing. Upon achieving starch kernel swelling, the enzymatic degradation of starch begins. α-glucans are allowed to be dissipated by this process so as to render a starch negative result before reaching desired attenuation limits. With the aid of the amylases, limit dextrinase is able to hydrolyse the β-limit dextrins i.e., 1, 6-α-glucosidic branch points in low molecular weight dextrins formed by amylases action on starch (Esslinger, 2009; Gupta *et al.*, 2010). Solubilization of starch, and in turn extract, increases as mash temperatures are increased up to 70 ºC i.e., towards the optimum activity range of the liquefying enzyme α amylase. However, the fermentability of the wort begins to decline as temperatures exceed 65 ºC and β-amylase is rapidly inactivated (Ullrich, 2011).

During mashing, only a limited amount of β-glucan hydrolysis occurs. Endo-β-glucanase shows maximum activity levels at 40 – 45 ºC, and is very rapidly inactivated at temperatures above 50 ºC (Jin *et al.,* 2004). Additional hydrolysis of β-glucans can be accomplished with low mash-in temperatures, but this can result in excessive proteolysis. As such, it is very important that breakdown of β-glucans be accomplished in the malt house. Well modified malts promote the ease of mash-profile planning as both the glucan and proteolytic rests are no longer necessary in the brew house (Briggs *et al.*, 2004). Coghe *et al.,* (2004), selected unboiled wort as study material with the purpose of excluding flavour-active compound effects originating from boiled wort, hops and the fermentation process. A colour of 20 EBC units was also preferred by considerations of difficult flavour profiling for low-coloured worts and burnt/bitter flavour masking problems for darker worts.

1.3.2. Free amino nitrogen in malt and wort

Maltability, foam, yeast nutrition and beer stability all depend on malt-derived protein content. The use of unmalted cereals and adjuncts in any practical brewing scenario dilutes the soluble nitrogen (protein) content of the wort (Esslinger, 2003; Fontana and Buiatti, 2009). Americans however brew with protein levels of 13.5 %, the high nitrogen barley is invariably accompanied by the use of significant levels of unmalted grains and syrups that, in effect, dilute nitrogen levels (Fix, 1999; Esslinger, 2009).

Worts from North America pilsner-type beers (25 % adjuncts) have been reported to contain 255 mg/l amino acids, 225 mg/l peptides and 195 mg/l protein. A considerable amount of higher molecular weight protein precipitates during wort boiling. Approximately 17 amino acids derived from malt, with proline as the predominant one (not utilized by yeast), are found in wort. The term free amino nitrogen (FAN) became a customary parameter in using a single number to characterize the total amino acid content of wort. This is a measure of the nitrogen contributed by the amino acids in the wort, irrespective of type, and is expressed in terms of mg/l (Fix, 1999; Ullrich, 2011).

Table 1.1 below depicts the classified importance of all malt-derived amino acids to a typical brewing yeast strain. The first class of proteins in order of complexity are the peptides, which are combinations of 2 – 30 amino acids bound by peptide links. Proteins are nitrogen sources and therefore a formula for representing % proteins as % nitrogen has come to be based on the fact that % protein is directly proportional to % nitrogen .

$$(\%\ \text{protein}) = 6.25 \times (\%\ \text{nitrogen})$$

Another class of malt proteins are enzymes. Enzymes are relatively high-molecular-weight proteins consisting of about 300 – 400 amino acids connected by peptide links.

Table 1.1. Classification and absorbance groups of wort amino acids (Fix, 1999).

	ABSORPTION RATE			
	Group A: Rapid	Group B: Moderate	Group C: Slow	Group D: Largely unabsorbed
Class1: Unimportant amino acids	Glutamic acid			Proline
	Glutamine			
	Aspartic acid			
	Asparagine			
	Serine			
	Threonine			
Class 2: Vital amino acids		Valine	Glycine	
		Isoleucine	Phenylalanine	
			Tyrosine	
			Alanine	
Class 3: Crucial amino acids	Lysine	Leucine	Tryptophan	
	Arginine	Histidine		

Proteins comprise about 8 – 15 % of the total dry weight of the mature barley grain, the total amount depending primarily on the availability of nitrogen (Fix, 1999; Ullrich, 2011).

Lipid transfer protein 1 (LTP1), protein Z, hordein, non-specific lipid transfer protein (ns-LTP), and glutelin, are soluble protein material associated with beer foam stability and formation (Hiralal *et al.*, 2013). Protein Z, 50 – 200 mg/l in beer, is partially homologous to serine protease inhibitors and this property might be the reason why it is not degraded by proteolytic enzymes during malting and mashing (Fontana and Buiatti, 2009; Steiner *et al.*,

2011). The hydrophobic LTP1 is concentrated in beer foam and is between 50 – 90 mg/l in beer. LTP1 is also homologous to protease inhibitors and is able to inactivate cysteine protease and serine protease in malt. The manipulation of native barley LTP1 structure by chemical and thermal modifications i.e., Maillard reaction glycosylation, partial proteolysis and denaturing during the malting and brewing stages, help improve its poor foaming properties to become a foam-promoting component in wort. Many LTPs are potent food allergens, and this is the case for barley LTP1 whose high stability to heating and proteinases results in its presence as an allergen in beer. The addition of wheat LTP to beer has been shown to reduce lipid-induced foam destabilization. However, modified forms of barley LTP1, called LTPb, which are reduced and glycated to promote foam formation are also contained in beer foam (Leisegang and Stahl, 2005; Ullrich, 2011). The hydrophobicity of the LTP1 which is enhanced by wort boiling, together with protein Z and an α-amylase inhibitor, gives a positive correlation with beer foam stability (Bamforth, 2011).

Non-specific lipid transfer proteins found in plants possess the ability of transferring numerous lipids between membranes (Douliez *et al.*, 2001). These proteins share a stabilized α-structure and are differentiated from other proteins by hydrophobic cavities which can accommodate lipid binding molecules in plants, formed by the folding of the four helix bundle (i.e., covered by a long C-terminal arm) (Van Nierop *et al.*, 2004). This multigene family of ns-LTPs is subdivided into two subfamilies; ns-LTP1 which is a prominent protein in barley grain, malt and beer, and ns-LTP2 which is expressed mainly in plant roots, but is also available in the plant grain and these have molecular masses of 10 and 7 kDa, respectively (Hippeli and Elstner, 2002; Stanislava, 2007).

Based on their activity against fungi *in vitro* and in transgenic plants, ns-LTPs have been suggested to contribute to plant defence systems because of their synthesis in the formation of some LTPs being induced by infection or damage. The LTPs‟ ability to transfer cutin and

suberin monomers to the site of cuticle synthesis in epidermal cells has also been suggested (Ullrich, 2011). It has been observed that vast efficient defense strategies have been developed by evolving plants to resist pest and phytopathogenic microorganism attack. The accumulation of Pathogenesis Related (PR) proteins which hinder microorganism and insect-gut enzyme activity is one of the many defensive strategies that evolving plants have adopted. Most barley variety proteins have been confirmed to fall under the PR protein group due to the barley‟s genetic resistance against pests and microorganisms. The brewing and malting sectors find this property of barley very crucial and useful for commercial gain. The benefit of such proteins is the suppression of gushing properties that come with fungal-infected malt, making beer stabilization almost impossible (Hippeli and Elstner, 2002; Stanislava, 2007).

Lipids are only partially used up during malting, the remainder staying mainly in the spent grains (Esslinger, 2003). Barley grain contains approximately 2 – 3 % lipid, which exists predominately as triacylglycerides in the embryo. Lipase catalyzes the hydrolysis of triacylglycerides to yield free fatty acids (Kunze, 1996). Increases in lipase activity of 40 – 80 fold have been reported following 7 days of germination. The free fatty acids liberated during the early phases of germination are metabolized through β-oxidation, and are an important source of energy. Alternatively, some linoleic acid is metabolized by the pathway. Beer flavour stability is influenced greatly by the alternative metabolic pathway of lipoxygenase as it acts on some malt linoleic acid (Ullrich, 2011).

1.4 Hop Chemistry and Wort Boiling

Hops (*Humulus lupulus*) are a major ingredient in brewing due to their cultivated hop cone‟s hard and soft resin content. The soft resins comprise of the crucial brewing compound groups α and β acids. The α acid group (humulone) consists of humulone, cohumulone and adhumulone, while the β acid group (lupulone) consists of lupulone, colupulone and adlupulone as the important acids in brewing, respectively (Lewis and Young, 2001). Hop harvesting, drying and storage in compressed bales, prior to pelleting and extraction, is very important as soft resin fractions may be lost in the store house. It has been noted over the years that α acids decrease linearly with storage time where this decrease is further promoted by exposure to air, lack of refrigeration and high moisture content. As more α acids are lost by oxidation and moisture hydration/hydrolysis, the hard resins (containing hop oils, polyphenols, acids, etc.) increase and eventually a cheesy aroma becomes pronounced due to old (or ill-treated) hops that contain excessive low molecular weight acids (SABMiller, 2014).

The α acid: β acid ratio is used in the brewing industry to differentiate hops. High alpha hops are intended for more efficient bittering and are characterized by ratios of greater than 0.8 and some such as the SAB Southern star exceed 1.0. American high alpha varieties are known to exceed 3.0. Aroma hops on the other hand, have more β acid content in their soft resin matrix hence lowering the ration to as low as 0.5. In South Africa, Southern passion and Southern aroma are some of the new hop breeds that are used because of the granadilla/citrus aroma they possess (SABMiller, 2014).

The use of aroma hops i.e., hops with more hard resin content has been noted to improve beer flavour stability. Although these hops are lower in bittering ability, it is known however that their rich polyphenol profiles act as strong antioxidants that give beer its sensorial flavour stability (Mikyška *et al.*, 2011). For efficient extraction of the aromatic essential oils in hops,

dry hopping was found to be the best brew house technique to use. Major components of the oxygenated fraction such as humulene and cystene (sesquiterpenes), were rarely found in beer brewed using the late kettle hopping technique. Oxygenated compounds derived from hops and synthesized during the boiling step were instead the ones responsible for the hoppy aroma of beer boiled using the late hopping technique (Lermusieau *et al.*, 2001).

Isomerization and extraction of hop α acids is the most important reaction that comes with wort and hop boiling. Other known reasons for boiling wort are inactivation of malt enzymes, sterilization of wort, concentration of wort through evaporation, coagulation of protein-polyphenol content in wort, and the reduction in wort pH (Briggs *et al.*, 2004).

Figure 1.2 below illustrates different forms of the desired hop-derived α acid during its natural state in hops, extracted and isomerized state in boiled wort, and light struck state in UV-visible light exposed beer. The desirable bitter taste is imparted by the iso-α acid from hops and the harsh lingering bitterness is usually imparted by other bittering compounds that come mainly as hop by-products of boiling e.g. oxidized β acids. To reduce the magnitude of these astringent boil by products, hop farmers now process dried hops into a pre isomerized humulone (PIH) product. Hop pellets or pure hop extract is now available in the industry where the pre-isomerized α acids increase hop utilization in the brew house and reduce energy dissipation. This PIH product usage has led to the creation of modified PIH hops which contain light-stable iso-α-acids such as Tretrahydro-, Hexahydro-, and Dihydroiso-α-acids which possess enhanced bittering and foam stabilizing properties. This light stabilized property inhibits the formation of the skunky light-struck off flavour in beer derived from H_2S forming the 3-methyl-2-butene-1-tiol compound. This has seen the clear-bottled products such as Miller Genuine Draft and Redd‟s emerging in the South African beer industry (Lewis and Young, 2001; SABMiller, 2014).

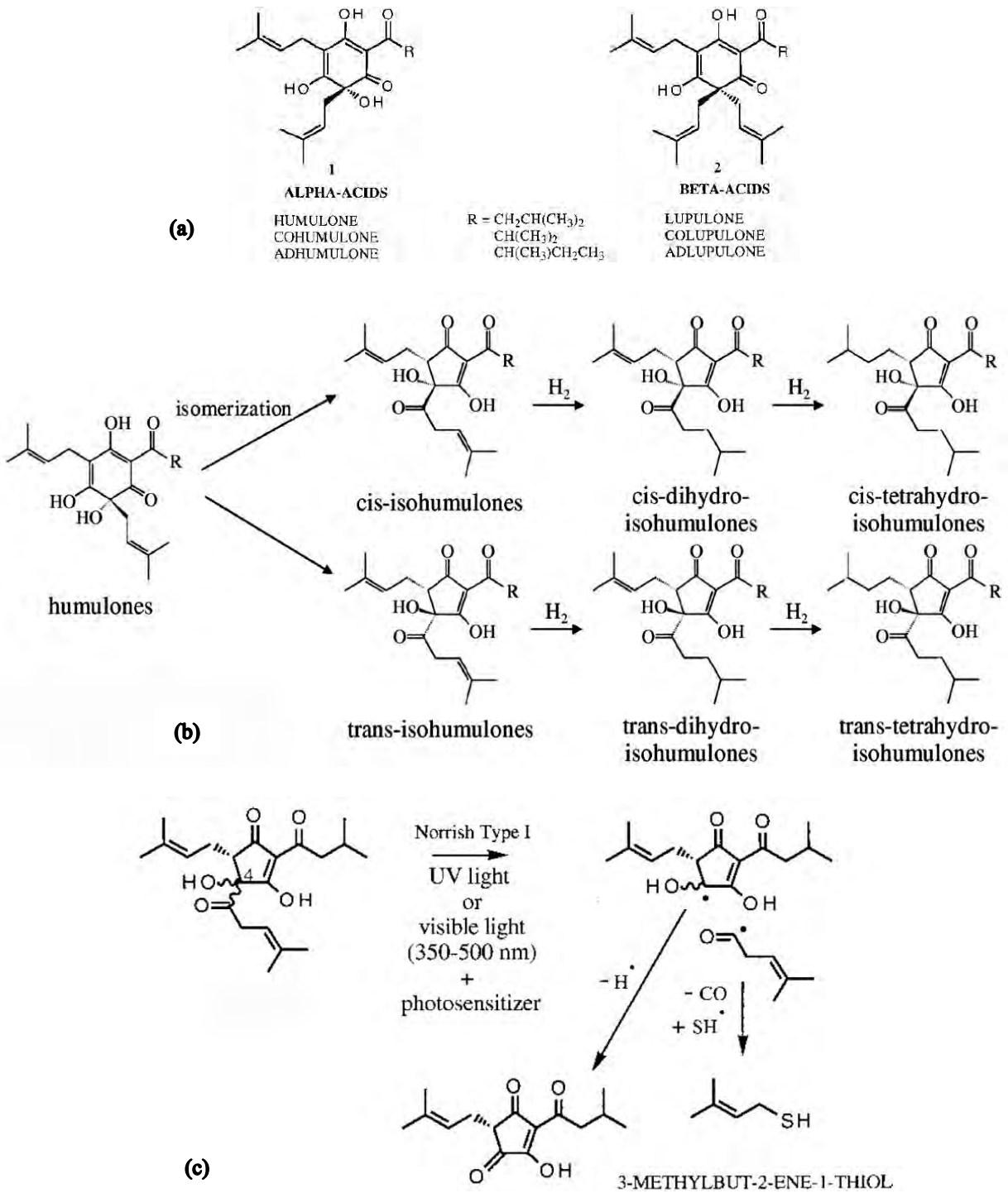


Figure 1.2. The chemical structures of the bittering hop acids in (a) α and β acid form, (b) isomerized α acid forms, and (c) light struck form (De Keukeleire, 2000; Rodrigues and Gil, 2011).

Although light stable, PIH hops are susceptible to oxidation, and this deterioration is associated with beer aging accompanied by a decrease in beer bitterness. Beer aging and flavour degradation rates have been quantified by the use of decomposing pathways of iso-α-acids i.e., their transformation to allo-iso-α-acids, acetylhumulinic acids, and humulinic acids. The more sensitive *trans*-iso-α-acid is the one largely responsible for these oxidative pathways and the decrease in beer bitterness. The greatly reduced hop extracts such as the tetrahydro-iso-α-acids are said to be more resistant to oxidative deterioration compared to dihydro-iso-α-acids (Cooman *et al.*, 2000; De Schutter *et al.*, 2008).

1.5 Brewer's Yeast

Brewing yeast strains belong to the *Saccharomycetaceae* (Fungi) family and are eukaryotes. For uniformity in all scientific notations, brewers‟ yeast strains are denoted with the genus *Saccharomyces* (sugar fungus) and species *cerevisiae*. *Saccharomyces carlsbergensis* (lager yeast), a bottom-fermenting yeast strain, and top-fermenting yeast strain *Saccharomyces cerevisiae* (ale yeast) are the brewing industry nomenclature used to separate the two. Due to the fact that the major properties of yeast strains depend on raw material use paired with manufacturing procedures, it is therefore acknowledged that this distinction is artificial (Lewis and Young, 2001). Healthy yeast cells are observed by distinct singular vacuoles when viewed under a microscope. The typical life-span of a cell is between 10 – 30 generations, but an average of 25 generations is normal. The effect of repitching harvested yeast cells can be observed in as little as 5 generations primarily because of mutations which directly affect the yeast‟s fermentation performance and physiology (Fermentis, 2010). A large filling vacuole together with small numerous vacuoles which are granular in appearance are visual signs of a dying yeast cell. The cells lyse, and the process of lysis results in the

release of cell contents leaving empty shells of predominantly cell walls (autolysis being the act of self-digestion by the cells). Stains such as methylene blue may be used to determine and evaluate the viability of a yeast sample under a microscope. Viability (the proportion of living cells in a sample) is a means of describing the ability of cells to grow, interact and reproduce within their environment by counting all dead cells in the sample stained blue (by methylene) or pink (by eosin). Normally a healthy culture would contain ≥ 95 % viable cells and it would be advisable not to ferment with a culture possessing viability of < 85 % (Lewis and Young, 2001; Guido *et al.*, 2004).

It has been accepted in general terms that investigating viability alone (by staining) is inadequate to provide a true indication of fermentative ability since systems with rapid yeast growth reaching up to 226 * 10^6 cells/ml within 36 hours per stage, at viability > 98 % are now available for use in the industry (Andrews *et al.*, 2011). The concept of very high gravity (VHG) fermentation technology was defined and extended on grounds that provided research with new perspective on yeast-ethanol toxicity tolerance. *Saccharomyces cerevisiae* has been reported to have higher ethanol concentration tolerances than previously assumed and without need for genetic modifications (Pires *et al.*, 2014). VHG is defined in this case as substrates containing 27 g or more of dissolved solids per 100 g mash i.e., gravity > 18 ºP. However, the yeast cells are exposed to extreme osmolaric stresses at the propagation phase of fermentation when the sugar level of the medium increases above their normal tolerance limits (> 30 % w/v). The composition of this sugars profile together with FAN content and pitching rate drop ethanol production efficiency due to sluggish fermentations occurring. High osmotic pressure, low water activity and toxic effects of higher ethanol levels are the key factors, along with high temperatures, pressure and extremes of pH, responsible for inhibition of yeast growth and decreased fermentation ability and viability (Puligundla *et al.*, 2011).

1.5.1 Yeast handling

The collective management process that includes yeast physical treatments is termed yeast handling. Yeast handling procedures involve the recovering of yeast slurry from cylindroconical vessel cones by cropping (Lodolo and Cantrell, 2007). Due to various fermentation by-products the yeast experiences vast amounts of stress contributed by DO concentration fluctuations, CO_2, head space pressure build up, pH, ethanol concentration, nutrient limitations and temperature. With high mortality rates for the yeast cells emerging as first flocculants in the vessel cone, a significant amount of the first yeast crop is scrapped and discarded to maintain integrity of the harvested crop. The remainder of the crop is collected into collection vessels, where it is treated with dilution liquor in order to decrease potential negative impacts of ethanol toxicity (Lodolo *et al.*, 2008). The cropped yeast is held at refrigeration temperatures and will remain healthy for a week or so. Even at these low temperatures, it is not recommended to store yeast outside its nutritious medium (wort/beer) for long periods as this may drop glycogen levels resulting in slow future fermentations upon repitching. Yeast kept in suspension and gently agitated has a longer healthy life as "hot spots" due to yeast metabolic activity are prohibited from accelerating cell death and autolysis (Lewis and Young, 2001; Fermentis, 2010).

Yeast recovery, storage, propagation and repitching must be done in a manner that conforms to QA targets that regulate phenotypical homogeneity (metabolism, flocculence and age), correct strain integrity, freedom from contaminants and high viability and vitality. A detrimental impact on yeast fermentation performance is deemed imminent in a brewing environment that does not recognize and follow good cropping practices i.e., cold storage at 4 ºC, effective agitation for continuous homogeneity, effective sterilization and cleaning (Lodolo *et al.*, 2008). Yeast that has bacterial contamination can be washed at acidic pH to reduce/eliminate contamination. Various acids i.e., tartaric, sulphuric phosphoric, may be

used where typical pH values would be in the range 2.1 – 2.4 and contact at temperatures 2 – 4 ºC for 30 – 60 min. Under normal conditions, yeast is resistant to these acidic conditions, but if other physico chemical properties change, this resistance may decline rapidly. An increased alcohol content (> 8 % v/v) and acid washing at temperatures greater than 5 °C come with the disadvantages of not eliminating all bacteria efficiently and negatively impact on the yeast‟s fermentative ability, even though they remain viable by staining (Cunningham and Stewart, 2000).

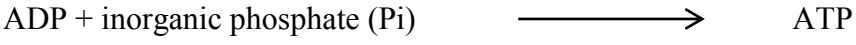
1.5.2 Yeast biochemistry and beer flavours

Brewer‟s yeast needs a diverse combination of nutrients for its lifecycle metabolism. These nutrients are made available to the yeast in the wort medium in the form of trace elements from brewing water and malt, malt derived amino acids, fermentable sugars (mainly maltose), and vitamins from malt. Oxygen is a brewer‟s direct input to the wort by means of aeration, where desirable concentrations are in the range of 9.0 ± 2.0 mg/l. Upon pitching the yeast in wort, nutrients are depleted for energy provision purposes and by so doing, forms alcohol and carbon dioxide (Briggs *et al.*, 2004). Synthesis of new yeast substance comes in the form of nicotinamide adenine dinucleotide phosphate (NADPH), a yeast metabolism-derived reducing power. The nutrients are also either directly assimilated into new cell components or used to generate intermediates for this process. All these reactions are made possible by the catalytic means of enzymes, which come in the form of complex polypeptide chains having high affinities for different substrates they are meant to catalyze (Fermentis, 2010; White, 2012).

The major brewing sugar in wort which accounts for 50 – 55 % of total wort carbohydrate content is maltose. Maltose uptake by yeast involves the use of maltase i.e., hydrolyzing

action of the α-glucosidase enzyme to yield two glucose molecules. The energy dependent maltose permease is also used for maltose uptake by converting ATP to ADP to achieve transportation energy for the permease molecules located in the yeast membrane(Lodolo *et al.*, 2008; Obasi *et al.*, 2014).

Yeast cells have phosphorus as an essential component in their deoxyribonucleic acid (DNA) together with phospholipids within their membranes. Cell replication and nutrient metabolism is facilitated by phosphorous-containing compounds. Without phosphates, yeast cells cannot replicate nor complete metabolic pathways that produce energy resulting in stuck or incomplete fermentations. Chemical pathways during fermentation that serve to generate energy achieve this goal by chemically oxidizing target substrates. Such oxidations involve an enzyme cofactor NAD^+, which upon receiving a hydride ion converts to NADH, the reduced form, which is performed and achieved by dehydrogenate enzymes. In metabolism, the oxidation process is carefully controlled so that some of the energy released is retained by the cell in the form of adenosine triphosphate (ATP).



By hydrolyzing the ATP in a reverse reaction, energy needed to synthesize new products is released. The experienced temperature increases in primary fermentations is proof that these energy releases are not 100 % efficient, as some of this energy is dissipated in the form of heat, raising the green beer temperature. Larger fermentation vessels need more efficient cooling systems than smaller ones since their contact surface area is smaller with respect to the liquid inside (Lewis and Young, 2001; White, 2012; Malik *et al.*, 2014).

Fast yeast growth is stimulated by high temperature and DO concentrations which lead to high FAN utilization, resulting in products that quickly express flavour imbalance. Amino

acid utilization has been noted to follow a sequence that is independent of all fermentation conditions (Lodolo *et al.*, 2008). Although know pathways exist, the abundance and therefore the sequential use of amino acids is directly affected by the technological processes of the brew house (Gorinstein *et al.*, 1999). Wort amino acids were characterized into four groups based on evolving observations over the years i.e., table 1. Amino acids utilized first are aspartate, glutamine, serine, arginine, glutamate, threonine, lysine, and asparagine belonging to the amino group A. The next combination of amino acid uptake involves isoleucine, methionine, histidine, valine, and leucine which are termed amino group B (Faria-Oliveira *et al.*, 2013). Group C amino acids made up of glycine, tyrosine, alanine, ammonia, phenylalanine, and tryptophan are utilized after total consumption of group A amino acids from the wort has been completed (Boulton, 2013). Group D has proline as the only amino acid which tends to be abundant in the wort because of the absence of free amino groups on its structure, and therefore is not utilized by yeast (Lodolo *et al.*, 2008).

Amino nitrogens are deemed essential components of yeast nutrition, implying that their absence directly points to imminent disordered fermentations. The carbon skeletons embedded in amino acid structures play an important role later in fermentation, particularly in the metabolic pathways of by-products like diacetyl and fusel alcohols (Fix, 1999).

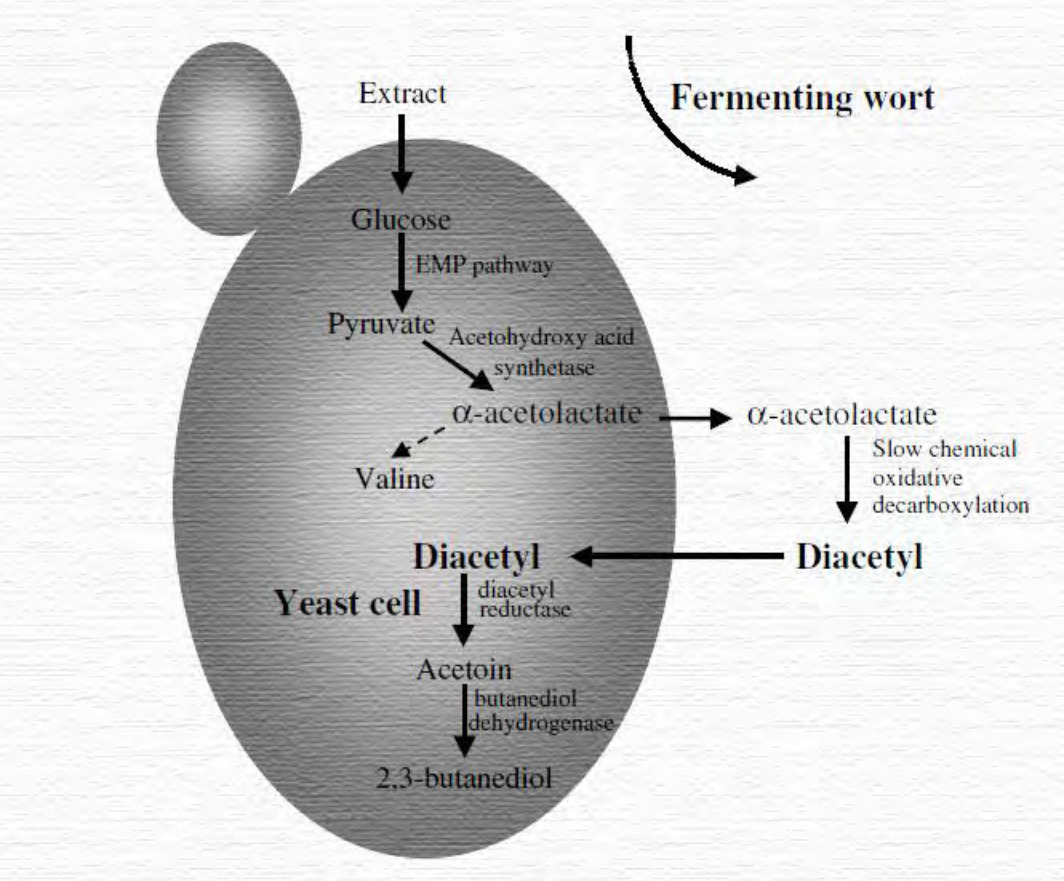


Figure 1.3. The metabolic pathway of diacetyl in budding yeast by means of a glycolytic metabolism involving a sugars Embeden Meyerhof Parnas (EMP) pathway (Willaert and Nedovic, 2006).

Figure 1.3 shows a detailed metabolic pathway used by brewing yeast when synthesizing diacetyl and 2, 3-butanediol. As shown above, diacetyl formation is a result of excess α-acetolactate leakage from the valine-isoleucine biochemical pathway, now experiencing oxidative decarboxylation in the yeast cell‟s external environment i.e., leaked into the green beer. Amongst the vicinal diketones (VDK) formed as a byproducts in the primary fermentation stage, diacetyl is the most flavour active (Brányik *et al.*, 2008). Diacetyl has a very low taste threshold concentration of approximately 0.15 mg/l and is characterized by an unclean, sweet-like taste in beer, which sums up to a butterscotch off-flavour in higher concentrations. The precursor of diacetyl, α-acetolactate, is an intermediate of the valine

synthesis pathway and hence diacetyl is formed when yeast synthesizes valine. This diacetyl production is amplified by low pitching and removal rates of yeast, as well as the deficiency of FAN in the wort particularly malt-derived valine. Brewing yeast however doesn‟t possess α-acetolactate decarboxylase, an enzyme capable of hydrolyzing α-acetolactate to produce acetoin, a significantly less flavour-active compound. This setback leads to the assimilation of α-acetolactate back into the yeast as diacetyl, which is then reduced enzymatically to acetoin and further to 2, 3-butanediol during the time consuming maturation (Brányik *et al.*, 2005; Fermentis, 2010).

During the anaerobic growth of yeast there are organic acids with short carbon skeleton structures derived both from the incomplete turnover of the tricarboxylic acid (TCA) cycle and from the amino acid catabolism in beer (Blanco *et al.*, 2014). These organic acids (acetate, citrate, pyruvate, oxo-acids, succinate, lactate, and malate) play an important role in pH reduction during fermentation by imparting to beer a sour taste. The toxic medium chain fatty acids ($C_6 - C_{12}$), result from long-chain fatty acid anabolism under anaerobic conditions and are imparted into beer by means of yeast cell autolysis. As far as foam stability and taste are concerned, these long chain fatty acids originating mostly from wort, are considered undesirable in beer (Brányik *et al.*, 2008).

During fermentation, young yeast cells require lipid synthesis to grow. These lipids are observed as key cell membrane components where both saturated and unsaturated fats are used. Also common with the membranes of all eukaryotes, those of yeast contain sterols (mainly ergosterol). Acetyl coenzyme A (acyl CoA) is the essential beginning of fat and sterol synthesis in the production of unsaturated and saturated acyl CoA sterols and molecules (Lewis and Young, 2001; Pires *et al.*, 2014). A certain portion of the hydrophobic foam-active protein fraction in beer is known to be hydrolyzed by proteinases resulting in

decreased foam stability. An aspartyl proteinase enzyme encoded by the PEP4 gene and located in the vacuoles of *Saccharomyces cerevisiae* is responsible for protein fraction hydrolysis. This enzyme is leaked under stressful conditions for living yeast cells and also released from dead yeast cells by autolysis (Brey *et al.*, 2002; Leisegang and Stahl, 2005). Characteristics of oxygen deprived yeast are reduced transport capabilities coupled with reduced osmotic tolerance, inferior membrane integrity and high exterior ethanol levels (Hiralal *et al.*, 2013). Reactive oxygen species (ROS) are produced endogenously by cells under aerobic conditions due to the moderately abundant oxygen intended for cell propagation soon after yeast pitching. The superoxide radical (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and even non-radicals with the potential to oxidize or convert to oxidizing radicals are all classified as ROS which can damage cell components, contribute to cellular ageing and ultimately lead to cell death. Known effects revealed in past works include lipid peroxidation, protein inactivation and nucleic acid damage, including damage to mtDNA, which can lead to the generation of respiratory deficient "petites" (Gibson *et al.*, 2007; Aron and Shellhammer, 2010). The yeast however possesses a defense mechanism which is both mechanical and enzymic, purposed for the relief of such oxidative stress. Peroxidase and catalases are some of the enzymic mechanisms whilst antioxidants such as thioredoxin and glutathion are known to be the mechanical defense mechanisms against yeast oxidative stress (Berner and Arneborg, 2012).

Aroma active compounds play a major role in defining the organoleptic character of beer. The relationship between an aroma compound's absolute concentration and its sensory threshold value in the beer matrix, and in the presence of other compounds, is the defining factor in characterizing overall beer flavours. For the nearly 250 volatile components identified in malts, not all of them contribute considerably to the overall flavour profiles.

Esters are known for imparting fruity and flowery aromas to beer and they are classified into two main groups (Blanco *et al.*, 2014). Acetate (ethanoate) esters belong to the first group and include ethyl acetate, isoamyl acetate and phenyl ethyl acetate. The second ester group is made up of ethyl (medium chain fatty acid) esters i.e., ethyl caproate and ethyl caprylate, just to name a few (Verstrepen *et al.*, 2003; Coghe *et al.*, 2004; Saerens *et al.*, 2008). Ester formation was found to be derived from lipid metabolism by yeast as it grows during fermentation. Esters are products of yeast ATF activities catalyzing the condensation reaction between either acyl CoA and higher alcohols or ethanol (Brányik *et al.*, 2006; Segura-García *et al.*, 2015). Alcohol acyltransferases (AATase) make up most of the different enzymic profile responsible for the formation of esters; however, esterases are capable of dictating final esters levels in beer. Lodolo *et al* (2008), showed gene disruption evidence and expression analyses of ATF gene family members (i.e., ATF1, Lg-ATF1, ATF2) indicating that different ester syntheses are involved in the synthesis of esters during fermentation. Fatty acid metabolism regulations were observed to be linked closely with the yeast mechanisms controlling the under pinning of oxygen-mediated regulations as far as ATF1 gene transcription was concerned. Formation of esters in yeast is believed to occur as a means of free CoA regeneration, medium fatty acid detoxification and formation of analogues of unsaturated fatty acids (Brányik *et al.*, 2008; Lodolo *et al.*, 2008; Saerens *et al.*, 2008).

Basically two components determine ester formation rates i.e., substrate abundance (acyl CoA and alcohols), and enzyme activity (AATases) (Yilmaztekin *et al.*, 2013). Acetate esters are synthesized by *Saccharomyces cerevisiae* by the intracellular enzyme AATase whose activity is vital in the amount and rate of ester production. The acetate esters are lipid soluble and hence readily diffuse into the fermenting medium i.e., green beer. On the other hand, long chain fatty acid-ethyl esters are not as abundant in the beer as acetate esters due to their

long structures that make their transfer to the medium (and abundance) structure, temperature and strain dependent (Verstrepen *et al.*, 2003; Mallouchos *et al.*, 2007). For instance, it is known that high DO levels in wort affect ester formation due to availability of acyl CoA and suppression of AATase. Although there‟s an overlap of the effects of different factors, common ester synthesis factors are gene transcription regulation and AATase activity. Due to numerous factors involved in the regulation of activity and the gene expression of AATase and regulation of substrate availability to the yeast, it is practically difficult to fully control ester formation during fermentation (Brányik *et al.*, 2008).

Table 1.2. Major aroma active compounds found in different gravity beers (Saerens *et al.*, 2008).

Aroma compound	12°P 12°C (mg/l)±SD	12°P 15°C (mg/l)±SD	16°P 15°C (mg/l)±SD	14°P 20°C (mg/l)±SD	14°P 24°C (mg/l)±SD	18°P 24°C (mg/l)±SD	Threshold (mg/l)
Alcohols							
Propanol	11.37±0.10	12.82±0.28	13.43±0.30	30.95±1.09	27.32±0.02	42.02±0.00	600
Isobutanol	7.13±0.13	7.55±0.07	8.20±0.12	35.07±0.09	33.78±1.28	37.75±0.99	100
Isoamyl alcohol	58.29±0.59	59.98±1.25	58.08±0.18	126.66±1.26	116.6±0.50	123.15±0.93	50
Phenyl ethanol	25.37±1.10	28.11±1.15	27.18±1.13	64.82±0.35	60.96±1.13	66.16±1.65	40
Total alcohols	102.16±1.92	108.46±2.76	106.88±1.72	257.50±2.79	238.42±2.93	269.08±3.57	
Acetate esters							
Ethyl acetate	23.02±0.13	22.01±1.87	29.71±0.16	78.86±0.50	72.23±0.40	129.23±0.59	30
Isoamyl acetate	1.20±0.01	1.73±0.07	1.84±0.00	8.50±0.07	7.98±0.03	9.32±0.02	1.2
Phenyl ethyl acetate	0.59±0.01	0.66±0.01	0.73±0.01	2.43±0.09	2.85±0.04	3.38±0.04	3.8
Total acetate esters	24.81±0.15	24.40±1.95	32.28±0.17	89.79±0.66	83.06±0.47	141.93±0.65	
Ethyl esters							
Ethyl hexanoate	0.154±0.002	0.186±0.008	0.232±0.012	0.210±0.007	0.209±0.019	0.254±0.001	0.21
Ethyl octanoate	0.463±0.003	0.532±0.001	0.504±0.009	0.430±0.001	0.271±0.028	0.395±0.020	0.9
Ethyl decanoate	0.103±0.005	0.109±0.013	0.082±0.005	0.035±0.010	0.029±0.001	0.019±0.002	1.5
Total ethyl esters	0.720±0.010	0.827±0.022	0.818±0.026	0.675±0.018	0.509±0.048	0.668±0.023	
Vicinal diketones							
Diacetyl	0.332±0.002	0.267±0.008	0.144±0.003	0.109±0.001	0.066±0.002	0.049±0.001	0.15
Pentanedione	0.279±0.003	0.257±0.005	0.184±0.006	0.044±0.001	0.054±0.002	0.015±0.001	0.9
Total vicinal diketones	0.611±0.005	0.525±0.013	0.328±0.009	0.153±0.002	0.120±0.004	0.064±0.002	

Table 1.2 contains a list of the common beer aromatic compounds which characterize on or off flavours if produced in excess. Minute ester concentrations observed in some immobilized cell processes have been suggested to be linked with low cellular metabolic activities in these systems. Extremely low oxygen concentrations in other immobilized systems have been

found to be the reason for the reduction in ester synthesis (because of mass transfer limitations), hence causing reduced cellular growth. Due to the low oxygen levels which tally with high CO_2 levels and pressure, the cellular acetyl CoA pool can be more available for ester synthesis instead of channeling for fatty acid biosynthesis (Renger *et al.*, 1992; Willaert and Nedovic, 2006).

Fusel (higher) alcohols, are produced by yeast during primary fermentation and have an inverse relationship with amino acids i.e., they reach their optimal concentration in green beer when the amino acids are at their minimal. Fusel alcohols can be grouped into two classes namely aliphatic [n-propanol, isobutanol, 2-methylbutanol (active amyl alcohol) and 3-methyl butanol (isoamyl alcohol)] and aromatic (2-phenylethanol, tyrosol and tryptophol) higher alcohols. The alcoholic (solvent) aroma and warm mouthfeel of beer is contributed by aliphatic higher alcohols. The sweet aroma contributed by 2-phenylethanol is a positive on-flavour in most beer styles, but tryptophol and tyrosol alcohol aromas are highly undesirable. Yeast utilizes wort amino acids during the catabolic pathway, to produce an α-keto acid and a corresponding glutamic acid via a transamination reaction (Aguilera *et al.*, 2010). The excess oxo-acid (α-keto acid) is then decarboxylated to aldehydes and further reduced to a higher alcohol (Renger *et al.*, 1992; Willaert and Nedovic, 2006; Kordialik-Bogacka and Antczak, 2011). Fusel alcohols are produced from the carbon skeletons of amino acids in the catabolic pathway, or from sugars in the biosynthetic anabolic pathway, and the effect of fusel alcohols on finished beer flavours is quite negative if present above or near their flavour thresholds. It is therefore desirable to keep FAN concentrations below 350 mg/l in wort so as to minimize this potentially negative effect. The importance of the anabolic pathway decreases as the number of carbon atoms in the alcohol increases and increases in the later stage of a

conventional batch fermentation as wort amino acids are depleted (Fix, 1999; Willaert and Nedovic, 2006; Erten *et al.*, 2007).

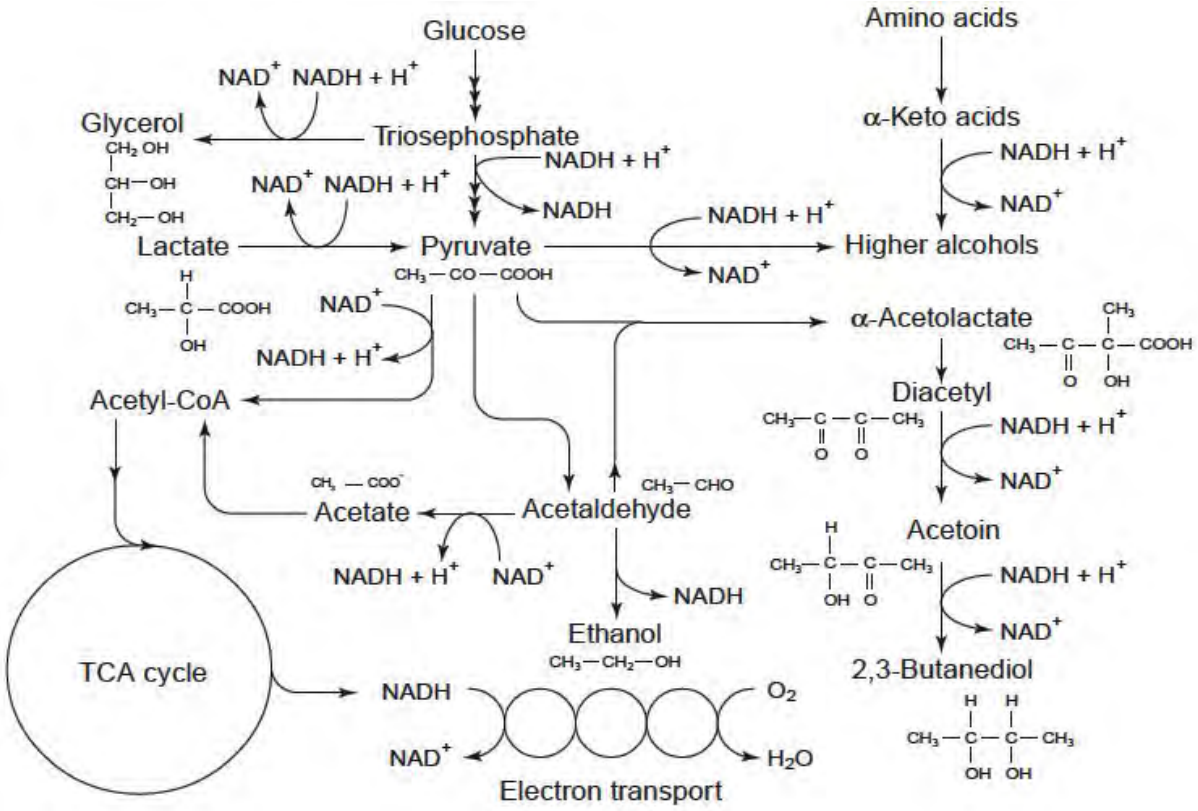


Figure 1.4. The anabolic and catabolic pathways of fusel alcohol synthesis in brewing yeast during fermentation (Briggs *et al.*, 2004).

With comparison between immobilized and free cell system beers, it is seen that the uneven higher alcohol formation can be assigned to levels of amino acid utilization, different yeast growth rates, and mass transfer limitations. For entrapped cells (i.e., immobilization by alginate, carrageenan, and calcium pectate) the reduction of fusel alcohol synthesis seemed to be proportional to the reduction in FAN utilization. By carefully choosing appropriate yeast strains, wort composition, fermentation conditions, immobilization method and reactor design the control of continuous fermentation systems can be very precise with respect to fusel alcohol formation (Brányik *et al.*, 2008; Klose *et al.*, 2011; Yilmaztekin *et al.*, 2013).

Hydrogen sulphide (H_2S), sulphur dioxide (SO_2), mercaptans [e.g. dimethyl sulphoxide (DMSO)], and dimethyl sulphide (DMS) are the main sulphur components impacting on beer flavour. SO_2 and H_2S are influenced the most by yeast metabolism. H_2S can be synthesized metabolically by brewer‟s yeast from either inorganic sulphur compounds or from organic compounds i.e., amino acids, and is known for being problematic in breweries by its possession of a highly volatile sulphur compound that imparts a „rotten egg‟ aroma in beer. When H_2S reacts with ethanol or acetaldehyde, it forms ethanethiol, which displays an onion-like aroma (Lodolo *et al.*, 2008).

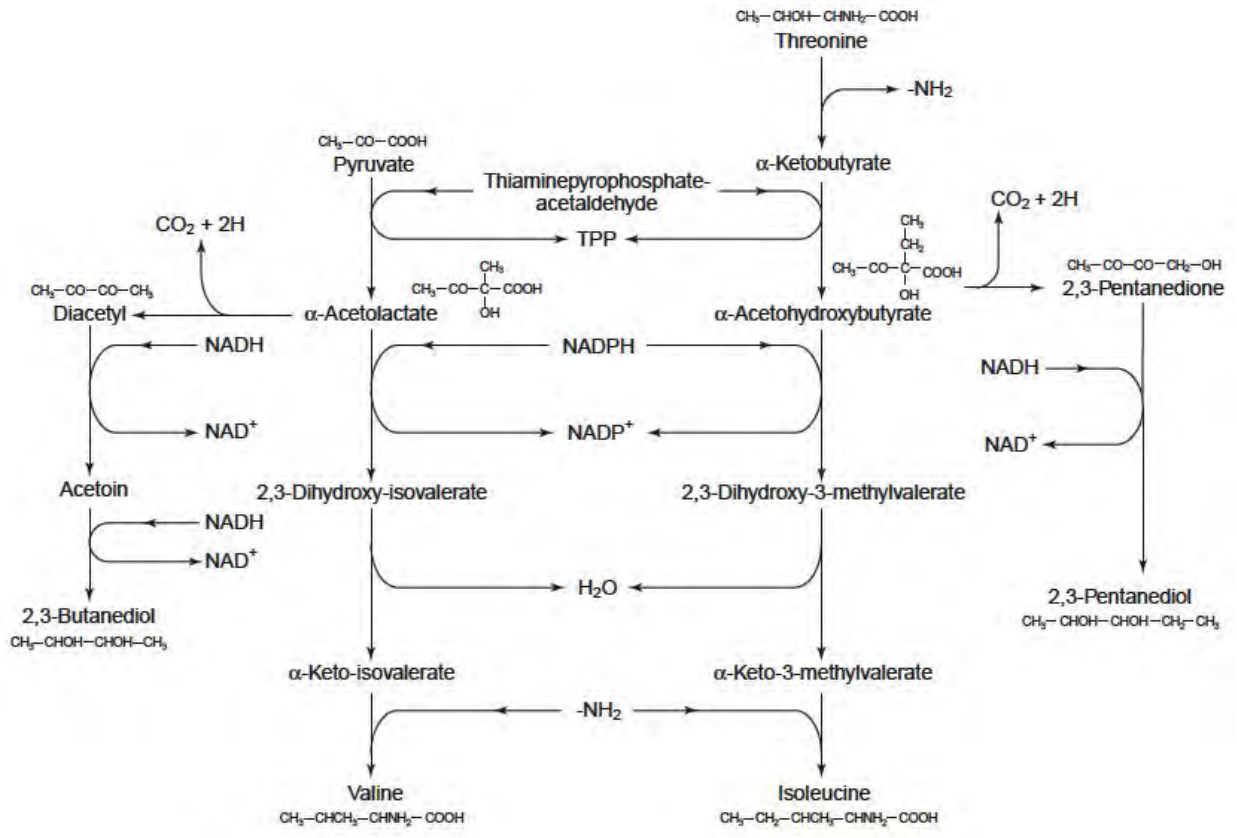


Figure 1.5. The influence of amino acids on the biosynthesis of sulphurs during the anaerobic stage of primary fermentation (Briggs *et al.*, 2004).

Figure 1.5 reveals the intermediates that play an important role in achieving the final sulphur-containing compounds required by the yeast during fermentation. DMS is synthesized as a

byproduct of two pathways i.e., the thermal disintegration of S-methyl methionine (SMM) during wort kettle boiling and DMSO reduction by fermenting yeast (nee‟Nigam *et al.*, 2009). The final concentration of DMS in packed beer is a result of DMSO present in wort at pitching where the DMS formed from reduction of DMSO during fermentation is lost by CO_2 stripping of the green beer (Lodolo *et al*., 2008).

Polyphenols and their condensed products, mostly a combined class of malt- and hop-derived polyphenols, possess antioxidative properties that help preserve beer during its maturation and distribution stages. Flavan-3ol and proanthocyanidin have the ability to improve product oxidative stability and are acknowledged in other food sectors, hence gaining significant consideration as reliable beer stabilizers. As a failsafe mechanism for beers with potentially permanent haze, the establishment of colloidal stability in the form of commercial stabilization treatment has become acceptable. A common commercial additive is polyvinylpolypyrrolidone (PVPP) to finished beer. PVPP effectively removes Polyphenols (haze precursors) and haze from beer (Rehmanji *et al.*, 2000; Aron and Shellhammer, 2010).

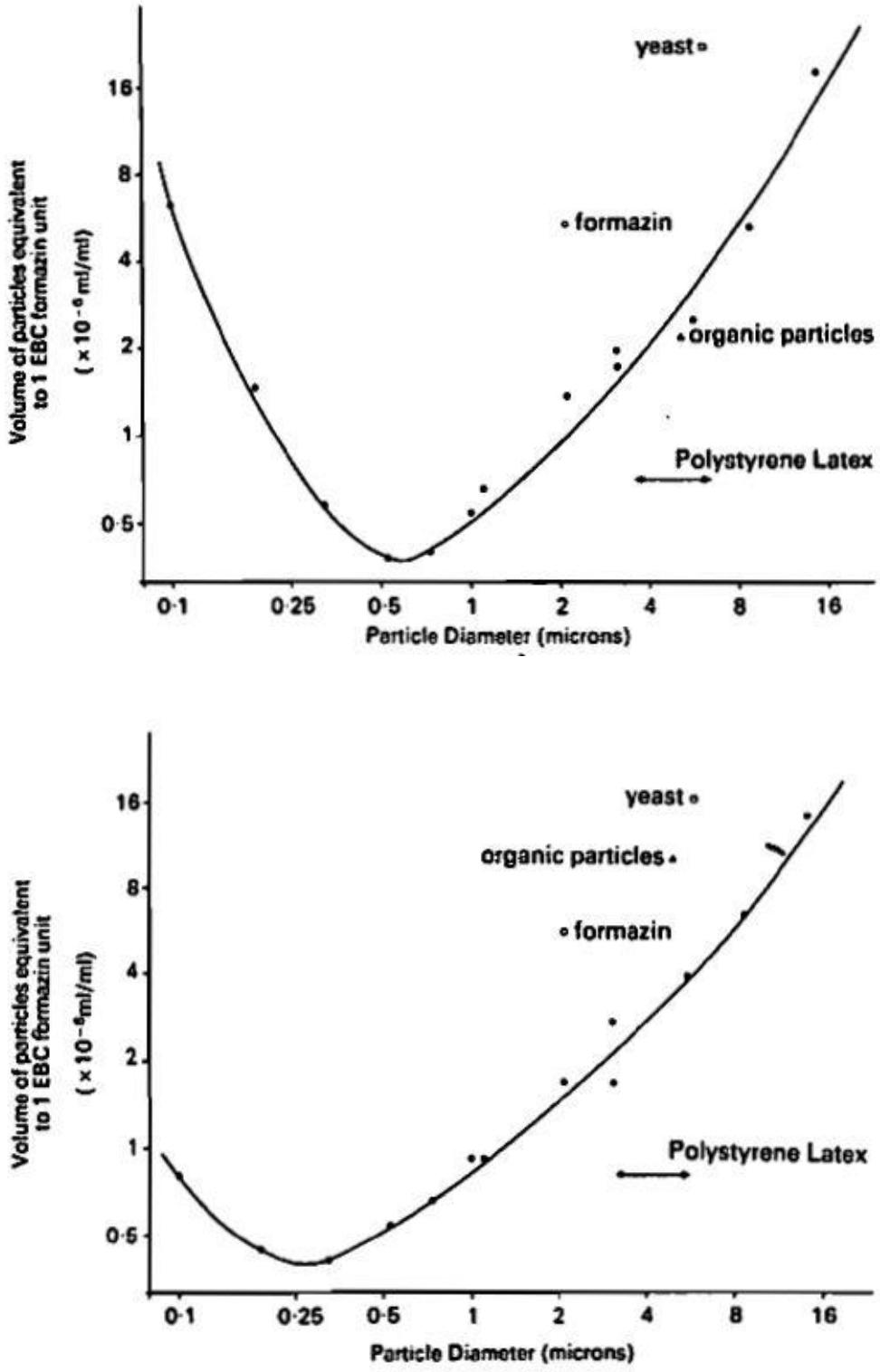


Figure 1.6. Particulate size and concentration in solution for imparting **(a)** 1 EBC colour unit and **(b)** 1 EBC haze unit to beer (Morris, 1987).

According to Morris, 1987, invisible haze is attributed by low concentrations of yeast in finished beer where by the naked eye cannot detect any colour or clarity deviations. Techniques such as those demonstrated in figure 1.6 were considered for invisible haze detection. Yeast fermentation patterns which in-cooperate fermentation yield efficiencies, yeast flocculation characteristics, maximum working yeast generations, etc. are included in determining yeast scrapping protocols, filtration methods and process durations as well as optimum beer clarity. Figure 1.7 illustrates a typical combination of such yeast characteristics.

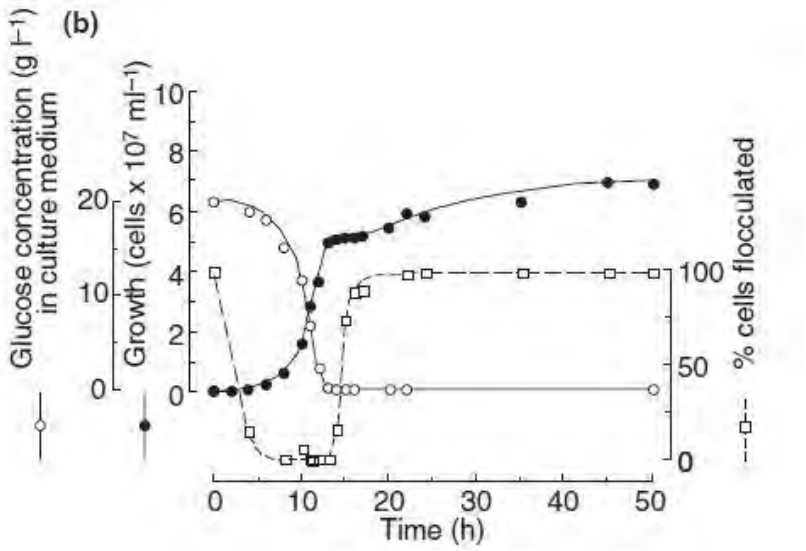


Figure 1.7. Fermentation trend of the yeast strain NCYC 1195 with modified flocculation genes in low – medium gravity wort, (Soares, 2011).

1.6 Instrumentation, Systems and Automation Society (ISA)

In 1988, a standards committee was formed by the Instrumentation, Systems and Automation Society (ISA) to formulate principles of batch systems controlling in process industries as far as specification and designs were concerned (Osif, 2011; Lipták, 2013). An architectural standard batch control was also defined, outlining a structural hierarchy relating data communication and equipment required for physical areas in need of batch controlling (Fisher, 1990; Bosquet, 2004). This standard also had the purpose of outlining function oriented models showcasing the common ground between the control activities of recipe management, production scheduling, batch management, and process control required in batch control. World Batch Forum (WBF), a non-profit professional organisation was also established in 1994 to promote the exchange of information related to the management, operation, and automation of batch process manufacturing (Jensen, 2006).

Model-based strategies put to use qualitative and quantitative process modelling approaches. The popularity of knowledge-based approaches has grown with the introduction and usage of computational tools. Fuzzy logic for instance, is a batch controlling tool that utilizes the popular knowledge-based approach in fermentation automation systems primarily due to the uncertainties it can represent. Data-driven approaches use process data from similarly operated batches to construct models of the process, which may then be used for supervisory control applications. Other ways of data-driven process controlling are found in trends and/or symbolic abstracts of process data (Muthuswamy and Srinivasan, 2003).

Processes that are event-driven and vary with time, and are of a finite or discontinuous manner are known as batch processes. Examples of sequential events in time requiring corresponding control actions are heating, agitating, charging, discharging, reacting, and cooling, just to name a few (Lipták, 2013). Batch processing can be viewed through three perspectives i.e., a process point view, an equipment view (by which products are processed),

and a product-based (where regulatory and discrete controlling may be implemented) or recipe-based view. Chemical compositions, substance amount or final product quality can be used to classify batch processes. The three existing classifications are; a single procedure/single formula process, a single procedure/multiple formula process, and a multiple procedure/multiple formula process (Fisher, 1990; Jensen, 2006).

1.6.1 **Hierarchical modelling and control**

ISA S88 batch standards are terminologies and models that apply to all types of control systems. They are modular structures that promote the full understanding of a process and therefore apply to both automated and manually-controlled batches (Nelson and Shull, 1997). The implementation of ISA S88 has many documented improvements with the emphasis of process optimization being converted to added value in profit within several areas i.e., savings in implementation expenditure, higher percentage guarantee of re-usability, quality and execution consistency, production capacity increase and reduced manual labour. All these areas come together to elevate business optimization at Enterprise level through plant systems integration (Asish, 2000; De Sousa, 2010).

The incorporation of standard operating procedures on the process design ensures identical process conditions during all production runs which in turn eliminate variability in plants. Production departments in the industry tend to benefit since the production process is executed in a very reproducible way i.e., due to thorough feasibility preliminary runs and tests performed in the modelling and planning stages. This is essential for obtaining a production licence (Moosbrugger *et al.*, 1993; Dorresteijn, 1997). Operating procedures are fundamental in batch processes and are an important source of process knowledge due to their scientific, functionality and methodological approaches. The ISA S88 standards for

batch control systems, developed in 1995 by the ISA, also advocate a hierarchical model of operating procedures. (Holý and Požívil, 2002; Muthuswamy and Srinivasan, 2003).

As the urge and experience in harnessing applied hierarchical control developed, engineers developed a knowledge of best practices regarding the structuring, safety and distributed applications of processes. This knowledge was incorporated in the ISA S88 standard, which defines a consistent model for automation and control applications for batch processes. The safety aspect of this knowledge-based approach had an investigative aim with respect to accident/incident causations so as to protect operators from harm, and the enterprise from costs. The acceptance of this standard expanded the core ideas in IS S88 to the continuous and discrete manufacturing industries known to date (Mill, 1992; De Sousa, 2010).

In order to implement good manufacturing practices in a production facility, plant automation through rigorous trial and error becomes an essential. To this end manufacturing execution systems have been developed that control all operations inside a production facility. The introduction of these recipe-driven control systems that follow the ISA S88 standards for batch processes, regardless of the extent of automation in that particular enterprise, has fulfilled good manufacturing practice ideas in the control strategy of biological production processes (Dorresteijn, 1997). In the control of bioreactor production processes, major improvements have been achieved by the introduction of computerized measurement and control units. Although faced with plan-wide integration and economic challenges, this has made the on-line modelling of the production process a possible application, thereby improving both the yield and the consistency of the process (Dorresteijn, 1997; De Prada *et al.*, 2009).

Table 1.3. ISA S88 batch standard model illustration (Erickson and Hendrick, 1999).

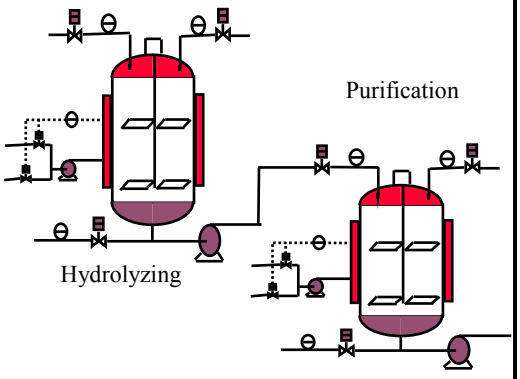
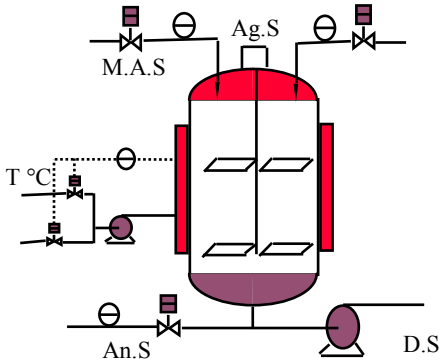
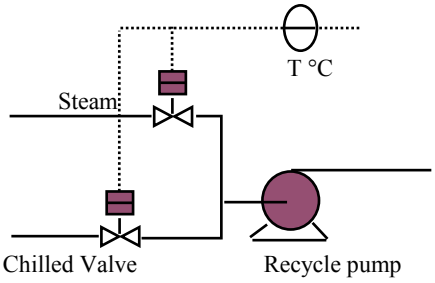
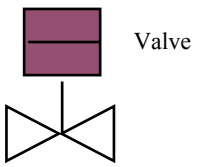
Equipment	Recipe Level	Example	Illustration
Process Cell	Procedure	Production area consists of: 1. Hydrolysing reactor. 2. Purification reactor.	
Unit	Unit Procedure	Reactor consisting of: 1. Temperature control system (T °C) 2. Agitation system (Ag.S) 3. Material addition systems (M.A.S) 4. Discharge system (D.S) 5. Analysis system(An.S)	
Equipment Module	Operation	Temperature Control System consisting of: 1. Recycle pump 2. Chilled water valve 3. Steam valve 4. Temperature Controller	
Control Module	Phase	Valve Pump Motor Pressure Controller	

Table 3 is a top-down illustration of the hierarchical implementation of the ISA S88 modelling principle. The system depicted in the table is essentially built from the control module level up to the process cell. This is an industrial approach that helps in simplifying

equipment commissioning as well as defining operating protocols. Automation of batch production includes batch planning, batch control and real-time monitoring and control. Batch automation (full or semi-) by means of computerized PLC, SCADA and DCS component installations, top-down planned and implemented bottom up. The models outlined by the ISA S88 Committee and Standards are designed to be relevant in batch processing facilities, regardless of the level of automation involved. The different control levels are designed to be replaceable in case the functions are no longer applicable or need maintenance/upgrading (Lipták, 2013). These designs are meant to be fail-safe for equipment/operator safety and protection. Safety interlocks protect plant equipment, and protect the environment whilst prioritizing the safety of operating personnel. These types of interlocks are embedded in the process program or SOP and are initiated by equipment malfunction and usually cause shutdown (Mill, 1992; Liu and Liptak, 1999; Jensen, 2006; Andrews *et al.*, 2011).

Table 1.4. Control Activity Model adapted from (Liu and Liptak, 1999; Jensen, 2006).

Level	Function	Activity
Planning	Process/product management	Product planning, inventory planning, general recipe management, etc.
	Production management	Recipe management, production scheduling, batch history management, etc.
Batch control	Batch management	Recipe generation/selection, batch execution supervision, unit activities coordination, log and report generation, etc.
	Unit supervision	Unit allocation management, unit coordination, etc.
Monitoring and control	Process control	Sequential/regulatory/discrete control: device, loop, and equipment module control, predictive control, model based control, process interlock, etc.
	Safety interlocking	–

Much attention has been paid to advanced bioprocess and biotechnological automation where research clearly shows that the reproduction of these processes is mainly dependent on the environmental conditions for the cells and on the quality of the equipment, pointing out all respective pros and cons of the modelling (Dorresteijn, 1997; Holý and Poživil, 2002). Operational benefits such as fast process delivery, improved product quality, short process turn-around-times, reduced utility costs, etc. are some of the contributing factors towards investments on batch process automation. For batch reactors, reducing batch cycle times, minimized turnaround time between bathes, minimal CIP and down times, and better scheduling for reactor use are amongst the benefits acquired by increased production through automation (Jensen, 2006; Lipták, 2013).

The use of standard operating procedures ensures that all processes are executed identically. This way highly consistent production results can be obtained that mainly depend on the quality of the raw materials (Lalor and Goode, 2009). This can be achieved by the so called "difficult" to reach steady state in batch-oriented automations. Engineers have now countered this phenomenon over time, for example, by using a brew master"s best practices as a default optimum setting for a brewing model. With this development, the batch-to-batch variation caused by small inconsistencies in the operations during the production period will be minimized (Dorresteijn, 1997; Asish, 2000).

1.6.2 Recipes

ISA S88 distinguishes the notions of recipes and equipment. A recipe is a sequence of operational commands that need to be executed in order to transform raw materials to final product. However, equipment consists of hardware or machines used to implement all procedural commands depicted by the recipe (De Sousa, 2010). ISA S88 defines four types of recipes i.e., general recipe, site recipe, master recipe, and control recipe. General recipes define how to produce a specific product without giving an account of any equipment and are most applicable at cooperate top-management level. Site recipes define the part of the product that is produced locally at a specific site/plant and are created from general recipes. Formulated from site recipes are the master recipes which define how to create products with known specifications. Control recipes, which are the closest form of operation control, may be viewed as extracted details of the master recipes. One new control recipe is instantiated for each batch that is desired to be produced (Nelson and Shull, 1997; De Sousa, 2010).

A recipe may therefore be viewed as a sum of parallel or sequenced commands to be executed in a modelled manner using a procedure function chart defined in ISA S88.02, which is similar to a sequential function chart defined in the International Electritechnical Commission (IEC) 61131-3 (De Sousa, 2010).

1.7 Scope of the study

Globally it has been reported that overall beer sales, by volume, have declined as craft beer sales by volume increased. This trend has led to the establishment of more entrepreneurial type of craft brewers who seek to dominate this emerging niche market. McGrath and O'Toole, (2013), reported that a tendency to desire full ownership of businesses as well as maintain personal relationships with customers has seen most craft brewers venturing into independent and unique brewing projects. Craft brewers in the USA and New Zealand have noted that brand loyalty, brand consistency, accessibility to the brand, conformation to local traditions as well as low transportation costs are some of the growth promoting factors of the craft brewing industry (Kleban and Nickerson, 2011; McGrath and O'Toole, 2013). As brewing scientists such as Branyik, Briggs and Soares have advised, a high quality crispy fresh beer is produced by carefully monitoring brewing parameters hence implying the need for combining strict brewing engineering practices with standard brewing styles. The literature reviewed presents segments of beer batch production or control and therefore it is the main aim of the research work at hand to use the brewing engineering standards across all processes from raw material intake to product storage. A handful of the experienced brewers‟ advice, techniques and parameter controlling/monitoring strategies are to be followed as a means of investigating quality integrity on craft beer produced under several known conditions. Furthermore, a target market favouring major SAB products such as Castle lager, Hansa pilsner, Miller genuine draft, etc. is to be used as the general population within which an informal panel of tasters will be selected. A brewing style more inclined to a smooth drinkable beer product will be followed for research purposes.

1.8 Hypothesis

It is hypothesized that the implementation of ISA S88 batch control standards on a traditional microbrewery system will provide better process control, improve beer quality and promote beer quality consistency across batches.

1.9 Objectives and Aims

The following objectives and their respective aims were established for research purposes;

1.9.1 To define a basic traditional recipe to be followed for the brewing style investigated.

- To choose a beer type and style based on the BJCP styling guideline.
- To decide on all raw material type and quantity for use per brewing batch.
- To formulate a basic brewing procedure that contains process parameters relevant to the brewing equipment at hand.

1.9.2 To implement ISA S88 batch control standards across experimental brews.

- To identify brewing and beer quality with respect to physico chemical properties as per process/storage stage.
- To define an ISA S88 batch controlling standard that is applicable to the brewing process by means of a hierarchical and procedural model.
- To produce experimental brews with respect to the defined handling procedures.
- To identify different experimental conditions and measure their effect on beer quality.
- To collect samples at each quality measuring point for correlation with analytical results.

1.9.3 To perform quality analyses of all collected samples as a means of measuring consistency and improvement.

- To perform crude estimation analyses on all collected samples for reducing sugars and FAN content.
- To analyze samples for simple sugars content.
- To analyze samples for aroma-active components i.e., esters and alcohols.
- To correlate quality defining physico chemical values with analytical results obtained.
- To measure quality consistency and improvement of the brewing process by statistical means.

CHAPTER TWO: MATERIALS AND METHODS

2.1. Introduction

Prior to controlled experimental work, the micro-brewing system at hand had to go through some engineer-defining steps before being considered as designed, modelled and structured well enough for consistent batch production. The designs to be assumed had to be centred on process efficiencies, losses and safety before being implemented. Deviations from the traditionally known microbrewery designs were also to be kept at a minimum if this new system was to function at par or better than its peers, giving an ease of performance comparisons with other microbreweries of the same capacity and built. Mashing, lautering and boiling steps in brewing are known to be batch processes, therefore this gave an indication that the hierarchical S88 batch standards were a good start in designing an efficient microbrewery (Moosbrugger *et al.*, 1993). Another crucial property of the system to be designed was its conformity to the microbiological, biochemical and brewing-style requirements. This meant that the final microbrewery equipment together with the defined brewing recipes and SOPs, were meant to satisfy the consumer requirements at all times, and produce a consistent product with a guaranteed shelf life.

The proposed aims and objectives were achieved by the protocols discussed in this chapter. With quality as one of the major investigations in this research, it was noted beforehand that products with lengthy distribution cycles and high consumer demands needed to have a shelf life of 6 months or more. Appearance and flavour are amongst the important quality factor determinants in beer production with beer and wort parameters noted being EBC colour and clarity, pH, particulate matter, and storage temperature amongst other factors (Leather, 1998). The experimentally brewed beer was to be subjected to a shelf life determining experiment together with other aging investigations.

Beer aging is physically noted by the clearing out of the colour which Vanderhaegen *et al.*, (2006), describes as caramels oxidation, or reversible redox reactions that occur in aging beer. The colourimetric scale for beer and wort established by the EBC was one of the quality tools used in the research so providing a platform to compare the experimental brews with known industrial standards.

Consistency across different brew batches was proposed and so with respect to yeast handling, a fairly high pitching rate was deemed necessary so as to avoid osmolarity pressure on yeast and sluggish fermentations which will result in poor batch consistency (Puligundla *et al.*, 2011). Yeast strains ferment different sugars at specific affinity and inhibition rates (Rautio and Londesborough, 2003) and so the beer produced by the Safale s-04 strain had to go through a sugars profile analysis.

2.2. Materials and Methods

2.2.1. Physical and procedural implementation of the ISA S88 model

A hierarchical flow of actions in terms of physical equipment and intended operations from the working areas all the way down to the smallest command/action had to be established. The University of KwaZulu Natal, established in 2004 as the University of Natal (est. 1910) and the University of Durban Westville (est. 1972) merged, is an enterprise with 5 campuses (UKZN, 2014). Three of the five campuses (sites) have active microbreweries i.e., Howard, Pietermaritzburg and Westville. The campus of interest in this study is the Westville site and below is a table with a broken-down view of the site‟s physically intended hierarchical structure. The site layout is a generalised format where the bottom four levels apply to the ISA S88 physical terminology and modelling which impart directly to the procedural design of the project. The hierarchical representation and definition of the optimised system is modelled and illustrated by figure 2.2 i.e., an interpretation of the lower four levels of the model which also group lower process equipment levels into higher grouped levels with specific tasks. The procedural levels of the bottom four layers are shown in detail in the next section. S88 implementation in this study was focused on the brew house equipment, even though the standards were observed for other site areas as well. The brew house was subdivided into two cells i.e., the milling and brewing cells.

Table 2.1. Structural hierarchy and ISA S88 model implementation on the available areas/equipment. Adapted from (Erickson and Hendrick, 1999).

Site Area	Cell	Unit	Equipment module	Control module
Store house	Grain and Hops	Walk-in Fridge	Cooling system	Cooling fan Temperature probe Temperature controller
	Dehumidifier	Walk-in incubator 1	Cooling system Dehumidifier system	Cooling fan Moisture absorber Temperature probe Temperature controller
Brew house	Mill	Miller	Twin roller mill	Induction motor Twin rollers
	Microbrewery	Mash tun	Heating system Agitator Loader	Temperature probe Heating belt Temperature controller Induction motor Mixing flaps Transfer valve
		Lauter tun	Recirculation Cleaning Discharge	Water pump Variable valve Transfer valve
		Boiler Kettle	Heating system Cleaning Loader	Temperature probe Heating belt Temperature controller Water pump Transfer valve
Yeast house	Fermenters	Walk-in incubator 2	Temperature regulator	Cold/hot air fan Temperature probe Temperature controller
	Fermenter beer handlers	Walk-in incubator 3	Temperature regulator	Cold/hot air fan Temperature probe Temperature controller
		Racking	Cooling system	Cooling fan Temperature probe Temperature controller
	Beer handling Conditioner	Bottling	Bottler	
		Walk-in incubator 4	Temperature regulator	Cold/hot air fan Temperature probe Temperature controller
Laboratory	Quality test	Physico chemical analyser	HACH hq 40d system	Conductivity probe D.O probe Multimeter pH probe
	Beer handling quality	Absorbance analyser	Spectrophotometer	Cuvette positioner Light bulb Temperature probe Temperature controller
		Compound analyser	GC-MS system	Gas chromatography Mass spectrometer Enhanced MSD software
	Yeast handling Yeast Propagation	Purification	YPD spread plates YPD streak plates	
		Incubation and Isolation	Temperature regulator	Cold/hot air fan Temperature probe Temperature controller
		Bio-Freezer	Bio-freezing system	Cooling fan Compressed gas Temperature probes Temperature controller
		Shaking Incubator	Incubating system	agitating motor RPM controller Temperature probe Temperature controller

2.2.2. Brew House Area

2.2.2.1 Milling cell

This one-unit cell handles dry malted barley only. The malt is loaded for grinding through rotating rollers that are 0.4 cm apart and collected in a bucket at the bottom of the mill. With only one unit i.e., twin roller system, it implies that when this unit is running, so is the cell, and when the unit is idle the cell is idle as well.

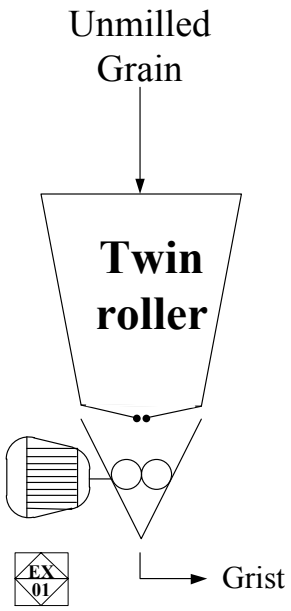


Figure 2.1. Standard Piping and Instrumentation Diagram of the brew house milling cell.

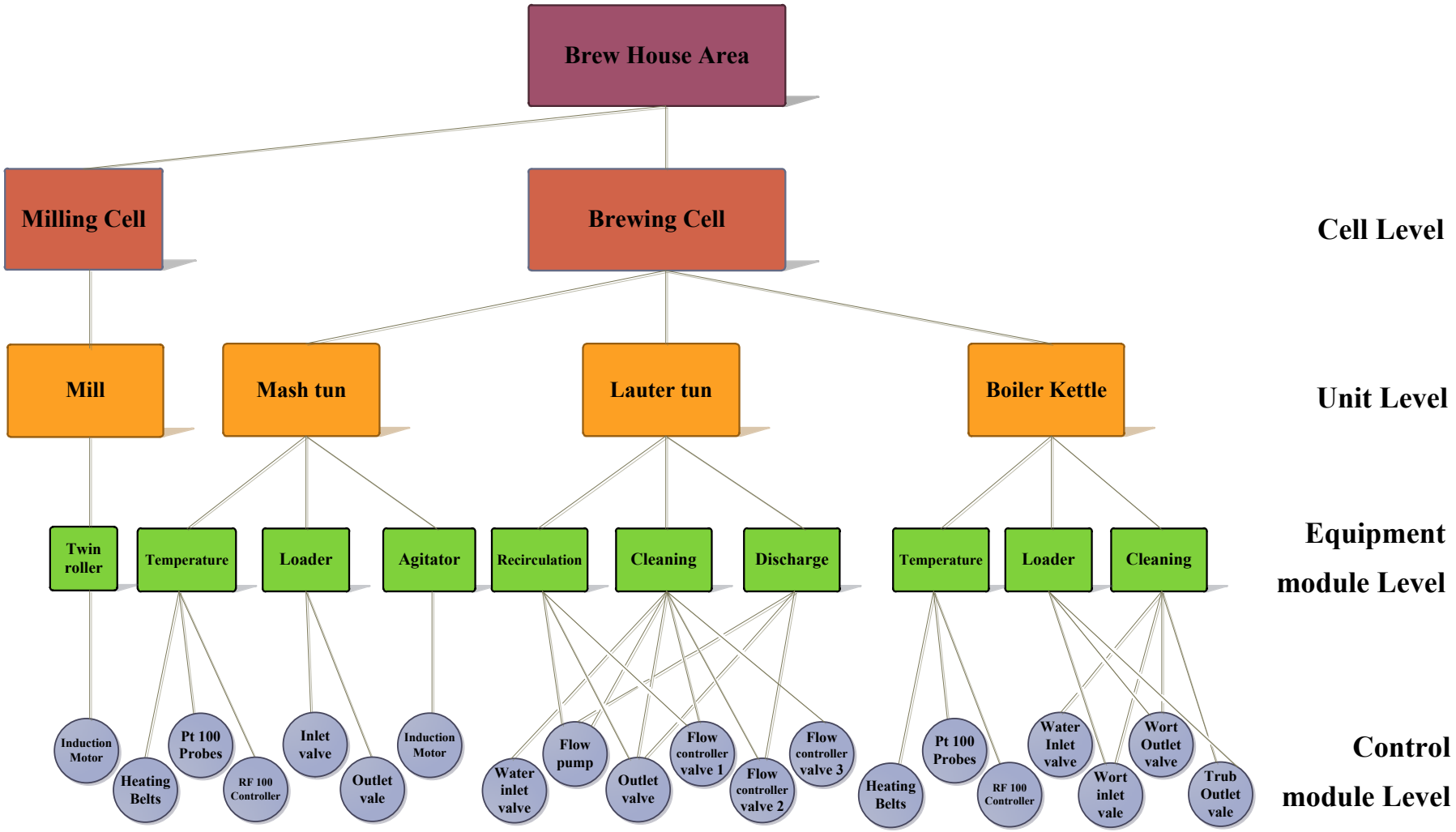


Figure 2.2. Hierarchical layout of the ISA S88 model with respect to the available instruments and units. Emphasis is given only to the bottom four levels of the brew house where most of the designing and implementation was carried out.

2.2.2.2 Miller unit

The mill is a batch-controlled unit which needs a finite grain amount before being allowed to run. Dry and pre-weighed malt is loaded into the top receiving silo whilst the rollers are stationary and the bottom hatch closed. Once filled up, the rollers are set in motion and the hatch open to gear 3. The grain is milled until the rollers reach a constant steady rate, which then allows the hatch to be opened wider i.e., between gears 4 – 6. Milled grain is collected by means of gravity at the base of the mill in a bucket and is sent to the microbrewery cell as raw material. The operating states of twin roller unit are shown in the table below.

Table 2.2. Procedural oriented states of the miller unit during its different stages of operation. Process objective and exceptional handling critically determine the efficiency of such models. Adapted from (Erickson and Hendrick, 1999).

	Operating State Name			
	Idle	Starting	Running	Shutting down
Routine Activity	Clean equipment	Prestart checks;	Malt loaded	Batch complete
	Inspect equipment	Close bottom hatch	Start rollers	Stop rollers
Exception Handling	None	Shut down on:	Shut down if:	None
		Power failure	Rollers malfunction	
		Hatch malfunction	Metal found in malt	
Primary Control Objective	None	None	Amount of malt	None
			processed per batch	
Performance Information	None	None	Kg malt/ batch	None
			g malt losses/ batch	
State End Conditions	Start-up request	Hatch shut	Shut down request	Power off
		Load malt in silo		

Table 2.3. Standard operating procedures and detailed control strategy of the milling unit during operation. Adapted from (Mill, 1992; Erickson and Hendrick, 1999).

Device	**Strategy**	**Interlocks**
Mill Silo	Fill/empty by operator	Do not fill if leaking/dirty. Do not fill if hatch cannot open/close properly.
Silo Hatch	Open/close by operator	Do not open if rollers are not running. Do not open if the grain in the silo contains stones, metals, etc.
Milling equipment module	Start/stop by operator	Do not start if collecting bucket is not in position. Do not stop mid-run whilst there is grain going through the rollers.
Collecting bucket	Fill/empty by operator	Do not remove during milling. Do not overfill.

2.2.2.3 Microbrewery cell

The microbrewery cell consists of three units namely the mash tun, lauter tun, and boiler kettle. In this cell, milled malt (modified barley) and filtered water are fed in as raw materials in the mashing stage. The grain is then cooked gently using a single infusion temperature until the grain starch is completely hydrolysed. As the brewing progresses from the mash tun to the lauter tun the mash is recirculated and clarified so as to separate the spent grain from the sugar-rich wort. Additional water is used to wash remaining sugars off the grain bed as well as make up the required final brew length. Finally the wort is boiled together with adjuncts and hops to give the final bitter-sweet product which is required for fermentation.

The operating states of the described system are listed in table 2.4. This cell is batch-operated and no single unit can run two or more batches concurrently. The ISA S88 methodologies implemented in this cell are meant to promote and maintain adequate and consistent quality control of raw materials and products produced.

Table 2.4. An overview of the microbrewery cell operation states through the mashing, lautering and boiling stages. Process parameters which serve as quality checks are included in this modelling step to guarantee efficiency of the implementation. Adapted from (Fleming *et al.*, 1998; Erickson and Hendrick, 1999).

	Operating State Name			
	Idle	Starting	Running	Shutting down
Routine Activity				Empty all vessels.
	Clean equipment	Prestart checks.	Execute mash recipe.	Rinse vessels.
	Inspect equipment	Start Mash tun	Execute lauter recipe.	Shutdown Mash tun
		Start Lauter tun	Execute boiling recipe.	Shutdown Lauter tun
		Start Boiler kettle.		Shutdown Boiler kettle.
Exception Handling		Shut down if:	Shut down if:	
	None	Pneumatic pressure drops.	Pneumatic pressure drops.	None
		Units malfunction.	Units malfunction.	
Primary Control Objective			Mashing temperature	
	None	None	Lautering flow rate	None
			Boiling temperature	
Performance Information			Runnings volume (litres)	
	None	None	Wort specific gravity	None
			Wort temperature (°C)	
			Wort final volume (litres)	
State End Conditions		Mash tun ready		
	Start-up request	Lauter tun ready	Shutdown request	All units shut down.
		Boiler kettle ready		

2.2.2.4 Mashing

The beer brewing style followed was that of an English pale ale i.e., 8C – extra strong/bitter English pale ale (Strong *et al.*, 2008), and the customized Westville campus micro-brewing system was used for all experimental mashing. The desired target brew length and original gravity across batches was 26 l and 12 °P, respectively. 5.775 kg pale malt and 20.00 g roasted malt was milled through a 0.4 cm milling gap. Milled malt was added to 25 l of treated water which was pre heated to 64 °C prior to malt addition. The mashing process was carried out by stirring the water and malt mixture in the mash tun at 50 rpm for 90 min at 64 °C to allow saccharification. The mash was then heated to 76 °C and held constant for 5 min whilst stirring at 50 rpm to inactivate all starch hydrolysing enzymes. On completion, the cooked mash was transferred into the lauter tun. The mashing recipe was a result of optimised advice given to the researcher by the SAB Company in Prospecton, Durban (SABMiller, 2013).

2.2.2.5 Lautering

The transferred wort was recirculated through a false bottom, sprinkler system in the lauter tun at a constant flow rate of 8 l/min. Wort was recirculated for 50 min until it became a clear copper colour as it seeped through the now compacted grain bed. Recirculated wort was then transferred in the boiling kettle. A first sparging run was performed by adding 8 l of treated water, pre heated to 80 °C, to the grain bed in the lauter tun and recirculated for 45 min. The first runnings were transferred into the boiling kettle. A second sparging run was performed by adding 8 l of treated water, pre heated to 80 °C, to the grain bed in the lauter tun and recirculated for 40 min. The second runnings were transferred into the boiling kettle. A third sparging run was performed by adding 5 l of treated water, pre heated to 80 °C, to the grain bed in the lauter tun and recirculated for 30 min. The third runnings were transferred into the

boiling kettle. The lautering recipe was a result of optimised advice given to the researcher by the SAB Company in Prospecton, Durban (Hiralal *et al.*, 2013, SABMiller, 2013).

2.2.2.6 Boiling

The collected worts from the lautering stage were mixed in the boiling kettle and heated to boiling point i.e., 100 °C. On reaching boil, 50 g of southern hop pellets were added together with 300 ± 50 ml of concentrated maltose syrup as adjuncts. The boil was held constant and vigorous for 50 min before adding 5 g of saaz hop pellets. The boil was allowed to continue for 10 min before it was bought to rest. A manual stir was performed on the boiled wort for 5 – 10 min to create a whirlpool effect and then the wort was allowed to rest for 90 min whilst being simultaneously cooled. When the wort reached 17 ± 1 °C it was transferred into a sanitized bucket and the cloudy trub was discarded. The boiling recipe was a result of optimised advice given to the researcher by the SAB Company in Prospecton, Durban (SABMiller, 2013).

2.2.2.7 Centrifuging and aeration

A continuous centrifuging system was used to clarify the cooled wort. A sanitized submersible pump was immersed in the wort and used to pump the boiled wort. The pump drew wort out of the bucket and passed it through a centrifuging core running at 35 000 rpm, and pumped it into a sanitized 50 l stainless steel cylindrical keg. The particulate matter was collected at the bottom of the centrifuging core in a discard bottle. Filtered air was forced to bubble through the centrifuged wort, from the bottom up, until the dissolved oxygen content in the wort reached 8 ± 1 mg/l.

2.2.3. Brewing water treatment

All brewing water was passed through a ceramic cartridge filtering unit containing 5 micron and 1 micron filter pads which possess granular activated carbon properties. A conditioning ratio of 80 µl lactic acid per 1 l of water and 80 mg calcium sulphate dihydrate per 1 l of water was used for pH adjusting and salting of the filtered water (SABMiller, 2013).

2.2.4. Yeast cultivation and propagation conditions.

The yeast strain *Saccharomyces cerevisiae* Safale s-04 was obtained from the National Food Products Company, Johannesburg, and used for all experimental fermentations. A propagation method according to (SABMiller, 2013) was used with a few modifications (Erten *et al.*, 2007). 15 ml of sterile wort at 7 °P was inoculated with 2.5 g of dry yeast and shaken at 120 rpm for 24 h at 25 °C. After activation of the dry yeast, now suspended in solution, the solution was scaled up to 100 ml using sterile wort at 7 °P and shaken at 120 rpm. After 24 h, the cloudy wort solution was up scaled to 1 l using sterile wort at 7 °P and shake at 120 rpm for 48 h at 22.5 °C. After 24 h, the solution was up scaled to a final propagation volume of 5 l using sterile wort at 7 °P and shake at 100 rpm whilst maintain 20 °C for 48 h or until the desired OD_{600} nm value is reached. A pitching rate of 10×10^6 cfu/ml/°P was used for all experimental batches, and the inoculum size varied with respect to the activated yeast concentration.

2.2.5. Brewing particulate matter

Particulate matter suspended in wort was investigated by this method. Empty 1.5 ml tubes supplied by Eppendorf were weighed, and subsequently filled with 1 ml samples from the mashing, lautering and boiling stages, respectively. The filled tubes were centrifuged at 12000 rpm for 10 min, emptied and left open in an upside down position over night to dry. The dry tubes with solid matter pellets stuck at the base were reweighed and particulate matter present calculated in mg/ml. A method by (Hiralal *et al.*, 2013) was observed and used together with modifications done to compensate for the brewing design and scope at hand.

2.2.6. Wort fermentation

Propagated wort from method 2.2.4 was used as an active yeast source for the strain *Saccharomyces cerevisiae* Safale s-04. The inoculum size was calculated based on the concentration of yeast suspended in the wort solution. A constant pitching rate of 10×10^6 cfu/ml/°P was used throughout all experimental batches brewed. Fermentation was performed in 3.5 l sterile glass vessels and wort was filled up to the 3.0 l mark. Fermentation was monitored every 24 hours by aseptically drawing 200 ml of wort from the control and experimental fermenters and using a hydrometer to measure specific gravity. Each experimental brew had a control fermentation kept at 18 °C until specific gravity reached 3.8 ± 0.2 °P. Experimental fermentation temperatures were 14 °C and 16 °C for batches 1 – 3 and 4 – 6, respectively. These batches were considered fermented once their final gravity values were below 5.0 ± 1.0 °P (Briggs *et al.*, 2004).

2.2.7. Beer bottling and conditioning

A separate propagation batch was prepared for bottle conditioning. 1 sachet of Safale s-04 yeast (2.5 g) was propagated up to the second stage i.e., 100 ml solution and stopped when the $OD_{600\,nm}$ value equivalent to $30 \pm 2 \times 10^6$ cfu/ml was reached. The fermented and matured wort, now termed beer, was carefully tapped out and distributed evenly into sanitized 750 ml brown beer bottles. The beer was then spiked with 4 ml of a 50 % maltose solution and 3 ml of the activated yeast. This was left to stand for 30 min under an air extractor before being capped and mixed by gentle shaking. Capped bottles were labelled according to their unique experimental codes and were all incubated at 25 °C for 7 days and were left standing up right and allowed to clear out whilst carbonating (Hiralal *et al.*, 2013). After the 7 days, all bottles were taken to a 0 °C fridge where they were stored for 14 days and allowed to further clear out in appearance and lager. The conditioned bottles were finally stored at their respective experimental temperatures i.e., 1: 0 °C, 2: 4 °C, and 3: 18 °C.

2.2.8. Beer tasting

An untrained panel of general tasters was selected for periodical beer tasting sessions. The criterion for participation was age, ethnic groups, beer drinking experience and preference i.e., so as to have a very diverse tasting group. The tasters were given an initiation demonstration as to how to taste, characterise and judge beer, as according to (Strong *et al.*, 2008). Tasters were given score charts to enter their critics of each beer bottle based on appearance, foam colour and size, aroma, taste, mouth feel and overall impression. The tasting sequence was as follows: pour beer; observe colour, clarity, beading, and foam head; cover glass top with palm and swirl gently; nose (inhale) the emitted aroma; sip and hold beer in mouth; spit out then sip again from glass and swallow; rinse mouth and glass with water; move to the next beer bottle.

2.2.9. Wort and beer physico chemical analyses

A HACH HQ 40d multimeter probe was used for all physico chemical measurements with its various probes. During brewing 3 sampling points were defined where wort underwent quality checks by means of pH and specific gravity measurements within a sampling temperature range of 22 ± 3 °C. In all the sampling stages i.e., mash tun output, lauter tun output, and boiling output; 10 ml of wort was drawn in triplicate and measured for pH and 200 ml was drawn in triplicate for specific gravity measurement. For all beer samples, the freshly boiled wort (per respective batch) was used as a reference for all physico chemical analyses. The periodically tasted beer simultaneously had the following parameters measured; conductivity, dissolved oxygen, pH, salinity, and total dissolved solids. 5 ml of beer sample was drawn in duplicate for these measurements, and dissolved oxygen always came first, as this was to minimise ambient oxygen from dissolving into the carbon dioxide rich beer.

2.2.10. Wort and beer colourimetry

Wort and beer at various sampling and tasting points was simultaneously analysed for colour scores in the EBC and SMR colour charts. Samples were put into a 1 ml cuvette and measured at a wavelength of 430 nm by a Shimadzu UV – 1800 spectrophotometer coupled with a Shimadzu CPS temperature controller (Hudson, 1969). The blank for the entire analyses was distilled water and samples were measured in duplicate.

2.2.11. Reducing sugars content

An estimation method according to (Sadasivam and Manickam, 1996) was used with a few modifications (Hiralal *et al.*, 2013). Different 1 ml glucose standards ranging from 100 – 400 mg/ml were made up to 3 ml with distilled water in a test tube. 3 ml DNS reagent was added to the standards and the tubes were covered with foil paper and places in a boiling water bath for 5 min. While still warm after boiling, 1 ml of 40 % Rochelle salt solution was added and the solution was allowed to cool further. The now varying red concentration colours in the different tubes were measured with a Shimadzu UV – 1800 spectrophotometer coupled with a Shimadzu CPS temperature controller at 510 nm wavelength. The resulting absorbance values created a calibration graph with which unknown concentrations were determined. For sample treatment, 1 ml of wort diluted between 100 – 600 times and 1 ml of beer diluted 12.5 times was made up to 3 ml with distilled water in a test tube. 3 ml DNS reagent was added and the same procedure followed.

2.2.12. Free amino nitrogen content

A 0.1 % w/v glycine solution (1 g/l) was prepared as a stock calibration source (Sadasivam and Manickam, 1996). A volume range of 50 – 200 µl was pipetted into labelled test tubes, and made up to 4 ml with distilled water i.e., 12.5 – 50 mg/l concentration range. 1 ml of 8 % w/v Ninhydrin reagent was added to each test tube including a blank sample containing 4 ml distilled water. Foil paper was used to cover the test tubes, followed by vortexing and boiling in a water bath for 15 min. The different shades of purple generated in the tubes were cooled in cold water before 1 ml of 50 % v/v ethanol was added to each test tube and mixed well. Absorbance of the individual tubes was measured using a Shimadzu UV – 1800 spectrophotometer coupled with a Shimadzu CPS temperature controller at 570 nm. For

experimental work, 4 ml beer and wort samples diluted 4 times were mixed with 1 ml of 8 % w/v Ninhydrin reagent before being vortexed, boiled and viewed at OD_{570} nm wavelength.

2.2.13. Volatile esters and fusel alcohols analysis

Samples acquired from the different sampling stages were kept frozen at -30 °C until needed for analysis. For ester and fusel alcohol concentration analysis, 5 ml of sample were drawn into 20 ml headspace glass vials and 1.5 g of Sodium chloride was added i.e., 30 % w/v salting. Headspace gas chromatography (HS-GC) was used for volatile gas separation and a mass spectrometer (MS) was used as a detector i.e., (HS GC-MS). An Agilent 7890 A GC system coupled with an Agilent GC 80 sampler and an Agilent 5975 C inert MSD was used according to Jelen *et al*., (1998) with modifications (Pinho *et al.*, 2006; Mallouchos *et al.*, 2007; Charry-Parra *et al.*, 2011). Samples were heated in a rotating heating block at 80 °C while shaking at 800 rpm for 20 min. 1 ml of hot headspace sample was drawn by the Agilent auto sampler and injected into the front inlet port of the GC kept at 250 °C. The GC program used was as follows; CTC Pal ALS auto sampler needle was purged 5 times with the hot air sample and 1 ml was injected into the GC in a split ratio of 1: 15. The oven temperature was held at 60 °C for 4 min, raised at 5 °C/min to 100 °C followed by 10 °C/min to 220 °C and kept for 2 min at 220 °C. The MS detection temperature was kept at 250 °C; carrier gas used was Helium with a flow rate of 1.1 ml/min and a pressure of 10 psi.

2.2.14. Beer and wort ethanol analysis

Analysis was carried out using the HS GC-MS technique with beer and wort samples being diluted 10 and 5 times, respectively before 5 ml aliquots were prepared in 20 ml headspace glass vials (Buckee and Mundy, 1993; Jelen *et al.*, 1998). A 30 % w/v salting ratio with Sodium chloride was used and the samples were shaken at 800 rpm for 10 min at 70 °C using the Agilent 7890 A GC system coupled with an Agilent GC 80 sampler and an Agilent 5975 C inert MSD. 1 ml of hot headspace sample was drawn by the Agilent CTC Pal ALS auto sampler and injected into the front inlet port of the GC kept at 250 °C. The GC program used was as follows; auto sampler needle was purged 5 times with the hot air sample and 1 ml was injected into the GC in a split ratio of 1: 20. The oven temperature was held at 40 °C for 4 min, raised at 5 °C/min to 100 °C. The MS detection temperature was kept at 250 °C; carrier gas used was Helium with a flow rate of 0.8 ml/min and a pressure of 12 psi.

2.2.15. Analysis of sugars

2.2.15.1. Oximation reaction

An oximating reagent was prepared in water free glassware using gas-tight glass syringes. 2.5 g of hydroxlammonium chloride was added in 100 ml of pyridine and mixed using a stirring bar unit until the salt dissolved entirely. 550 µl of 2-(Dimethyl amino)-ethanol was added to the solution creating a 24 h stable activated oximation reagent. 15 µl of sample were drawn into dedicated 4 ml reaction vials which were in a clean and water free condition, followed by 2 µl of Arabinose which was used as the internal standard. 500 µl of the activated oximating reagent was added into the vials containing 15 µl sample and capped tightly. The reaction vials were hand-shaken vigorously and placed in a sonicating water bath for 20 min at 70 °C, and were removed and allowed to cool afterwards. All mixing and heating was done under a fume hood extractor. This oximation method and sylilation method below were adapted and optimised from the Sugar Milling Research Institute, in Durban (Cason *et al*., 1987).

2.2.15.2. Sylilation reaction

The exterior of the vials was wiped dry prior to opening and adding 0.45 ml of 1,1,1,3,3,3-Hexamethyl-disilazane (HMDS) and 50 µl of trifluoroacetic acid (TFA) to the cool contents in this respective order. Vials were capped and shaken vigorously before being sonicated for 20 min at 70 °C. Whilst the vials were still warm, they were shaken 3/4 times and opened in between each shake so as to release the ammonia from solution during the sylilation reaction. The vials were capped, and centrifuged at 4800 rpm for 10 min, placed up right and the supernatant drawn to the dedicated 1.5 ml GC vials. Acetone was used to clean the transferring syringe 3/4 times in between each sample vial. All transfers and mixtures were done under a fume hood extractor (Cason *et al*., 1987).

2.2.15.3. Gas chromatography analysis

A method by Cason *et al*., (1987) with modifications done by the Sugar Milling Research Institute, (1993) in Durban, and (Bogo and Mantle, 2000; Nogueira *et al.*, 2005) was used. The Agilent 7890A GC system coupled with the 5975C inert MSD was used for the identification and quantification of the sylilated sugar complexes created. The GC program used was as follows; auto sampler needle (AGC-inj R1) was pre-cleaned and post-cleaned 2 times in acetone and cleaned twice with the respective sample. The needle was purged 3 times with the sample solution and 1 µl sample was injected into the GC front inlet kept at 250 °C with a split ratio of 1: 25. The oven temperature was held at 160 °C for 2 min, then raised at 10 °C/min to 275 °C and held for 4 min at 275 °C. The MS detection temperature was kept at 250 °C; carrier gas used was Helium with a flow rate of 1 ml/min and a pressure of 10 psi.

CHAPTER THREE: RESULTS FOR BREWING, WORT FERMENTATION AND BEER STORAGE.

3.1. Introduction

This chapter deals with the brew house activities undertaken experimentally to practically prove the benefits of implementing ISA S88 batch control modelling in a traditional microbrewery system. This chapter includes all modifications made on the microbrewery system as well as day to day brewing results that were observed per stage and recorded for further analysis. All good brewing practices such as rinsing all vessels and pipe lines with hot water before brewing, sanitizing all working areas and equipment before use, sanitizing of hands before coming into contact with wort/beer, wearing protective clothing in the brew house, just to name a few, were observed during the entire duration of the experimental work. Although not mentioned much in the modelling of the brewing protocol (chapter 2), these small but important practices ensured that no unwanted deviations of a particular process were experienced due to handling errors. The mashing, lautering and boiling stages were done at room temperature which averaged 20.8 ± 2.37 °C, as well as at ambient (atmospheric) pressure. The figures reported in this chapter are all averages ± standard deviation with participants ranging from n = 3 – 9 samples, unless otherwise stated. GraphPad Prism 5 for windows (2009 edition) was used for all statistical analysis. For the averaging of numerous sample values, a descriptive analysis of the table rows was used where the focus was on the mean, standard deviation, confidence level range and sample size. The measure of consistency/reproducibility of a process by means of recorded physico chemical properties was done with the aid of a two-way ANOVA coupled with multiple t-tests at $p \leq 0.05$, unless otherwise stated. Properties with wider acceptable ranges and very sensitive characteristics had their significance bound more to the literature and known industrial brewing trends than to the strict value of $p \leq 0.05$. The measure of decay/depletion of substrate such as reducing

sugars was analyzed by the use of a one-phase decay nonlinear regression analysis and the one-phase association nonlinear regression was used for the analysis of substance build up e.g. ethanol. Calculations used in this chapter for yield, extraction efficiencies; losses, etc. are found in the appendix-G.

Samples collected from the various brewing stages and storage periods were analyzed for simple sugars and flavour compounds. All analyses and results produced were obtained by using the Agilent 7890A GC system coupled with the Agilent 5975C inert MSD. This analytical instrument was remotely controlled by Agilent 5975C MSD ChemStation software coupled with the Enhanced Data Analysis software version E.02.02.1431 for protocol runtime and chromatogram analysis, respectively. Sample preparations and runtime protocols were according to Jelen *et al.*, (1998) and Hiralal *et al.*, (2013) where modifications are stated in chapter two. The simple sugars investigated as substrate for the yeast strain Safale s-04 were fructose, glucose, sucrose, and maltose. Maltotriose, an important brewing sugar after maltose and glucose, was undetectable in the optimized oximation and sylilation methods used coupled with GC-MS analysis, and this was acknowledged as a major missing component of the results expected. Flavour compounds investigated in this chapter were ethanol, propanol, ethyl acetate, isoamyl alcohol, isoamyl acetate, ethyl hexanoate, and ethyl octanoate. Ethanol concentrations, expressed as percentage v/v, were of great concern and were closely monitored for proof of beer consistency within the brewing style intended. Statistical means of testing consistency was a two-way ANOVA coupled with multiple post Bonferroni tests at $p \leq 0.05$.

3.2. Microbrewery system design and modification

The Westville Campus microbrewery equipment donated by SABMiller came in the form of three 50 l capacity stainless steel kegs. These kegs had their bottoms cut open and placed in an inverted position on a rectangular frame in series, so as to mimic the three respective brewing vessels i.e., mash tun, lauter tun, and boiler kettle. The piping and instrumentation shown in figure 3.1 was designed and implemented by the researcher with help from the UKZN‟s Academic Instrumentation Unit, and was completed with available and procured instruments shown in table 3.1. The following sub-sections are brief overviews of how each brewing unit, i.e., mash tun, lauter tun, and boiler kettle was optimized for operation as far as instrument and piping designs are concerned.

Table 3.1. Instrument list dedicated to the final Westville microbrewery design.

Instrument	Quantity	Rating
Heating belts	4	230 V, 50 Hz
Pt. 100 temperature probe	4	0 – 10 V DC, 4 – 20 mA
Pneumatic valves	6	8 bar, -40 – 80 °C range
Solenoid valves	6	2 – 7 kgf/cm^2 range
Water pumps	2	220 – 240 V AC, 0.25 A
Single phase relays	4	250 V AC, 10 A (n.o), 5 A (n.c)
Single phase motors	2	220 – 240 V AC, 0.3 – 0.6 KW
RF 100 temperature controllers	4	100 – 240 V AC, 47 – 63 Hz

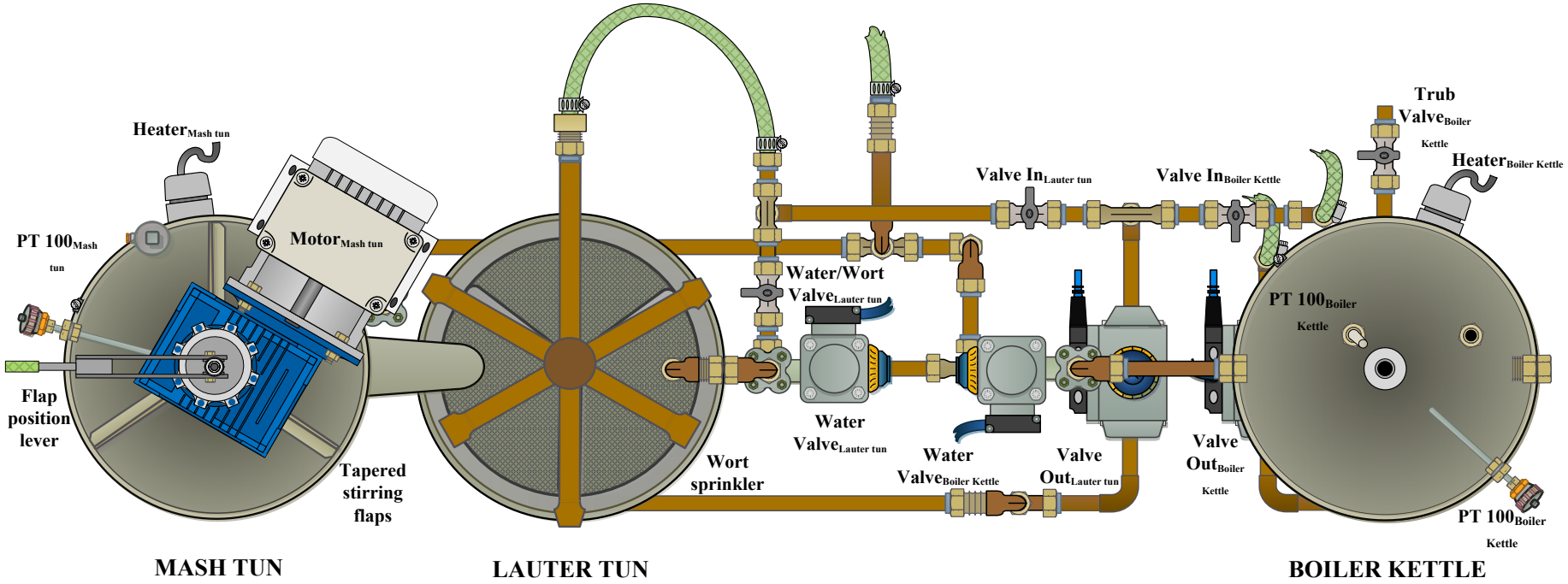


Figure 3.1. Optimized top-view design of the 50 l capacity microbrewery system i.e., after S88 batch control implementation and hierarchical considerations of all equipment and modelled processes.

3.2.1. Mash tun

The mash tun was equipped with a single phase induction motor set at 1380 rpm. The motor worked as a stirrer and the stirring rod had three flaps attached perpendicular to it and set at 120 ° apart. The heat controlling equipment consisted of two 230 V AC heating belts coupled with two Pt. 100 temperature probes via two RF 100 temperature controllers. Each probe, belt and controller formed a closed loop system which was monitored by means of a fine-tuned PID scheme embedded in the controller program. During its trial and preliminary runs, the mash tun as a unit was found to have temperature value deviations, and total average mash temperature inaccuracies. This was caused mainly by low mixing efficiency of the thick mashes using only one set of flaps. To mix much quicker, and also increase the efficiency of heat distribution across the mash, an additional temperature probe was installed to increase temperature reading accuracy. Another set of three flaps were installed above the first set on the mixing rod, and all six flaps were tapered by 45 ° to promote simultaneous horizontal and vertical circular mixing during mashing. A mixing rod lever was attached on the top end of the mixing motor to give the operator an option to mix the mash further by manually pushing the lever up and down before returning it to its down default position. A two state 8 bar pneumatic valve coupled with a solenoid was used as an outlet regulator at the bottom of the mash tun and this valve was designed to be manually switched open or shut by the operator.

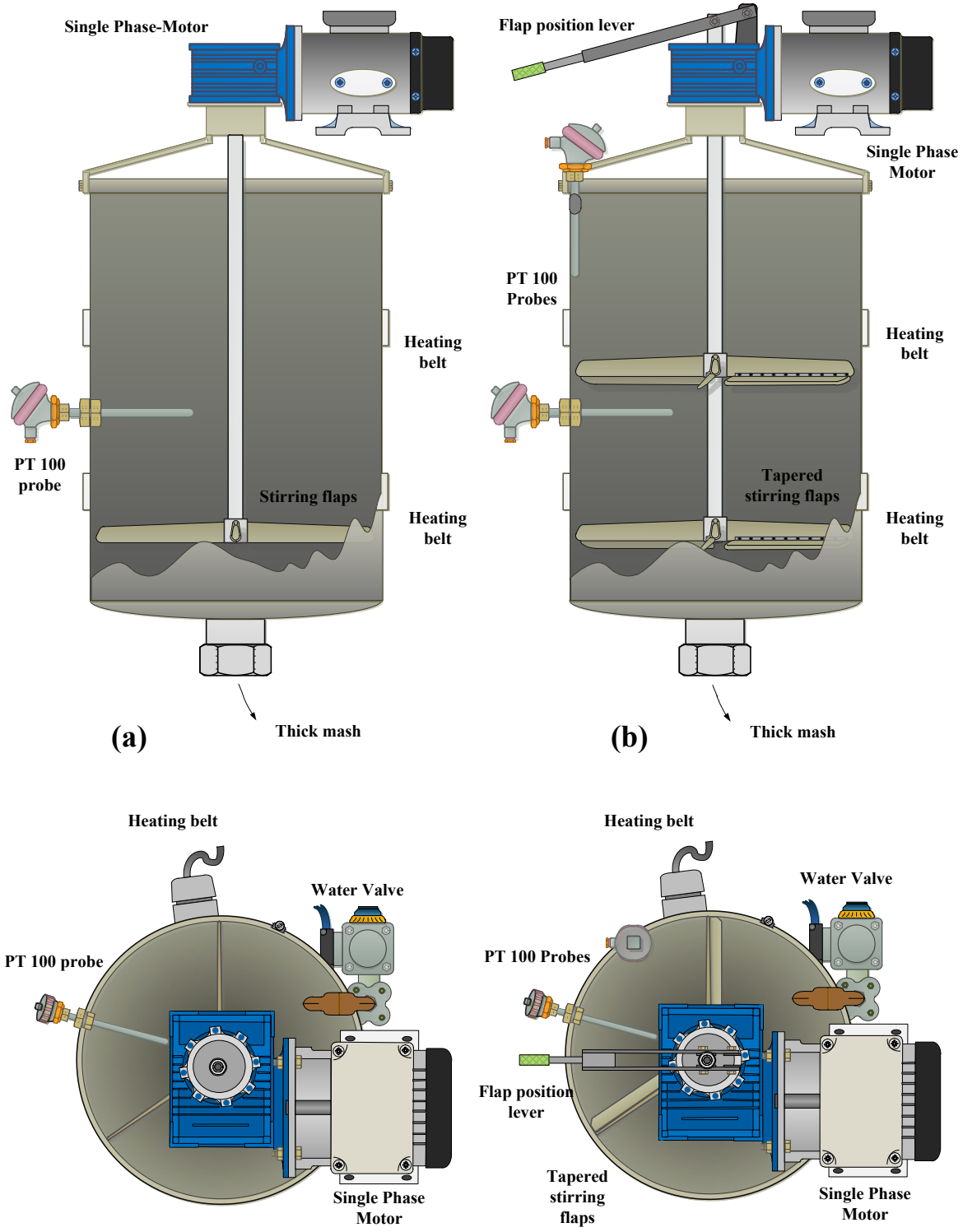
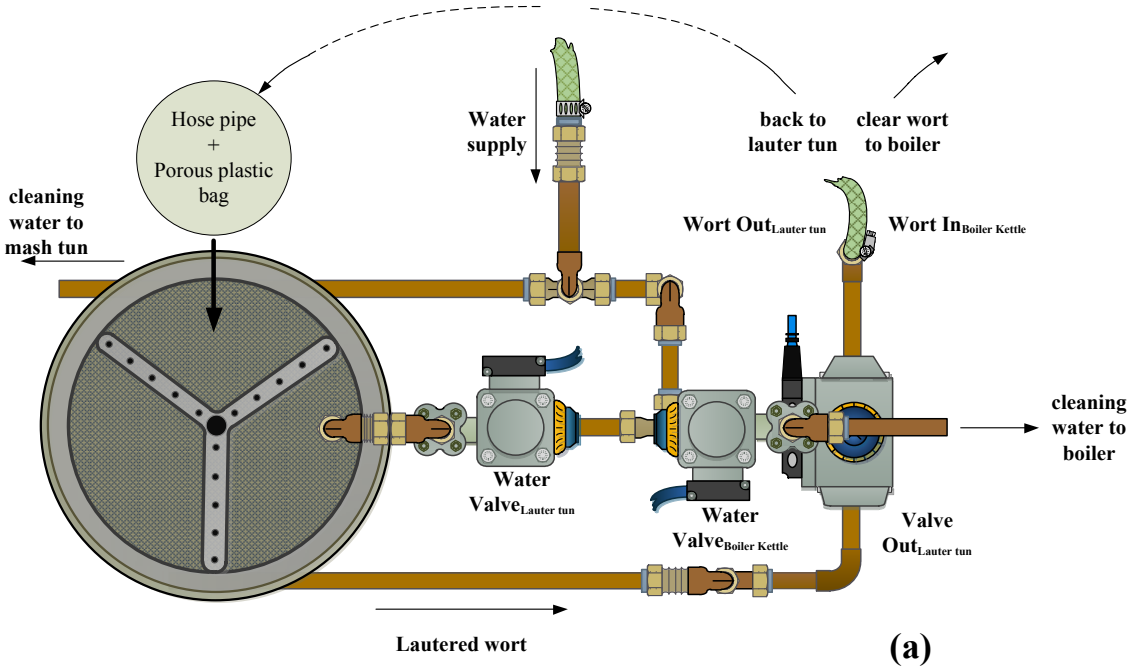


Figure 3.2. Side- and top-view of (a) the default mash tun design and (b) the optimized design after implementation of the batch control-enhancing modifications. Even temperature and mash distribution within the vessel were the primary influences of this final design.

3.2.2. Lauter tun

The lauter tun was positioned lower than the mash tun as gravity was used to transfer the mashed wort into the lauter unit. A false bottom made of porous stainless steel sheeting was placed at 15 cm above the real bottom of the vessel. A two state 8 bar pneumatic valve coupled with a solenoid was used to open and close the outlet point of the vessel situated at the bottom. A standard 240 V AC water pump was used for suction of the lautered wort back to the top of the spent grain bed, either for recirculation or, transfer into the boiling kettle. The recirculating piping system was not available prior to this study and recirculated wort was passed through a rubber pipe tied to a porous plastic bag which served as a sprinkler, and had to be held by hand throughout the process by the operator. This method was highly tedious and inaccurate and hence a two way path for the lauter output piping system was designed, i.e., one path leading back to the sprinkler mechanism installed and placed overhead of the spent grain bed, and the other path leading to the boiler kettle. This two way piping system was designed such that only one path could function at a time i.e., either the lautering pathway during sparging, or the pump-out path way during wort transfer to the kettle. This design was included in the model as a way of eliminating inconsistencies in lauter batch volumes, spent grain bed compression, false bottom differential pressure, percentage wort recovery, and wort clarity.



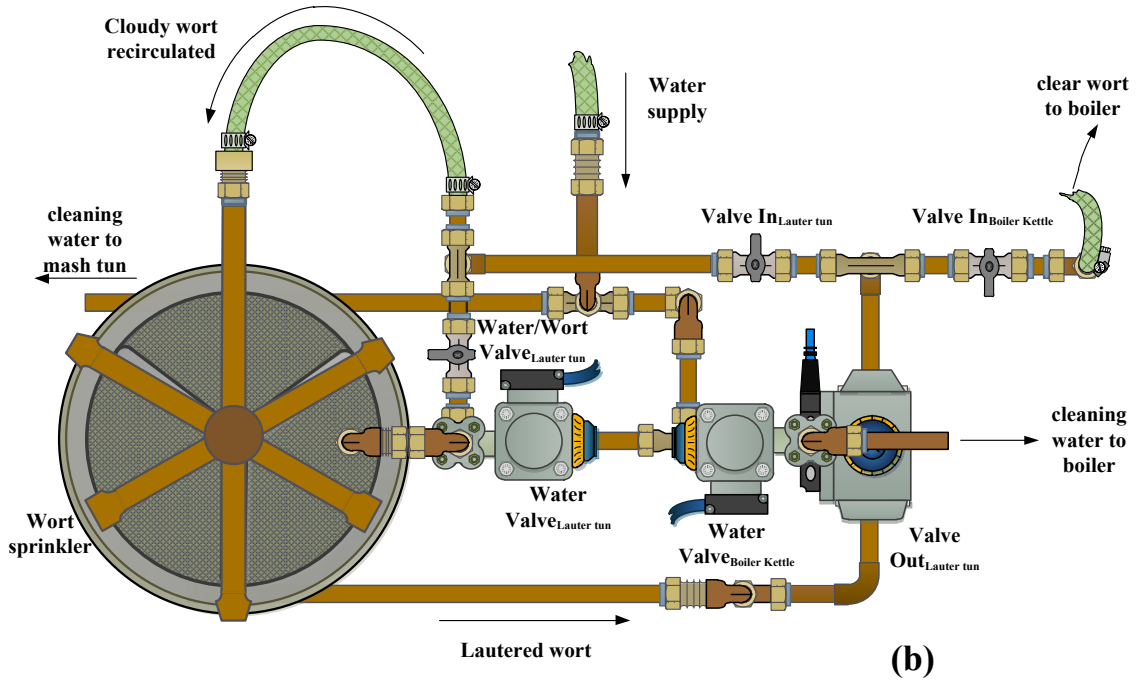


Figure 3.3. Top-view (a) of the lauter unit design (b) with an implemented wort recirculation piping system for accurate and reproducible lauter batches and filterability.

3.2.3. Boiler kettle

Initially the boiler kettle was designed with one Pt. 100 temperature probe regulating the two heating belts installed. However, due to the temperature variations in wort from the bottom up, the probe gave an under estimated temperature value for the top belt and an over estimate for the lower belt, hence inaccuracies were noted in boiling. A modification identical to the mash tun was then implemented where each heating belt had its own Pt. 100 probe assigned to it through an RF 100 temperature controller. The initially fixed cooling coils were observed to be a cleaning obstruction because of their structure and size; hence a modification on their piping was made. These coils were removed from the boiling kettle and were fitted on to a lid making them a removable part of the boiler which only came to use during the wort cooling stage. The coils were connected to the cold water pipes thus promoting heat exchange between the hot wort in the kettle and cold water running through the coils during cooling. Two bottom outlet points were used in this vessel i.e., a centre outlet for trub and cleaning water, and a slightly displaced outlet to the side for clear wort and water. The clear wort outlet had a pneumatic valve and a pumping system regulating wort flow, whilst the trub outlet had a variable manual valve as the only instrument thus relying on gravity for flow.

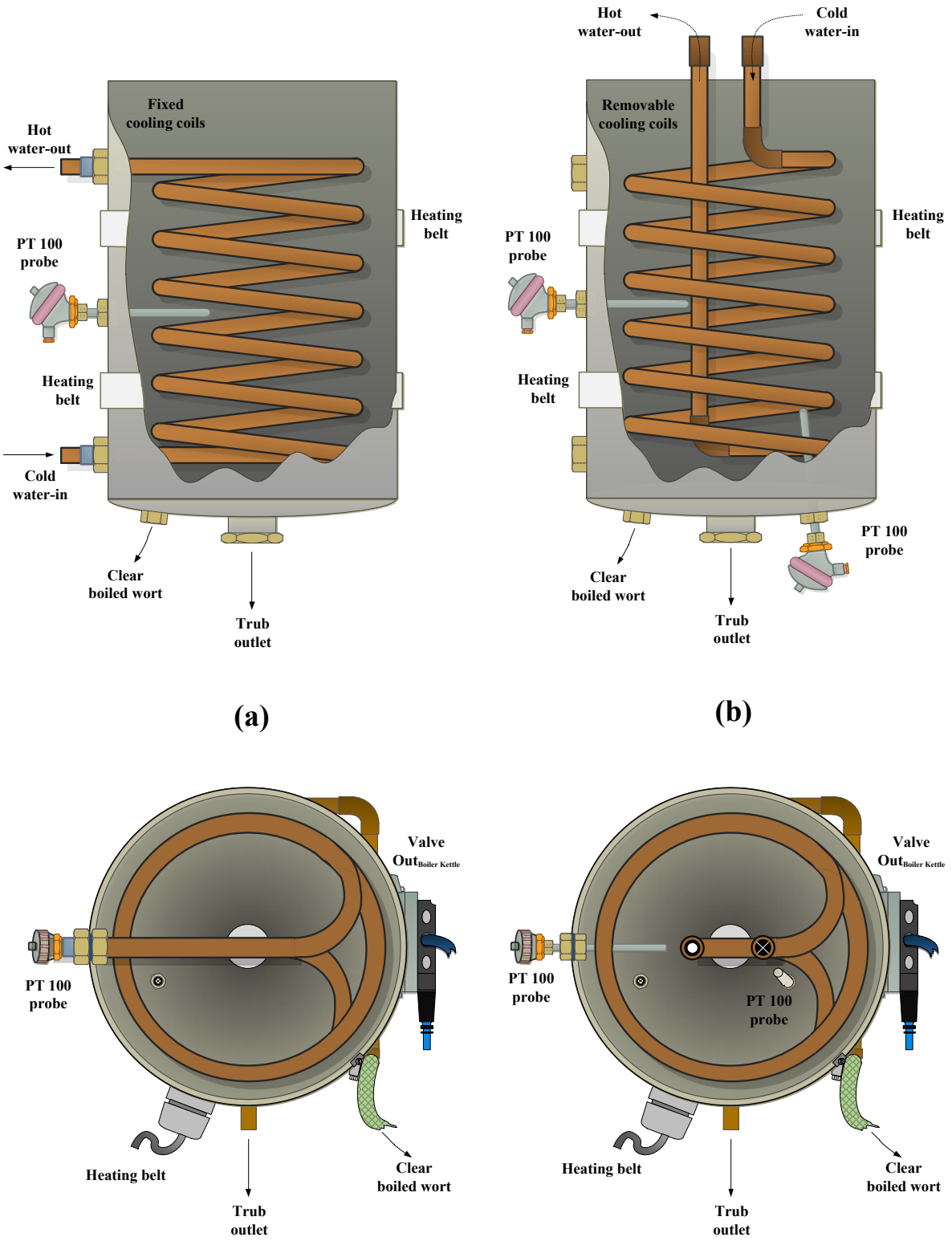


Figure 3.4. Side- and top-view of (a) the default kettle design and (b) optimized design with improved temperature controlling, cooling system and accessibility modifications.

3.3. Brewing process and product quality analysis

After the implementation of all modelled designs, the microbrewery system was used to brew beer under constant conditions as a means of validating the proposed hypothesis. This beer production plan was followed to produce six English ale batches that were experimentally partitioned from the fermentation stage onwards. Although many preliminary batches were brewed before optimizing the recipe, this section deals only with the consistently brewed batches that were regulated by the ISA S88 standards.

3.3.1. Brewing water

All brewing water was treated with lactic acid and $CaSO_4$ as described in section 2.2.3. Physical properties were then monitored to note the extent and effect of these additives on filtered untreated water. Filtered municipality water was found to have a decrease in pH from 7.53 ± 0.26 to 5.13 ± 0.50 after adjustments with lactic acid. The addition of $CaSO_4$ resulted in an increase in physico chemical properties i.e., from 125.02 ± 11.48 µS/cm to 282.33 ± 65.67 µS/cm, 0.055 ± 0.005 $‰_0$ to 0.146 ± 0.0370 $‰_0$, and 62.69 ± 4.86 mg/l to 145.56 ± 36.13 mg/l for conductivity, salinity and total dissolved solids (TDS), respectively. However, it was noted that both the acid and salt additions had no significant effect on dissolved oxygen content, with values observed ranging from 8.13 ± 0.63 mg/l to 8.10 ± 0.77 mg/l for untreated and treated water, respectively.

The figure 3.5 below shows five physico chemical properties investigated and used to monitor the quality of untreated-, treated water and final boiled wort during the brewing stage.

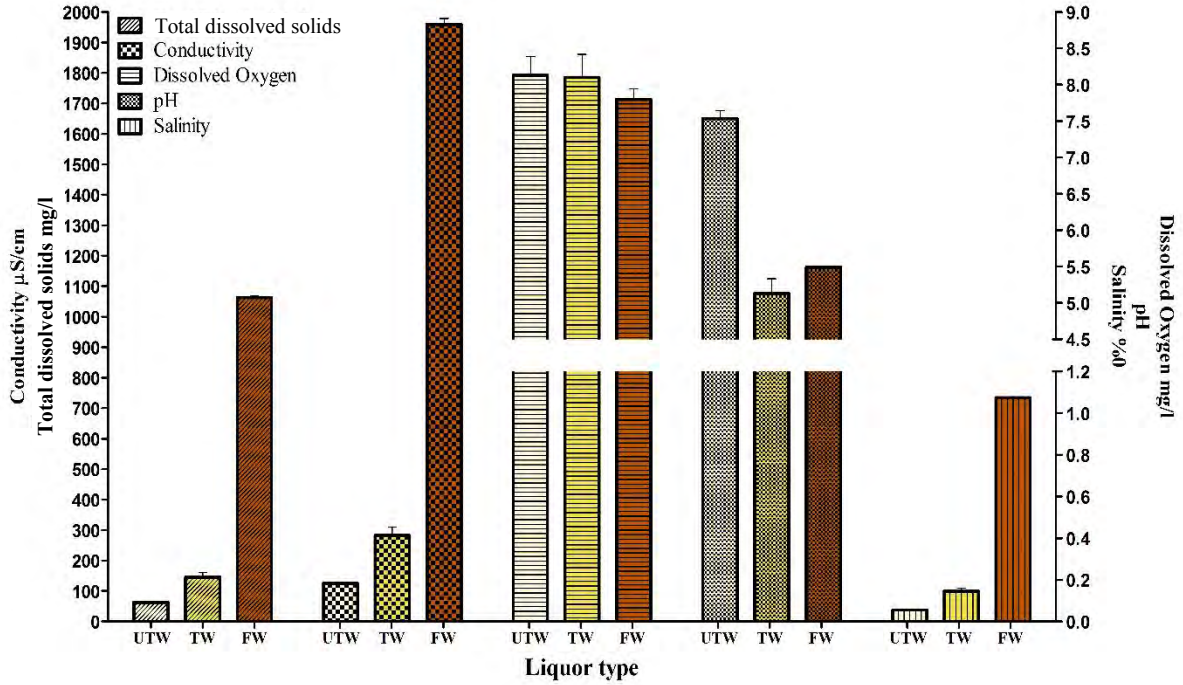


Figure 3.5. Comparison of the salting and pH treatment effects on untreated water (UTW), treated water (TW), and boiled final wort (FW).

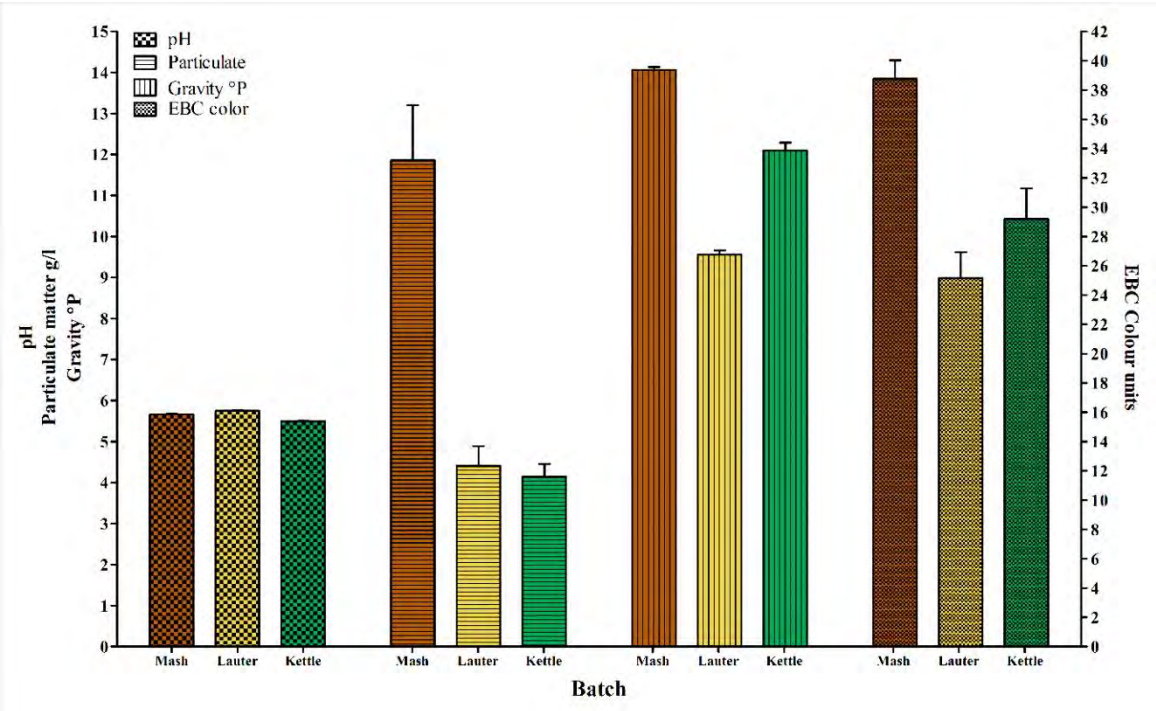


Figure 3.6. Physico chemical trends across the mashing, lautering and boiling stages repeated under identical brewing conditions.

3.3.2. Brewing process

3.3.2.1. Physico chemical analyses of water and wort

For the entire ISA S88 brewing model, six identical batches were brewed and analyzed for trends and consistency as described in chapter 2. In the mashing process, the final gravity of the wort was observed to be 14.06 ± 0.18 °P, a value very close to the anticipated mash control gravity i.e., 14.72 °P. The volume of the mashed wort was found to be 15.38 ± 0.60 l and it had an EBC colour of 38.76 ± 3.10 EBC units. Particulate matter in solution (excluding spent barley husks) was very easy to observe with the naked eye and amounted to 11.86 ± 3.30 g/l. Mash pH was 5.66 ± 0.03 and the total grain losses during malt transfer into the mash tun amounted to 20.76 ± 4.74 g.

In the lauter tun sparging process, the gravities were observed to decrease as the spent grain was rewashed, from 7.92 ± 0.51 °P, 3.95 ± 0.60 °P and 1.67 ± 0.15 °P for the first, second and third runnings, respectively. The corresponding volumes were 7.67 ± 0.55 l, 7.58 ± 0.48 l and 5.45 ± 0.42 l, respectively. When recirculated wort plus the three runnings were collected in one vessel and observed for overall properties, the wort was observed to be clearer, more dilute and less acidic. The wort now amounted to 35.71 ± 0.51 l in volume, had an EBC colour of 25.16 ± 4.33 EBC units, 4.39 ± 0.16 g/l particulate matter in solution, a pH value of 5.75 ± 0.03, and an overall gravity of 9.56 ± 0.25 °P. At the boiling kettle stage wort became concentrated, darker and more acidic. The gravity increased from the lauter value by 26.57 % to 12.10 ± 0.46 °P whilst the colour darkened by 15.97 % to 29.20 ± 5.12 EBC units. The concentrating effect of the boiling process simultaneously reduced particulate matter to 4.14 ± 0.76 g/l, pH value to 5.49 ± 0.03 and the final volume to 26.45 ± 0.35 l i.e., a reduction of 5.70 %, 4.52 %, and 25.93 %, respectively.

3.3.2.2. Chemical analyses of wort

Reducing sugars and free amino nitrogens (FAN) were used as chemical estimates for the investigation of quality in the brewing process i.e., as described in chapter 2. For the reducing sugars, the initial extraction amount in the mashing stage was found to be 1376 ± 2.57 g in 15.38 ± 0.60 l of wort. The lauter stage recovered 167.61 ± 1.71 g reducing sugars from the spent grain after the three consecutive runnings giving a total sparging recovery volume of 20.33 ± 1.44 l. Total reducing sugars lost together with spent grain, after performing a lab-scale rewashing of the discarded grain amount to 27.15 ± 0.00 g. This loss was calculated and found to be 2.13 % of the total extracted reducing sugars. The recovered reducing sugars from the lauter stage however bought the expected total reducing sugars extract in the boiling kettle to 1543.79 ± 2.57 g. After addition of 462 ± 68.87 g maltose syrup and boiling, the final reducing sugars amount was found to be 1632.97 ± 12.64 g in 26.45 ± 1.34 l of wort. Considering 78 % utilization of the maltose syrup, and excluding the maltose added during the boiling stage, it can be seen that the malt-derived reducing sugars reached a final value of 1272.61 ± 56.23 g (refer to appendix-G for calculations). A combination of the theoretically expected final reducing sugars and the total volume of liquor (wort and water) lost during the boiling stage gave rise to the formulation of a brew house efficiency tool. This tool was a simple calculation which gave an account of liquor lost during the brewing process and also specified in which stage the liquor was lost. As far as reducing sugars analysis is concerned and with reference to appendix-G, it was observed that the Westville microbrewery was 91.67 % efficient with respect to converting all raw materials to the final wort product, where losses due to evaporation were noted to be 2.98 ± 0.14 l (8.33 % volume lost as vapour), and total liquor lost was 9.26 ± 0.74 l. Depending on each batch‟s clarity, different volumes of wort were discarded intentionally after the boiling stage as trub,

and in the continuous centrifuge as particulate waste. These discards amounted to a final overall value of 6.29 ± 1.23 (17.88 % volume lost as wort/trub discard).

Total FAN in the mashing stage was 5368.10 ± 24.22 mg in 15.38 ± 0.60 l of wort. 2938.17 ± 52.23 mg of FAN was recovered with 20.33 ± 1.44 l of wort during the lautering stage, hence these two brewing stages giving a boiled wort FAN expectation of 8306.27 ± 76.45 mg. 2247.58 ± 0.23 mg of FAN was lost together with spent grain i.e., 27.06 % of the boiled wort expectation. Due to the very effective coagulation of protein-polyphenol complexes during boiling, the FAN in the final boiled wort amounted to 7452.95 ± 47.22 mg, which gave an indication that 853.32 ± 29.23 mg of FAN was trapped in the precipitate.

3.3.2.3. Instrument analyses of wort

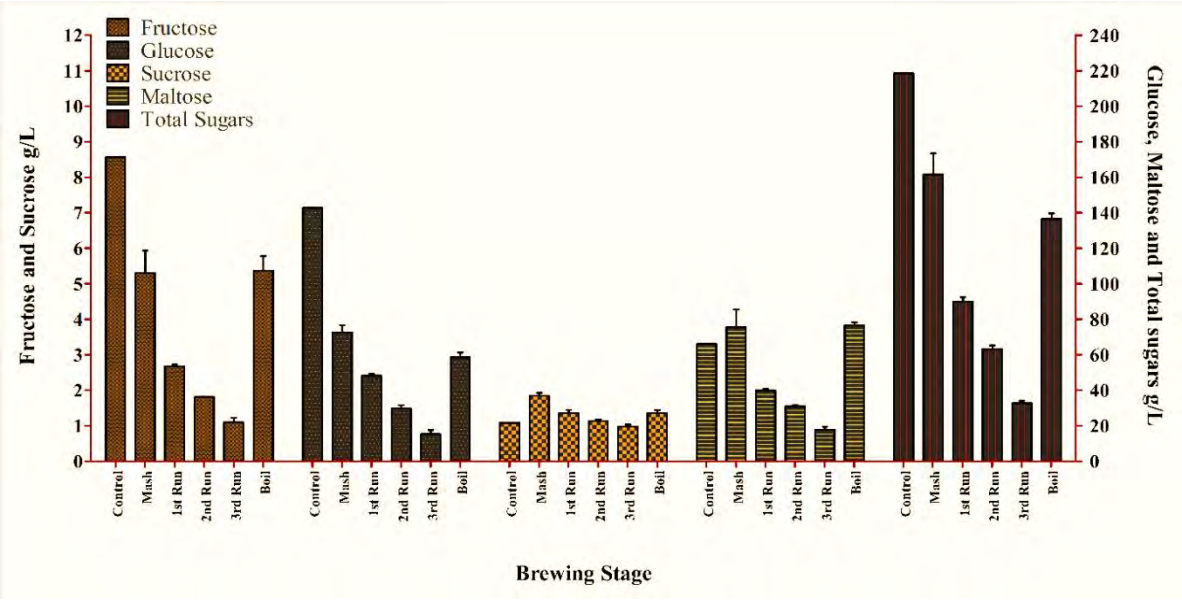


Figure 3.7. Simple sugar concentrations across different brewing stages.

The six participating batches that were analyzed using physico chemical means also had simultaneous instrumental analysis for specific compounds performed. During the brewing

phases, only simple sugars which constitute part of the malt extract were investigated. In the mashing stage, the total simple sugars concentration amounted to 155.41 ± 29.28 g/l where the individual simple sugars had concentrations of 5.31 ± 1.26 g/l, 72.72 ± 7.92 g/l, 1.85 ± 0.16 g/l, and 75.53 ± 19.91 g/l for fructose, glucose, sucrose, and maltose, respectively. In the lauter stage the total sugars recovered from the spent grain were 1380.24 ± 8.38 g across the three sparging runs. The corresponding recoveries for fructose, glucose, sucrose and maltose were 40.15 ± 0.18 g, 679.13 ± 4.86 g, 24.38 ± 0.21 g, and 636.59 ± 3.13 g, respectively. Residual sugars lost with discarded spent grain were investigated and amounted to a total of 10.52 ± 0.01 g. This result pointed that only 0.28 % of the total extracted simple sugars i.e., 3770.91 ± 25.80 g, were lost together with spent grain (refer to appendix-G). Figure 3.7 above shows the overall sugars trend observed across all brewing stages for each individual sugar concentration.

The final boiled wort had 141.98 ± 1.11 g fructose, 1546.45 ± 7.69 g glucose, 36.03 ± 0.25 g sucrose, 2023.88 ± 5.92 g maltose, and 3748.34 ± 14.96 g total simple sugars in solution. Excluding the maltose adjuncts added during boiling, it was noted that the malt derived total simple sugars in solution amounted to 3387.98 ± 38.76 g. The difference between the boiled and lautered malt derived sugars was 382.93 ± 12.96 g implying that these were the total sugars lost in the boiling stage as trub. Further calculations confirmed that with respect to simple sugars analysis, 3.63 ± 0.67 l of wort were lost as trub and 5.63 ± 0.07 l of water were lost as vapour. This gave an indication that the Westville microbrewery was 84.23 % efficient with respect to liquor retention during the entire brewing process.

3.3.3. Wort fermentation

Boiled wort from the six batches was pitched with a consistent amount of Safale s-04 yeast culture at a rate of ten million cells per litre. The individual batches were apportioned into smaller equal volumes before pitching for purposes of investigating the effect of different fermentation temperatures as described in chapter 2. Gravity in degree Plato (°P) was used to estimate depletion of sugars as fermentation was monitored from the start to the final intended gravities. On average, the fermentations took 19 days, 12 days and 10 days at 14 °C, 16 °C and 18 °C, respectively. At 14 °C, 7.48 ± 0.23 °P of gravity was utilized (i.e., difference between Initial and Final gravities), and 7.40 ± 0.11 °P and 8.28 ± 0.30 °P were utilized for 16 °C and 18 °C, respectively. Using the Balling factor and estimations made by Harris (SABMiller, 2013), it is seen that the above utilized gravities potentially yielded 4.83 ± 0.15 % v/v, 4.77 ± 0.07 % v/v and 5.34 ± 0.19 % v/v alcohol content for 14 °C, 16 °C and 18 °C, respectively.

Chemical estimations for reducing sugars and FAN were also measured every 24 hours during fermentation. It was observed that 1598.78 ± 128.57 g, 1468.23 ± 79.50 g, and 1587.64 ± 113.99 g of reducing sugars were utilized by the yeast culture during the 14 °C, 16 °C and 18 °C fermentations, respectively. For the FAN, 4633.65 ± 312.29 mg, 2470.52 ± 73.99 mg, and 3080.76 ± 224.43 mg were used during the 14 °C, 16 °C and 18 °C fermentations, respectively. Figure 3.8 gives a graphical representation of the fermentation results.

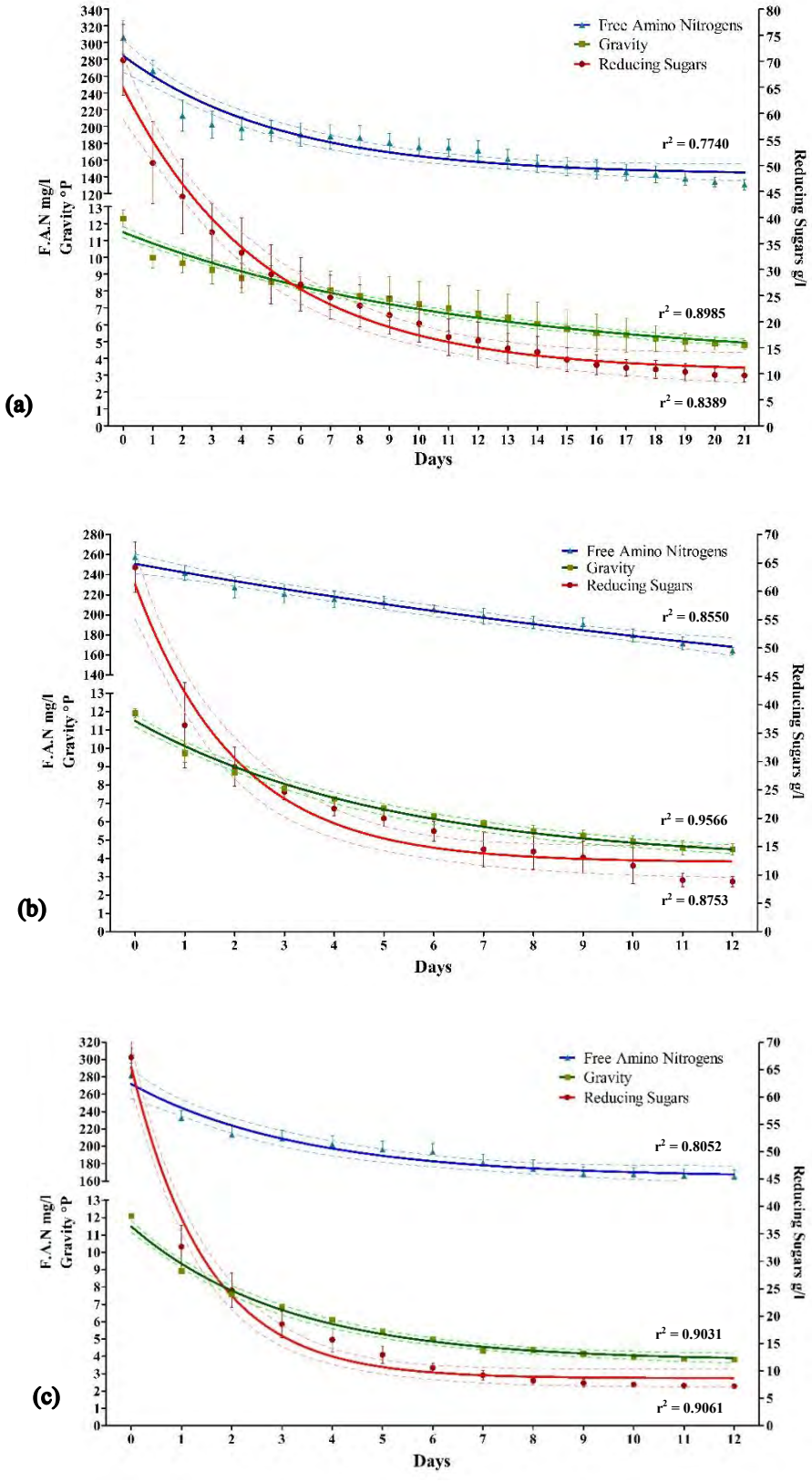


Figure 3.8. The depletion of wort FAN, reducing sugars and gravity in the (a) 14 °C, (b) 16 °C, and (c) 18 °C fermentation temperature batches.

The fermentation batches discussed above were simultaneously sampled for simple sugars and flavour compounds. All fermentation batches at different temperatures experienced a depletion of the simple sugars as ethanol and other flavour compounds emerged. Wort fermented at 14 °C showed a decrease in the total simple sugars concentration which ranged from 151.84 ± 19.32 g/l to 16.27 ± 6.17 g/l i.e., 3570.18 ± 28.46 g total sugars were utilized in the 26.40 ± 1.22 l batch. The corresponding flavour compounds produced accumulated up to concentration levels of 4.53 ± 0.58 % v/v, 120.60 ± 10.20 mg/l and 60.72 ± 14.44 mg/l for ethanol, total fusel alcohols and total esters, respectively. At 16 °C fermentation temperature, the total simple sugars utilized were 3295.92 ± 185.42 g in 26.50 ± 1.74 l of wort. Flavour compounds formed during this fermentation period were found to be at concentration levels of 4.52 ± 0.24 % v/v, 119.05 ± 9.66 mg/l, and 64.02 ± 7.72 mg/l for ethanol, total fusel alcohols and total esters, respectively. A 7.68 % drop in total sugar utilization in the 16 °C batches caused the ethanol concentrations to remain significantly the same between the 14 °C and 16 °C batches, as opposed to the theoretical concentration increase. The increase in fermentation temperature promoted a fusel alcohol concentration decrease and an ester concentration increase of 1.29 % and 5.44 %, respectively. In the 18 °C fermentation batches, a significant increase in sugar utilization and flavour concentrations was noted in comparison to the 16 °C fermentation. A 7.17 % sugar utilization increase brought the total sugars consumed at 18 °C to 3532.37 ± 120.52 g. For ethanol, total fusel alcohols and total esters, an increase of 9.96 %, 17.10 %, 19.79 % gave final concentration values of 4.97 ± 0.28 % v/v, 139.41 ± 15.73 mg/l, and 76.69 ± 7.06 mg/l, respectively. The individual simple sugars, fusel alcohols and esters that make up these total values are listed in table 3.2 below.

Table 3.2. The depletion of sugar concentrations and formation of flavour compounds across experimental fermentation temperatures of boiled wort.

Compound	**14 °C ≈ 19 days**	**16 °C ≈ 12 days**	**18 °C ≈ 10 days**
Substrate consumed (g)			
Fructose	101.19 ± 1.78	144.53 ± 21.02	116.30 ± 18.30
Glucose	1901.78 ± 20.87	1309.98 ± 135.67	1546.45 ± 151.85
Sucrose	24.29 ± 0.17	20.41 ± 4.51	22.35 ± 0.45
Maltose	1542.92 ± 5.64	1821.00 ± 24.22	1847.27 ± 70.44
Total Sugars	3570.18 ± 28.42	3295.92 ± 185.42	3532.37 ± 120.52
Alcohol produced (% v/v)			
Ethanol	4.53 ± 0.58	4.52 ± 0.24	4.97 ± 0.28
Fusel alcohols produced (mg/l)			
Propanol	17.86 ± 1.86	17.42 ± 1.81	23.43 ± 4.97
Isoamyl alcohol	102. 74 ± 8.38	101.63 ± 7.85	115.98 ± 10.76
Total fusel alcohols	120.60 ± 10.20	119.05 ± 9.66	139.41 ± 15.73
Esters produced (mg/l)			
Ethyl acetate	58.29 ± 14.07	62.18 ± 6.97	74.23 ± 6.20
Isoamyl acetate	0.57 ± 0.27	0.86 ± 0.43	1.06 ± 0.43
Ethyl hexanoate	0.41 ± 0.04	0.27 ± 0.05	0.34 ± 0.07
Ethyl octanoate	1.45 ± 0.06	0.71 ± 0.27	1.06 ± 0.36
Total esters	60.72 ± 14.44	64.02 ± 7.72	76.69 ± 7.06
Batch volume (l)	26.40 ± 1.22	26.50 ± 1.74	26.45 ± 1.34

3.3.4. Beer quality

3.3.4.1. Physico chemical analyses of beer

Three main parameters were monitored for bottled beer i.e., pH, TDS and EBC colour. As explained by the experimental design, bottled beer from each respective fermentation temperature was partitioned further into three storage temperatures namely 0 °C, 4 °C, and 18°C. For beer stored at 0 °C it was noted that as it aged the trend was a drop in pH from 4.375 ± 0.096 to 4.360 ± 0.065, no change in pH i.e., 4.393 ± 0.098 to 4.395 ± 0.100, and a rise in pH from 4.388 ± 0.117 to 4.409 ± 0.111 for fermentation temperatures 14 °C, 16 °C and 18 °C, respectively. At 4 °C storage temperature the pH trend amongst aging beer was a drop from 4.367 ± 0.090 to 4.344 ± 0.066, no change i.e., 4.388 ± 0.102 to 4.386 ± 0.099, and a rise from 4.383 ± 0.114 to 4.407 ± 0.108 for fermentation temperatures 14 °C, 16 °C and 18 °C, respectively. Beer stored at 18 °C was observed to have a pH trend which drops from 4.373 ± 0.080 to 4.359 ± 0.073, has no change i.e., 4.392 ± 0.096 to 4.391 ± 0.098, rises from 4.380 ± 0.113 to 4.411 ± 0.118 for fermentation temperatures 14 °C, 16 °C and 18 °C, respectively. Figure 3.9 illustrates the results reported above.

TDS were observed to gradually increase with higher storage temperatures implying more solubility of the solids. Beer fermented at 14 °C was seen to have an increase in TDS from 988.83 ± 16.97 mg/l to 1030.83 ± 8.12 mg/l, 992.00 ± 19.19 mg/l to 1038.58 ± 7.52 mg/l, and 1002.67 ± 24.50 mg/l to 1049.00 ± 13.15 mg/l for 0 °C, 4 °C, and 18 °C, respectively. For beer fermented at 16 °C the rising concentrations in TDS were from 1067.22 ± 40.66 mg/l to 1076.67 ± 42.41 mg/l, 1056.44 ± 30.20 mg/l to 1087.89 ± 35.69 mg/l, and 1068.94 ± 30.32 mg/l to 1102.17 ± 34.16 mg/l for 0 °C, 4 °C, and 18 °C, respectively. At 18 °C fermentation temperature, TDS concentrations increased from 1020.33 ± 64.34 mg/l to 1048.61 ± 68.58 mg/l, 1019.79 ± 73.45 mg/l to 1055.89 ± 70.33 mg/l, and 1025.50 ± 71.06 mg/l to 1063.78 ± 67.47 mg/l for 0 °C, 4 °C, and 18 °C, respectively. The increase in TDS concentrations was

noted to have a linear relationship with storage time (shelf life), fermentation temperature and storage temperature. Figure 3.9 illustrates the results reported above.

EBC colour was observed to have a slight decrease as beer aged at different storage temperatures. However, beer fermented at 16 °C was darker than beer fermented at 18 °C, which in turn was darker than beer at 14 °C. These different shades of the golden-brown colour were almost identical to the naked eye, but were significantly different in some cases by means of spectrophotometry. At 14 °C fermentation temperature, beer colour clarified from 14.33 ± 0.36 EBC units to 13.08 ± 0.58 EBC units, 14.42 ± 0.34 EBC units to 12.89 ± 0.67 EBC units, and 14.65 ± 0.38 EBC units to 13.48 ± 0.61 EBC units for 0 °C, 4 °C, and 18 °C, respectively. For beer fermented at 16 °C the colour changed from 14.84 ± 0.72 EBC units to 13.65 ± 0.82 EBC units, 14.95 ± 0.96 EBC units to 13.78 ± 0.48 EBC units, and 15.34 ± 0.83 EBC units to 14.00 ± 0.41 EBC units for 0 °C, 4 °C, and 18 °C, respectively. Beer fermented at 18 °C had EBC colour changes varying from 14.48 ± 0.93 EBC units to 12.98 ± 0.81 EBC units, 14.67 ± 0.99 EBC units to 13.25 ± 0.77 EBC units, and 15.21 ± 1.15 EBC units to 13.66 ± 0.95 EBC units for 0 °C, 4 °C, and 18 °C, respectively. Figure 3.9 is a comparison of the effect of different handling and storage temperatures of beer on the beer quality over time. The impact of these storage conditions was measured against colour, dissolved solids and pH. Figure 3.9 illustrates the results reported above.

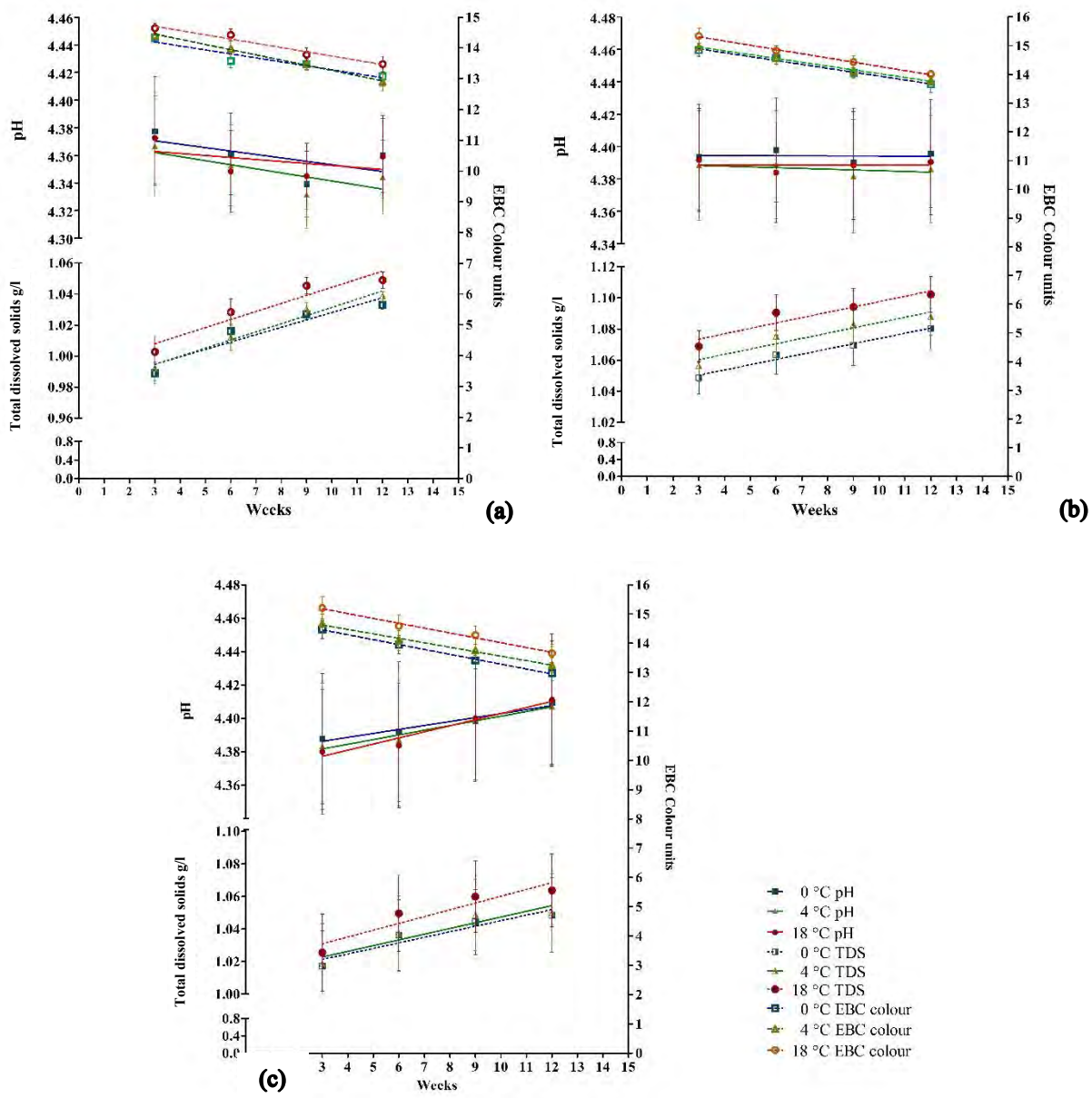


Figure 3.9. The effect of accumulating total dissolved solids on the colour and pH of beer stored at (a) 0 °C, (b) 4 °C, and (c) 18 °C.

3.3.4.2. Chemical analyses of beer

Reducing sugars and FAN concentrations were monitored simultaneously with the physico chemical properties, and used as beer quality assessment tools. The concentration of reducing sugars for beer fermented at 14 °C was observed to decrease from 5.46 ± 0.73 g/l to 2.14 ± 0.29 g/l, 5.12 ± 0.88 g/l to 1.83 ± 0.24 g/l, and 4.18 ± 0.71 g/l to 1.68 ± 0.46 g/l for the storage temperatures 0 °C, 4 °C, and 18 °C, respectively. At the fermentation temperature of 16 °C, the respective beer was found to have a decrease in reducing sugar concentrations ranging from 4.35 ± 0.30 g/l to 1.96 ± 0.27 g/l, 4.10 ± 0.63 g/l to 1.95 ± 0.26 g/l, and 3.72 ± 1.06 g/l to 1.82 ± 0.23 g/l for 0 °C, 4 °C, and 18 °C storage temperatures, respectively. For beer fermented at 18 °C and stored at 0 °C, 4 °C, and 18 °C, the decrease in reducing sugar concentrations was from 3.26 ± 0.70 g/l to 1.83 ± 0.45 g/l, 3.47 ± 0.38 g/l to 1.81 ± 0.33 g/l, and 2.97 ± 0.62 g/l to 1.85 ± 0.69 g/l, respectively.

FAN concentration for the stored beer was observed to decrease with time as well. Beer fermented at 14 °C had a decrease in FAN concentration ranging from 183.46 ± 9.01 mg/l to 149.43 ± 13.22 mg/l, 183.47 ± 6.66 mg/l to 157.50 ± 7.43 mg/l, and 179.23 ± 14.44 mg/l to 154.37 ± 11.50 mg/l for 0 °C, 4 °C, and 18 °C storage temperatures, respectively. At 16 °C fermentation temperature, beer stored at 0 °C, 4 °C, and 18 °C was found to have a decrease in FAN concentration varying from 206.00 ± 20.19 mg/l to 167.95 ± 14.00 mg/l, 208.09 ± 16.15 mg/l to 169.31 ± 14.07 mg/l, and 199.32 ± 19.82 mg/l to 166.35 ± 12.93 mg/l, respectively. The concentration of FAN for beer fermented at 18 °C decreased from 190.89 ± 22.87 mg/l to 162.46 ± 16.21 mg/l, 191.25 ± 12.64 mg/l to 163.49 ± 16.62 mg/l, and 189.06 ± 16.54 mg/l to 157.57 ± 16.42 mg/l for the storage temperatures 0 °C, 4 °C, and 18 °C, respectively.

An observation on stored beer and aging beer trends revealed that there was significant positive correlation between salinity and TDS for all beer storage temperatures for the 14 °C

and 18 °C ($p \leq 0.05$). Significant positive correlations were observed between beers‟ EBC colour, FAN, and reducing sugars for all batches fermented at 16 °C and 18 °C, respectively (refer to appendix-F).

3.3.4.3. Instrument analyses of beer.

In this section beer quality at different storage temperatures was measured by means of residual sugars and flavour compound concentrations. The ideal case as beer aged was that there should be a minimum amount of flavour compound change as this would imply flavour stability and consistent taste of the product across its shelf life. Beer fermented at 14 °C had a slow but gradual depletion of residual sugars in solution. Total simple sugar concentrations were observed to have a decreasing trend ranging from 14.48 ± 2.14 g/l to 5.66 ± 0.54 g/l, 11.62 ± 2.05 g/l to 5.78 ± 0.43 g/l, and 9.88 ± 1.50 g/l to 5.14 ± 0.10 g/l for beer stored at 0 °C, 4 °C, and 18 °C, respectively. The corresponding ethanol changes due to this sugars utilization were 4.49 ± 0.44 % v/v to 5.31 ± 0.14 % v/v, 4.66 ± 0.36 % v/v to 5.37 ± 0.26 % v/v, and 4.81 ± 0.25 % v/v to 5.51 ± 0.19 % v/v, respectively. Fusel alcohol concentration in the beer was observed to increase in a similar manner as ethanol, with values ranging from 95.17 ± 12.29 mg/l to 122.29 ± 17.94 mg/l, 93.53 ± 15.52 mg/l to 120.37 ± 12.14 mg/l, and 102.14 ± 13.28 mg/l to 127.72 ± 11.23 mg/l for 0 °C, 4 °C, and 18 °C storage temperatures, respectively. The impact of these changes on the total aroma active ester concentration was a decrease which ranged from 31.06 ± 7.42 mg/l to 23.97 ± 6.42 mg/l, 35.65 ± 7.16 mg/l to 29.99 ± 8.08 mg/l, and 36.62 ± 7.46 mg/l to 30.13 ± 8.31 mg/l for beer stored at 0 °C, 4 °C, and 18 °C, respectively. A graphical summary of the results is shown in Figures 3.10 and 3.11.

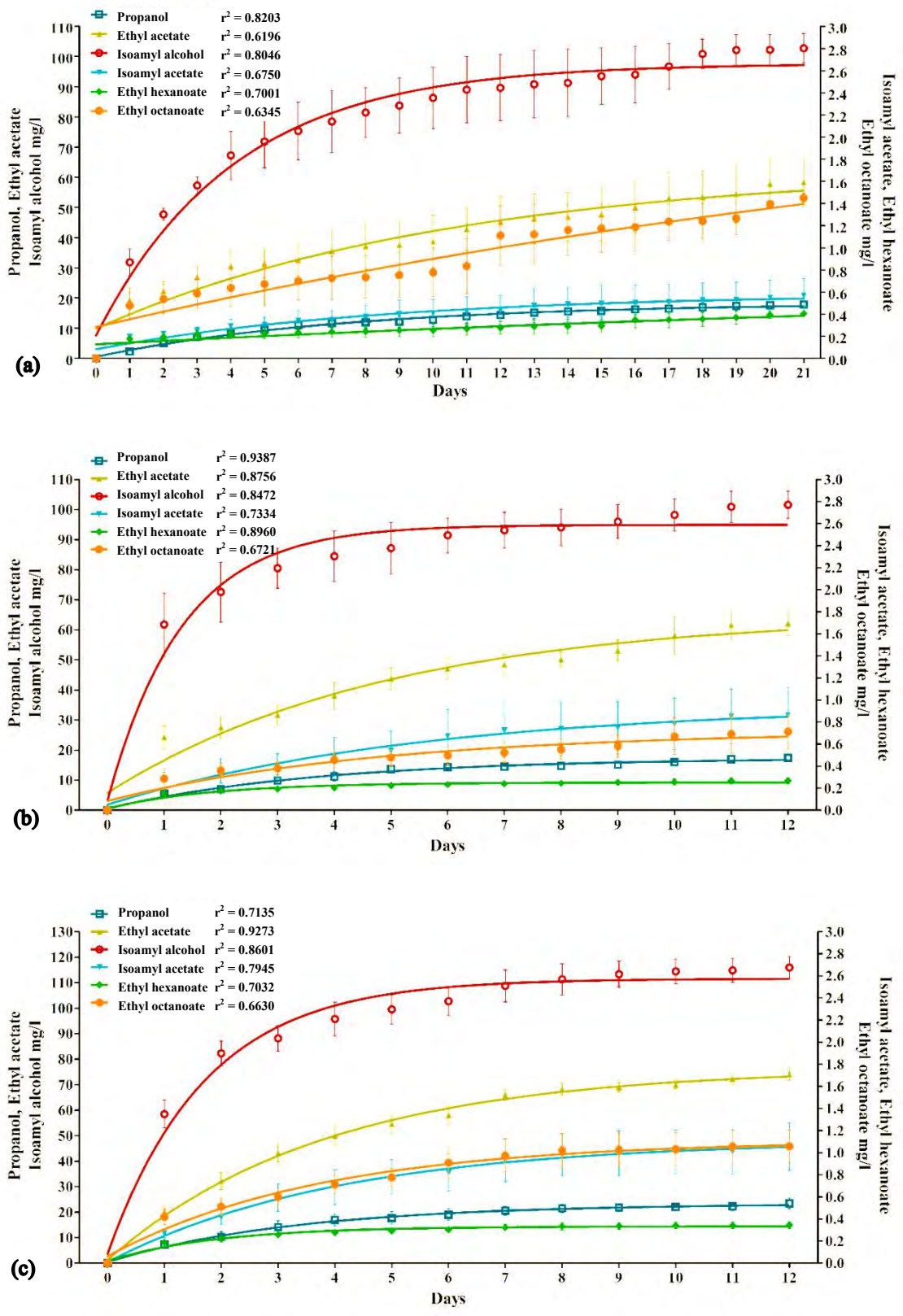


Figure 3.10. Aroma active flavour formation across the three fermentations temperatures of (a) 14 °C, (b) 16 °C, and (c) 18 °C, respectively.

At 16 °C fermentation temperature, the general trends observed were the same as those of the 14 °C fermentation temperature profile. The depletion of the total simple sugars in this bottled beer fermentation profile was observed to range from 12.99 ± 1.25 g/l to 5.23 ± 0.24 g/l, 10.61 ± 1.61 g/l to 5.24 ± 0.29 g/l, and 8.56 ± 3.12 g/l to 4.84 ± 0.47 g/l for beer storage temperatures of 0 °C, 4 °C, and 18 °C, respectively. The ethanol concentrations increased during the storage period from 4.57 ± 0.39 % v/v to 5.12 ± 0.43 % v/v, 4.70 ± 0.37 % v/v to 5.24 ± 0.29 % v/v, and 4.82 ± 0.43 % v/v to 5.39 ± 0.22 % v/v for beer stored at 0 °C, 4 °C, and 18 °C, respectively. Total fusel alcohol concentration simultaneously increased from 93.23 ± 8.31 mg/l to 109.85 ± 11.11 mg/l, 91.83 ± 9.67 mg/l to 111.87 ± 13.26 mg/l, and 89.89 ± 12.71 mg/l to 110.43 ± 12.49 mg/l, respectively. Due to the storage time duration and flavour changes, the active aroma esters were observed to decrease from 27.75 ± 3.45 mg/l to 23.57 ± 2.73 mg/l, 27.39 ± 3.53 mg/l to 24.24 ± 2.37 mg/l, and 30.01 ± 2.62 mg/l to 24.88 ± 2.07 mg/l at 0 °C, 4 °C, and 18 °C storage temperatures, respectively. A graphical summary of the results is shown in Figures 3.10 and 3.11.

Beer fermented at the control temperature of 18 °C had similar trends as observed for the last two fermentation profiles. The concentration of total simple sugars was found to decrease from 10.45 ± 1.12 g/l to 5.29 ± 0.26 g/l, 10.19 ± 1.32 g/l to 5.31 ± 0.28 g/l, and 7.52 ± 1.53 g/l to 4.87 ± 0.53 g/l across the storage temperatures of 0 °C, 4 °C, and 18 °C, respectively. The simultaneous ethanol concentration changes ranged from 4.96 ± 0.26 % v/v to 5.32 ± 0.13 % v/v, 5.12 ± 0.17 % v/v to 5.39 ± 0.10 % v/v, and 5.23 ± 0.14 % v/v to 5.57 ± 0.15 % v/v, respectively. A significant increase in total fusel alcohol content was observed as well and the concentrations increased from 99.64 ± 9.68 mg/l to 123.79 ± 16.27 mg/l, 101.57 ± 17.71 mg/l to 128.60 ± 20.47 mg/l, and 101.40 ± 8.52 mg/l to 128.32 ± 16.40 mg/l for 0 °C, 4 °C, and 18 °C storage temperatures, respectively. Total ester concentrations were observed to decrease in this fermentation profile at the different storage temperatures from 32.10 ± 3.01

mg/l to 29.53 ± 2.82 mg/l, 34.16 ± 3.12 mg/l to 31.86 ± 4.99 mg/l, and 35.96 ± 2.69 mg/l to 30.99 ± 2.61 mg/l for beer stored at 0 °C, 4 °C, and 18 °C, respectively.

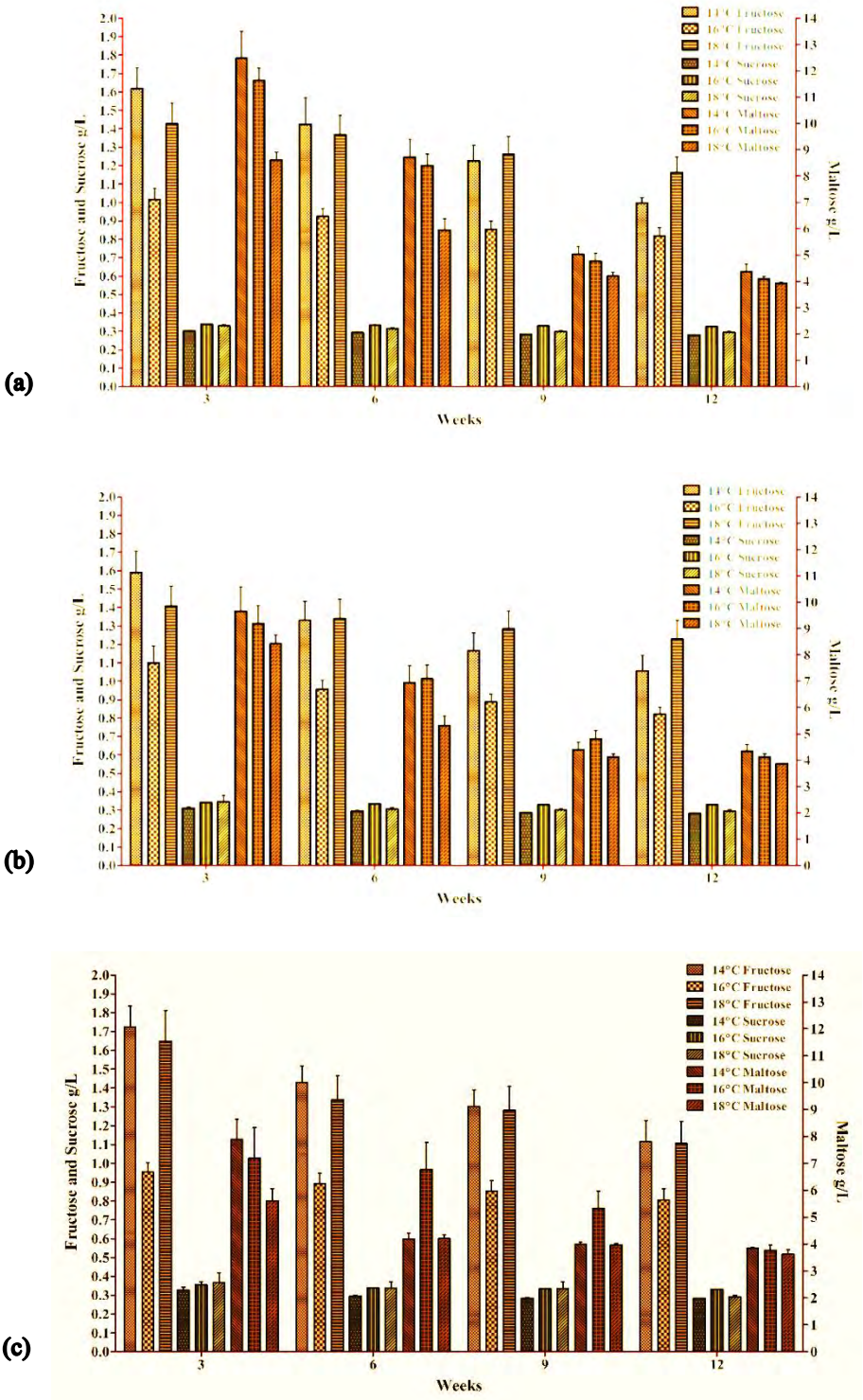


Figure 3.11. Residual sugars profile in beer stored at (a) 0 °C, (b) 4 °C, and (c) 18 °C during 12 weeks of aging.

Table 3.3. Residual sugar and flavour compound changes during beer storage at different temperatures over a period of 12 weeks.

Fermentation (°C)	14						16						18					
Storage (°C)	**0**		**4**		**18**		**0**		**4**		**18**		**0**		**4**		**18**	
	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%	Conc.	%
Sugars (g/l)																		
Fructose	- 0.62	38.32	- 0.53	33.52	- 0.61	35.36	- 0.20	19.67	- 0.28	25.39	- 0.15	15.41	- 0.26	18.50	- 0.18	12.78	- 0.54	32.97
Glucose	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Sucrose	- 0.02	7.29	- 0.03	9.62	- 0.04	13.46	- 0.01	3.83	- 0.01	3.51	- 0.03	7.28	- 0.04	10.64	- 0.05	14.70	- 0.08	20.87
Maltose	- 8.12	65.08	- 5.33	55.12	- 4.05	51.18	- 7.55	64.82	- 5.07	55.15	- 3.42	47.55	- 4.69	54.44	- 4.55	54.04	- 2.00	35.59
Total Sugars	- 8.82	60.90	- 5.84	50.29	- 4.74	48.01	- 7.76	59.75	- 5.36	50.57	- 3.73	43.53	- 5.17	49.43	- 4.88	47.87	- 2.66	35.32
Alcohol (% v/v)																		
Ethanol	+ 0.82	18.18	+ 0.71	15.27	+ 0.69	14.38	+ 0.55	12.10	+ 0.54	11.57	+ 0.57	11.86	+ 0.36	7.28	+ 0.27	5.29	+ 0.35	6.66
Fusel (mg/l)																		
Propanol	+ 3.59	23.84	+ 3.08	20.28	+ 2.26	13.95	+ 2.06	14.56	+ 2.60	18.10	+ 2.05	13.97	+ 3.53	18.56	+ 3.34	17.17	+ 3.59	18.89
Isoamyl alcohol	+ 20.55	27.68	+ 19.25	23.18	+ 20.22	24.19	+ 17.82	23.51	+ 18.87	24.76	+ 16.20	21.41	+ 20.95	26.86	+ 24.92	30.15	+ 26.23	32.51
Total alcohol	+ 24.14	25.36	+ 22.33	23.88	+ 22.48	22.01	+ 19.88	21.33	+ 20.04	21.83	+ 18.25	20.30	+ 24.15	24.23	+ 27.03	26.61	+ 29.82	29.41
Ester (mg/l)																		
Ethyl acetate	- 6.97	20.25	- 6.97	18.32	- 1.40	4.02	- 4.58	16.24	- 5.17	19.05	- 4.77	16.79	- 3.47	10.94	- 6.76	18.87	- 3.79	11.19
Isoamyl acetate	- 0.07	15.93	- 0.06	12.86	- 0.13	25.29	- 0.01	2.68	- 0.05	8.95	- 0.11	20.48	- 0.11	20.74	- 0.07	12.84	- 0.08	16.60
Ethyl hexanoate	- 0.04	6.77	- 0.06	9.82	- 0.04	7.82	- 0.02	3.80	- 0.03	4.99	- 0.04	8.63	- 0.07	12.27	- 0.04	7.98	- 0.07	12.5
Ethyl octanoate	- 0.15	30.85	- 0.11	20.88	- 0.16	30.77	- 0.03	9.28	- 0.05	12.89	- 0.12	31.01	- 0.02	5.16	- 0.06	12.13	- 0.22	47.78
Total esters	- 7.09	22.84	- 5.66	15.87	- 1.73	4.73	- 4.18	15.05	- 5.30	9.37	- 5.13	17.10	- 3.67	11.44	- 6.93	20.31	- 4.16	11.56

Conc.: Concentration change
%: Percentage change of concentration with respect to initial value (t = 0 weeks)
+/-: implies increase/decrease of the respective concentration.

3.4. Discussion

With an estimate concentration of 50 mg/l Ca^{2+} ion, the Durban municipality water used during the pale ale brewing required additional calcium treatment. According to Briggs *et al.,* (2004) reasonable Ca^{2+} ion concentrations in brewing water should be between 20 – 150 mg/l and they also stated that there was no significant gain from exceeding the 100 mg/l mark. This overdose was observed to slowly introduce a negative alternative effect on the final wort properties and also resulted in yeast phosphate deficiencies. Gibson (2011) stated the importance of the Mg^{2+} ions being greater than the Ca^{2+} ions as far as yeast physiology is concerned. Due to the trace amounts of Mg^{2+} ions in the malt, Gibson (2011) warned brewers that overdosing brewing liqour with Ca^{2+} would outweigh the presence of Mg^{2+} and would eventually lead to the replacement of Mg^{2+} in some vital biochemical pathways. These are some of the contributing factors that led to the final $CaSO_4$ dosage of 80 mg per liter of water for a Ca^{2+} ion concentration increase of 23.52 mg/l.

Different salting and acid treatment doses were experimented on (refer to appendix-B) where a comparison between $CaSO_4$ and $CaCl_2$ was a key point. Although $CaCl_2$ had a more accurate salting effect due to its ability to totally dissolve in water, it carried an even bigger risk of extremely surpassing the concentration limit of the Cl^{2-} ions recommended by most brewing scientists to be aound 150 mg/l. This is due to the main fact that brewers do not necessarily have control over the chlorination of municipality water, and so apointing a constant dose of $CaCl_2$ to the brewing water would not solve the variation and inconcistency that came with the municipality supply. Higer concentrations of chlorine are known to increase beer mouth feel especially around the palate, and eventually tilt the saline/bitterness ratio entirely on the saline side making the beer quite salty.

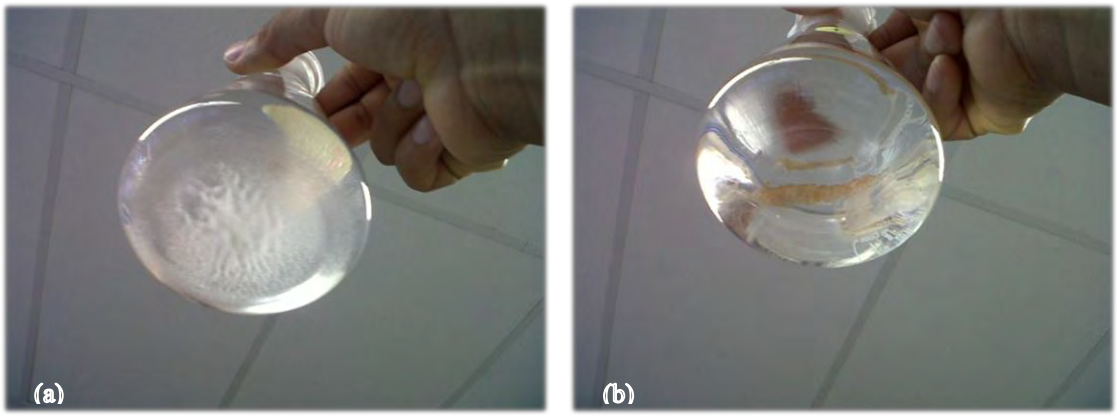


Figure 3.12. The solubility differences between (a) $CaSO_4$ and (b) $CaCl_2$ in filtered municipality water after 2 min of addition and stirring.

This flavour inclination would have seen entire batches in the experimetal work fall out of the extra special English pale ale style 8C, as stipulated by the BJCP style guide (Strong *et al.*, 2008). On the other hand, the SO_4^{2-} ions were deemed necessary so as to balance out the already existing Cl^{2-} ions acquired during the municipality chlorination process. These SO_4^{2-} ions according to Briggs *et al.,* (2004), contribute a drier and more bitter flavour to the final beer and also help cancel out the saline effect of the Cl^{2-} ions in beer. This is why gypusm salt became the salt of choice and the total SO_4^{2-} ion concentration increase in water amounted to 56.47 mg/l. This concentration fell within the recommended SO_4^{2-} ion concentration range of 10 – 250 mg/l. The water profile which was intended by the above salt treatment was the London water which is known for having 90 mg/l, 58 mg/l, and 18mg/l concentrations of the Ca^{2+}, SO_4^{2-}, and Cl^{2-} ions, respectively. This was deemed necessary as the pale ale style being brewed was of English origin, and it would make the product coform greatly to the prerequisites if an English water profile was assumed.

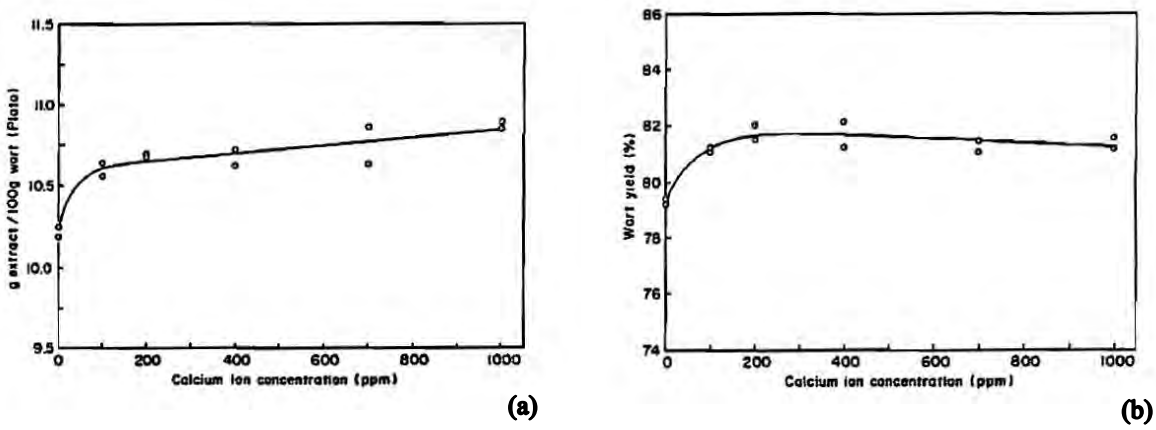


Figure 3.13. The effect of Ca^{2+} ion concentration on (a) wort gravity and (b) percentage yield for a brew with a grist-water ratio of approximately 1 : 5 (Taylor and Daiber, 1988).

For lactic acid pH treatment, a dosage that would bring down the initial pH value to fall in the range of 5.0 – 5.5 pH units was used. It was noted that above the dosage rate of 1 ml per liter of water, no significant change with brewing benefits was made in water pH adjustment. During the mashing stage, it is also in the brewer‟s interest to keep mash-in water above the pH of 5 as lower pH values would have a negative impact on the amylase enzymes, which are key to the gelitization of starch to sugars. The final pH range desired for the treated water was 5.0 – 5.5 pH units, as this range will rise slightly once the malt is added during mash-in to 5.2 – 5.7 pH units. The later range is actually considerd optimum (Steiner *et al.*, 2012), for many biochemical aspects in the mashing process such as proteolysis (Celus *et al.*, 2006), saccharification , and numerous more.

In the individual brewing stages concistency across the six experimental brews was closely monitored. During the mashing process, the average gravity amounted to 14.06 ± 0.18 °P where all six participating brews fell within the 95 % confidence band i.e 15.33 – 12.80 °P . This meant that even though the gravities were not identical, they were proven by statistical means, to be significantly the same/indifferent ($p \leq 0.05$). The mashing recipe in this study was made up of a grits-water ratio of 1 : 5.2 where the grain was made up of 99.66 % pale

malt (4.775 kg) and 0.34 % black malt (0.020 kg). The calculated EBC colour range with respect to this combination was found to be 16 – 34 EBC colour units for boiled wort and the average wort colour during the mashing stage was 38.76 ± 3.10 EBC units. Although the mashing colour was out of range, it was obsevered by the naked eye that the wort had significantly high turbidity (cloudy white colour) due to the powdery dust from the milling stage now suspended in solution. Such observations showed the importance of the lautering stage, as this is where clarification and filtration of the wort would take place giving the observer a much more accurate EBC colour value to work with. Although the EBC colour value was high due to turbidity, descriptive statistical analysis showed that the six batches had insignificant deviations from this value, and individually gave values that were within the 95 % confidence band ranging from 30.75 – 46.76 EBC colour units. This analysis showed that the mash colour across batches was concistant and reproducible. The pH overall value of the mashing process was found to be 5.66 ± 0.03 at 25 °C. Lewis and Young, (2001) and Briggs *et al.,* (2004) seperately discuss the mash pH range and optima values. The average pH value observed for the brews is almost identical to the optimum value for α-amylase activity optimum value i.e., 5.7 pH units. But again literature goes on to stress the point that at a cooled wort temperature, the pH value rises from its "mashing" pH value, where Briggs *et al* suggests that the 5.7 pH value is actually a 5.3 if it were measured at the mashing temperature of 65 °C. None the less, the pH value found for the mashing stage correlates with many scientific brewers‟ findings and is reproducible statistically ($p \leq 0.05$), where deviation betweeen batches was found to be less than 1 % of the mean value. Mash-out volume concistency was found to be reproducible statistically using $p \leq 0.05$, with deviations only amounting to 3.9 % of the average mean i.e., 15.38 ± 0.60 l.

The simple sugars profile in wort was a major area of investigation in this research where maltose was considered as the main contributing sugar or brewer‟s sugar. It was

acknowledged though at an early analytical stage, that the GC-MS protocol assumed could not cater for maltotriose which is one of the five major brewing sugars. The single infusion mashing protocol adopted and set at 64 °C made it possible to liberate maltose as the main sugar during the mashing stage, just as Briggs *et al.*, (2004) and Montanari *et al.*, (2005) had revealed. This was made possible by mashing at the optimum activity temperature range of the β-amylase enzyme. The results however showed that the glucose levels in the mash were significantly above the 8 – 15 % w/v range reported in the literature for similar maltose promoting mash profiles (MacWilliam, 1968; Tenhunen *et al.*, 1994; Briggs *et al.*, 2004).

The three main reasons for this deviation were the decline of the heating rate from saccharification to inactivation, the minor hydrolysis of maltose by water to produce glucose in solution, and the exclusion of maltotriose from the total fermentable sugars value. For the heating equipment, due to the open vessel system design in all units (mash tun, lauter tun and kettle), the heating belts efficiency and sensitivity declined with rising batch temperature. This was evidently noted by the decrease in heating rate performance of the belts from the 1 °C/min rate to 0.79 °C/min when heating a batch above 60 °C. This meant that the heated wort was exposed for approximately 12 min plus an extra 3 min to the α-amylase optimum temperature range before the enzyme inactivation temperature of 76 °C was reached. The 15.17 min heating duration was more than enough to hydrolyze any remaining traces of starch into the DP1 glucose sugars by the enzyme. Even at 64 °C a very significant amount of α-amylase was active and was simultaneously liberating glucose as the β-amylase optimally liberated maltose (Lewis and Young, 2001; Briggs *et al.*, 2004). This suggested, even though not desired at high levels, that 64 °C is a temperature value that promotes the moderate accumulation of glucose in the mash solution even though the α-amylase enzyme is not yet in its optimum activity status. The hot mash promotes the further dissolution of the not-so-soluble $CaSO_4$ salt, and this salt increases the conductivity of the mash. With a lot of free

electrons and Ca^{2+} ions in solution, the dissociation of a small percentage of water molecules from their stable form i.e., H_2O, to the ionic fragments of H^+ and OH^-, and vice-versa, becomes more possible and frequent. These water ions are responsible for the hydrolysis of the maltose molecule at the α-1-4 position hence liberating two glucose molecules. The third reason for the glucose deviation, was a systematic percentage increase of the four sugars analyzed (fructose, glucose, sucrose and maltose) in the fermentable sugars total, due to the absence of maltotriose in the analysis. Considering five reported maltotriose ranges (MacWilliam, 1968; Tenhunen *et al.*, 1994; Fix, 1999; Goode *et al.*, 2005; Montanari *et al.*, 2005) it is approximated that the theoretically expected percentage value in this study is 15.95 %, in the 14 – 18 % range. This then indicated that the concentrations of all four sugars investigated with respect to total fermentable sugars were above their actual values by 18.98 % (refer to appendix-G for calculations). After error considerations and recalculations, it was found that glucose was approximately 39.33 % of the total fermentable sugars, which is a 27.43 % rise in concentration, and is triple the percentage previously reported (MacWilliam, 1968; Tenhunen *et al.*, 1994; Fix, 1999; Goode *et al.*, 2005; Montanari *et al.*, 2005) i.e., 11.90 % in the 9 – 14 % range. It became evident at this stage that the α-amylase enzyme had a larger contribution in starch hydrolysis than anticipated, both during the 64 °C single infusion mash and also during the 15.17 min heating up period hence tripling the liberated glucose amount found in wort as extract. The maltose hydrolysis by water, although acknowledged, was not believed to be of great magnitude in the mash. But similar calculations from literature estimations (MacWilliam, 1968; Tenhunen *et al.*, 1994; Fix, 1999; Goode *et al.*, 2005; Montanari *et al.*, 2005) showed that the maltose measured during the investigative work was actually 40.95 % of the total simple sugars, and this suggested a 22.49 % decrease from the expected maltose levels. The rise in glucose concentration (42.63 g/l = 27.43 %) was deemed a repercussion of the drop in maltose concentration (35.11 g/l = 22.49 %) plus

the extra liberation of more glucose and other monosaccharide sugars by the α-amylase enzyme. The calculated estimations conclusively suggested that the maltose hydrolysis contributed a 36.97 g/l gain and the α-amylase activity contributed a 5.66 g/l gain in the total 42.63 g/l concentration deviation observed in glucose.

This final explanation is deemed misleading from a brewer, microbiologist and biochemist‟s point of view due to the following three assumptions previously made. Firstly it was assumed, although not stated, that across batches the potential beta and alpha amylases activity profiles due to grain modification in the malting stage are consistent for all the grain used, and this grain comes from only one barley farm, modification process and storage batch. Secondly, an assumption was made that any decrease of the extracted maltose concentration from the theoretically calculated value, was due to hydrolysis by the action of water ions on the maltose in solution to liberate two units of glucose per unit of maltose. The third and most inaccurate assumption, was that the glucose and maltose concentrations in each batch were expected to be identical to (or be within 95 % confidence levels of) the theoretical values calculated. These three assumptions left no room for other possible permutations of physico chemical and enzymic conditions that might have resulted in the significantly different sugars profile observed in this chapter. Rather than literally taking the calculated values as precise answers to deviation questions, these calculations together with the numerous assumptions made were seen as a new direction of thought which further brewing investigations should take in order to acquire more data for solving this situation. The current research work had insufficient data regarding enzyme activities, real time starch hydrolysis – sugars accumulation, and malted barley chemical and enzymic properties prior to milling and mashing. A new investigation can therefore be taken in that perspective and further the current work for more accurate explanations.

In the lautering stage, the slight difference in grain age and producer batch origin was felt, as these grains did not have identical friability and filtration properties. This resulted in significant deviations of the physico chemical properties as far as measurement by the p-value of 0.05 was concerned. Even so, not all quality parameters deviated across batches as seen with pH and overall final lautering gravity which had average values at 5.75 ± 0.03 and 9.56 ± 0.25 °P, respectively. These were the only two properties reproducible across batches with a confidence level of 95 % and higher. In terms of the individual runnings, batch 5 was the only outlier with a 1st runnings final volume of 6.6 l. This significantly raised the standard deviation of the average 1st runnings volume to be wider than the 95 % confidence band hence statistically implying that the intended 1st runnings volume of 8.0 l was not consistent. In the 2nd runnings analysis, batch 2 and 6 were outliers with their collected volumes amounting to 8.4 l and 7.0 l, respectively. These also deviated from the intended 8.0 l recovery volume to give 7.58 ± 0.48 l, and so raised the average standard deviation to be above the 5 % limit of the regulating p-value. The 3rd runnings were the most difficult to manage as sugars intended to be washed off the grain and recovered were very low, and an even slower extraction flow rate was required. Batches 1 and 2 had extremely low recovery volumes which were way below the intended recovery volume of 5.0 l, hence they were considered as extreme outliers and were not included in the statistical computations of the average values. The remaining four batches analysed had batch 4 as the computed outlier i.e., recovery volume of 6.0 l. At 95 % confidence level, batch 4 was found to be the only significantly different batch from the other 3 participating batches. This implied indirectly that there was 50 % chance of reproducing the 5.0 l recovery volume using the proposed ISA S88 model (considering all six batches), and directly implied that with respect to the four participants, there was a 75 % chance of reproducing the intended recovery volume. Looking at the overall effect of the lautering process it is noted that by effectively using 20.33 ± 0.64 l

of sparging water across three runnings, the overall wort volume accumulated in the boiling kettle increased to 35.71 ± 0.62 l. This process clarified the wort by means of filtration and simultaneously reduced the wort EBC colour and particulate matter by 35.09 % and 62.95 %, respectively, , resulting in a reduction to 25.16 ± 4.33 EBC units and 4.39 ± 0.16 g/l respective final values. There was no reported case to compare and contrast with, as different brewers and brewing scientists use different recipes, equipment, procedures as well as brew different beer styles. However, the overall extract recovered during lautering, i.e., the sugars intended for conversion into ethanol and other by products, was used as a measure of the accuracy and overall reproducibility of this complex stage, as shall be seen in later analysis.

The boiled wort had an average gravity of 12.10 ± 0.46 °P. This gravity value, as explained by Navarro *et al.,* (2007), was a format that could be used to calculate the extract in solution with respect to water i.e., w/w or percentage. For the final wort which contained malt-derived exract and maltose syrup-derived extract, it was calculated and seen that the extracts from these two sources amounted to 3194.69 ± 307.90 g. Considering the contribution of the mashing and lautering stages, plus the 462.00 ± 68.87 g maltose syrup addition, the extract accumulated in the wort prior to trub losses was found to be 3768. 09 ± 153.48 g in 35.71 ± 0.62 l. After the 6.16 ± 1.23 l trub losses the extract in solution decreased to 3104.38 ± 186.58 g and a theoretical gravity of this extract value in a total volume of 29.42 ± 0.51 was found to be 10.57 ± 0.37 °P. The final calculation was the concentrating effect the evaporation losses had on the 29.42 ± 0.51 volume as it decreased to its final value of 26.45 ± 1.34 l. The final theoretically calculated gravity value was 11.65 ± 0.14 °P. The accuracies of the final wort extract and gravity estimates were found to be 97.18 % and 96.30 %, respectively, when compared to the measured values (refer to appendix-G). A trend across the six participating batches was noted in relation to gravity and volume. The batch with the highest gravity i.e., batch 2 with 12.85 °P, had the lowest final volume value of 24.50 l whilst

batch 5 with the lowest gravity of 11.90 °P had the highest final volume of 27.60 l (refer to appendix-D). This trend although it was not entirely linear across participants, correlated with the theoretical expectations that the boiling process had a concentrating effect by means of water loss due to evaporation. Another common analysis at this stage of brewing is the brew house yield obtained i.e., the amount of extract produced related to the adjuncts and grain used. Looking back at the mashing process, it was seen that the extraction yield was 62.03 %. The final wort extract was calculated from the wort gravity as 3194.69 ± 307.90 g, but in the case of maltose a maximum weight contribution of 360.36 ± 56.74 g to the total malt-extract weight used was possible. The total grist amount used during mashing was 5795.10 ± 0.08 g and this grain had moisture level ranging between 6 – 8 % of the dry weight. Taking the largest possible moisture percentage and compensating for the correct dry weight of the grain, it is noted that the total grist amounted to 5331.40 ± 0.07 g. Expressing the final brew house extraction yield as a percentage of the total malt-extract used i.e., 5331.40 ± 0.07 g, it is noted to be 70.68 % and 60.03 % before and after trub losses, respectively. It was noted that the 17.25 % volume loss as trub for wort clarity reasons came at a cost of losing 10.04 % extraction yield. This meant a drop in the potential final gravity and also loss of fermentable sugars as well as higher weight carbohydrates that would have helped enhance the beer‟s mouth feel, foam stability and overall flavour profile. The yield value at the mashing stage was a very good conversion value as similar yield values were observed to be 63.6 % and 62.7 % from Briggs *et al.,* (2004) and Navarro *et al.,* (2007), respectively (table 3.4). With relation to the utilization limits for both barley malt and maltose syrup i.e., 78 %, it is easy to see that the brew house extraction efficiency was actually 90.04 % and 76.47 % before and after trub losses. Evaporation losses were experimented on by Meilgaard, (2001) using a new pressurised boiling invention, observing that boil losses were usually between 6 – 12 % and 3 – 6 % for ambient and pressurised boils, respectively. The evaporation losses for the

Westville microbrewing system were found to be 8.68 % which was within the ambient boil range stipulated by literature.

Table 3.4. Mashing extraction yield expectations for different mashing durations and temperatures (Briggs *et al.*, 2004).

Mashing period (min.)	15	30	60	120	180
60 °C (140 °F)					
Extract (%)	50.2	53.4	57.2	60.7	62.2
Fermentable extract (%)	36.0	39.0	43.1	47.9	50.2
65 °C (149 °F)					
Extract (%)	60.6	62.2	62.8	63.6	63.6
Fermentable extract (%)	44.2	46.6	48.5	50.7	51.7
70 °C (158 °F)					
Extract (%)	61.2	62.5	62.9	63.4	63.6
Fermentable extract (%)	40.9	42.0	41.6	42.2	42.7

The same calculations were made in this chapter with respect to total simple sugars from the GC analytical protocol, and the reason being consistency measures and also creation of an average efficiency calculating tool. The first calculations were based on crude estimation reactions i.e., specific gravity and the DNS reducing sugars reaction whilst the second set of calculations were based on the precise analytical measuring ability of the GC technology. Appendix-G shows that the reducing sugars and simple sugars analysis rate the Westville microbrewery as 91.32 % and 89.37 % efficient, respectively, and on average they both imply 90.35 % efficiency as far as the evaporative index-liquor retention balance is concerned in the boiling stage. This average deduction then imply an overall trub loss in the system to be 5.83 ± 1.33 l and vapour losses to be 3.43 ± 0.66 l i.e., 16.33 % and 9.61 % losses by volume, respectively.

Variation sources in the brewing process were investigated statistically by means of the two-way ANOVA coupled with post Bonferroni tests ($p \leq 0.05$). The test was a comparison between brewing batch number and brewing stages across the six participating batches. For

the total simple sugars batch variations were considered to be statistically insignificant i.e., no inconsistencies were observed across different batches at the same brewing stage. The percentage of total variation contributed by batch differences was 3.25 % and $p = 0.2118$, hence not satisfying the test condition of ($p \leq 0.05$) and hence insignificant. With respect to the individual simple sugars, there was no significant variation across batches although batch 5 was considered an outlier in all tests and was excluded statistically. This outlying behavior of batch 5, and batch 6 for maltose and sucrose tests, gave percentages of total variations of 6.42 %, 1.24 %, 11.67 %, and 0.02 %, and p values of 0.0985, 0.8960, 0.0705, and 0.9684 for fructose, glucose, sucrose, and maltose tests, respectively. In all four tests the p value condition was not satisfied and so the sugars were individually regarded as of consistent quality. The percentage of total variation for the total simple sugars across the brewing stages was found to be 88.50 % with a value of $p < 0.0001$. This result proved that the brewing stages i.e., mashing, lautering and boiling, were significantly different from each other and the statistical tool at hand was sensitive enough to note the differences.

The concentrating effect of the boil increased the colour of the wort significantly to 29.20 ± 5.12 EBC units. Across all participating batches, it was expected that the higher volumetric batches to possess a lighter (dilute) EBC colour whilst the lower volumetric batches a darker (concentrated) EBC colour. This was not the case across all batches, due to the unidentical extraction efficiencies for the individual batches, grain moisture, friability, FAN and pH values. All these parameters influenced directly how much of the colour defining compounds and temporary haze made it into the final wort solution. A transition in wort colour however, saw the golden brown colour observed during the lautering stage change into a dark coppery brown colour which had a glossy effect once viewed against incident light. The final wort colour was also key to scoring the final EBC beer colour required by the BJCP style guideline

i.e., 11.8 – 35.4 EBC colour, which is very similar to the grain mixture EBC wort colour range calculated theoretically to be 16 – 34 EBC units. The simple descriptive and ANOVA comparison tests found that most of the batches were significantly different even though with respect to literature and theory, they were well within the acceptable range. This is one result that showed the weakness of solely using a strict and sensitive comparison tool as means of bench marking beer/wort quality. This is due to the fact that statistics at this level (not yet considering correletion, impact factors, multivariable comparisons, PCA, etc) did not consider all the above mentioned phsico chemical parameters which have more than enough potential to alter EBC colour values significantly. Smythe *et al.,* (2002) expressed the importance of beer/wort appearance as being a direct impact on its perception with regards to acceptability and likelihood for purchase by consumers, hence beer/wort colour being extremely important to brewers as far as consumer and recipe requirements are concerned.

The 5.49 ± 0.03 final pH value obtained at the end of the boil correlated with a number of literature citings. Values of 5.30 and 5.28 pH units were reported by Navarro *et al.,* (2007) and Steiner *et al.,* (2012) for their pH treated worts, respectively. Statistically the pH values were found to be highly reproducible as they were significantly indiferrent ($p \leq 0.05$). The combined buffering effect of the lactic acid and $CaSO_4$ added during the mashing stage prevented the final wort pH from increasing rapidly during the addition of the maltose syrup adjunct, and during the use of a significant amount of untreated water during lautering. As expected theoretically, the pH value of the boiled wort was lower than that of the lautered wort i.e., a fall in wort pH was deemed inevitable. Briggs *et al.,* (2004) claimed this fall in pH to be between 0.1 – 0.2 pH units and also adviced that boiled wort pH should be kept above 5.0 pH units. This came with the advantage of concistency as far as eliminating flavour-unstable polyphenols and higher weight proteins through precipitation of their complex links was concerned. This is a key feature in brewing which comes with the benefits

of having clarified wort, which in turn will produce permanet haze-free beer in the pH range of 4.2 – 4.6 pH units.

During wort fermentation, a combined effect of yeast concentration, fermentation temperature, FAN concentration and wort gravity determined how fast the wort sugars were depleted and also to which extent. With the *Saccharomyces cerevisiae* Safale s-04 strain kept at a constant pitching rate of 10 million cells/ml/ºP, the only experimental factors were temperature and fermentation time. A look at the brewing and fermentation FAN concentration values shows some variation amongst batches (refer to appendix-D). Although these were intended to be consistent, Jones, (2005) reported that very little or no inactivation of the endoproteinases enzymes during malt kilning and mash protein rest was achived by maltsters and brewers. This meant that the soluble protein fraction of the wort i.e., comprising of dissolved proteins, peptides, and amino acids (FAN), was always increasing during mashing and lautering. Therefore the different malt batches used for the experimental brews gave slightly different FAN concentrations due to their unique soluble protein profiles and enzyme activities. Pickerell, (1986) highlighted the importance of FAN in yeast growth and physiology. The initial FAN concentration in the three experimental temperatures i.e., 281.78 ± 35.21 mg/l was classified as very high FAN content. This according to brewing guidelines and literature was found to be within acceptable limits for an English pale ale brew. Temperature was the biggest controlling factor of FAN utilization, with the best comparison being between the 16 ºC and 18 ºC fermentation profiles, where 2470.52 ± 73.99 mg, and 3080.76 ± 224.43 mg were consumed respectively in 12 days. The higher temperature promoted a faster yeast metabolic growth and hence a faster consumption rate of the FAN i.e., nitrogen source. The larger amount of consumed FAN for the averaged 14 ºC fermentation was due to more exposure of the yeast to the medium i.e., a 21 day fermentation period. Total sugars consumed were very similar at the end of the fermentation process

irregardless of the consumption rates. This meant that sugar consumption was consistent due to a targeted final gravity value and also due to the reproducible original gravities produced at the end of each boiled wort batch. Although none of the batches were identical, as far as FAN, reducing sugars and gravity profiles are concerned, the great similarities and key reproducible properties across all six batches meant that the pitched yeast experience the same stress levels during fermentation. The stresses in this research, even though not investigated, were identified as osmotic stress, anaerobic shift, nutritional stress, ethanol toxicity, and cold sock. These stresses were associated respectively with high gravity/high FAN wort medium, change from propagative to anaerobic fermentation, depletion of nutrients in green beer, build up of ethanol in green beer, and racking at chilled temperatures as low as 0 ºC. Due to the fact that all these steps during primary and secondary fermentation were closely regulated, the yeast cells in all six batches were assumed to exhibit a very similar general stress response until investigated further (Gibson *et al.*, 2007).

The tall cylindrical fermentation vessel designs i.e., where the diameter to height ratio was > 3 : 1, were used for all partitioned fermentation vessels (FVs) of 3.0 l capacity. Briggs *et al.,* (2004) stated that such FV designs, with emphasis on cylindroconical shapes, promoted higher alcohol formation at the expense of esters during fermentation. This development, as stated by Briggs, was observed in the form of vigorous beeding of the fermenting wort, due to rapid CO_2 production, as the yeast utilized high amounts of FAN in all three fermenting temperatures. Considering the brewing guideline (Strong *et al.*, 2008), style 8C depicted a fairly strong alcoholic ale with an estery and alcoholic aroma. Therefore the proposed design was used in the ISA S88 modelling of the brewing process so as to further conform to the brewing style and flavour profile.

Wort fermentation in the 14 °C, 16 °C, and 18 °C batches lasted on average 19, 12, and 10 days, respectively. The purpose of these different temperatures was to note the Safale s-04 yeast strain‟s performance in different physical conditions. The governing factor for ending all fermentations in this research work was the final gravity, which was acceptable once in the 1.015 – 1.020 specific gravity range (3.83 – 5.09 °P). Sugar utilizations were expected to increase linearly across increasing fermentation temperatures and duration. The results obtained in this study however did not fully reflect this theoretical idea, implying that more than one factor was responsible for sugars uptake. With a constant pitching rate of 10×10^6 cfu/ml/°P, sources of slight variation in this strictly controlled investigation were found to be wort initial FAN and sugars. The initial values for FAN were 306.16 ± 34.31 mg/l, 257.39 ± 11.78 mg/l, and 281.78 ± 35.21 mg/l and the total initial sugars were 151.84 ± 19.32 g/l, 136.39 ± 7.45 g/l, and 136.60 ± 6.09 g/l for the 14 °C, 16 °C, and 18 °C batches, respectively. It has been proven in past brewing investigations that the initial FAN concentration in wort influences the rate of sugar consumption by the yeast. The higher the FAN concentration, the faster the uptake of sugars by the yeast, where the overall demand of this concentrated FAN content is governed by the initial sugars concentrations i.e., nitrogen required to help the yeast metabolize the sugars and bud quicker (Pickerell, 1986). So considering the total sugars consumed, i.e., 3570.18 ± 28.46 g, 3295.92 ± 185.42 g, and 3532.37 ± 120.52 g for 14 °C, 16 °C, and 18 °C, the calculated consumption rates of total sugars in each fermentation duration are 187.91 ± 1.50 g/day, 274.66 ± 15.45 g/day, and 353.24 ± 12.05 g/day, respectively. A look at these simple estimation models for the respective batches would give an impression that the fermentations do follow the anticipated linear behavior as fermentation temperatures increase. However, the more accurate non-linear regression models governing the single phase sugars-decay/depletion profiles across time, will give a better overview of the consumption rates. The regressions gave the time dependent decay rates K of 0.9526 day^{-1},

0.6178 day^{-1}, and 1,036 day^{-1} which implied fermentation half-lives of 0,7276 days, 1,1220 days, and 0,6689 days for the 14 °C, 16 °C, and 18 °C fermentations, respectively. The half-lives clearly showed that the yeast strain *Saccharomyces cerevisiae* Safale s-04 took 17 hours 28 min (0.7276 days) to consume 2004.25 ± 11.78 g, 26 hours 22 min (1.1220 days) to consume 1807.17 ± 6.46 g, and 16 hours 1 min (0.6689 days) to consume 1806.47 ± 80.20 g of total sugars at 14 °C, 16 °C, and 18 °C fermentation temperatures, respectively. These three non-linear regression models gave slightly lower values at the respective half-life times due to them being models, and not actual graph point outlines. The observed sugars consumed for the models at $t_{1/2} = 1$ were 1847.07 ± 9.19 g, 1614.82 ± 5.77 g, and 1678.65 ± 74.53 g for fermentation temperatures of 14 °C ($r^2 = 0.8863$), 16 °C ($r^2 = 0.9551$), and 18 °C ($r^2 = 0.9325$), respectively.

The ethanol production rates after such observations, were then thought to have a similar trend by the simple reasoning that rapid sugar metabolism would imply faster ethanol production, and vice-versa to be true as well. The ethanol non-linear regressions however gave a slightly different trend. The calculated half-life ethanol concentrations for the fermentations were 2.43 ± 0.27 % v/v, 2.35 ± 0.12 % v/v, and 2.54 ± 0.14 % v/v for the fermentation temperatures of 14 °C ($r^2 = 0.7803$), 16 °C ($r^2 = 0.9442$), and 18 °C ($r^2 = 0.8663$), respectively. The respective half-life times for these profiles were 3.74 days, 2.10 days, and 1.56 days. These half-lives together with the final ethanol concentrations of 4.53 ± 0.58 % v/v, 4.52 ± 0.24 % v/v, and 4.97 ± 0.28 % v/v in 14 °C, 16 °C, and 18 °C batches, respectively, gave theoretically anticipated half-life concentrations of ethanol that deviated from the modelled values above by 7.08 %, 3.98 %, and 2.04 %, respectively. The ethanol production rates between the 14 °C and 16 °C batches governed by the regression association constants K i.e., 0.1853 day^{-1} and 0.3305 day^{-1}, did not correlate with their total sugars consumption rates observed above. Ethanol production rate at 16 °C was approximately twice

that of 14 °C, which now deviated from the observations regarding high initial FAN contents and total sugars in the wort (Pickerell, 1986). Although the yeast strain Safale s-04 was found to be a strong attenuator according to (Fix, 1999), it still fell under the category of many ale yeast strains which are medium attenuators. This meant that the tendency to produce other fermentation by-products such as aldehydes, fusel alcohols, esters, etc. during different metabolic pathways of available substrates was high, and as known, fermentation is not a single reaction process (White, 2012). The production of aroma active flavour compounds as well as combinations of specific amino acids, vitamins, minerals and fatty acids affected the rate of ethanol production to follow the initial linear behavior assumed.

Fusel alcohols are a major part of the volatiles formed during wort fermentation. Aliphatic higher alcohols were investigated in this study i.e., propanol and isoamyl alcohol. It has been reported in the past that fusel alcohol production is dependent and directly affected by nutrient levels, temperature change, and yeast cell growth and fermentation vessel shape (Briggs *et al.*, 2004; Willaert and Nedovic, 2006; Brányik *et al.*, 2008). Amino acid uptake efficiency and the utilization rate of sugars, at an assumed constant temperature, became the dominant determinants of fusel concentrations found in beer. The FAN levels in all fermentation batches were in the "high" concentration region (Pickerell, 1986), and so the slight differences amongst them was not enough to impact negatively in amino acid utilization during both anabolic and catabolic pathways of fusel alcohol formation (Briggs *et al.*, 2004; Willaert and Nedovic, 2006). Total fusel alcohols in the 14 °C fermentation exceeded theoretical expectations of being lower than the 16 °C due to the elongated fermentation time, and also due to the much higher growth promoting FAN and initial sugars concentration in the wort. The ratio of propanol: isoamyl alcohol was noted to be 1: 5.75, 1: 5.84, and 1: 4.95 for the 14 °C, 16 °C, and 18 °C batches, respectively. These very similar

ratios implied that the flavour balance of the combined fusel alcohols across the three fermentation temperatures was approximately the same, and considering that propanol is way below its threshold value of ≈ 800 mg/l, the only aroma active flavour imparted to the beer at this stage was the solvent/alcoholic flavour of the isoamyl alcohol. The brewing style followed depicted a strong alcoholic ale beer; therefore ethanol productivity was also of great concern in the research work. Ramirez and Maciejowski, (2007), modelled an optimum fermentation experiment and observed that for their yeast strain ethanol production was optimum within the range of 12 – 13.5 ºC i.e., with minimum fusel alcohol production. A simple ratio of fusel alcohols: ethanol was done and at the end of each fermentation profile it was noted that the ratios were 1: 0.0375, 1: 0.0379, and 1: 0.0357 for the fermentation temperatures 14 ºC, 16 ºC and 18 ºC. A quick glance at the three ratios shows that there is no significant difference between the fusel alcohols: ethanol flavour profiles. The magnitude of the ratio differences however gives an indication that 16 ºC is the optimum temperature for highest ethanol production paired with the most suppressed fusel alcohol production and 18 ºC being the least of the three. Lower temperature effects on yeast growth coupled with CO_2 pressure were noted to have a more prominent impact at 14 ºC and 16 ºC, respectively (Willaert and Nedovic, 2006). At 18 ºC the temperature was in favour of rapid yeast growth hence a greater extent of fusel alcohol formation. Production of ethanol within the shortest period was also deemed optimum and resourceful, and therefore calculating the same ratio for fermentation temperature 14 ºC on day 12 resulted in a ratio of 1: 0.0376. This ratio gave an indication that the optimum range for high ethanol: low fusel alcohol production, was approximately 15 – 16 ºC for the yeast strain Safale s-04. Standardized brewing practices such as the ISA S88 investigated in this research, together with modelled strategies performed by Ramirez and Maciejowski, (2007), help improve batch to batch consistencies

with respect to slight variations in initial conditions such as gravity, inoculum, pitching temperature, etc.

Four ester compounds were investigated in the research work, where these comprised of two acetate esters i.e., ethyl acetate and isoamyl acetate, as well as two ethyl esters i.e., ethyl hexanoate (caproate) and ethyl octanoate (caprylate). The acetate ester concentration was observed as having a linear relationship with fermentation temperature i.e., where final concentrations at the end of primary fermentation were found to be 58.29 ± 14.07 mg/l, 62.18 ± 6.97 mg/l, and 74.23 ± 6.20 mg/l for ethyl acetate, as well as 0.57 ± 0.27 mg/l, 0.86 ± 0.43 mg/l, and 1.06 ± 0.43 mg/l for isoamyl acetate at 14 ºC, 16 ºC and 18 ºC, respectively. Ethyl ester concentrations however behaved in an inverse Gaussian distribution manner when compared to rising fermentation temperature, with the lowest values observed at 16 ºC. Ethyl hexanoate concentrations were 0.41 ± 0.04 mg/l, 0.27 ± 0.05mg/l, and 0.34 ± 0.07 mg/l whilst ethyl octanoate concentrations were 1.45 ± 0.06 mg/l, 0.71 ± 0.27 mg/l, and 1.06 ± 0.36 mg/l for fermentation temperatures 14 ºC, 16 ºC and 18 ºC, respectively. Willaert and Nedovic, (2006) pointed out the need for availability of fatty acyl CoA in the presence of an alcohol so as to produce an ester. However, the different behaviors in the ester groups make it evident that the ethyl group, being the medium-chain fatty acid group, is directly affected by the amount of fusel alcohols available. At 16 ºC more ethanol is produced at the expense of fusel alcohols, therefore only small-chain esters i.e., acetate esters, will be synthesized more whereas the ethyl ester concentration will drop due to less fusel alcohols being available for the lipid metabolic pathway.

Considering all literature cited and handling SOPs of the beer samples, the only evident source of the high standard deviation values of isoamyl acetate concentration was the Agilent 5975C inert MSD coupled with the capillary column. This observation was confirmed by the non-zero conforming and insensitive isoamyl acetate calibration graph at low concentrations below 1 ppm (refer to appendix-C). No other concentration above 1 ppm had such great deviations implying that the Agilent 5975C inert MSD was accurate for all concentrations greater than 1 mg/l. The volatility and threshold values of individual esters played a role in influencing instrument sensitivity and accuracy. It was noted that for compounds whose concentration values were significantly below their threshold values, or almost equal to the threshold, lower accuracies were experienced as far as the Agilent Enhanced Data Analysis software was concerned when integrating ionic peak areas into the concentration calibration curve. Ethyl octanoate and isoamyl acetate experienced such difficulties as they had concentration values mostly below 1 ppm and also below their respective threshold values.

A similar but unidentical trend was shown by statistically analyzing the goodness-of-fit and consistencies of linear regression models for all flavour compounds i.e., figure 3.10 above and table 3.5 below. Considering work done by Landaud *et al.,* (2001), and also noting the green beer beading (effervescence of CO_2) that increased with increasing fermentation temperature, it was conclusively accepted that CO_2 production, and therefore, fermentation vessel top pressure due to CO_2 accumulation in the head space, had a linear relationship with temperature. Although not quantified, the effect of this increasing top pressure with increased temperature promoted certain flavour profiles during primary fermentation. At 14 ºC and with low – medium CO_2 top pressure, ethyl hexanoate (r^2 = 0.8061) and ethyl octanoate (r^2 = 0.6345) were found to have their highest consistency values. These two compounds together with ethyl acetate and isoamyl alcohol were significantly above their threshold values and attributed a gentle apple-aniseed aroma laced with an alcoholic-solvent scent to the green

Table 3.5. A comparison of flavour compound final concentrations during primary fermentation and their modelled consistency of production factors i.e., r^2.

Compound	Aroma	Threshold	14 °C		16 °C		18 °C	
			Final Conc.	r^2	Final Conc.	r^2	Final Conc.	r^2
		mg/l	mg/l		mg/l		mg/l	
Ethanol	Alcoholic	14000.00	36057.30 ± 4.58	0.7803	35662.80 ± 1.89	0.9442	39213.30 ± 2.21	0.8663
Propanol	Weak-solvent	800.00	17.86 ± 1.86	0.8203	17.42 ± 1.81	0.9387	23.43 ± 4.97	0.7135
Ethyl acetate	Fruity solvent-like	30.00	58.29 ± 14.07	0.6196	62.18 ± 6.97	0.8756	74.23 ± 6.20	0.9273
Isoamyl alcohol	Solvent	60.00	102.74 ± 8.38	0.8046	101.63 ± 7.85	0.8472	115.98 ± 10.76	0.8601
Isoamyl acetate	Banana, pear drop	1.20	0.57 ± 0.27	0.3750	0.86 ± 0.43	0.4334	1.06 ± 0.43	0.4945
Ethyl hexanoate	Apple-aniseed	0.21	0.41 ± 0.04	0.8061	0.27 ± 0.05	0.7886	0.34 ± 0.07	0.7032
Ethyl octanoate	Apples	0.90	1.45 ± 0.06	0.6345	0.71 ± 0.27	0.5721	1.06 ± 0.36	0.5630

Conc. = Concentration

beer. At 16 ºC as the CO_2 top pressure increased, the flavour profile of the green beer changed. Ethanol (r^2 = 0.9387) and propanol (r^2 = 0.9442) had their highest consistencies at this temperature with ethyl acetate (r^2 = 0.8756) and isoamyl alcohol (r^2 = 0.8472) following closely behind. Out of the seven investigated flavour compounds, only ethanol, ethyl acetate and isoamyl acetate were significantly above their threshold values hence imparting a strong alcoholic/ solvent-like aroma paired with a faint fruity scent onto the green beer. At 18 ºC top CO_2 pressure was regarded as high and the green beer was noted to have strong solvent and fruity aroma paired with gentle aniseed notes. This was mainly due to ethyl acetate (r^2 = 0.9273) and isoamyl alcohol (r^2 = 0.8601) having their highest consistencies at this temperature, and together with ethyl hexanoate, were significantly above their threshold values.

It was noted and acknowledged that most of these flavour compounds had non-linear regression models that were statistically difficult to reproduce i.e., most r^2 values were below the r^2 = 0.9 value. A few key compounds i.e., ethanol, propanol and ethyl acetate had significant models with respect to the less sensitive value of $p \leq 0.1$, but still failed to be of

significance at $p \leq 0.05$. This deviation to expectations was contributed by the insensitivity of the Agilent 5975C inert MSD to minute concentrations discussed above, but also came as a revelation to the fact that no matter how many iterations performed, no fermentation batches were identical. This line of thought insinuated that future work with the strain Safale s-04 had to incorporate detailed yeast life cycles/biomass, vitality, viability, and activity analysis as well as amino acid profiles so as to correctly identify the different biochemical pathways and conditions that gave rise to such beer flavour profiles.

The drop in beer EBC colour during storage and forced aging was primarily due to the clarifying effect of the process. Although no analytical means were used to quantify and distinguish between haze and EBC colour, it was noted that after bottling and conditioning, the beer ranged between 14.33 – 15.34 EBC units in colour. Considering all particulate elimination stages in the brewing process i.e., continuous centrifuging, primary fermentation arrest (flocculation), beer maturation (racking and further flocculation); the only soure of particulate matter and haze in the finished product was the conditioning yeast culture. Each bottled unit of beer has a concetration of 3 ml culture in 750 ml matured beer i.e., $4 * 10^{-3}$ ml/ml. Morris, (1987) reported that yeast in suspension with a concentration of approximately $24 * 10^{-6}$ ml/ml contributes 1 EBC colour unit to beer and yeast concentration of approximately $15 * 10^{-6}$ ml/ml contributes 1 EBC haze unit to beer i.e., figure 1.6. A yeast strain genetically modified for high gravity fermentation and delayed flocculation was used by Soares, (2011) to investigate the amount of flocculation in a cylindrical non-agitated fermentation vessel with different nutrient combinations. The findings gave an indication that at the end of the primary fermentation approximately 99 % of the fermenting culture flocculates and sediments at the bottom i.e., figure 1.7. Considering these two literature reports, and acknowledging the two yeast removal stages during fermentation and maturation,

plus an elongated bottle conditioning period, the flocculated amount of yeast culture in the conditioned bottles during the experiment can be approximated to be 99.5 ± 0.2 %. With the initial conditioning dose set at 3 ml culture per 750 ml of beer, it implies that only 0.5 % of the 4 * 10^{-3} ml/ml yeast concentration attributed by Morris, (1987) remained in solution as invisible haze or additional EBC colour. This approach to suspended yeast, according to Morris, (1987) and Soares, (2011); implied that the experimental beer had 0.83 EBC colour units in all beer bottles contributed by suspended traces of yeast, and contained 1.33 EBC units of invisible haze. These findings correlate with feedback given to the researcher by an informal parnel of beer tasters who ranked the beer clarity to be between 3 – 4 of 5 marks i.e., ranging between "fairly-clear" and "clear". The majority of the beer bottles tasted potrayed an invisible haze characteristic as tasters could not visually see any cloudy suspension of yeast. As the beer aged, the difference was observed through spectrophotometry due to the naked eye failing to note the differences in colour. This observation supported the hypothesis in the sense that adhering to the proposed fermentation, maturation and conditioning S88 model produced beer with an above average colour clarity, reproducible colour range and was visually acceptable to the targeted market.

The other two physico chemical properties measured for stored beer, i.e., pH and TDS, were reported to correlate with bottled yeast physiology and flocculation characteristics. Lodolo *et al*., (2008) stated that calcium concentration, medium ionic strength and pH affect the FLO gene activity as well as cell – cell interaction during floc formation. As the experimental beer aged, it became more conductive (i.e., more ionic strength) due to more solids slowly dissolving into the beer. With Ca^{2+} as one of the main solids, this meant that more cell – cell interactive lectins found on the yeast cell walls were activated, hence more cells being sedimented out of solution as active flocculent yeast (Soares, 2011). Considering the rising bottle pressure due to the produced CO_2 during conditiong and storage, and also pH values of

the numerous bottles that were all within the optimum flocculation range i.e., 3.0 – 5.0 pH units, it meant that the conditions were very conducive hence beer clarification was inevitable. The pH and TDS trends were highly reproducible amongst all six batches with variations being statistically insignificant ($p \leq 0.05$), and hence showed that the S88 model was sufficient in producing beer with consistent colour and mouth contributed by pH and TDS.

During the 12 week storage period, all beer experienced a very significant drop in residual sugars in solution. This implied that fermentable sugars were being slowly consumed by the carbonating culture in the bottle leaving only the unfermentable sugars in solution. The total simple sugar concentration drop across all bottles was in the range of 35 – 60 % of all initial concentration values i.e., table 3.3. Maltose was the main contributor to this significant drop in total simple sugars due to the fact that it was the most abundant and most utilized sugar in wort/beer by the ale strain Safale s-04. Fermentation batches 14 °C and 16 °C had more or less the same amount of residual sugars after racking i.e., 16.27 g/l and 12.34 g/l, respectively. This meant that during each sampling point, CO_2 was released, traces of oxygen were reintroduced, and upon capping and re-carbonating, the settled yeast had more sugars to consume. This is one phase of the bottle-sampling and tasting process that gave room for a significant reduction hence some of the total sugar profiles dropping as much as 60 % (i.e., $week_3$ – $week_{12}$). For the beers bottled from the 18 °C fermentation batches, a smaller amount of residual sugars were left at the end-of-racking stage, hence the reduction of the respective sugar profiles across the 12 storage weeks did not exceed 50 % of the initial concentration.

The ethanol content in the green beer at the end of fermentation for the three fermentation temperatures was observed to be 4.53 ± 0.58 % v/v, 4.52 ± 0.24 % v/v, and 4.97 ± 0.28 % v/v i.e., 14 °C, 16 °C, and 18 °C. These concentrations were ideally kept below 5.0 % v/v due to considerations of extra ethanol to be produced in the bottle by the conditioning and lagering/storage stages. During the spiking of bottles in preparation for bottle conditioning, 743 ± 1 ml of matured beer was added into each 750 ml cot. These beers each had a different mass due to the slight differences in end-of-ferment gravities i.e., 5.24 °P, 4.50 °P, and 3.82 °P for the 14 °C, 16 °C, and 18 °C fermentation batches. The 4 ml of the 50 % maltose solution used as carbonation sugar contributed an extra 0.318 °P, 0.319 °P, and 0.320 °P to the 14 °C, 16 °C, and 18 °C fermented beers, respectively. According to Harris‟ law and the Balling factor of converting extract into ethanol concentrations in % v/v (SABMiller, 2013), it was theoretically expected that there would be an ethanol concentration increase in all beer types after bottle conditioning and lagering by 0.205 ± 0.001 % v/v. Within the 3 ml of carbonation culture added, a small unquantified amount of ethanol produced during the three/four propagation steps was also expected to increase further the bottled beer‟s ethanol concentration. A closer look at the 14 °C and 18 °C fermented beers stored at 0 °C at $week_3$ revealed results in contrast with this theoretical expectation. More than one factor was at play during the bottle conditioning and lagering of the beers which gave a different meaning and reasoning to this particular phase of production. The diluting effect of the wort medium containing the propagated yeast culture plus that of the 50 % maltose solution was more than the ethanol-promoting effect of the 7 day carbonation plus the added traces of ethanol from the propagated biomass medium. This is supported by the fact that the propagation medium was made up of ≈ 80 – 85 % water, whilst the 50 % maltose was made up of ≈ 80 % water plus other non-fermentable material. Hence at 0 °C storage temperature there‟s little/no increase in ethanol concentration as opposed to the significant diluting effect of the water in

the two additives. Other than the beers mentioned above, all the other ferment-storage permutations of the beers met the theoretical expectation as their ethanol contents % v/v significantly increased and differed from $week_0$ (end-of-racking) till $week_{12}$. At $week_{12}$ beer ethanol concentrations were in the range of 5.12 ± 0.43 – 5.57 ± 0.15 % v/v for all nine beer iterations of this experiment. This range was well within the depicted brewing style 8C (Strong *et al.*, 2008) i.e., a 4.6 – 6.2 % v/v ethanol content range. The three fermentation temperature products maintained their ethanol content trend as they aged. Initially the end of rack trend showed that the 16 °C beer had the lowest ethanol content (4.52 ± 0.24 % v/v) followed by 14 °C (4.53 ± 0.58 % v/v) then 18 °C beer with the highest (4.97 ± 0.28 % v/v). The same trend was observed after 12 weeks i.e., appendices-D and -E, where all stored beer ethanol concentrations drifted within that trend pattern. This reflected the fact that although aging was inevitable and did eventually occur, the uniform handling and storage procedures gave no room for other anomalies hence maintaining the existing flavour profiles and patterns as far as ethanol is concerned i.e., a correlation of the results with the proposed hypothesis. As it was logically expected, all beer expressed the least ethanol flavour drift at 0 °C storage temperature and the most flavour drift at 18 °C storage temperature. Fermentable residual sugars in green beer were noted to decrease with increasing fermentation temperature i.e., the ethanol/residual sugar ratios were 3.10, 3.51, and 4.75 for 14 °C, 16 °C, and 18 °C fermentation beers stored at 0 °C at $week_3$. An increase in ethanol content against a decrease in residual sugars was implied by these ratios which was an effect also expressed by increasing pitching rate (Edelen *et al.*, 1996). This implied that for the carbonating cultures in the beer, the yeast pitched in beer derived from the 14 °C fermentation batches by default had the most abundant substrate than yeast in other beers. This was supported by the observation of the ethanol concentrations in table 3.3 i.e., all stored beer from the 14 °C fermentation had the highest percentage flavour drift. If the storage time was allowed to exceed 12 weeks, the

14 °C beers would have eventually surpassed the 18 °C fermentation beers‟ ethanol content due to the yeast being exposed much longer to the residual sugar-rich beer.

The two fusel alcohols (FAs) investigated in this study were propanol and isoamyl alcohol. A significant drop in these alcohols was noted when comparing green beer FAs and bottled beer FAs at any stage of storage. As discussed above, the same diluting effect of the bottle conditioning additives was acknowledged and identified as the main source of this concentration drop when progressing from the racking to the bottle conditioning production phases. The additives, being weak fermented wort with biomass (3 ml) and a 50 % maltose solution (4 ml) were fairly rich in sugars and were extremely deprived of FAN content. The only source of FAN was the wort-biomass solution. Considering the fact that the 7 °P wort for lab-scale biomass propagation was a dilution of 12 °P microbrewery-scale wort, it implied that after dilution and the four-stage propagation steps, wort derived FAN was depleted from the 260 – 300 mg/l range to the 60 – 100 mg/l range approximately. The only additional source of FAN content in this low concentration solution would be protein mixtures and enzymes from autolysed yeast cells (which was unlikely of the highly vital propagates possessing viability approximately ≥ 90 %).

According to literature and SAB SOPs (Lodolo *et al.*, 2008; SABMiller, 2013), minimum FAN content for a healthy fermentation is ≈ 180 mg/l, hence the nutrients in the bottle did not favour FA production. Furthermore, it was reported (Lodolo *et al.*, 2008) that in the carboxylic pathway of yeast metabolism, the FA branch of the pathway was mostly supported by sulphur/amino substrates in solution. With sugars having an upper hand during bottle conditioning and storage, it implied that the carbonating yeast felt the imbalance between the available sugars and the not-so-abundant amino acid/keto acid substrate hence a slower production of the FAs when compared with ethanol production in the bottles post-racking

(Renger *et al.*, 1992). Branyik *et al*, (2008) pointed out that FA production can be inhibited by immobilization conditions (cold storage) of yeast as well as CO_2 toxicity coupled with its hydrostatic effect in bottled cultures. Landaud *et al.*, (2001), argued that the rate of formation of FAs was not inhibited by the absence of FAN content as the carboxylic pathway had the ability to also synthesize FAs by the reduction of aldehydes derived from their α-keto-acid and sugar precursors.

A closer look at the appendix-E revealed that the biggest drift in flavour stability with respect to the two FA was in beers fermented at 14 °C. This drift however, even not significant enough to impact on the overall FA flavour profile, correlated with the observation made earlier that the 14 °C fermentation batch had the most abundant residual material (i.e., fermentable sugars, FAN content, and gravity) and hence gave the conditioning yeast culture a better environment and nutrient combination for FA production, when compared to the beers from the 16 °C and 18 °C batches in their respective storage temperatures. An observation on the end of ferment FAN concentrations i.e., 145.91 ± 36.93 mg/l, 164.16 ± 6.20 mg/l, and 170.03 ± 24.18 mg/l for 14 °C, 16 °C, and 18 °C fermentations, respectively; plus the fact that the 14 °C batch had the most abundant initial FAN content, 306.16 ± 34.31 mg/l, supported the expectation of more FA production in beers derived from this fermentation batch even under storage conditions. Due to considerations of yeast autolytic material, higher level and long-chain poly peptide/protein presence in racked green beer, which all test positive for the ninhydrin FAN method used, it was acknowledged that a lower FAN concentration (especially consumable amino acids) was left as residual which was evident by the yeast‟s performance during conditioning. A consistent FA flavour profile was displayed by the stored beer with the slight drifts being representative of each other. The isoamyl alcohol: propanol ratios in bottle during all tasting points were closely related and deviations noted were insignificant. The ratios as noted in table 3.6 ranged from 4.73 – 5.63

at the end of storage week$_{12}$. The fairly similar ratios meant that the combination of propanol: isoamyl alcohol, especially for the 16 °C fermented beer, was consistent as far as taste and mouth feel were concerned. The combined aroma was governed more by the actual concentrations vs. the respective threshold frequencies. As observed under the results section, only isoamyl alcohol was above the threshold hence its contribution could be smelt and tasted, although the subtle concentration differences were too minute for the human senses to distinguish.

Table 3.6. A representation of isoamyl alcohol: propanol concentration ratios across fermentation batches and storage temperatures at week$_{12}$.

Storage	**End-of-rack**	**0 °C**	**4 °C**	**18 °C**
Fermentation				
14 °C	5.75	5.08	5.60	5.63
16 °C	5.83	5.77	5.60	5.48
18 °C	4.95	5.09	4.73	4.73

The consistency of the FA profiles was contributed mainly by the storage temperatures used in the experiment and the consistent handling and sampling of the beers proposed by the modelled S88 standards. As discussed in literature (Kaneda *et al.*, 1995), a decrease in incubation temperature by 10 °C decreases the production rate of any first order chemical reaction by up to 50 %. This effect of the cold temperatures on the bottled yeast resulted in a significant decrease in FA production rate in storage when compared to the warmer primary fermentation rates. The optimum fermentation and storage conditions for the English pale ale with regards to FA flavour and profile stability was 16 °C and 0 °C, respectively which reflected the least concentration drifts, and the most stable FA ratios across the experimented 3 month shelf life.

Ester analysis and observations in bottled beer met basic expectations. Both ethyl and acetate esters are known to be highly volatile, and so were expected to be lost during every beer sampling point. This was evident for the four investigated esters by their significant concentration drops across the 12 weeks of storage. This gave many scientific implications with the major one being the fact that the rate of ester formation in bottle by the conditioning culture was less than the rate and extent of ester loss during each sampling point. For all experimental beers total ester losses ranged from 1.73 – 7.07 mg/l in concentration. These losses equated to flavour drift of 4.73 – 22.84 % where all the outlying participants were noted to be from fermentation batch 14 °C. Acetate esters consisting of ethyl acetate and isoamyl acetate were found to be drifting significantly with time, as their maximum deviations were 20.25 % and 25.29 % of the original concentration values. These percentages statistically present massive flavour drifts, but practically and with respect to sensory evaluations, these drifts are only 6.97 mg/l and 0.13 mg/l. Ethyl acetate was found to be lingering around its threshold frequency value (30 mg/l) whilst isoamyl acetate was way below its 1.20 mg/l threshold value. This implied that the collective mouth feel and taste might have felt the flavour drift impact, but with regards to aroma, no significant difference could be felt by human senses.

For a brewing ale strain like the Safale s-04 yeast, which has a very high maltose metabolism viability/vitality, it was noted from literature that levels of about 0.6 mg/l isoamyl acetate and 18 – 20 mg/l ethyl acetate were produced for low to medium gravity wort. This was explained by the low levels of the acyl CoA enzyme which mostly favoured the monosaccharides (Stewart, 2006). This correlates with the sugar profile of the experimental wort, as they possessed maltose as the main sugar, and maltose syrup (containing dextrose) as the only adjunct used in the brews. This observation suggests that wort with higher concentrations of glucose and fructose (with gravity ≥ 15 °P) will produce beer with elevated

levels of ethyl acetate and isoamyl acetate (Verstrepen *et al.*, 2003). The bigger ethyl esters of this experiment however (ethyl hexanoate and ethyl octanoate) which are products of longer sugar and alcohol structures, were found to drift very insignificantly. Concentration drifts were noted to be 0.03 – 0.07 mg/l and 0.02 – 0.22 mg/l for ethyl hexanoate and ethyl octanoate, respectively. The collective apple – aniseed notes of these ethyl esters remained fairly unaltered during storage due to the fact that ethyl hexanoate remained significantly above its threshold concentration whilst ethyl octanoate was about half its threshold value. These drifts however looking at table 3.3 as well as the appendix-E on the individual esters across the different permutations show a reflection of uniform handling and have closely related trends. This is another observation of the experimental work that supports the proposed hypothesis of consistent quality products upon implementation of the S88 batch control standards.

Another crucial aspect of this study that might have assisted in explaining the different FA – ester drifts during storage, is the consideration of staling compounds. Staling, which is commonly known by the formation of off-flavour long chain carbonyl compounds such as the radical 1-hydro-ethyl (from ethanol and the hydroxyl radical), is an inevitable process in aging beer. The handling and processing of beer is done in a way that suppresses the stale-promoting factors i.e., heat, light, oxygen, free radicals, free radical catalysts, and staling precursors amongst others (Uchida *et al.*, 1996). A staling relationship has to be established for the storage profiles 0 °C, 4 °C, and 18 °C, with respect to the three fermentation profiles. This profile has to have a storage time-temperature relation with critical contributors such as oxygen, CO_2 top pressure and flavour profiles being closely monitored (Kaneda *et al.*, 1995).

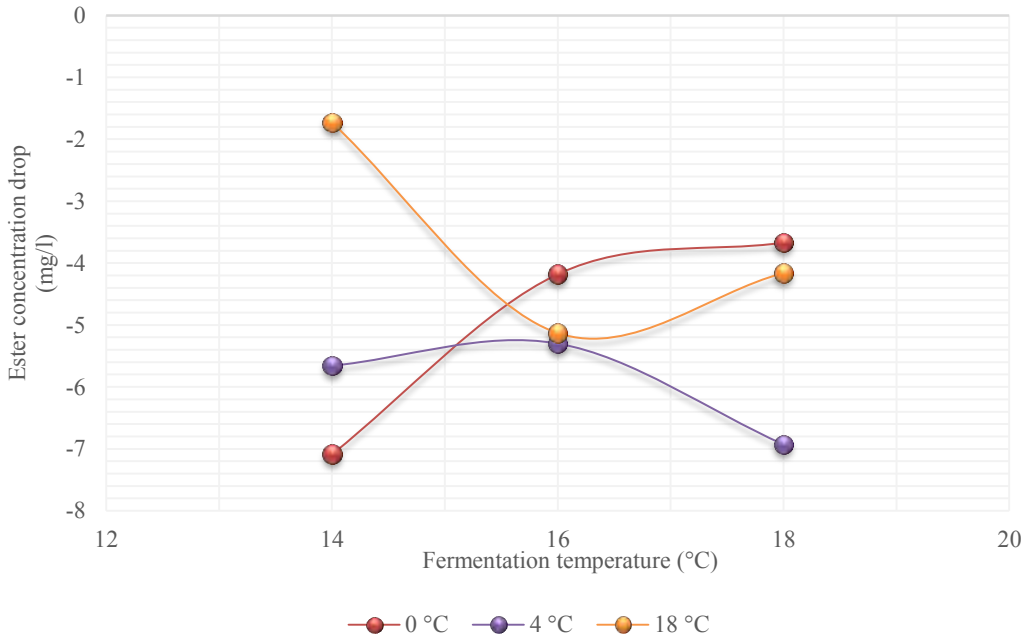


Figure 3.14. Total ester concentration reduction for the nine storage permutations over the 12 week period.

The figure above shows the total ester drift across the three temperatures with time. This ester drift is a balance of esters lost through volatility at each sampling point with ester formation and replacement during re-carbonation period. It was simple to note that since the beers were mostly in cold storage (0 °C and 4 °C), ester formation was going to be way less than ester losses as aroma, hence explaining the total ester concentration drop. Storage at 0 °C and 4 °C provides very little deviation for the 14 °C and 16 °C fermented beers. 16 °C fermentation batches are viewed to be at the optimum temperature as all storage conditions give more or less the same total ester content drop. This observation correlates with FA and ethanol analysis as well as the sugar analysis which all agree to the 16 °C fermentation batch and the 0 °C storage temperature to be optimum.

The dormant state that the cold storage temperatures induce on the yeast is likely the reason that other dormant-favouring metabolic pathways may have been followed instead of the lipid-alcohol pathway to produce more esters on re-carbonating. Formation of glycogen as a

future energy source during dormant stages is one known behavior that brewer‟s yeast possess and therefore an analytical evaluation of glycogen levels in the carbonating biomass as well as in the beer could have further explained these different ester concentration drops per fermentation batch per storage temperature (Lodolo *et al.*, 2008). A small variation in sampling times i.e., minutes the beer bottles stood open, might have been a source of different O_2 exposure to the different beer batches. It is known that too much oxygen in fermenting wort suppresses ester encoding (AATase encoding genes of the yeast) and also, too little oxygen (≤ 1 mg/l) means insufficient biomass growth resulting in slow-still fermentations (Verstrepen *et al.*, 2003; Stewart, 2006). Oxygen also activates the reactive oxygen species (ROS) radicals which cause a chain reaction with flavour compounds to give off flavour carbonyl compounds. For the different storage temperatures, different carbonating rates and therefore different CO_2 top pressures were experienced by the beers. The anti-oxidation property of the CO_2 in solution and on the head space was not equally expressed in the experimental bottles, hence different flavour formation pathways as well as different flavour staling pathways were exhibited per storage temperature. With some beer possessing more FAN and sugar content than others, these might have acted as staling catalysts for certain ROS with respect to the different orientation of the 14 °C, 16 °C, and 18 °C fermentation batches. This then resulted in different rates of ester and staling compound formation throughout the experimental population hence the significant scatter across expected concentrations with respect to esters depicted in figure 3.14 (Kaneda *et al.*, 1995; Landaud *et al.*, 2001).

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Another flavour indication characteristic is the ester: FA ratio. In literature, it was noted that primary fermentation done under atmospheric pressure resulted in an ester: FA ratio that was in the range of 2.81 – 4.30. In pressurized fermentations, i.e., where CO2 top pressure was permitted to reach approximately 1.5 bars, the ratio range increased to 6.87 – 7.5 (Landaud *et al.*, 2001).

Table 3.7. A representation of the ester: FA ratios at racking and across the nine experimental permutations after 12 weeks of storage.

Storage	**End-of-rack**	**0 °C**	**4 °C**	**18 °C**
Fermentation				
14 °C	2.00	5.10	4.04	4.24
16 °C	1.86	4.66	4.62	4.44
18 °C	1.82	4.19	4.03	4.14

Table 3.7 above shows that the end of rack ratios for the experimental work ranged more closely to the lower end of the cited ratio range, and the end of storage ratios were expressed ester suppression/depletion with FA concentration increase. Although it was previously thought that the CO_2 top pressures in the stored beers was significantly different and not consistent, it is evident from table 3.7 that the impact of this pressure in dictating and governing the flavour ratio outcome was uniform across beer of the same fermentation batch. Fermentation batch 16 °C had the least ester: FA ratio drift across the cold storages 0 °C and 4 °C followed by the 18 °C fermentation batch.

Table 3.8. Average total ester flavour drifts in percentage (%) across the nine storage permutations after 12 weeks. All drifts in the table are depicted as flavour concentration decrease.

Storage	0 °C	4 °C	18 °C
Fermentation			
14 °C	22.92	19.90	20.04
16 °C	15.50	16.76	17.67
18 °C	16.84	16.91	21.42

This observation correlated with the total ester average flavour profile drift depicted in table 3.8. Again fermentation batch 16 °C was viewed as the optimum ferment temperature, and 0 °C as the optimum storage temperature. These observations tally with the FA, ethanol, sugar and gravity analyses done on the primary fermentation and storage phases of the maturing and finished beer. This was also a reminder that even though consistency was postulated by the proposed hypothesis, flavour drifts due to staling, yeast biochemical pathway changes, exposure of beer to oxygen and light, etc. all were inevitable and could only be suppressed/delayed, not avoided (Kopsahelis *et al.*, 2007).

Table 3.9. Correlation of the alcohols‟ suppressing ability (r^2) on esters for beer fermented at 16 °C and stored at 0 °C over a period of 12 weeks.

	Ethanol		Propanol		Isoamyl alcohol	
	p	**r^2**	**p**	**r^2**	**p**	**r^2**
Ethyl acetate	0.012	-0.988	0.008	-0.992	0.007	-0.993
Isoamyl acetate	0.552	-0.448	0.530	-0.470	0.696	-0.304
Ethyl hexanoate	0.005	-0.995	0.014	-0.986	0.012	-0.988
Ethyl octanoate	0.338	-0.662	0.186	-0.814	0.186	-0.814

The table above is a summarized depiction of the inverse relationship between alcohols and esters in finished beer and is adapted from appendix-F. It is well known and established by brewing scientists (Bishop, 1971; Briggs *et al.*, 2004; Esslinger, 2009; Bamforth *et al.*, 2011) that ester synthesis requires an alcohol as part of the substrate constituent to the respective metabolic pathway and hence the inverse relationship. The negative r^2 values in table 3.9 give an implication that as the alcohols in the beer becomes more prominent; ester production by the yeast is significantly suppressed by possibly the most obvious reason, ethanol (alcohol) toxicity. Ethyl acetate and ethyl hexanoate were found to have statistically significant suppression by all three alcohols investigated. This was drawn from the observation that their r^2 values were approximately $\approx$ -1 whilst satisfying the statistical condition of $p \leq 0.05$. Although Isoamyl acetate and ethyl octanoate failed to satisfy the statistical p-value condition of this correlation, it was acknowledged that no single alcohol existed without the influential presence of the other alcohols in the entire beer-flavour matrix. Therefore it was deemed correct to note that the collective suppressing effect of the three investigated alcohols on any individual ester was greater than the results displayed in table 3.9, which in this study accounted for individual correlations. These correlation figures were reflective of one another, and looking at appendix-F, it is noted that a similar statistical trend can be drawn. This spoke directly to the very similar ester: FA ratios found in table 3.7 which suggested that the statistically significant suppressive effects observed in the study were some of the major reasons why ester concentrations dropped during storage. The high resemblance amongst these ratios gave the total ester flavour drifts found in table 3.8 a similar trend across temperature and storage profiles. Credit for these consistent trends was given to the adherence of the ISA S88 batch controlling standards even at post-production stages implying that good quality beer was not dependent only on brewing and bottling expertise, but also on the storage and handling/transportation aspect of the process flow.

Amongst the three fermentation temperatures, the 16 ºC primary fermentation temperature was found to be the most ideal, as it produced a very steady and predictable fermentation trend. There were no pH changes in the beer fermented at 16 ºC implying that no mouth feel changes in the product‟s taste were significantly possible. TDS increase in beer fermented at 16 ºC and stored at 0 ºC was found to be the least i.e., 9.45 mg/l, implying that under these conditions the product experienced minimum physico chemical changes during storage, hence maintaning customer and product specification requirements for a longer time. These conditions were observed as optimum for quality concistency and preservation of the product. It is evident at this stage that the hypothesized theorem of uniform processing, handling and storage in the microbrewery does give consistent beer quality that consumers may acquire a taste for, and be guaranteed that no significant flavour and appearance drifts will occur. The flavour trends noted for the volatile aroma esters, FA, ethanol and residual sugars all gave good trends most of which were found to satisfy the very sensitive ($p \leq 0.05$) statistical tool. All deviations acknowledged and observed in the study pointed to parameters that were outside the experiment‟s reach to control such as raw material quality, equipment limitations, and minute human handling inconsistencies during tasting.

CHAPTER FOUR : GENERAL DISCUSSION

4.1. Perspective of the research

The craft brewing industry has been defined as a fast-growing niche market which has a very diverse culture that challenges monopolistic large scale breweries. This fast-growing craft industry is said to belong to the long tail section of a popularity distribution graph (Baginski and Bell, 2011). These market related findings correlated with studies by Dowler, (2013) and McGrath and O'Toole, (2013) which suggest that craft brewing was a market to be reckoned with and harnessed into food, beverage and fast moving commercial goods (FMCG) standards and policies. With a very diverse consumer base, and with multiple small to mid-scale producers all over the globe, brewing gurus and scientists have shown in numerous works that a minimal level of standardised practices, protocols, raw materials, etc. were observed and used in order to keep the customers satisfied (Brown and Hammond, 2003; Strong *et al.*, 2008; Lungu *et al.*, 2010; Andrews *et al.*, 2011). A closer look at microbreweries globally reflected the fact that most of them were operated on a small scale, hence not affording superior technology that helps with process control and automation, as used by their large-scale brewing counterparts. The mode of operation for such facilities is manual or semi-automated, and therefore poses questions around process and product quality integrity. In this study, a hypothetical approach was used to investigate and prove that the use of ISA S88 batch controlling standards in a small scale traditional microbrewery, could produce good quality and consistent results in such a system. Questions raised by studies around brand loyalty (Smythe *et al.*, 2002), micro-brewing equipment capabilities (Andres-Toro *et al.*, 2004; Lehnert *et al.*, 2009), and process/product quality were addressed in this research (Anderson and Kirsop, 1975; Meilgaard, 2001; Zangue S. C. *et al.*, 2011).

In this study, the English pale ale recipe for a 26 l brew length showed an overall brew house efficiency of 87.78 %, implying that all losses inclusive of extract, liquor and hops accounted for the 12.22 % efficiency drop. Nonetheless, this figure in the brew house was generated and supported by consistency trends across the brewing stages that showed insignificant variations, implying a true reflection of the system‟s performance. The inclusion and implementation of the ISA S88 model defined in chapter 2 produced a mash of 14.06 ± 0.18 °P gravity with a pH of 5.66 ± 0.03, which was lautered to give a final boiled wort of 12.10 ± 0.46 °P gravity and a pH of 5.49 ± 0.03. This final wort was produced by a brewing process that had an extract yield efficiency of 62.03 % which is very similar to brewing literature expectations (Briggs *et al.*, 2004). The use of the advised salt and acid dosing rates in the literature (Taylor and Daiber, 1988; Lewis and Young, 2001; Durand *et al.*, 2009) ensured that this wort contained 281.78 ± 35.21 mg/l FAN, 136.60 ± 6.09 g/l total simple sugars and a colour amounting to 29.20 ± 5.12 °EBC units. Total simple sugar variations across the six participating batches per brewing stage had an overall value of 3.25 % and $p = 0.2118$. This implied that the variations were insignificant, and could not satisfy the $p \leq 0.05$ test conditions. Although the ISA S88 standards served their purpose by guaranteeing quality across six brews, they also provided evidence that no two brews were identical. This is a major fact that day to day brewers struggle to keep up with as different raw material batches, ambient temperature/pressure/humidity, and municipality water treatments are all sources of variation in the process. If not monitored and dealt with effectively, these parameters amongst others, can directly affect the process and brand quality, thereby instilling doubt in customer loyalty and satisfaction, which is not good for the brewing business. This observation comes with a key suggestion that for all micro-brewing facilities, a standby optimization protocol on existing recipes, with quick-fixing strategy abilities, must be

available as a fail-safe method of handling quality parameters (e.g., pH, gravity, EBC color, and malt moisture content) that fall "out-of-range" in the brew.

In the fermentation phase, significant FAN concentration variations were observed per fermentation temperature, which was the only acknowledged source of deviation. However, despite this variation, the highly monitored initial gravity, total simple sugars and pitching yeast concentration resulted in fermentation patterns with similar trends. The combination of FAN content and fermentation temperature gave an unexpected model for 14 °C, but very similar models for the 16 °C and 18 °C fermentation patterns. These sugar-based models discussed in chapter 3, had r^2 values of 0.8863, 0.9551 and 0.9325, respectively implying optimal fermentation and reproducibility at 16 °C. Ethanol production had a different behaviour with production rates at each model's first half-life being 2.43 ± 0.27 % v/v, 2.35 ± 0.12 % v/v, and 2.54 ± 0.14 % v/v for the fermentation temperatures of 14 °C, 16 °C, and 18 °C, respectively. This suggested an indication that the depletion of substrate was not equal to the production of ethanol, and further investigations showed that different fermentation temperatures favoured different aromatic by-product concentrations. At a fermentation temperature of 14 °C, ethyl hexanoate and ethyl octanoate were optimally produced to final concentrations of 0.41 ± 0.04 mg/l and 1.45 ± 0.06 mg/l, respectively, whilst at 18 °C, propanol, ethyl acetate, isoamyl alcohol and isoamyl acetate were optimally produced to final concentrations of 23.43 ± 4.97 mg/l, 74.23 ± 6.20 mg/l, 115.98 ± 10.76 mg/l, and 1.06 ± 0.43 mg/l, respectively. These various fermentation trends are vital for brand-defining fermentations as far as appearance, aroma, taste, and the mouth feel of the final beer are concerned. The well modelled ISA S88 batch standards helped define a fermentation recipe resulting in the desired green beer depicted by the brewing style 8C (Strong *et al.*, 2008).

In the storage phase, bottled beer produced flavour drifts as far as the residual sugar, ethanol, FAs and esters were concerned. Residual sugar on average across the three storage temperatures were found to be consumed by the conditioning culture to a concentration of approximately ≈ 5.50 g/l giving an average increase in ethanol concentration of ≈ 0.8 % v/v across all beers. Fusel alcohols consisting of propanol and isoamyl alcohol were found to be more prominent in stored beer and exhibited a concentration increase across storage time when compared with aromatic esters ethyl acetate, isoamyl acetate, ethyl hexanoate and ethyl octanoate. The propanol: isoamyl alcohol ratios were found to be in the range of 4.73 – 5.63 at the end of the 12 weeks of storage. This ratio proved that the FA flavour profile was consistent across the beer shelf life and supported the proposed hypothesis. This was termed the maximum potential drift for all beer produced using the proposed ISA S88 model over a shelf life period of 12 weeks. Fusel alcohols were found to be suppressed at storage temperature 16 °C, and promoted mostly at 18 °C. Generally, FA flavour in stored beer was observed to increase over the 12 weeks. However, esters decreased during storage where losses at each sampling point as active aroma was attributed to be the main factor of this trend. Overall total ester flavour drifts were found to be reflective of each other across all storage permutations and ranged between 16.76 – 22.92 %. The volatile aroma flavour balance, or the ester: FA ratio, was found to range between 4.03 – 5.10 for all storage conditions with the 16 °C fermentation batch producing the most consistent beer which was consistent with reported findings (Kaneda *et al.*, 1995; Landaud *et al.*, 2001). Statistical correlations of these esters with all alcohols also suggested that 16 °C was the optimum fermentation batch temperature and 0 – 4 °C the optimum storage condition.

In this study, physical and chemical property trends, statistical analyses, and literature comparison of the produced wort and beer proved that ISA S88 batch controlling standards even in a basic traditional microbrewery can improve process-product quality and guarantee

product quality consistency. All objectives stated and proposed were achieved by means of the research work demonstrated in this study.

4.2. Future work

The various aspects investigated clearly demonstrated that there is a need for improved methods of experimental research. The first aspect was with regard to the profiling of sugars in the sweet wort produced during brewing. A very significant deviation from literature trends was observed and acknowledged. A new approach involving malt-enzymic load and activity, enzyme activity during mashing, lautering and boiling, and the effect of the milling/handling damage index on malt-derived enzymes, is required in order to accurately solve the mystery behind the various sugar profiles observed in the study. Barley genus type, breed type and farm location would also provide useful information necessary in such future work.

With regard to yeast performance and fermentation kinetics, a new approach involving yeast cell viability, vitality, and detail on wort amino acid, minerals and fatty acid content would alleviate the task of determining factors affecting flavour profile formation at different temperatures.

Other flavour impacting compounds such as the hop-derived iso-α-acids as well as polyphenol amount will help future studies address the issue of invisible haze, slight colour drifts and beer bitterness profiles. The inclusion of foam studying in future works will also help explain the uptake of some potential body-flavour active compounds into the beer head (foam) structure and also give revelation on flavour impact of over carbonated, carbonated and flat beer. Beer foam investigations would by default need carbonation detail such as forced pressure amounts coupled with carbonation temperature and duration details. Primary fermentation vessel design and CO_2 top pressure quantification would also give no room for

unknown anomalies in such a study. This kind of perspective in the new study would influence fermentation vessel design, fermentation vessel head space, wort aeration or oxygenation method, beer filtration, inline beer carbonation or kegerating force carbonation, amongst other process parameters.

A detailed investigation on primary and secondary fermentation flavour formation pathways needs to be investigated. Per yeast strain, preferred anabolic and catabolic pathways at different fermentation temperatures, pitching rates and initial wort nutrient matrices needs to be broken down into experimental trials. Inclusion of more flavour active compounds such as aldehydes, ketones, flavonoids, as well as off flavours such as sulphurs needs to be accounted for. The conditions and respective pathways of staling compound formation in wort and beer needs investigation where identification of basic precursors and promoting catalysts should be a priority. These different flavour investigations will help brewers account better for substrate utilization and strain performance during different process stages. A good flavour bench mark of the brewer‟s yeast in this manner will make it easier to identify the presence of wild yeasts in the inoculating culture or other foreign bacteria presence due to great flavour profile deviations or production of bacteria-based flavour compounds e.g. high production of lactic acid in stored beer due to *lactobacilli* presence.

Investigation of yeast generations for the purpose of defining maximum number culture re-pitching regimes is necessary for yeast mutation studies. This kind of investigation may be paired with yeast life cycle studies where attention should be focused on the yeast dormancy promoting condition and resulting metabolic pathway changes. The build-up of compounds such as trehalose and glycogen in yeast cells should also be studied as a measure that can be related to yeast activity, fermentation efficiency of a yeast generation, duration of the fermentation process and viability/vitality status.

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APPENDICES

APPENDIX A: LIST OF MEDIA AND REAGENTS USED

1. Yeast peptone dextrose (YPD) agar

1.1. Yeast Extract	10 g
1.2. Peptone	20 g
1.3. Dextrose (glucose)	20 g
1.4. Agar	20 g
1.5. Distilled water	100 ml

2. Yeast peptone dextrose (YPD) broth

2.1. Yeast Extract	10 g
2.2. Peptone	20 g
2.3. Dextrose (glucose)	20 g
2.4. Distilled water	100 ml

3. 50 % Maltose solution

3.1. Maltose syrup concentrate	50 ml
3.2. Distilled water	50 ml

4. Dinitrosalicyclic acid (DNS) reagent

4.1. Dinitrosalicyclic acid	1 g
4.2. Crystalline phenol	200 mg
4.3. Sodium sulphite	50 mg
4.4. 1 % Sodium hydroxide	100 ml

5. 40 % Rochelle salt

5.1. Potassium sodium tartrate	40 g
5.2. Distilled water	100 ml

6. Ninhydrin reagent

6.1. Ninhydrin	8 g
6.2. Acetone	100 ml

7. 50 % Ethanol

7.1. Ethanol	50 ml
7.2. Distilled water	50 ml

8. Salting assay (30 % w/v)

8.1. Sodium chloride	1.5 g
8.2. Sample	5 ml

9. Oximating reagent

9.1. Hydroxyl ammonium chloride	2.5 g
9.2. Pyridine	100 ml
9.3. 2-(Dimethyl amino)-ethanol	55 µl

10. Sylilation reagent (per 500 µl of oximation reagent)

10.1. 1. 1. 1. 3. 3. 3. - Hexamethyl-disilazane (HMDS)	450 µl
10.2. Trifluoroacetic acid (TFA)	50 µl

APPENDIX B: WATER DOSING EXPERIMENTS

Table B1. The effect of calcium and acid dosing on brewing water pH and conductivity.

	Salt amount	pH					Conductivity				
		1	2	3	Average	SD	1	2	3	Average	SD
$CaCl_2$ (g)	0.30	7.86	7.84	7.83	**7.84**	**0.02**	600.7	600.3	600.0	**600.33**	**0.35**
	0.60	7.83	7.83	7.80	**7.82**	**0.02**	1079.0	1079.4	1079.2	**1079.20**	**0.20**
	1.20	7.82	7.81	7.78	**7.80**	**0.02**	1533.0	1533.0	1533.2	**1533.07**	**0.12**
	1.80	7.81	7.79	7.76	**7.79**	**0.03**	2740.0	2740.0	2740.0	**2740.00**	**0.00**
	2.40	7.79	7.77	7.74	**7.77**	**0.03**	3603.3	3603.4	3602.9	**3603.20**	**0.26**
	3.00	7.75	7.68	7.72	**7.72**	**0.04**	4450.0	4450.0	4460.0	**4453.33**	**5.77**
$CaSO_4$ (g)	0.30	7.85	7.87	7.86	**7.86**	**0.01**	812.0	814.0	815.0	**813.67**	**1.53**
	0.60	7.83	7.85	7.85	**7.84**	**0.01**	1310.0	1309.0	1308.0	**1309.00**	**1.00**
	1.20	7.82	7.85	7.84	**7.84**	**0.02**	1824.0	1821.0	1819.0	**1821.33**	**2.52**
	1.80	7.82	7.84	7.86	**7.84**	**0.02**	2182.0	2182.0	2182.0	**2182.00**	**0.00**
	2.40	7.80	7.84	7.85	**7.83**	**0.03**	2350.0	2340.0	2340.0	**2343.33**	**5.77**
	3.00	7.79	7.80	7.83	**7.81**	**0.02**	2340.0	2330.0	2340.0	**2336.67**	**5.77**
Lactic Acid (µl)	0.00	7.53	7.51	7.54	**7.53**	**0.02**	107.40	108.20	107.70	**107.77**	**0.40**
	1.00	7.16	7.18	7.16	**7.17**	**0.01**	112.10	112.40	112.50	**112.33**	**0.21**
	2.00	6.98	6.99	7.01	**6.99**	**0.02**	115.10	115.30	114.50	**114.97**	**0.42**
	3.00	6.92	6.92	6.93	**6.92**	**0.01**	115.20	115.60	116.30	**115.70**	**0.56**
	4.00	6.22	6.18	6.23	**6.21**	**0.03**	119.70	118.40	117.00	**118.37**	**1.35**
	5.00	4.46	4.49	4.45	**4.47**	**0.02**	124.30	124.50	125.70	**124.83**	**0.76**
	10.00	4.07	4.07	4.06	**4.07**	**0.01**	134.90	133.30	134.40	**134.20**	**0.82**
	15.00	3.76	3.77	3.75	**3.76**	**0.01**	163.40	164.10	163.00	**163.50**	**0.56**
	20.00	3.58	3.58	3.58	**3.58**	**0.00**	200.20	200.50	200.00	**200.23**	**0.25**
	25.00	3.38	3.38	3.37	**3.38**	**0.01**	241.00	242.00	240.00	**241.00**	**1.00**
	30.00	3.31	3.30	3.29	**3.30**	**0.01**	260.00	260.00	261.00	**260.33**	**0.58**
	35.00	3.24	3.26	3.24	**3.25**	**0.01**	290.00	290.00	290.00	**290.00**	**0.00**
	40.00	3.16	3.15	3.17	**3.16**	**0.01**	326.00	326.00	326.00	**326.00**	**0.00**
	45.00	3.12	3.12	3.11	**3.12**	**0.01**	369.00	369.00	368.00	**368.67**	**0.58**
	50.00	2.88	2.87	2.88	**2.88**	**0.01**	391.00	390.90	391.10	**391.00**	**0.10**
	100.00	2.79	2.79	2.78	**2.79**	**0.01**	668.30	668.00	668.60	**668.30**	**0.30**
	150.00	2.73	2.72	2.73	**2.73**	**0.01**	788.70	788.20	788.60	**788.50**	**0.26**
	200.00	2.69	2.69	2.69	**2.69**	**0.00**	818.30	817.90	818.10	**818.10**	**0.20**
	250.00	2.58	2.58	2.58	**2.58**	**0.00**	1026.70	1027.60	1026.90	**1027.07**	**0.47**
	300.00	2.53	2.53	2.54	**2.53**	**0.01**	1103.30	1103.60	1103.50	**1103.47**	**0.15**
	350.00	2.49	2.48	2.47	**2.48**	**0.01**	1208.30	1208.30	1208.30	**1208.30**	**0.00**
	400.00	2.44	2.43	2.43	**2.43**	**0.01**	1355.60	1355.80	1355.10	**1355.50**	**0.36**
	450.00	2.40	2.40	2.40	**2.40**	**0.00**	1466.40	1466.80	1466.60	**1466.60**	**0.20**
	500.00	2.39	2.38	2.37	**2.38**	**0.01**	1483.00	1483.20	1483.40	**1483.20**	**0.20**
	550.00	2.37	2.37	2.37	**2.37**	**0.00**	1551.00	1551.30	1550.80	**1551.03**	**0.25**
	600.00	2.34	2.34	2.35	**2.34**	**0.01**	1676.30	1676.50	1676.30	**1676.37**	**0.12**

~~*123.45*~~ = statistically excluded value

Table B2. The effect of calcium and acid dosing on brewing water salinity and total dissolved solids (TDS).

	Amount	Salinity					Total Dissolved Solids				
		1	2	3	Average	SD	1	2	3	Average	SD
$CaCl_2$ (g)	0.30	0.29	0.28	0.29	**0.29**	**0.01**	295.0	295.0	297.0	**295.67**	**1.15**
	0.60	0.55	0.55	0.55	**0.55**	**0.00**	544.0	545.0	544.0	**544.33**	**0.58**
	1.20	0.81	0.80	0.82	**0.81**	**0.01**	807.7	807.1	807.6	**807.47**	**0.32**
	1.80	1.49	1.49	1.49	**1.49**	**0.00**	1472.3	1472.6	1472.2	**1472.37**	**0.21**
	2.40	2.01	2.01	2.01	**2.01**	**0.00**	1974.3	1974.5	1974.0	**1974.27**	**0.25**
	3.00	2.52	2.52	2.51	**2.52**	**0.01**	2470.0	2470.0	2480.0	**2473.33**	**5.77**
$CaSO_4$ (g)	0.30	0.40	0.39	0.39	**0.39**	**0.01**	393.0	393.0	394.0	**393.33**	**0.58**
	0.60	0.66	0.66	0.66	**0.66**	**0.00**	662.0	662.0	662.0	**662.00**	**0.00**
	1.20	0.93	0.93	0.93	**0.93**	**0.00**	919.0	920.0	921.0	**920.00**	**1.00**
	1.80	1.15	1.15	1.15	**1.15**	**0.00**	1133.0	1134.0	1134.0	**1133.67**	**0.58**
	2.40	1.23	1.23	1.23	**1.23**	**0.00**	1219.0	1218.0	1219.0	**1218.67**	**0.58**
	3.00	1.24	1.24	1.24	**1.24**	**0.00**	1227.0	1224.0	1226.0	**1225.67**	**1.53**
Lactic Acid (µl)	0.00	0.05	0.06	0.05	**0.05**	**0.01**	54.80	54.60	55.20	**54.87**	**0.31**
	1.00	0.06	0.05	0.06	**0.06**	**0.01**	58.20	57.50	58.80	**58.17**	**0.65**
	2.00	0.06	0.06	0.05	**0.06**	**0.01**	59.76	59.23	58.74	**59.24**	**0.51**
	3.00	0.05	0.06	0.06	**0.06**	**0.01**	59.50	58.50	59.30	**59.10**	**0.53**
	4.00	0.06	0.06	0.05	**0.06**	**0.01**	59.10	59.30	59.60	**59.33**	**0.25**
	5.00	0.06	0.05	0.06	**0.06**	**0.01**	62.40	62.90	62.30	**62.53**	**0.32**
	10.00	0.07	0.06	0.07	**0.07**	**0.01**	71.10	72.00	70.80	**71.30**	**0.62**
	15.00	0.08	0.08	0.07	**0.08**	**0.01**	85.40	84.50	85.00	**84.97**	**0.45**
	20.00	0.10	0.11	0.10	**0.10**	**0.01**	105.50	105.50	105.50	**105.50**	**0.00**
	25.00	0.13	0.13	0.13	**0.13**	**0.00**	128.40	128.50	128.60	**128.50**	**0.10**
	30.00	0.14	0.14	0.13	**0.14**	**0.01**	139.10	139.60	138.70	**139.13**	**0.45**
	35.00	0.15	0.15	0.15	**0.15**	**0.00**	155.30	155.60	155.00	**155.30**	**0.30**
	40.00	0.17	0.17	0.16	**0.17**	**0.01**	175.10	175.00	174.90	**175.00**	**0.10**
	45.00	0.19	0.19	0.20	**0.19**	**0.01**	189.60	189.60	189.40	**189.53**	**0.12**
	50.00	0.21	0.20	0.21	**0.21**	**0.01**	202.30	202.50	202.20	**202.33**	**0.15**
	100.00	0.45	0.44	0.46	**0.45**	**0.01**	452.00	452.00	452.00	**452.00**	**0.00**
	150.00	0.35	0.35	0.35	**0.35**	**0.00**	355.00	355.10	355.40	**355.17**	**0.21**
	200.00	0.43	0.43	0.42	**0.43**	**0.01**	427.00	427.00	427.10	**427.03**	**0.06**
	250.00	0.54	0.54	0.54	**0.54**	**0.00**	538.00	538.30	537.90	**538.07**	**0.21**
	300.00	0.58	0.57	0.58	**0.58**	**0.01**	579.00	579.00	578.90	**578.97**	**0.06**
	350.00	0.64	0.65	0.66	**0.65**	**0.01**	638.00	638.00	638.00	**638.00**	**0.00**
	400.00	0.72	0.71	0.71	**0.71**	**0.01**	715.00	715.00	715.00	**715.00**	**0.00**
	450.00	0.78	0.78	0.78	**0.78**	**0.00**	777.00	770.50	770.20	**772.57**	**3.84**
	500.00	0.79	0.77	0.80	**0.79**	**0.02**	785.00	785.00	785.00	**785.00**	**0.00**
	550.00	0.83	0.84	0.83	**0.83**	**0.01**	829.00	829.20	829.00	**829.07**	**0.12**
	600.00	0.90	0.90	0.90	**0.90**	**0.00**	895.00	895.00	895.10	**895.03**	**0.06**

~~*123.45*~~ = statistically excluded value

APPENDIX C: INSTRUMENT CALIBRATIONS

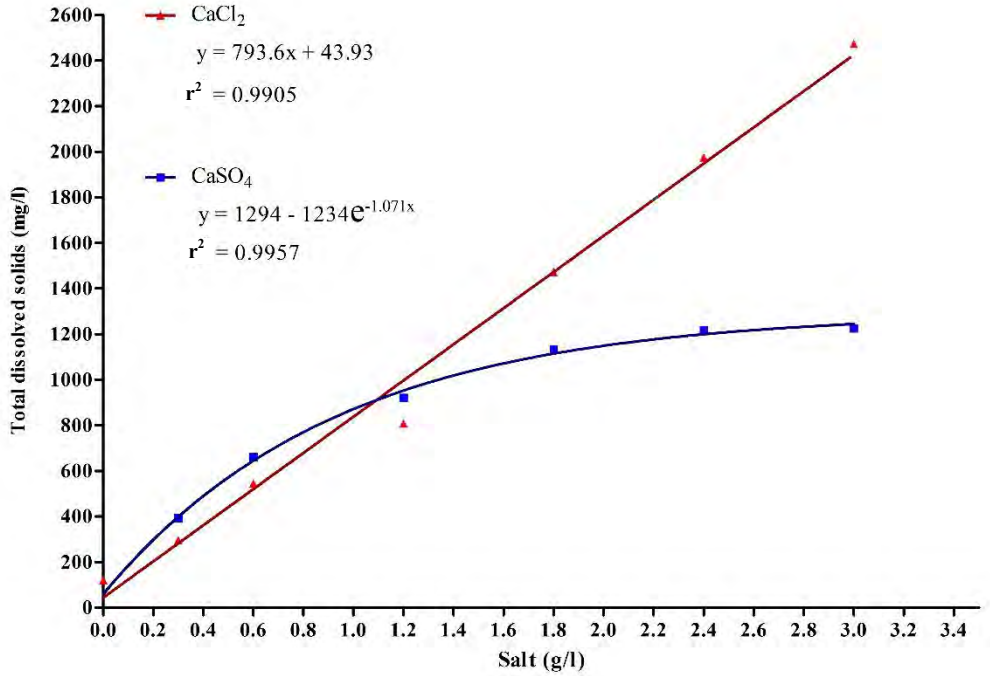


Figure C1. $CaSO_4$ and $CaCl_2$ water dosing calibration for the HACH HQ 40d multimeter probe.

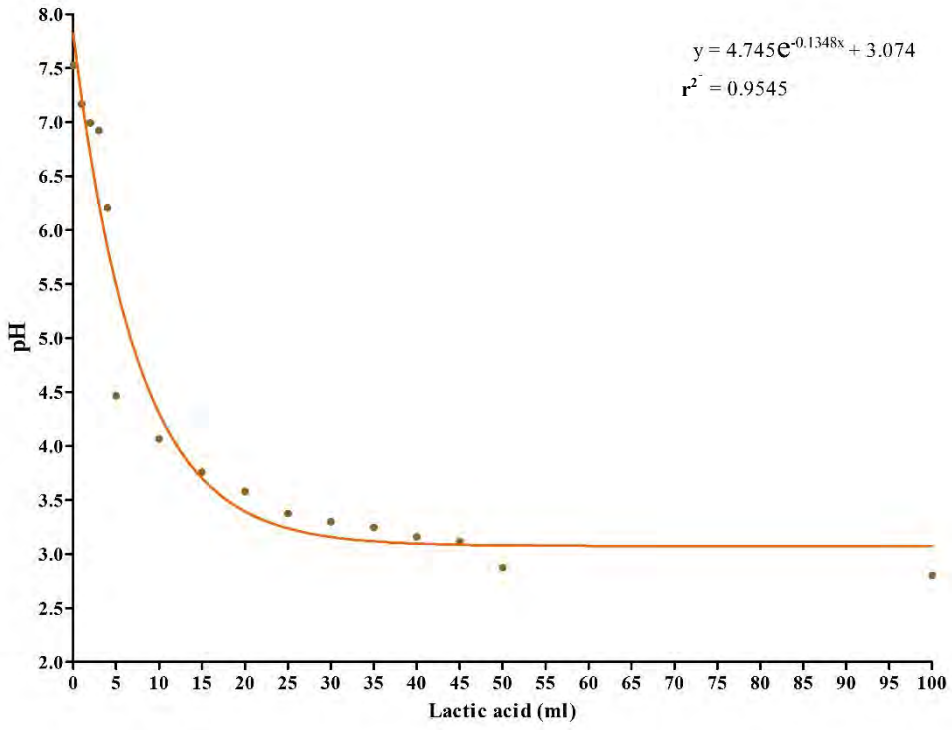


Figure C2. Lactic acid water dosing calibration for the HACH HQ 40d multimeter.

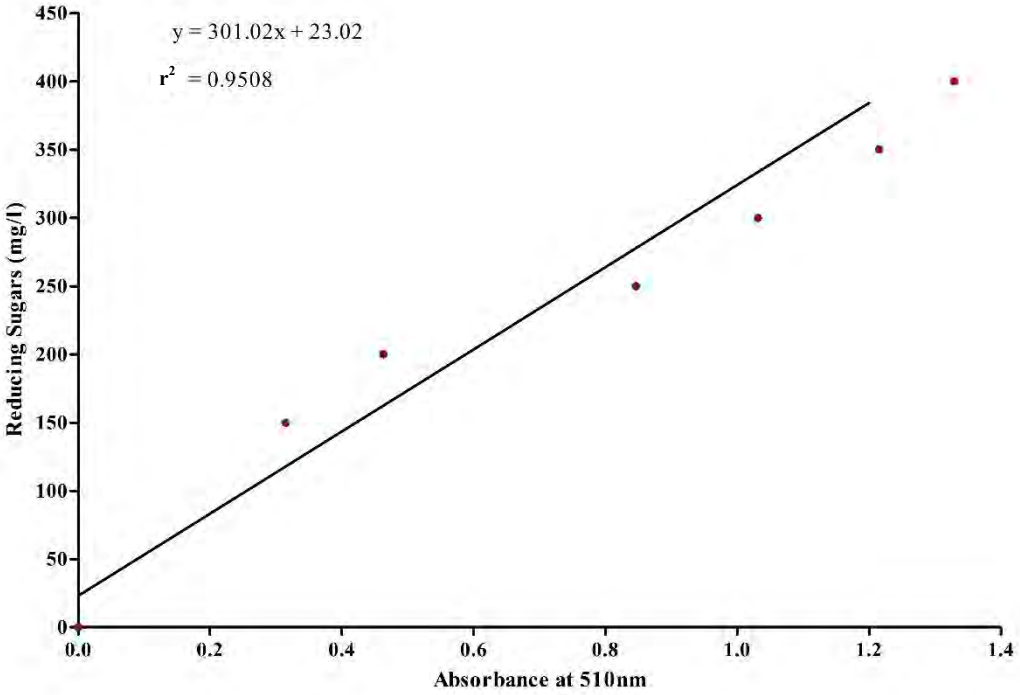


Figure C3. Wort and beer reducing sugars calibration for the Shimadzu UV-1800 spectrophotometer DNS reagent assay.

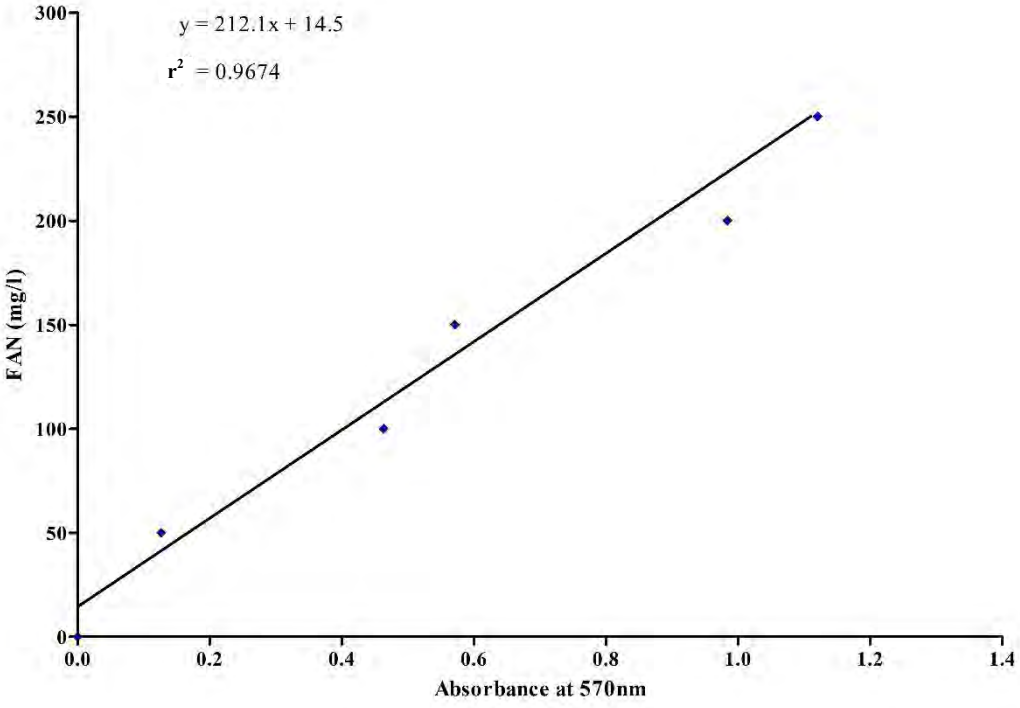
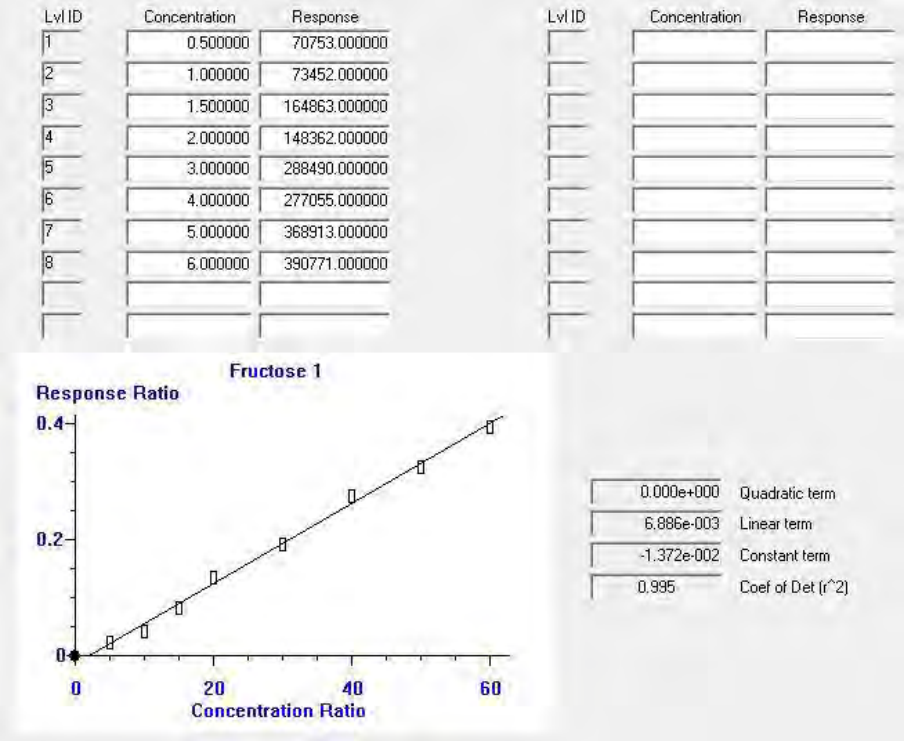
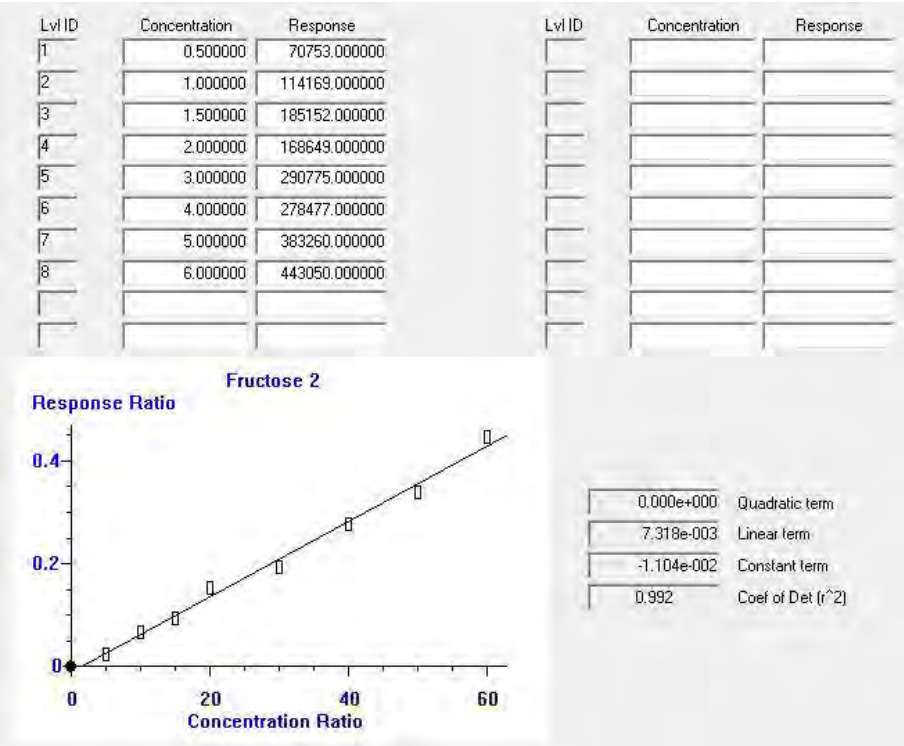


Figure C4. Wort and beer free amino nitrogen calibration for the Shimadzu UV-1800 spectrophotometer ninhydrin reagent assay.

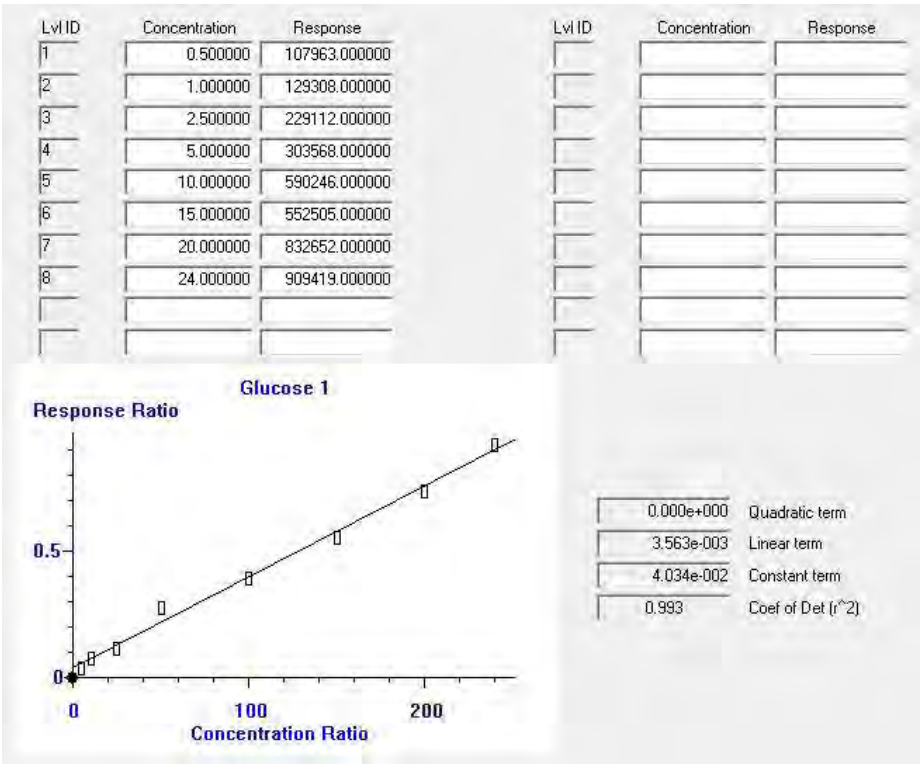


(a)

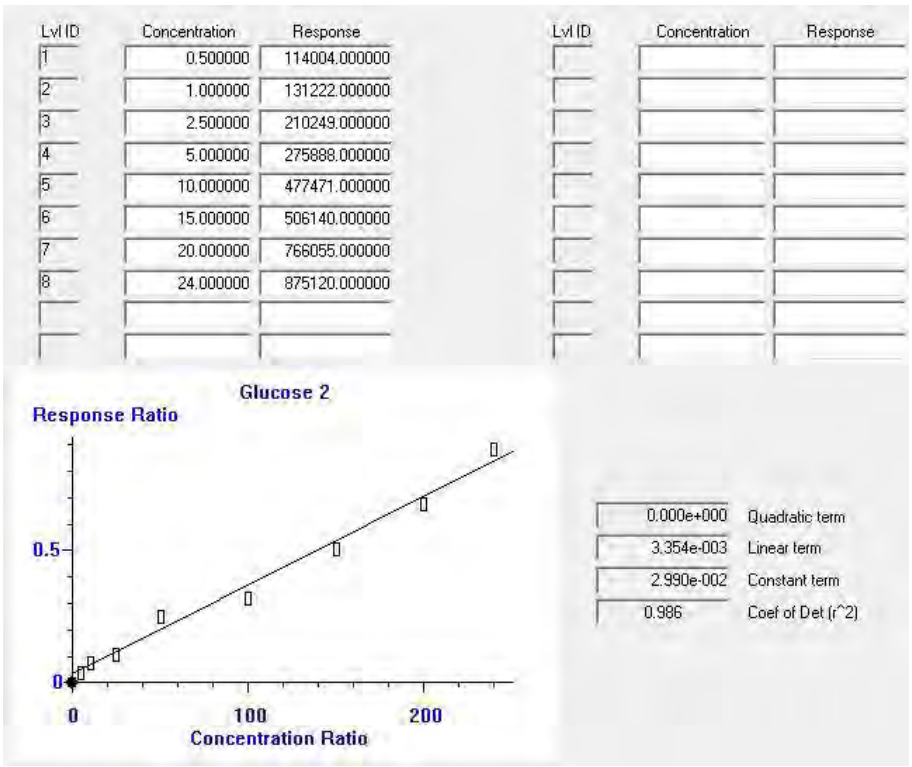


(b)

Figure C5. Fructose concentration calibration for the Agilent 7890A GC system using the two chromatogram peaks (a) and (b).

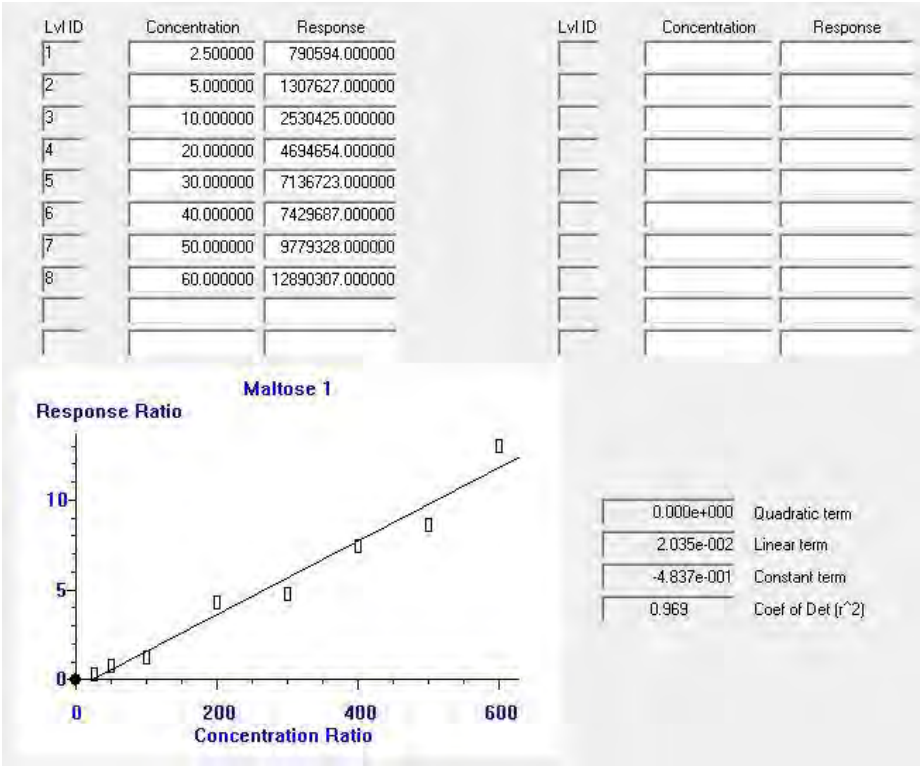


(a)

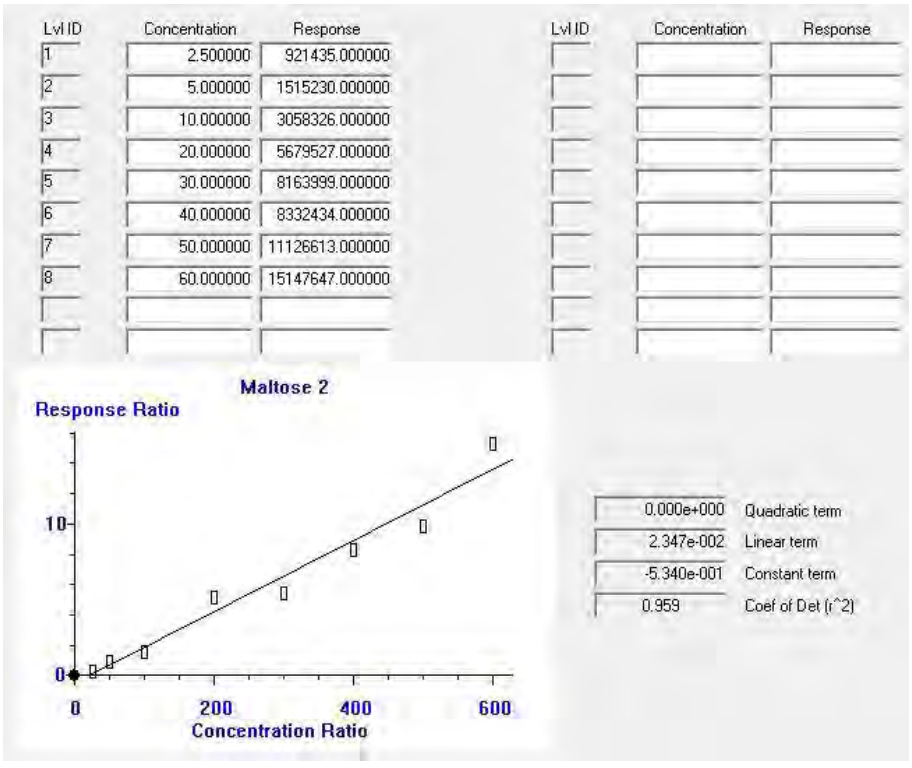


(b)

Figure C6. Glucose concentration calibration for the Agilent 7890A GC system using the two chromatogram peaks (a) and (b).



(a)



(b)

Figure C7. Maltose concentration calibration for the Agilent 7890A GC system using the two chromatogram peaks (a) and (b).

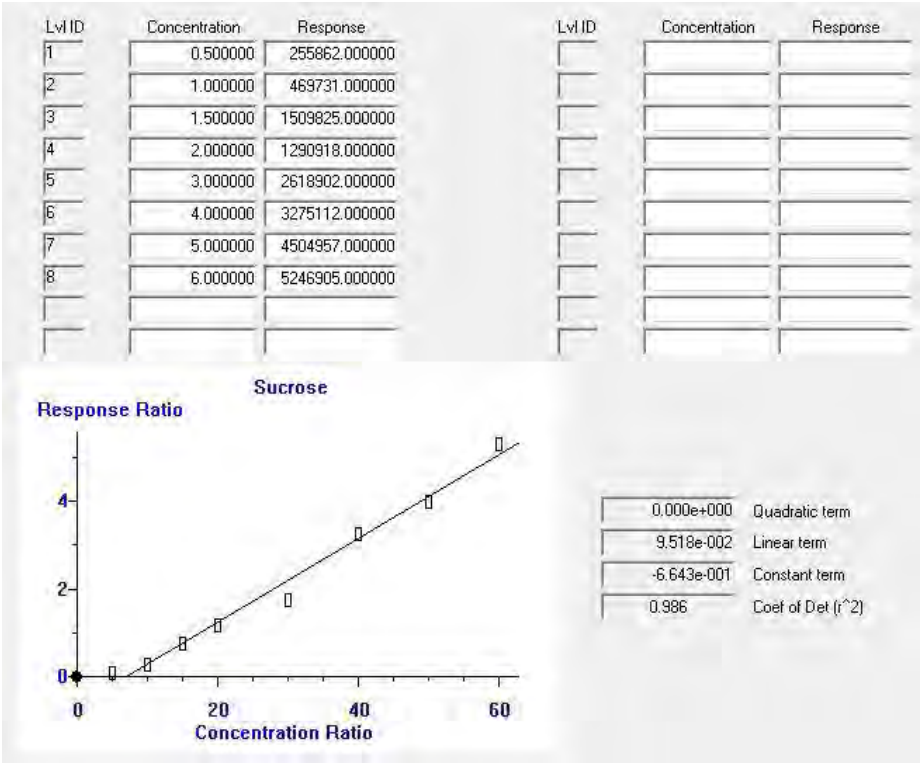


Figure C8. Sucrose concentration calibration for the Agilent 7890A GC system.

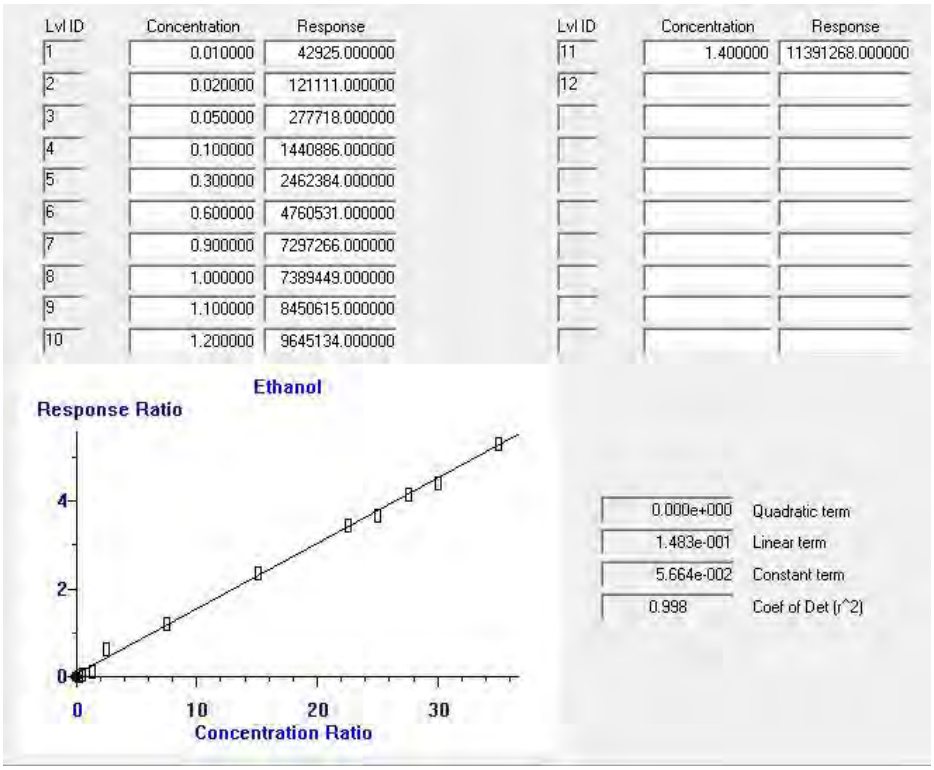


Figure C9. Ethanol concentration calibration for the Agilent 7890A GC system.

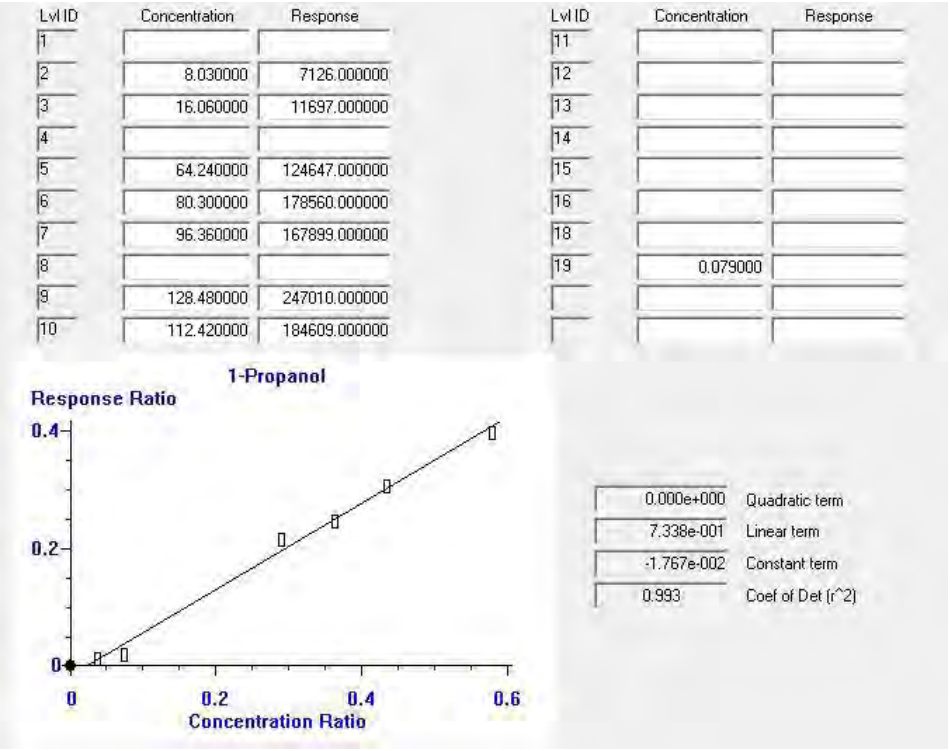


Figure C10. Propanol concentration calibration for the Agilent 7890A GC system.

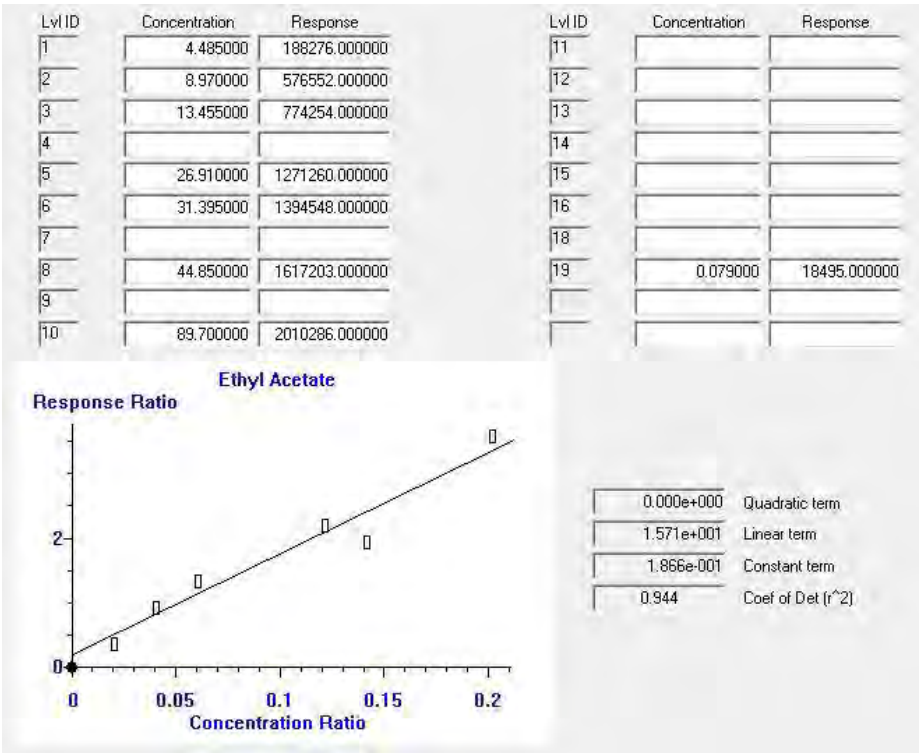


Figure C11. Ethyl acetate concentration calibration for the Agilent 7890A GC system.

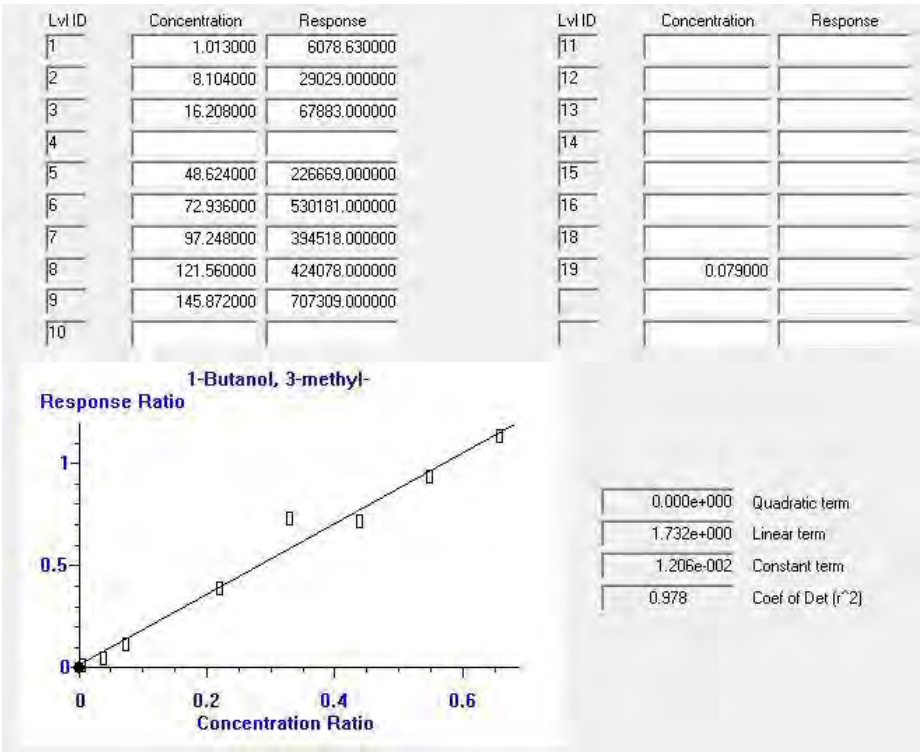


Figure C12. Isoamyl alcohol concentration calibration for the Agilent 7890A GC system.

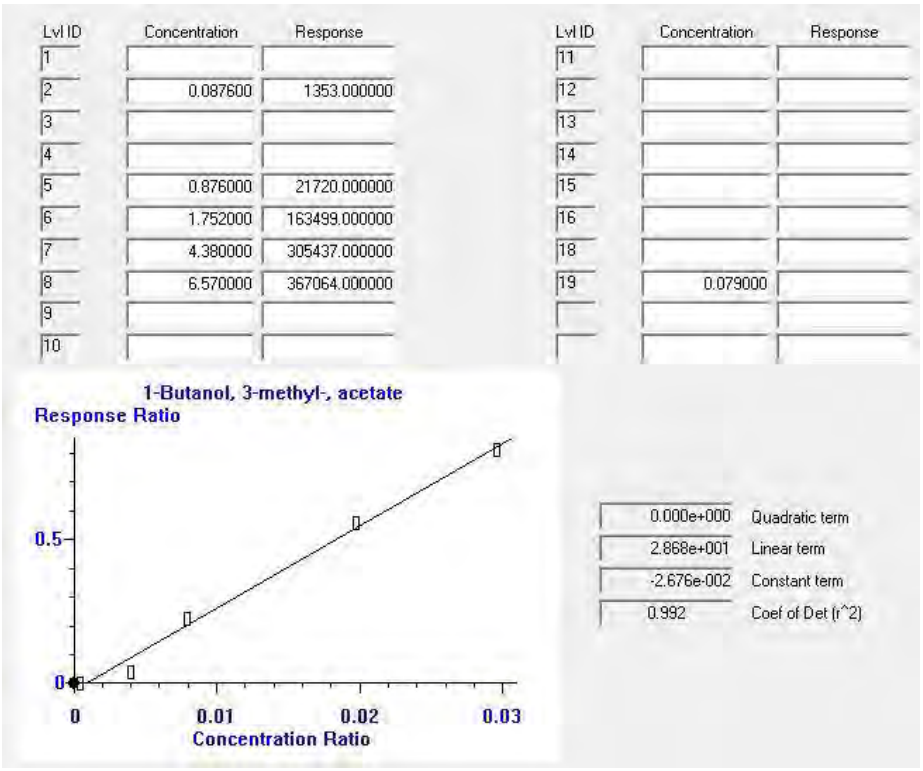


Figure C13. Isoamyl acetate concentration calibration for the Agilent 7890A GC system.

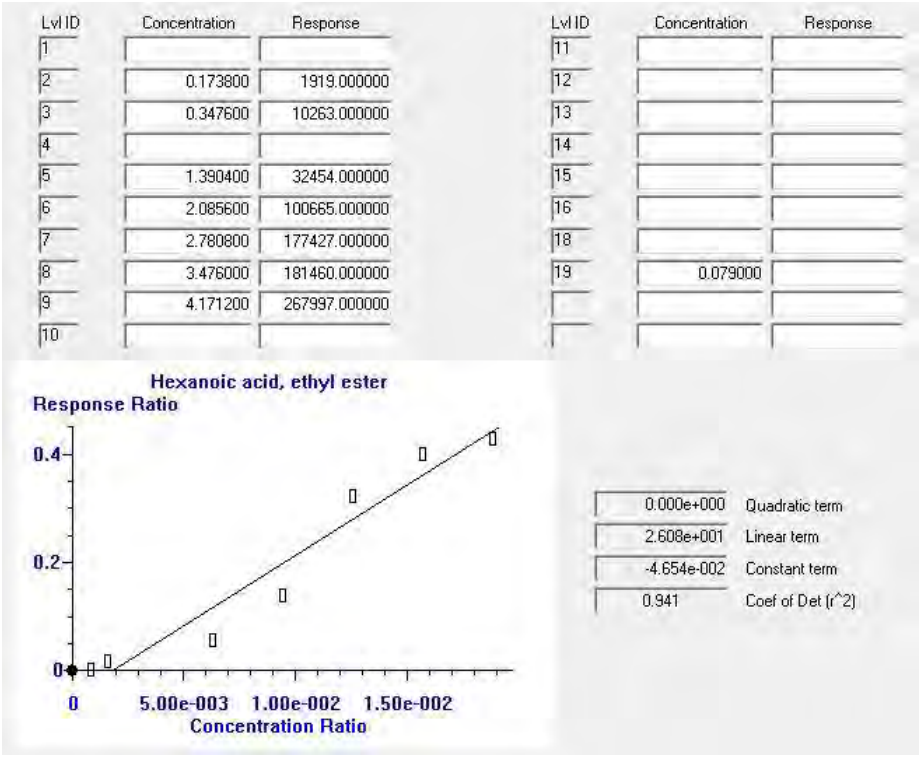


Figure C14. Ethyl hexanoate concentration calibration for the Agilent 7890A GC system.

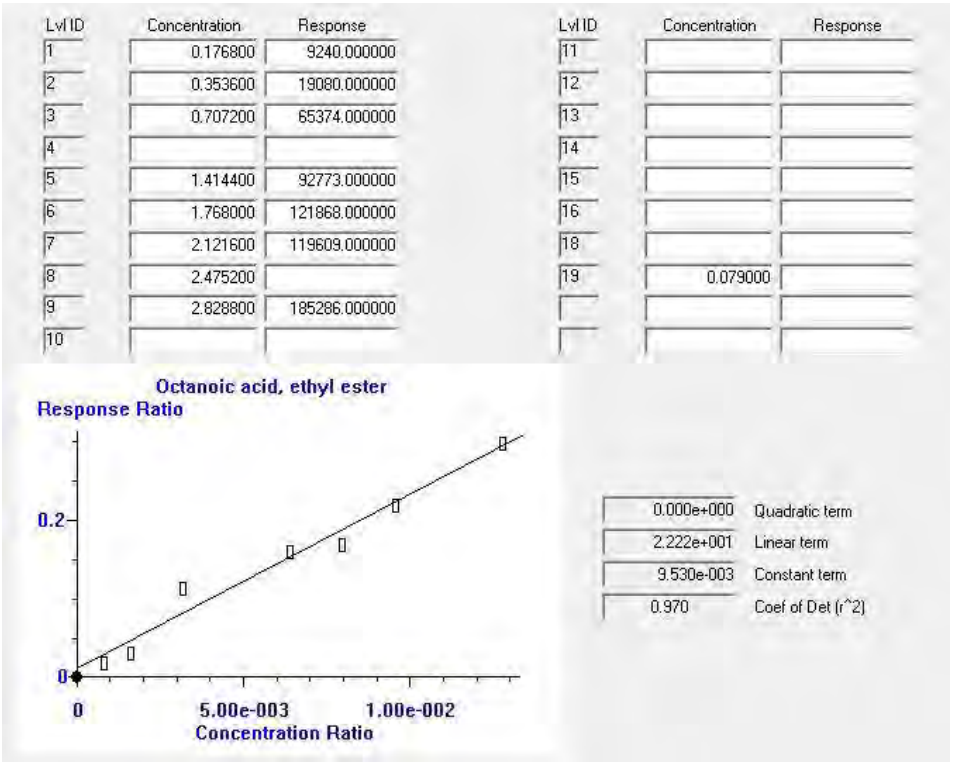


Figure C15. Ethyl octanoate concentration calibration for the Agilent 7890A GC system.

APPENDIX D: BREW HOUSE PROCESS QUALITY PARAMETERS

Table D1. Wort physico chemical properties across different brew house stages.

	Parameter	Batch	1	2	3	4	5	6	Average	SD
		Stage								
Reducing Sugars **(g/l)**	Brew house	Mash	85.99	86.00	87.38	91.12	97.33	88.95	**89.46**	**4.32**
		1st Run	26.73	29.10	34.70	36.78	39.32	34.89	**33.58**	**4.76**
		2nd Run	~~5.52~~	4.38	4.37	~~2.79~~	3.19	3.54	**3.87**	**0.60**
		3rd Run	-	-	2.16	1.60	1.77	1.45	**1.74**	**0.30**
		Boil	63.03	~~83.77~~	63.87	66.26	~~55.86~~	70.64	**65.95**	**3.41**
	Spent Grain (Residual)	Mash	5.24	1.43	1.43	1.02	4.74	1.07	**2.49**	**1.95**
		1st Run	3.47	0.94	0.69	0.56	3.30	1.01	**1.66**	**1.35**
		2nd Run	1.89	0.80	0.36	0.51	2.07	0.92	**1.09**	**0.72**
		3rd Run	0.92	0.43	0.24	0.52	1.14	0.24	**0.58**	**0.37**
FAN **(mg/l)**	Brew house	Mash	316.59	320.45	313.79	344.93	407.92	390.10	**348.96**	**40.70**
		1st Run	130.07	206.68	171.69	169.14	206.98	167.83	**175.40**	**28.78**
		2nd Run	230.90	138.60	118.24	89.82	151.11	93.29	**136.99**	**51.98**
		3rd Run	-	-	136.73	71.41	89.09	109.80	**101.76**	**28.10**
		Boil	333.18	317.74	267.56	270.52	247.75	253.90	**281.78**	**35.21**
	Spent Grain (Residual)	Mash	406.96	134.41	82.00	550.89	107.14	111.55	**232.16**	**197.19**
		1st Run	118.12	60.79	50.17	177.00	55.26	65.82	**87.86**	**50.13**
		2nd Run	53.54	39.27	44.76	86.08	87.99	64.95	**62.77**	**20.72**
		3rd Run	48.64	31.59	41.98	51.29	38.52	39.50	**41.92**	**7.17**
Batch Volumes **(l)**	Brew house	Mash	15.00	16.10	15.00	16.20	15.00	15.00	**15.38**	**0.59**
		1st Run	7.80	8.00	8.00	6.60	8.00	7.60	**7.67**	**0.55**
		2nd Run	7.80	8.40	7.40	7.40	7.50	7.00	**7.58**	**0.48**
		3rd Run	~~3.00~~	~~1.80~~	5.30	6.00	5.50	5.00	**5.45**	**0.42**
		Boil	27.20	24.50	27.00	27.40	27.60	25.00	**26.45**	**1.34**
	Spent Grain (Residual)	Mash	0.086	0.086	0.087	0.086	0.085	0.086	**0.086**	**0.000**
		1st Run	0.028	0.028	0.027	0.028	0.028	0.028	**0.028**	**0.000**
		2nd Run	0.029	0.028	0.028	0.028	0.028	0.028	**0.028**	**0.000**
		3rd Run	0.014	0.014	0.014	0.013	0.014	0.014	**0.014**	**0.000**
Physical Props.	Batch Gravities **(°P)**	Mash	14.02	13.79	14.02	14.02	14.26	14.26	**14.06**	**0.18**
		1st Run	7.80	7.06	7.80	8.04	8.53	8.28	**7.92**	**0.51**
		2nd Run	~~2.56~~	3.07	4.33	4.08	~~5.08~~	4.33	**3.95**	**0.60**
		3rd Run	1.54	~~1.28~~	1.80	1.80	~~2.31~~	1.54	**1.67**	**0.15**
		Boil	12.14	12.85	11.90	12.14	11.42	12.14	**12.10**	**0.46**
	EBC Colour **(°EBC)**	Mash	38.92	42.65	35.90	40.88	34.38	39.82	**38.76**	**3.10**
		Lauter	25.31	25.91	33.15	20.93	23.18	22.46	**25.16**	**4.33**
		Boil	24.54	38.53	26.26	30.90	25.93	29.02	**29.20**	**5.12**
	Particulate Matter **(g/l)**	Mash	5.70	12.80	13.47	12.53	15.36	11.27	**11.86**	**3.30**
		Lauter	6.30	4.40	4.37	4.60	2.57	4.20	**4.41**	**1.19**
		Boil	3.30	3.97	3.30	4.40	5.20	4.67	**4.14**	**0.76**
	pH	Mash	5.64	~~5.71~~	5.68	5.65	5.65	5.64	**5.66**	**0.03**
		Lauter	5.72	~~5.80~~	5.75	5.74	5.74	5.75	**5.75**	**0.03**
		Boil	5.48	~~5.54~~	5.50	5.47	5.46	5.50	**5.49**	**0.03**

~~123.45~~ = statistically excluded value

Table D2. Wort and spent grain simple sugars (g/l) in each brewing stage respectively.

Parameter	Section	Batch / Stage	1	2	3	4	5	6	Average	SD
Glucose	Brew house	Mash	65.65	66.08	79.55	79.59	~~90.11~~	~~61.20~~	**72.72**	**7.91**
		1st Run	47.22	48.43	46.18	~~54.08~~	~~68.04~~	51.29	**48.28**	**2.21**
		2nd Run	~~36.98~~	25.04	~~16.09~~	29.53	30.13	34.25	**29.74**	**3.77**
		3rd Run	0.00	0.00	11.63	21.62	15.01	13.00	**15.32**	**4.43**
		Boil	60.68	~~91.76~~	63.67	~~38.78~~	50.32	59.20	**58.47**	**5.74**
	Spent Grain (Residual)	Mash	0.52	0.80	0.00	0.00	4.79	0.00	**0.66**	**0.20**
		1st Run	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
		2nd Run	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
		3rd Run	0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
Fructose	Brew house	Mash	~~4.04~~	4.45	4.08	~~7.96~~	6.75	5.94	**5.31**	**1.25**
		1st Run	2.59	2.60	~~2.15~~	2.66	~~3.44~~	2.83	**2.67**	**0.11**
		2nd Run	~~1.94~~	1.83	~~1.09~~	1.78	1.81	1.81	**1.81**	**0.02**
		3rd Run	0.00	0.00	0.92	1.48	0.95	1.03	**1.10**	**0.26**
		Boil	~~2.75~~	4.98	5.80	~~7.81~~	4.42	6.27	**5.37**	**0.83**
	Spent Grain (Residual)	Mash	1.05	1.15	0.55	0.54	0.85	0.43	**0.76**	**0.30**
		1st Run	0.78	0.74	0.52	0.54	0.72	0.41	**0.62**	**0.15**
		2nd Run	0.57	0.41	0.46	0.47	0.49	0.37	**0.46**	**0.07**
		3rd Run	0.43	0.35	0.41	0.41	0.41	0.37	**0.40**	**0.03**
Sucrose	Brew house	Mash	1.66	~~1.34~~	1.78	~~3.16~~	1.96	1.99	**1.85**	**0.16**
		1st Run	1.24	1.08	1.30	1.46	1.70	1.40	**1.36**	**0.21**
		2nd Run	1.20	1.05	0.95	1.18	1.16	1.24	**1.13**	**0.11**
		3rd Run	0.00	0.00	0.86	1.04	1.09	0.94	**0.98**	**0.10**
		Boil	1.23	1.40	1.12	1.66	1.31	1.45	**1.36**	**0.19**
	Spent Grain (Residual)	Mash	0.73	0.71	0.72	0.71	0.72	0.71	**0.72**	**0.01**
		1st Run	0.73	0.70	0.71	0.70	0.71	0.70	**0.71**	**0.01**
		2nd Run	0.72	0.70	0.70	0.70	0.70	0.70	**0.70**	**0.01**
		3rd Run	0.72	0.70	0.70	0.70	0.70	0.70	**0.70**	**0.01**
Maltose	Brew house	Mash	47.33	~~41.28~~	~~45.25~~	92.79	76.78	85.22	**75.53**	**19.90**
		1st Run	~~33.65~~	39.72	37.27	41.45	~~54.41~~	40.71	**39.79**	**1.82**
		2nd Run	30.21	32.51	~~19.82~~	29.17	~~28.55~~	31.88	**30.94**	**1.53**
		3rd Run	8.00	6.00	16.34	22.64	15.08	17.07	**17.78**	**3.34**
		Boil	72.55	75.60	72.97	79.58	83.92	74.48	**76.52**	**4.42**
	Spent Grain (Residual)	Mash	7.67	5.36	4.90	5.03	6.57	5.21	**5.79**	**1.10**
		1st Run	6.47	5.21	4.75	4.89	5.81	5.00	**5.36**	**0.66**
		2nd Run	5.67	5.02	4.73	4.80	5.67	4.85	**5.12**	**0.43**
		3rd Run	5.27	4.86	4.71	4.76	5.26	4.80	**4.94**	**0.25**
Total Sugars	Brew house	Mash	~~118.68~~	~~113.15~~	130.66	183.50	175.60	154.35	**161.53**	**23.81**
		1st Run	84.70	91.83	86.90	~~99.65~~	~~127.59~~	96.23	**89.92**	**5.16**
		2nd Run	~~70.33~~	60.43	~~37.95~~	61.66	61.65	69.18	**63.23**	**4.01**
		3rd Run	~~8.00~~	~~6.00~~	29.75	46.78	32.13	32.04	**35.18**	**2.95**
		Boil	137.21	~~173.74~~	~~143.56~~	127.83	139.97	141.40	**136.60**	**6.09**
	Spent Grain (Residual)	Mash	9.97	8.02	6.17	6.28	12.93	6.35	**8.29**	**2.71**
		1st Run	7.98	6.65	5.98	6.13	7.24	6.11	**6.68**	**0.79**
		2nd Run	6.96	6.13	5.89	5.97	6.86	5.92	**6.29**	**0.49**
		3rd Run	6.42	5.91	5.82	5.87	6.37	5.87	**6.04**	**0.27**

~~123.45~~ = statistically excluded value

Table D3. Wort gravity (°P) depletion across the three experimental fermentation temperatures.

Days	14 °C					16 °C					18 °C							
	1	2	3	**Average**	**SD**	1	2	3	**Average**	**SD**	1	2	3	4	5	6	**Average**	**SD**
0	12.14	12.85	11.90	**12.30**	**0.49**	12.14	11.42	12.14	**11.90**	**0.42**	12.14	12.85	11.90	12.14	11.42	12.14	**12.10**	**0.46**
1	10.22	10.46	9.26	**9.98**	**0.63**	10.46	8.77	9.98	**9.74**	**0.87**	9.98	8.28	8.53	9.74	8.04	9.01	**8.93**	**0.79**
2	9.98	9.98	9.01	**9.66**	**0.56**	9.26	8.04	8.77	**8.69**	**0.61**	9.01	6.81	6.57	8.28	7.06	7.80	**7.59**	**0.95**
3	9.74	9.74	8.28	**9.25**	**0.84**	8.04	7.55	7.80	**7.80**	**0.25**	8.53	6.81	5.57	6.81	6.32	7.06	**6.85**	**0.98**
4	9.50	9.01	7.80	**8.77**	**0.88**	7.30	7.06	7.30	**7.22**	**0.14**	8.04	5.57	5.08	5.82	5.82	6.32	**6.11**	**1.03**
5	9.50	8.53	7.55	**8.53**	**0.98**	6.81	6.57	6.81	**6.73**	**0.14**	7.55	4.58	4.58	5.08	5.08	5.82	**5.45**	**1.13**
6	9.26	8.04	7.30	**8.20**	**0.99**	6.32	6.07	6.57	**6.32**	**0.25**	6.81	4.06	4.08	4.58	4.83	5.57	**4.99**	**1.05**
7	9.26	7.80	7.06	**8.04**	**1.12**	5.82	5.57	6.32	**5.90**	**0.38**	6.07	3.82	3.82	4.08	4.33	5.33	**4.58**	**0.92**
8	9.01	7.30	6.81	**7.71**	**1.16**	5.33	5.08	6.07	**5.49**	**0.51**	5.82	3.57	3.82	3.82	3.82	4.83	**4.28**	**0.87**
9	9.01	7.06	6.57	**7.55**	**1.29**	5.08	4.83	5.82	**5.24**	**0.51**	5.33	3.57	3.82	3.82	3.82	4.58	**4.16**	**0.67**
10	8.77	6.57	6.32	**7.22**	**1.35**	4.58	4.58	5.57	**4.91**	**0.57**	4.58	3.57	3.82	3.82	3.82	4.33	**3.99**	**0.38**
11	8.53	6.32	6.07	**6.97**	**1.35**	4.08	4.33	5.33	**4.58**	**0.66**	4.08	3.57	3.82	3.82	3.82	4.06	**3.86**	**0.19**
12	8.28	5.82	6.07	**6.72**	**1.35**	4.08	4.33	5.08	**4.50**	**0.52**	4.08	3.57	3.82	3.82	3.82	3.82	**3.82**	**0.16**
13	8.04	5.57	6.07	**6.56**	**1.31**													
14	7.55	5.33	6.07	**6.32**	**1.13**													
15	7.06	5.08	6.07	**6.07**	**0.99**													
16	6.81	5.08	6.07	**5.99**	**0.87**													
17	6.57	4.83	6.07	**5.82**	**0.90**													
18	6.07	4.83	6.07	**5.66**	**0.72**													
19	5.28	4.58	6.07	**5.31**	**0.75**													
20	5.57	4.58	6.07	**5.41**	**0.76**													
21	5.08	4.58	6.07	**5.24**	**0.76**													

~~*123.45*~~ = statistically excluded value

Table D4. Free amino nitrogen (mg/l) content in wort during primary fermentation across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	333.18	317.74	267.56	**306.16**	**34.31**		270.52	247.75	253.90	**257.39**	**11.78**		333.18	317.74	267.56	270.52	247.75	253.90	**281.77**	**35.21**
1	204.77	189.38	264.37	**219.51**	**39.61**		256.53	234.98	234.00	**241.84**	**12.73**		209.78	252.20	244.35	252.62	204.18	232.09	**232.54**	**21.22**
2	204.05	185.56	248.76	**212.79**	**32.49**		239.94	207.53	234.17	**227.21**	**17.29**		195.10	178.05	239.30	237.78	203.03	228.15	**213.57**	**25.20**
3	188.91	183.31	248.30	**206.84**	**36.01**		238.07	206.89	217.63	**220.86**	**15.84**		188.57	176.69	236.59	232.43	201.80	227.38	**210.58**	**25.09**
4	188.70	180.13	225.05	**197.96**	**23.85**		230.52	200.83	215.63	**215.66**	**14.85**		178.73	170.16	236.93	209.86	198.33	222.98	**202.83**	**25.64**
5	187.04	178.18	218.31	**194.51**	**21.08**		222.40	200.91	214.45	**212.59**	**10.87**		176.14	169.18	230.99	203.34	184.33	215.25	**196.54**	**24.07**
6	181.15	171.94	217.92	**190.34**	**24.33**		207.78	197.31	211.86	**205.65**	**7.50**		172.72	168.97	227.04	197.80	182.16	213.92	**193.77**	**23.33**
7	180.72	168.17	215.68	**188.19**	**24.62**		200.07	184.07	211.39	**198.51**	**13.73**		168.55	161.59	204.26	194.34	146.70	208.85	**180.71**	**25.31**
8	179.36	165.92	213.98	**186.42**	**24.80**		194.08	181.61	202.06	**192.58**	**10.31**		166.21	151.83	204.26	182.72	137.88	205.58	**174.75**	**27.72**
9	179.36	162.06	210.42	**183.95**	**24.50**		194.42	179.53	198.88	**190.94**	**10.13**		165.35	151.83	204.26	182.72	137.88	194.85	**172.82**	**25.65**
10	178.94	156.03	202.06	**179.01**	**23.02**		170.20	177.12	191.71	**179.68**	**10.98**		164.43	151.83	204.26	182.72	137.88	192.98	**172.35**	**25.39**
11	178.94	154.72	201.30	**178.32**	**23.30**		160.15	171.30	182.42	**171.29**	**11.13**		161.25	151.83	204.26	182.72	137.88	187.72	**170.94**	**24.83**
12	177.12	148.19	188.48	**171.26**	**20.77**		160.15	171.30	161.04	**164.16**	**6.20**		161.25	151.83	204.26	182.72	137.88	182.25	**170.03**	**24.18**
13	176.10	139.07	188.48	**167.88**	**25.71**															
14	174.78	138.65	188.48	**167.30**	**25.74**															
15	173.64	136.50	188.48	**166.21**	**26.78**															
16	170.71	132.76	188.48	**163.98**	**28.46**															
17	163.24	129.83	188.48	**160.52**	**29.42**															
18	160.66	125.33	188.48	**158.16**	**31.65**															
19	149.37	122.44	188.48	**153.43**	**33.21**															
20	136.22	122.44	188.48	**149.05**	**34.84**															
21	126.81	122.44	188.48	**145.91**	**36.93**															

~~*123.45*~~ = statistically excluded value

Table D5. Reducing Sugars (g/l) content in wort during primary fermentation across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	63.03	83.77	63.87	**70.22**	**11.74**		66.26	55.86	70.64	**64.25**	**7.59**		63.03	83.77	63.87	66.26	55.86	70.64	**67.24**	**9.43**
1	50.98	63.91	36.59	**50.49**	**13.67**		51.25	26.66	31.30	**36.40**	**13.06**		48.70	22.35	29.34	39.11	26.56	29.87	**32.66**	**9.61**
2	47.40	54.37	30.24	**44.00**	**12.42**		35.89	24.51	26.80	**29.07**	**6.02**		39.01	18.26	18.26	25.99	22.03	24.74	**24.71**	**7.70**
3	45.01	40.06	26.38	**37.15**	**9.65**		27.01	22.18	24.78	**24.65**	**2.42**		29.20	13.17	13.63	18.33	16.50	20.23	**18.51**	**5.89**
4	44.91	32.86	21.87	**33.21**	**11.53**		20.43	20.49	24.20	**21.70**	**2.16**		25.17	12.14	12.29	13.97	12.17	18.42	**15.69**	**5.23**
5	39.62	27.48	20.13	**29.08**	**9.84**		18.71	18.40	22.82	**19.98**	**2.47**		20.57	9.60	11.33	12.29	11.12	12.84	**12.96**	**3.89**
6	36.57	25.76	19.09	**27.14**	**8.82**		16.25	15.68	21.24	**17.72**	**3.06**		13.73	7.91	9.21	10.51	10.75	11.47	**10.60**	**1.99**
7	32.86	22.21	18.94	**24.67**	**7.28**		14.59	9.15	19.82	**14.52**	**5.33**		12.85	7.80	7.00	9.12	8.24	10.22	**9.20**	**2.10**
8	30.78	21.25	17.15	**23.06**	**6.99**		13.86	8.90	19.63	**14.13**	**5.37**		11.40	7.27	7.00	6.06	7.24	9.73	**8.12**	**2.02**
9	28.35	18.40	16.99	**21.25**	**6.19**		11.98	8.79	18.49	**13.09**	**4.95**		10.42	7.27	7.00	6.41	7.24	9.20	**7.92**	**1.54**
10	26.74	16.45	15.77	**19.65**	**6.14**		8.99	8.02	17.87	**11.63**	**5.43**		9.14	7.27	7.00	6.41	7.24	8.76	**7.64**	**1.07**
11	24.04	12.54	14.56	**17.04**	**6.14**		8.00	7.86	11.42	**9.09**	**2.01**		8.14	7.27	7.00	6.41	7.24	8.58	**7.44**	**0.79**
12	23.42	11.85	13.87	**16.38**	**6.18**		8.00	7.86	10.68	**8.85**	**1.59**		8.14	7.27	7.00	6.41	7.24	8.05	**7.35**	**0.65**
13	20.49	11.31	13.87	**15.22**	**4.74**															
14	19.96	10.59	13.87	**14.81**	**4.75**															
15	17.12	9.70	13.87	**13.56**	**3.72**															
16	15.40	9.22	13.87	**12.83**	**3.22**															
17	14.09	8.80	13.87	**12.25**	**2.99**															
18	14.02	8.00	13.87	**11.97**	**3.43**															
19	13.19	7.38	13.87	**11.48**	**3.57**															
20	11.44	7.38	13.87	**10.90**	**3.28**															
21	11.11	7.38	13.87	**10.79**	**3.26**															

~~*123.45*~~ = statistically excluded value

Table D6. Glucose (g/l) content in wort during primary fermentation across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	60.68	91.76	63.67	**72.04**	**17.15**		38.78	50.32	59.20	**49.43**	**10.24**		60.68	91.76	63.67	38.78	50.32	59.20	**58.47**	**5.74**
1	0.00	3.12	9.12	**4.08**	**4.64**		30.42	1.76	13.87	**15.35**	**14.39**		0.88	2.19	1.28	2.41	0.11	1.92	**1.47**	**0.88**
2	0.00	0.38	0.84	**0.41**	**0.42**		1.19	0.40	0.36	**0.65**	**0.47**		0.58	0.81	1.17	0.66	0.00	0.52	**0.62**	**0.38**
3	0.00	0.07	0.00	**0.02**	**0.04**		0.35	0.00	0.00	**0.12**	**0.20**		0.40	0.58	1.05	0.39	0.00	0.12	**0.42**	**0.37**
4	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.22	0.17	0.37	0.35	0.00	0.00	**0.19**	**0.16**
5	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.12	0.00	0.30	0.26	0.00	0.00	**0.11**	**0.14**
6	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.09	0.00	0.26	0.14	0.00	0.00	**0.08**	**0.11**
7	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
8	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
9	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
10	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
11	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
12	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
13	0.00	0.00	0.00	**0.00**	**0.00**															
14	0.00	0.00	0.00	**0.00**	**0.00**															
15	0.00	0.00	0.00	**0.00**	**0.00**															
16	0.00	0.00	0.00	**0.00**	**0.00**															
17	0.00	0.00	0.00	**0.00**	**0.00**															
18	0.00	0.00	0.00	**0.00**	**0.00**															
19	0.00	0.00	0.00	**0.00**	**0.00**															
20	0.00	0.00	0.00	**0.00**	**0.00**															
21	0.00	0.00	0.00	**0.00**	**0.00**															

~~*123.45*~~ = statistically excluded value

Table D7. Fructose (g/l) content in wort during primary fermentation across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	2.75	4.98	5.80	**4.51**	**1.58**		7.81	4.42	6.24	**6.16**	**1.70**		~~2.75~~	4.98	5.80	~~7.81~~	4.42	6.24	**5.36**	**0.82**
1	1.68	3.46	3.65	**2.93**	**1.09**		5.88	3.63	4.25	**4.59**	**1.16**		1.97	2.88	3.39	3.46	2.29	4.74	**3.12**	**0.99**
2	1.59	2.51	2.37	**2.16**	**0.50**		3.31	2.61	2.58	**2.83**	**0.41**		1.79	1.49	1.71	1.07	1.33	2.16	**1.59**	**0.38**
3	1.51	2.17	1.98	**1.89**	**0.34**		2.06	1.78	1.77	**1.87**	**0.16**		1.42	1.35	1.39	1.03	1.40	1.71	**1.38**	**0.22**
4	1.51	1.97	1.68	**1.72**	**0.23**		1.82	1.60	1.77	**1.73**	**0.12**		1.37	1.33	1.33	0.95	1.10	1.24	**1.22**	**0.16**
5	1.44	1.33	1.61	**1.46**	**0.14**		1.25	1.33	1.59	**1.39**	**0.18**		1.33	1.31	1.25	0.93	1.01	1.07	**1.15**	**0.17**
6	1.27	1.11	1.43	**1.27**	**0.16**		0.96	1.05	1.02	**1.01**	**0.05**		1.06	1.14	1.16	0.92	0.99	1.05	**1.05**	**0.09**
7	1.20	1.00	1.21	**1.14**	**0.12**		0.74	0.95	0.99	**0.89**	**0.13**		1.04	1.13	1.14	0.91	0.90	1.05	**1.03**	**0.10**
8	1.15	0.96	1.14	**1.08**	**0.11**		0.74	0.89	0.93	**0.85**	**0.10**		1.02	1.09	1.14	0.84	0.88	1.03	**1.00**	**0.12**
9	1.08	0.95	0.93	**0.99**	**0.08**		0.74	0.86	0.88	**0.83**	**0.08**		1.01	1.09	1.14	0.84	0.88	0.99	**0.99**	**0.12**
10	1.04	0.95	0.75	**0.91**	**0.15**		0.65	0.86	0.87	**0.79**	**0.12**		1.00	1.09	1.14	0.84	0.88	0.97	**0.99**	**0.12**
11	1.04	0.93	0.65	**0.87**	**0.20**		0.65	0.78	0.87	**0.77**	**0.11**		0.88	1.09	1.14	0.84	0.88	0.96	**0.97**	**0.12**
12	0.99	0.91	0.54	**0.81**	**0.24**		0.63	0.78	0.70	**0.70**	**0.08**		0.88	1.09	1.14	0.84	0.88	0.95	**0.96**	**0.12**
13	0.96	0.87	0.54	**0.79**	**0.22**															
14	0.93	0.85	0.54	**0.77**	**0.21**															
15	0.93	0.84	0.54	**0.77**	**0.20**															
16	0.89	0.83	0.54	**0.75**	**0.19**															
17	0.84	0.80	0.54	**0.73**	**0.16**															
18	0.78	0.77	0.54	**0.70**	**0.14**															
19	0.77	0.76	0.54	**0.69**	**0.13**															
20	0.73	0.76	0.54	**0.68**	**0.12**															
21	0.73	0.76	0.54	**0.68**	**0.12**															

~~*123.45*~~ = statistically excluded value

Table D8. Sucrose (g/l) content in wort during primary fermentation across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	1.23	1.40	1.12	**1.25**	**0.14**		1.66	1.31	1.45	**1.47**	**0.18**		1.23	1.40	1.12	1.66	1.31	1.45	**1.36**	**0.19**
1	0.37	0.36	0.81	**0.51**	**0.26**		0.73	0.73	0.73	**0.73**	**0.00**		0.34	0.39	0.35	0.72	0.73	0.72	**0.54**	**0.20**
2	0.36	0.35	0.74	**0.48**	**0.22**		0.71	0.73	0.73	**0.72**	**0.01**		0.34	0.37	0.35	0.73	0.71	0.72	**0.54**	**0.20**
3	0.35	0.35	0.71	**0.47**	**0.21**		0.71	0.72	0.72	**0.72**	**0.01**		0.34	0.36	0.34	0.71	0.71	0.72	**0.53**	**0.20**
4	0.35	0.34	0.71	**0.47**	**0.21**		0.71	0.72	0.72	**0.72**	**0.01**		0.33	0.36	0.34	0.71	0.71	0.72	**0.53**	**0.20**
5	0.34	0.34	0.35	**0.34**	**0.01**		0.71	0.72	0.72	**0.72**	**0.01**		0.33	0.34	0.34	0.71	0.71	0.71	**0.52**	**0.20**
6	0.34	0.34	0.35	**0.34**	**0.01**		0.71	0.71	0.72	**0.71**	**0.01**		0.33	0.34	0.34	0.71	0.70	0.71	**0.52**	**0.20**
7	0.34	0.34	0.34	**0.34**	**0.00**		0.71	0.71	0.72	**0.71**	**0.01**		0.33	0.33	0.33	0.71	0.70	0.71	**0.52**	**0.21**
8	0.34	0.34	0.34	**0.34**	**0.00**		0.71	0.70	0.71	**0.71**	**0.01**		0.33	0.33	0.33	0.71	0.70	0.71	**0.52**	**0.21**
9	0.34	0.34	0.34	**0.34**	**0.00**		0.71	0.70	0.71	**0.71**	**0.01**		0.33	0.33	0.33	0.71	0.70	0.71	**0.52**	**0.21**
10	0.34	0.33	0.34	**0.34**	**0.01**		0.71	0.70	0.70	**0.70**	**0.01**		0.33	0.33	0.33	0.71	0.70	0.70	**0.52**	**0.20**
11	0.34	0.33	0.33	**0.33**	**0.01**		0.71	0.70	0.70	**0.70**	**0.01**		0.33	0.33	0.33	0.71	0.70	0.70	**0.52**	**0.20**
12	0.33	0.33	0.33	**0.33**	**0.00**		0.71	0.70	0.70	**0.70**	**0.01**		0.33	0.33	0.33	0.71	0.70	0.70	**0.52**	**0.20**
13	0.33	0.33	0.33	**0.33**	**0.00**															
14	0.33	0.33	0.33	**0.33**	**0.00**															
15	0.33	0.33	0.33	**0.33**	**0.00**															
16	0.33	0.33	0.33	**0.33**	**0.00**															
17	0.33	0.33	0.33	**0.33**	**0.00**															
18	0.33	0.33	0.33	**0.33**	**0.00**															
19	0.33	0.33	0.33	**0.33**	**0.00**															
20	0.33	0.33	0.33	**0.33**	**0.00**															
21	0.33	0.33	0.33	**0.33**	**0.00**															

~~*123.45*~~ = statistically excluded value

Table D9. Maltose (g/l) content in wort during primary fermentation across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	72.55	75.60	72.97	**73.71**	**1.65**		79.58	83.92	74.48	**79.33**	**4.73**		72.55	75.60	72.97	79.58	83.92	74.48	**76.52**	**4.42**
1	43.37	54.19	52.29	**49.95**	**5.78**		47.64	45.59	61.86	**51.70**	**8.86**		41.47	50.66	30.77	31.92	41.33	52.78	**41.49**	**9.14**
2	42.61	48.74	51.35	**47.57**	**4.49**		44.66	37.20	52.16	**44.67**	**7.48**		31.98	21.86	30.56	30.73	28.59	45.10	**31.47**	**7.59**
3	42.24	45.82	41.82	**43.29**	**2.20**		40.52	28.92	38.97	**36.14**	**6.30**		29.37	19.75	18.05	29.68	23.86	31.52	**25.37**	**5.65**
4	41.22	37.57	38.22	**39.00**	**1.95**		33.96	26.50	35.71	**32.06**	**4.89**		28.12	13.15	14.58	20.17	16.54	26.14	**19.78**	**6.19**
5	38.73	27.08	37.90	**34.57**	**6.50**		27.04	24.23	27.45	**26.24**	**1.75**		27.85	6.85	10.24	18.73	15.69	20.42	**16.63**	**7.51**
6	38.39	26.25	29.57	**31.40**	**6.27**		22.28	23.45	24.31	**23.35**	**1.02**		21.41	4.29	7.02	12.09	13.37	19.56	**12.96**	**6.73**
7	37.50	23.48	29.05	**30.01**	**7.06**		17.60	21.06	22.60	**20.42**	**2.56**		18.93	4.11	5.94	8.76	11.71	15.85	**10.88**	**5.74**
8	34.53	21.77	27.53	**27.94**	**6.39**		14.39	15.51	21.67	**17.19**	**3.92**		17.83	4.11	5.94	5.50	8.38	14.53	**9.38**	**5.54**
9	34.11	21.45	26.20	**27.25**	**6.40**		13.83	13.40	20.42	**15.88**	**3.93**		11.46	4.11	5.94	5.50	8.38	14.00	**8.23**	**3.83**
10	33.64	18.74	24.48	**25.62**	**7.52**		8.37	12.15	17.35	**12.62**	**4.51**		9.51	4.11	5.94	5.50	8.38	10.32	**7.29**	**2.47**
11	33.54	17.51	23.84	**24.96**	**8.07**		7.43	11.30	17.10	**11.94**	**4.87**		8.29	4.11	5.94	5.50	8.38	8.25	**6.75**	**1.81**
12	33.23	15.48	22.31	**23.67**	**8.95**		7.43	11.30	13.10	**10.61**	**2.90**		8.29	4.11	5.94	5.50	8.38	7.84	**6.68**	**1.75**
13	32.74	15.45	22.31	**23.50**	**8.71**															
14	29.19	14.87	22.31	**22.12**	**7.16**															
15	23.45	14.33	22.31	**20.03**	**4.97**															
16	23.59	14.10	22.31	**20.00**	**5.15**															
17	23.39	13.73	22.31	**19.81**	**5.29**															
18	21.64	12.04	22.31	**18.66**	**5.75**															
19	18.06	10.22	22.31	**16.86**	**6.13**															
20	15.93	10.22	22.31	**16.15**	**6.05**															
21	13.26	10.22	22.31	**15.26**	**6.29**															

~~*123.45*~~ = statistically excluded value

Table D10. Total simple sugars (g/l) content in wort during primary fermentation across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	137.21	173.74	144.56	**151.84**	**19.32**		127.83	139.97	141.37	**136.39**	**7.45**		137.21	173.74	143.56	127.83	139.97	141.37	**136.60**	**6.09**
1	45.42	61.13	65.87	**57.47**	**10.70**		84.67	51.71	80.70	**72.36**	**17.99**		44.66	56.12	35.79	38.51	44.46	60.16	**46.62**	**9.65**
2	44.56	51.98	55.30	**50.61**	**5.50**		49.87	40.94	55.83	**48.88**	**7.49**		34.69	24.53	33.79	33.19	30.63	48.50	**34.22**	**7.90**
3	44.10	48.41	44.51	**45.67**	**2.38**		43.64	31.42	41.46	**38.84**	**6.52**		31.53	22.04	20.83	31.81	25.97	34.07	**27.71**	**5.56**
4	43.08	39.88	40.61	**41.19**	**1.68**		36.49	28.82	38.20	**34.50**	**5.00**		30.04	15.01	16.62	22.18	18.35	28.10	**21.72**	**6.21**
5	40.51	28.75	39.86	**36.37**	**6.61**		29.00	26.28	29.76	**28.35**	**1.83**		29.63	8.50	12.13	20.63	17.41	22.20	**18.42**	**7.53**
6	40.00	27.70	31.35	**33.02**	**6.32**		23.95	25.21	26.05	**25.07**	**1.06**		22.89	5.77	8.82	13.86	15.06	21.32	**14.62**	**6.72**
7	39.04	24.82	30.60	**31.49**	**7.15**		19.05	22.72	24.31	**22.03**	**2.70**		20.30	5.57	7.58	10.38	13.31	17.61	**12.46**	**5.73**
8	36.02	23.07	29.01	**29.37**	**6.48**		15.84	17.10	23.31	**18.75**	**4.00**		19.18	5.53	7.58	7.05	9.96	16.27	**10.93**	**5.53**
9	35.53	22.74	27.47	**28.58**	**6.47**		15.28	14.96	22.01	**17.42**	**3.98**		12.80	5.53	7.58	7.05	9.96	15.70	**9.77**	**3.86**
10	35.02	20.02	25.57	**26.87**	**7.58**		9.73	13.71	18.92	**14.12**	**4.61**		10.84	5.53	7.58	7.05	9.96	11.99	**8.83**	**2.49**
11	34.92	18.77	24.82	**26.17**	**8.16**		8.77	12.78	18.67	**13.41**	**4.98**		9.50	5.53	7.58	7.05	9.96	9.91	**8.26**	**1.82**
12	34.55	16.72	23.18	**24.82**	**9.03**		9.73	12.78	14.50	**12.34**	**2.42**		9.50	5.53	7.58	7.05	9.96	9.49	**8.19**	**1.75**
13	34.03	16.65	23.18	**24.62**	**8.78**															
14	30.45	16.05	23.18	**23.23**	**7.20**															
15	24.71	15.50	23.18	**21.13**	**4.94**															
16	24.81	15.26	23.18	**21.08**	**5.11**															
17	24.56	14.86	23.18	**20.87**	**5.25**															
18	22.75	13.14	23.18	**19.69**	**5.68**															
19	19.16	11.31	23.18	**17.88**	**6.04**															
20	16.99	11.31	23.18	**17.16**	**5.94**															
21	14.32	11.31	23.18	**16.27**	**6.17**															

~~*123.45*~~ = statistically excluded value

Table D11. Ethanol (% v/v) accumulation in the primary fermentation of wort across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
1	0.82	1.76	1.14	**1.24**	**0.48**		1.49	2.24	1.82	**1.85**	**0.38**		1.78	2.45	2.86	1.95	1.87	2.58	**2.25**	**0.44**
2	1.87	2.22	2.11	**2.07**	**0.18**		2.59	2.35	2.55	**2.50**	**0.13**		2.01	3.50	3.92	2.82	2.57	3.12	**2.99**	**0.68**
3	2.05	2.72	2.97	**2.58**	**0.48**		3.15	2.67	2.62	**2.81**	**0.29**		2.38	4.70	3.97	3.99	3.76	3.37	**3.70**	**0.78**
4	2.07	2.76	3.07	**2.63**	**0.51**		3.46	3.12	3.08	**3.22**	**0.21**		2.51	5.11	4.06	4.00	3.80	3.83	**3.89**	**0.83**
5	2.12	3.11	3.20	**2.81**	**0.60**		3.69	3.23	3.58	**3.50**	**0.24**		2.99	5.16	4.53	4.44	4.00	3.84	**4.16**	**0.74**
6	2.27	3.17	3.43	**2.96**	**0.61**		3.87	3.60	3.65	**3.71**	**0.14**		3.02	5.33	4.55	4.65	4.46	3.97	**4.33**	**0.78**
7	2.36	3.73	3.60	**3.23**	**0.76**		4.29	3.64	3.70	**3.88**	**0.36**		3.60	5.34	4.58	4.97	4.55	4.19	**4.54**	**0.61**
8	2.37	4.12	3.84	**3.44**	**0.94**		4.32	3.65	3.84	**3.94**	**0.35**		4.18	5.36	4.58	5.14	4.99	4.62	**4.81**	**0.43**
9	2.41	4.15	3.86	**3.47**	**0.93**		4.62	3.91	4.02	**4.18**	**0.38**		4.41	5.36	4.58	5.14	4.99	4.76	**4.87**	**0.36**
10	2.46	4.25	3.92	**3.54**	**0.95**		4.74	4.18	4.16	**4.36**	**0.33**		4.72	5.36	4.58	5.14	4.99	4.77	**4.93**	**0.29**
11	3.00	4.50	3.93	**3.81**	**0.76**		4.78	4.47	4.26	**4.50**	**0.26**		4.75	5.36	4.58	5.14	4.99	4.78	**4.93**	**0.29**
12	3.11	4.70	3.93	**3.91**	**0.80**		4.78	4.47	4.30	**4.52**	**0.24**		4.75	5.36	4.58	5.14	4.99	5.02	**4.97**	**0.28**
13	3.15	4.84	3.93	**3.97**	**0.85**															
14	3.47	4.91	3.93	**4.10**	**0.74**															
15	3.48	4.94	3.93	**4.12**	**0.75**															
16	3.64	4.98	3.93	**4.18**	**0.71**															
17	3.81	4.99	3.93	**4.24**	**0.65**															
18	4.17	5.03	3.93	**4.38**	**0.58**															
19	4.35	5.08	3.93	**4.45**	**0.58**															
20	4.44	5.08	3.93	**4.48**	**0.58**															
21	4.57	5.08	3.93	**4.53**	**0.58**															

~~*123.45*~~ = statistically excluded value

Table D12. Propanol (mg/l) accumulation in the primary fermentation of wort across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
1	2.02	1.40	3.57	**2.33**	**1.12**		5.56	5.29	5.88	**5.58**	**0.30**		5.14	8.83	11.30	5.26	5.19	7.78	**7.25**	**2.52**
2	4.14	5.18	5.65	**4.99**	**0.77**		8.87	5.36	6.73	**6.99**	**1.77**		6.36	13.53	18.72	10.15	5.23	7.97	**10.33**	**5.06**
3	5.17	7.24	8.90	**7.10**	**1.87**		10.87	7.65	11.00	**9.84**	**1.90**		9.11	22.16	19.51	13.35	5.30	15.32	**14.13**	**6.30**
4	6.04	9.91	9.22	**8.39**	**2.06**		12.91	8.06	12.67	**11.21**	**2.73**		9.39	26.67	19.87	14.56	15.56	15.40	**16.91**	**5.83**
5	8.06	10.30	10.02	**9.46**	**1.22**		13.63	12.71	14.81	**13.72**	**1.05**		9.78	27.63	20.56	15.13	15.88	17.26	**17.71**	**5.99**
6	8.09	11.85	13.27	**11.07**	**2.68**		14.24	13.52	15.13	**14.30**	**0.81**		12.82	27.82	22.48	16.87	16.00	17.98	**19.00**	**5.34**
7	8.34	13.54	13.50	**11.79**	**2.99**		14.71	13.59	15.15	**14.48**	**0.80**		16.95	28.38	22.81	18.82	17.36	18.79	**20.52**	**4.37**
8	8.56	13.76	13.66	**11.99**	**2.97**		14.78	13.99	15.48	**14.75**	**0.75**		18.20	29.37	22.81	19.60	18.16	20.40	**21.42**	**4.25**
9	8.59	13.86	14.07	**12.17**	**3.11**		14.94	14.56	15.91	**15.14**	**0.70**		19.52	29.37	22.81	19.60	18.16	21.24	**21.78**	**4.05**
10	8.67	14.79	14.99	**12.82**	**3.59**		17.01	14.88	16.22	**16.04**	**1.08**		20.19	29.37	22.81	19.60	18.16	22.16	**22.05**	**3.97**
11	9.02	16.29	16.43	**13.91**	**4.24**		17.95	15.40	17.64	**17.00**	**1.39**		20.95	29.37	22.81	19.60	18.16	22.67	**22.26**	**3.91**
12	10.22	16.74	16.43	**14.46**	**3.68**		17.95	15.40	18.91	**17.42**	**1.81**		20.95	29.37	22.81	19.60	18.16	29.70	**23.43**	**4.97**
13	11.13	17.99	16.43	**15.18**	**3.60**															
14	11.82	18.50	16.43	**15.58**	**3.42**															
15	11.89	18.75	16.43	**15.69**	**3.49**															
16	12.99	19.22	16.43	**16.21**	**3.12**															
17	13.41	19.63	16.43	**16.49**	**3.11**															
18	14.45	19.81	16.43	**16.90**	**2.71**															
19	15.64	19.97	16.43	**17.35**	**2.31**															
20	16.26	19.97	16.43	**17.55**	**2.09**															
21	17.19	19.97	16.43	**17.86**	**1.86**															

~~*123.45*~~ = statistically excluded value

Table D13. Ethyl acetate (mg/l) accumulation in the primary fermentation of wort across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
1	11.72	19.81	25.98	**19.17**	**7.15**		22.70	31.49	18.53	**24.24**	**6.62**		10.80	20.99	25.74	16.82	22.18	24.31	**20.14**	**5.51**
2	16.78	22.47	27.79	**22.35**	**5.51**		24.00	34.59	23.88	**27.49**	**6.15**		24.05	32.63	44.02	21.40	36.83	34.39	**32.22**	**8.36**
3	20.25	28.03	32.42	**26.90**	**6.16**		29.85	38.64	26.60	**31.70**	**6.23**		31.22	46.49	46.46	35.22	54.10	44.80	**43.05**	**8.37**
4	20.72	35.46	35.50	**30.56**	**8.52**		38.59	45.23	30.32	**38.05**	**7.47**		35.94	59.13	45.91	45.60	58.92	53.94	**49.91**	**9.08**
5	21.99	34.27	37.54	**31.27**	**8.20**		43.74	50.11	37.59	**43.81**	**6.26**		39.06	60.91	56.21	52.71	59.42	59.08	**54.57**	**8.13**
6	22.32	37.36	37.74	**32.47**	**8.80**		46.22	53.40	41.59	**47.07**	**5.95**		41.24	66.74	58.17	60.00	60.56	61.41	**58.02**	**8.71**
7	22.47	45.54	38.75	**35.59**	**11.86**		49.48	53.69	42.12	**48.43**	**5.86**		58.78	72.21	64.37	69.42	69.12	61.94	**65.97**	**5.12**
8	22.60	48.15	40.64	**37.13**	**13.13**		50.65	54.24	45.50	**50.13**	**4.39**		60.46	75.27	64.37	72.19	72.06	65.17	**68.25**	**5.74**
9	22.61	49.37	40.68	**37.55**	**13.65**		58.28	54.72	46.41	**53.14**	**6.09**		65.88	75.27	64.37	72.19	72.06	64.48	**69.04**	**4.70**
10	22.61	52.47	40.82	**38.63**	**15.05**		67.99	59.64	46.68	**58.10**	**10.74**		69.23	75.27	64.37	72.19	72.06	67.03	**70.03**	**3.95**
11	29.52	53.34	45.82	**42.89**	**12.18**		69.50	61.41	53.82	**61.58**	**7.84**		80.54	75.27	64.37	72.19	72.06	70.02	**72.41**	**5.38**
12	29.84	59.93	45.82	**45.20**	**15.05**		69.50	61.41	55.62	**62.18**	**6.97**		80.54	75.27	64.37	72.19	72.06	80.93	**74.23**	**6.19**
13	32.27	60.80	45.82	**46.30**	**14.27**															
14	33.38	61.43	45.82	**46.88**	**14.05**															
15	33.43	63.78	45.82	**47.68**	**15.26**															
16	37.73	66.31	45.82	**49.95**	**14.73**															
17	43.26	69.90	45.82	**52.99**	**14.70**															
18	43.84	70.50	45.82	**53.39**	**14.85**															
19	43.96	73.54	45.82	**54.44**	**16.57**															
20	54.04	73.54	45.82	**57.80**	**14.24**															
21	55.51	73.54	45.82	**58.29**	**14.07**															

~~*123.45*~~ = statistically excluded value

Table D14. Isoamyl alcohol (mg/l) accumulation in the primary fermentation of wort across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
1	38.49	33.59	23.44	**31.84**	**7.68**		50.26	82.66	52.37	**61.76**	**18.13**		51.11	42.10	74.77	51.10	57.07	74.58	**58.46**	**13.44**
2	51.35	47.13	44.63	**47.70**	**3.40**		71.52	90.34	55.82	**72.56**	**17.28**		61.63	83.25	94.86	80.47	93.52	80.07	**82.30**	**12.00**
3	51.51	60.98	59.37	**57.29**	**5.07**		78.71	92.92	69.89	**80.51**	**11.62**		66.66	99.37	97.81	84.71	94.28	86.28	**88.19**	**12.12**
4	52.32	79.18	70.33	**67.28**	**13.69**		84.35	99.06	70.06	**84.49**	**14.50**		69.68	114.92	98.21	87.29	108.65	96.04	**95.80**	**16.07**
5	54.38	79.48	81.57	**71.81**	**15.13**		85.65	102.80	73.03	**87.16**	**14.94**		77.89	116.00	102.46	90.99	112.75	97.02	**99.52**	**14.16**
6	56.26	85.45	84.27	**75.33**	**16.52**		86.89	103.01	84.48	**91.46**	**10.07**		82.14	119.11	105.61	95.03	114.66	99.85	**102.73**	**13.49**
7	58.15	91.06	86.24	**78.48**	**17.77**		88.42	104.97	86.01	**93.13**	**10.32**		89.98	133.59	106.92	99.43	118.62	103.97	**108.75**	**15.36**
8	65.19	92.48	86.73	**81.47**	**14.39**		89.74	106.08	86.41	**94.08**	**10.53**		92.35	135.35	106.92	108.32	121.09	104.23	**111.38**	**14.91**
9	65.64	93.45	92.09	**83.73**	**15.68**		93.72	106.77	87.54	**96.01**	**9.82**		102.93	135.35	106.92	108.32	121.09	105.56	**113.36**	**12.49**
10	66.06	98.66	94.21	**86.31**	**17.68**		98.41	107.45	88.75	**98.20**	**9.35**		108.18	135.35	106.92	108.32	121.09	106.77	**114.44**	**11.61**
11	67.25	101.61	98.14	**89.00**	**18.92**		103.66	108.26	90.84	**100.92**	**9.03**		110.32	135.35	106.92	108.32	121.09	107.02	**114.84**	**11.37**
12	67.94	102.84	98.14	**89.64**	**18.94**		103.66	108.26	92.97	**101.63**	**7.84**		110.32	135.35	106.92	108.32	121.09	113.87	**115.98**	**10.76**
13	68.88	105.35	98.14	**90.79**	**19.31**															
14	69.47	106.22	98.14	**91.28**	**19.31**															
15	75.44	106.85	98.14	**93.48**	**16.22**															
16	76.16	107.72	98.14	**94.01**	**16.18**															
17	82.98	108.99	98.14	**96.70**	**13.06**															
18	94.09	110.35	98.14	**100.86**	**8.46**															
19	95.85	112.41	98.14	**102.13**	**8.97**															
20	96.16	112.41	98.14	**102.24**	**8.87**															
21	97.68	112.41	98.14	**102.74**	**8.37**															

~~*123.45*~~ = statistically excluded value

Table D15. Isoamyl acetate (mg/l) accumulation in the primary fermentation of wort across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
1	0.16	0.22	0.20	**0.19**	**0.03**		0.16	0.42	0.22	**0.27**	**0.14**		0.19	0.27	0.22	0.15	0.43	0.36	**0.27**	**0.11**
2	0.18	0.25	0.23	**0.22**	**0.04**		0.17	0.56	0.34	**0.36**	**0.20**		0.24	0.55	0.46	0.17	0.54	0.54	**0.42**	**0.17**
3	0.20	0.31	0.24	**0.25**	**0.06**		0.21	0.59	0.41	**0.40**	**0.19**		0.29	0.82	0.50	0.24	1.03	0.69	**0.60**	**0.31**
4	0.21	0.41	0.25	**0.29**	**0.11**		0.22	0.81	0.43	**0.49**	**0.30**		0.31	0.86	0.52	0.27	1.25	0.91	**0.69**	**0.38**
5	0.21	0.44	0.26	**0.30**	**0.12**		0.25	0.87	0.49	**0.54**	**0.31**		0.39	1.08	0.67	0.31	1.28	0.95	**0.78**	**0.39**
6	0.21	0.51	0.30	**0.34**	**0.15**		0.29	1.12	0.61	**0.67**	**0.42**		0.39	1.23	0.69	0.35	1.30	0.96	**0.82**	**0.41**
7	0.21	0.51	0.32	**0.35**	**0.15**		0.33	1.17	0.67	**0.72**	**0.42**		0.53	1.47	0.71	0.39	1.51	1.00	**0.94**	**0.48**
8	0.22	0.57	0.34	**0.38**	**0.18**		0.35	1.18	0.68	**0.74**	**0.42**		0.61	1.57	0.71	0.49	1.52	1.01	**0.99**	**0.47**
9	0.22	0.64	0.34	**0.40**	**0.22**		0.36	1.18	0.71	**0.75**	**0.41**		0.64	~~*1.57*~~	0.71	~~*0.49*~~	1.52	1.12	**1.00**	**0.41**
10	0.22	0.65	0.35	**0.41**	**0.22**		0.42	1.22	0.71	**0.78**	**0.41**		0.66	~~*1.57*~~	0.71	~~*0.49*~~	1.52	1.13	**1.01**	**0.40**
11	0.23	0.67	0.38	**0.43**	**0.22**		0.48	1.33	0.74	**0.85**	**0.44**		0.67	~~*1.57*~~	0.71	~~*0.49*~~	1.52	1.13	**1.01**	**0.40**
12	0.24	0.68	0.38	**0.43**	**0.22**		0.48	1.33	0.77	**0.86**	**0.43**		0.67	~~*1.57*~~	0.71	~~*0.49*~~	1.52	1.33	**1.06**	**0.43**
13	0.27	0.78	0.38	**0.48**	**0.27**															
14	0.29	0.79	0.38	**0.49**	**0.27**															
15	0.30	0.81	0.38	**0.50**	**0.27**															
16	0.31	0.82	0.38	**0.50**	**0.28**															
17	0.32	0.83	0.38	**0.51**	**0.28**															
18	0.36	0.84	0.38	**0.53**	**0.27**															
19	0.36	0.85	0.38	**0.53**	**0.28**															
20	0.39	0.87	0.38	**0.55**	**0.28**															
21	0.45	0.87	0.38	**0.57**	**0.27**															

~~*123.45*~~ = statistically excluded value

Table D16. Ethyl hexanoate (mg/l) accumulation in the primary fermentation of wort across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
1	0.16	0.21	0.16	**0.18**	**0.03**		0.11	0.19	0.15	**0.15**	**0.04**		0.19	0.24	0.18	0.13	0.18	0.20	**0.19**	**0.04**
2	0.17	0.23	0.18	**0.19**	**0.03**		0.14	0.22	0.16	**0.17**	**0.04**		0.19	0.30	0.25	0.15	0.23	0.21	**0.22**	**0.05**
3	0.18	0.29	0.18	**0.22**	**0.06**		0.18	0.23	0.17	**0.19**	**0.03**		0.22	0.36	0.26	0.20	0.28	0.21	**0.26**	**0.06**
4	0.18	0.30	0.18	**0.22**	**0.07**		0.20	0.23	0.18	**0.20**	**0.03**		0.25	0.38	0.27	0.23	0.29	0.24	**0.28**	**0.06**
5	0.18	0.32	0.18	**0.23**	**0.08**		0.22	0.26	0.19	**0.22**	**0.04**		0.27	0.40	0.28	0.25	0.30	0.25	**0.29**	**0.06**
6	0.19	0.33	0.18	**0.23**	**0.08**		0.24	0.26	0.20	**0.23**	**0.03**		0.28	0.43	0.28	0.27	0.30	0.25	**0.30**	**0.06**
7	0.19	0.34	0.19	**0.24**	**0.09**		0.25	0.27	0.20	**0.24**	**0.04**		0.33	0.45	0.30	0.29	0.31	0.26	**0.32**	**0.07**
8	0.19	0.35	0.19	**0.24**	**0.09**		0.25	0.28	0.20	**0.24**	**0.04**		0.33	0.48	0.30	0.31	0.32	0.26	**0.33**	**0.08**
9	0.20	0.37	0.19	**0.25**	**0.10**		0.28	0.28	0.20	**0.25**	**0.05**		0.34	0.48	0.30	0.31	0.32	0.27	**0.34**	**0.07**
10	0.20	0.37	0.19	**0.25**	**0.10**		0.29	0.28	0.20	**0.26**	**0.05**		0.36	0.48	0.30	0.31	0.32	0.27	**0.34**	**0.07**
11	0.21	0.38	0.21	**0.27**	**0.10**		0.30	0.29	0.21	**0.27**	**0.05**		0.37	0.48	0.30	0.31	0.32	0.27	**0.34**	**0.08**
12	0.23	0.39	0.21	**0.28**	**0.10**		0.30	0.29	0.21	**0.27**	**0.05**		0.37	0.48	0.30	0.31	0.32	0.28	**0.34**	**0.07**
13	0.24	0.41	0.21	**0.29**	**0.11**															
14	0.25	0.41	0.21	**0.29**	**0.11**															
15	0.26	0.41	0.21	**0.29**	**0.10**															
16	0.28	0.42	~~*0.21*~~	**0.35**	**0.10**															
17	0.28	0.42	~~*0.21*~~	**0.35**	**0.10**															
18	0.29	0.42	~~*0.21*~~	**0.36**	**0.09**															
19	0.31	0.43	~~*0.21*~~	**0.37**	**0.08**															
20	0.36	0.43	~~*0.21*~~	**0.40**	**0.05**															
21	0.38	0.43	~~*0.21*~~	**0.41**	**0.04**															

~~*123.45*~~ = statistically excluded value

Table D17. Ethyl octanoate (mg/l) accumulation in the primary fermentation of wort across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
1	0.49	0.67	0.27	**0.48**	**0.20**		0.19	0.46	0.21	**0.29**	**0.15**		0.51	0.63	0.56	0.22	0.28	0.31	**0.42**	**0.17**
2	0.58	0.74	0.29	**0.54**	**0.23**		0.34	0.48	0.26	**0.36**	**0.11**		0.58	0.78	0.64	0.38	0.38	0.31	**0.51**	**0.18**
3	0.61	0.81	0.34	**0.59**	**0.24**		0.36	0.49	0.29	**0.38**	**0.10**		0.66	0.88	0.70	0.56	0.48	0.32	**0.60**	**0.19**
4	0.63	0.93	0.35	**0.64**	**0.29**		0.48	0.57	0.32	**0.46**	**0.13**		0.77	0.95	0.83	0.80	0.51	0.41	**0.71**	**0.21**
5	0.65	1.01	0.35	**0.67**	**0.33**		0.51	0.58	0.34	**0.48**	**0.12**		0.78	1.00	1.00	0.80	0.51	0.56	**0.78**	**0.21**
6	0.66	1.02	0.42	**0.70**	**0.30**		0.55	0.60	0.34	**0.50**	**0.14**		0.81	1.21	1.41	0.81	0.56	0.65	**0.91**	**0.33**
7	0.67	1.09	0.42	**0.73**	**0.34**		0.59	0.63	0.35	**0.52**	**0.15**		0.91	1.31	1.51	0.81	0.59	0.71	**0.97**	**0.36**
8	0.67	1.09	0.44	**0.73**	**0.33**		0.63	0.65	0.37	**0.55**	**0.16**		0.92	1.45	1.51	0.87	0.61	0.76	**1.02**	**0.37**
9	0.67	1.14	0.45	**0.75**	**0.35**		0.71	0.65	0.38	**0.58**	**0.18**		0.93	1.45	1.51	0.87	0.61	0.78	**1.03**	**0.37**
10	0.67	1.20	0.46	**0.78**	**0.38**		0.96	0.67	0.38	**0.67**	**0.29**		0.96	1.45	1.51	0.87	0.61	0.79	**1.03**	**0.37**
11	0.71	1.31	0.48	**0.83**	**0.43**		0.98	0.71	0.38	**0.69**	**0.30**		1.10	1.45	1.51	0.87	0.61	0.80	**1.06**	**0.36**
12	0.84	1.38	~~*0.48*~~	**1.11**	**0.38**		0.98	0.71	0.45	**0.71**	**0.27**		1.10	1.45	1.51	0.87	0.61	0.81	**1.06**	**0.36**
13	0.85	1.39	~~*0.48*~~	**1.12**	**0.38**															
14	0.93	1.39	~~*0.48*~~	**1.16**	**0.33**															
15	0.96	1.39	~~*0.48*~~	**1.18**	**0.30**															
16	0.97	1.40	~~*0.48*~~	**1.19**	**0.30**															
17	1.07	1.40	~~*0.48*~~	**1.24**	**0.23**															
18	1.08	1.40	~~*0.48*~~	**1.24**	**0.23**															
19	1.12	1.41	~~*0.48*~~	**1.27**	**0.21**															
20	1.38	1.41	~~*0.48*~~	**1.40**	**0.02**															
21	1.49	1.41	~~*0.48*~~	**1.45**	**0.06**															

~~*123.45*~~ = statistically excluded value

Table D18. Total fusel alcohol (mg/l) accumulation in the primary fermentation of wort across the three experimental temperatures.

Days	14 °C						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
1	40.51	34.99	31.01	**35.50**	**4.77**		55.82	87.95	58.25	**67.34**	**17.89**		56.25	50.93	86.07	56.36	62.26	82.36	**65.71**	**14.83**
2	55.49	52.31	53.28	**53.69**	**1.63**		80.39	95.70	62.55	**79.55**	**16.59**		67.99	96.78	113.58	90.62	98.75	88.04	**92.63**	**15.01**
3	56.68	68.22	68.27	**64.39**	**6.68**		89.58	100.57	80.89	**90.35**	**9.86**		75.77	121.53	117.32	98.06	99.58	101.60	**102.31**	**16.28**
4	58.36	89.09	81.55	**76.33**	**16.02**		97.26	107.12	82.73	**95.70**	**12.27**		79.07	141.59	118.08	101.85	124.21	111.44	**112.71**	**21.20**
5	62.44	89.78	94.59	**82.27**	**17.34**		99.28	115.51	87.84	**100.88**	**13.90**		87.67	143.63	123.02	106.12	128.63	114.28	**117.23**	**19.32**
6	64.35	97.30	97.54	**86.40**	**19.09**		101.13	116.53	99.61	**105.76**	**9.36**		94.96	146.93	128.09	111.90	130.66	117.83	**121.73**	**17.80**
7	66.49	104.60	99.74	**90.28**	**20.74**		103.13	118.56	101.16	**107.62**	**9.53**		106.93	161.97	129.73	118.25	135.98	122.76	**129.27**	**18.85**
8	73.75	106.24	100.39	**93.46**	**17.32**		104.52	120.07	101.89	**108.83**	**9.83**		110.55	164.72	129.73	127.92	139.25	124.63	**132.80**	**18.20**
9	74.23	107.31	106.16	**95.90**	**18.78**		108.66	121.33	103.45	**111.15**	**9.20**		122.45	164.72	129.73	127.92	139.25	126.80	**135.15**	**15.52**
10	74.73	113.45	109.20	**99.13**	**21.23**		115.42	122.33	104.97	**114.24**	**8.74**		128.37	164.72	129.73	127.92	139.25	128.93	**136.49**	**14.47**
11	76.27	117.90	114.57	**102.91**	**23.13**		121.61	123.66	108.48	**117.92**	**8.24**		131.27	164.72	129.73	127.92	139.25	129.69	**137.10**	**14.11**
12	78.16	119.58	114.57	**104.10**	**22.61**		121.61	123.66	111.88	**119.05**	**6.29**		131.27	164.72	129.73	127.92	139.25	143.57	**139.41**	**13.78**
13	80.01	123.34	114.57	**105.97**	**22.91**															
14	81.29	124.72	114.57	**106.86**	**22.72**															
15	87.33	125.60	114.57	**109.17**	**19.70**															
16	89.15	126.94	114.57	**110.22**	**19.27**															
17	96.39	128.62	114.57	**113.19**	**16.16**															
18	108.54	130.16	114.57	**117.76**	**11.16**															
19	111.49	132.38	114.57	**119.48**	**11.28**															
20	112.42	132.38	114.57	**119.79**	**10.96**															
21	114.87	132.38	114.57	**120.61**	**10.20**															

~~*123.45*~~ = statistically excluded value

Table D19. Total ester (mg/l) accumulation in the primary fermentation of wort across the three experimental temperatures.

Days	14 ℃						16 °C						18 °C							
	1	2	3	**Average**	**SD**		1	2	3	**Average**	**SD**		1	2	3	4	5	6	**Average**	**SD**
0	0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	**0.00**	**0.00**		0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	**0.00**
1	12.53	20.91	26.61	**20.02**	**7.08**		23.16	32.56	19.11	**24.94**	**6.90**		11.69	22.13	26.70	17.32	23.07	25.18	**21.02**	**5.58**
2	17.71	23.69	28.49	**23.30**	**5.40**		24.65	35.85	24.64	**28.38**	**6.47**		25.06	34.26	45.37	22.10	37.98	35.45	**33.37**	**8.56**
3	21.24	29.44	33.18	**27.95**	**6.11**		30.60	39.95	27.47	**32.67**	**6.49**		32.39	48.55	47.92	36.22	55.89	46.02	**44.50**	**8.66**
4	21.74	37.10	36.28	**31.71**	**8.64**		39.49	46.84	31.25	**39.19**	**7.80**		37.27	61.32	47.53	46.90	60.97	55.50	**51.58**	**9.40**
5	23.03	36.04	38.33	**32.47**	**8.25**		44.72	51.82	38.61	**45.05**	**6.61**		40.50	63.39	58.16	54.07	61.51	60.84	**56.41**	**8.44**
6	23.38	39.22	38.64	**33.75**	**8.98**		47.30	55.38	42.74	**48.47**	**6.40**		42.72	69.61	60.55	61.43	62.72	63.27	**60.05**	**9.07**
7	23.54	47.48	39.68	**36.90**	**12.21**		50.65	55.76	43.34	**49.92**	**6.24**		60.55	75.44	66.89	70.91	71.53	63.91	**68.21**	**5.47**
8	23.68	50.16	41.61	**38.48**	**13.51**		51.88	56.35	46.75	**51.66**	**4.80**		62.32	78.77	66.89	73.86	74.51	67.20	**70.59**	**6.11**
9	23.70	51.52	41.66	**38.96**	**14.11**		59.63	56.83	47.70	**54.72**	**6.24**		67.79	78.77	66.89	73.86	74.51	66.65	**71.41**	**5.02**
10	23.70	54.69	41.82	**40.07**	**15.57**		69.66	61.81	47.97	**59.81**	**10.98**		71.21	78.77	66.89	73.86	74.51	69.22	**72.41**	**4.22**
11	30.67	55.70	46.89	**44.42**	**12.70**		71.26	63.74	55.15	**63.38**	**8.06**		82.68	78.77	66.89	73.86	74.51	72.22	**74.82**	**5.44**
12	31.15	62.38	46.89	**46.81**	**15.62**		71.26	63.74	57.05	**64.02**	**7.11**		82.68	78.77	66.89	73.86	74.51	83.35	**76.68**	**6.22**
13	33.63	63.38	46.89	**47.97**	**14.90**															
14	34.85	64.02	46.89	**48.59**	**14.66**															
15	34.95	66.39	46.89	**49.41**	**15.87**															
16	39.29	68.95	46.89	**51.71**	**15.41**															
17	44.93	72.55	46.89	**54.79**	**15.41**															
18	45.57	73.16	46.89	**55.21**	**15.56**															
19	45.75	76.23	46.89	**56.29**	**17.28**															
20	56.17	76.23	46.89	**59.76**	**15.00**															
21	57.83	76.23	46.89	**60.32**	**14.83**															

~~*123.45*~~ = statistically excluded value

APPENDIX E: STORED BEER QUALITY PARAMETERS

Table E1. pH levels for the differently fermented and stored beer over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	4.405	4.375	4.350	4.355	4.380	4.400	4.350	4.360	4.395	4.360	4.350	4.360
	2	4.465	4.470	4.415	4.430	4.430	4.440	4.420	4.440	4.450	4.460	4.440	4.480
	3	4.260	4.300	4.280	4.295	4.265	4.280	4.270	4.270	4.280	4.275	4.295	4.290
	4	4.265	4.270	4.260	4.270	4.255	4.260	4.260	4.270	4.275	4.260	4.275	4.280
	5	4.385	4.345	4.365	4.405	4.480	4.395	4.335	4.345	4.460	4.375	4.345	4.390
	6	4.485	4.405	4.365	4.405	4.390	4.340	4.355	4.380	4.375	4.360	4.365	4.355
	Average	**4.378**	**4.361**	**4.339**	**4.360**	**4.367**	**4.353**	**4.332**	**4.344**	**4.373**	**4.348**	**4.345**	**4.359**
	SD	**0.096**	**0.072**	**0.058**	**0.065**	**0.090**	**0.072**	**0.059**	**0.066**	**0.080**	**0.073**	**0.058**	**0.073**
16 °C	1	4.515	4.525	4.530	4.520	4.525	4.525	4.525	4.530	4.520	4.520	4.540	4.530
	2	4.475	4.475	4.475	4.475	4.465	4.465	4.470	4.400	4.465	4.465	4.480	4.465
	3	4.490	4.485	4.500	4.500	4.480	4.485	4.490	4.495	4.490	4.460	4.480	4.490
	4	4.340	4.360	4.335	4.340	4.330	4.340	4.330	4.335	4.360	4.355	4.355	4.350
	5	4.205	4.210	4.205	4.210	4.195	4.195	4.195	4.205	4.205	4.205	4.205	4.205
	6	4.310	4.315	4.315	4.300	4.300	4.310	4.280	4.295	4.320	4.310	4.320	4.310
	7	4.385	4.395	4.370	4.405	4.390	4.385	4.380	4.395	4.370	4.380	4.370	4.385
	8	4.415	4.405	4.390	4.410	4.420	4.395	4.385	4.425	4.410	4.395	4.380	4.390
	9	4.405	4.410	4.390	4.400	4.390	4.400	4.380	4.395	4.385	4.365	4.365	4.390
	Average	**4.393**	**4.398**	**4.390**	**4.396**	**4.388**	**4.389**	**4.382**	**4.386**	**4.392**	**4.384**	**4.388**	**4.391**
	SD	**0.098**	**0.096**	**0.101**	**0.100**	**0.102**	**0.100**	**0.105**	**0.099**	**0.096**	**0.093**	**0.100**	**0.098**
18 °C	1	4.435	4.455	4.470	4.500	4.415	4.445	4.455	4.480	4.390	4.440	4.470	4.490
	2	4.470	4.465	4.460	4.485	4.450	4.440	4.470	4.485	4.420	4.435	4.465	4.470
	3	4.315	4.340	4.375	4.360	4.320	4.350	4.370	4.350	4.325	4.350	4.360	4.350
	4	4.375	4.360	4.390	4.385	4.355	4.360	4.400	4.395	4.365	4.360	4.400	4.400
	5	4.295	4.265	4.280	4.295	4.305	4.270	4.285	4.320	4.300	4.270	4.290	4.315
	6	4.280	4.265	4.270	4.290	4.275	4.265	4.275	4.275	4.270	4.280	4.255	4.265
	7	4.650	4.670	4.615	4.630	4.650	4.660	4.630	4.630	4.655	4.640	4.635	4.665
	8	4.320	4.355	4.350	4.340	4.330	4.345	4.340	4.350	4.340	4.335	4.355	4.355
	9	4.350	4.355	4.375	4.400	4.350	4.350	4.370	4.380	4.355	4.345	4.370	4.390
	Average	**4.388**	**4.392**	**4.398**	**4.409**	**4.383**	**4.387**	**4.399**	**4.407**	**4.380**	**4.384**	**4.400**	**4.411**
	SD	**0.117**	**0.125**	**0.106**	**0.111**	**0.114**	**0.120**	**0.109**	**0.108**	**0.113**	**0.112**	**0.113**	**0.118**

Table E2. Total dissolved solid (mg/l) levels of beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	974.5	1012.5	1014.5	1032.0	969.5	987.0	1023.0	1028.5	976.0	1017.0	1027.5	1030.5
	2	964.0	981.5	1023.5	1024.5	971.5	984.0	1019.0	1048.5	984.0	995.5	1054.0	1054.0
	3	996.5	1021.5	1028.5	1034.0	990.5	1030.5	1035.0	1036.0	990.5	1034.0	1036.5	1040.0
	4	995.0	1028.0	1032.0	1019.5	994.0	1028.0	1033.0	1034.0	1001.5	1027.5	1041.0	1046.5
	5	1011.5	1037.0	1037.0	1043.0	1011.5	1026.0	1026.5	1038.5	1026.0	1057.0	1062.5	1068.5
	6	991.5	1024.0	1030.0	1032.0	1015.0	1017.0	1045.5	1046.0	1038.0	1039.0	1051.0	1054.5
	Average	**988.8**	**1017.4**	**1027.6**	**1030.8**	**992.0**	**1012.1**	**1030.3**	**1038.6**	**1002.7**	**1028.3**	**1045.4**	**1049.0**
	SD	**17.0**	**19.3**	**7.8**	**8.1**	**19.2**	**21.1**	**9.5**	**7.5**	**24.5**	**20.9**	**12.8**	**13.2**
16 °C	1	1138.0	1131.0	1090.0	1149.0	1096.0	1144.0	1146.0	1146.0	1105.5	1144.5	1154.5	1155.5
	2	1100.5	1082.5	1062.0	1121.0	1079.5	1081.0	1109.0	1123.0	1086.5	1121.5	1128.0	1132.0
	3	1111.5	1103.0	1095.0	1116.0	1077.0	1122.0	1128.5	1129.0	1095.5	1127.5	1127.5	1131.0
	4	1015.0	1009.0	997.5	1031.5	1001.0	1023.5	1038.0	1046.0	1018.5	1038.0	1043.5	1058.0
	5	1054.0	1048.5	1030.0	1061.0	1036.0	1051.5	1057.0	1063.0	1039.0	1067.0	1067.5	1082.0
	6	1027.0	1039.0	1020.0	1035.5	1025.0	1053.0	1055.0	1059.0	1035.0	1062.0	1072.0	1074.5
	7	1053.0	1061.0	1066.5	1037.5	1056.5	1057.5	1063.0	1067.0	1075.5	1087.0	1074.0	1072.5
	8	1049.5	1056.0	1053.5	1066.0	1070.0	1068.0	1072.0	1072.5	1082.0	1084.5	1087.0	1088.0
	9	1056.5	1061.0	1057.0	1072.5	1067.0	1077.0	1077.5	1085.5	1083.0	1083.0	1094.0	1126.0
	Average	**1067.2**	**1065.7**	**1052.4**	**1076.7**	**1056.4**	**1075.3**	**1082.9**	**1087.9**	**1068.9**	**1090.6**	**1094.2**	**1102.2**
	SD	**40.7**	**35.9**	**31.8**	**42.4**	**30.2**	**37.2**	**36.7**	**35.7**	**30.3**	**34.4**	**35.6**	**34.2**
18 °C	1	965.0	967.0	1011.5	1030.0	943.5	969.0	1008.5	1027.5	963.0	980.5	1033.0	1042.0
	2	994.0	1039.5	1037.5	1058.5	993.5	1036.0	1050.0	1058.5	998.0	1049.5	1062.0	1065.0
	3	986.0	1013.0	1027.0	1018.5	986.0	1015.5	1026.5	1020.0	984.0	1010.5	1026.0	1026.5
	4	997.5	1034.0	1039.0	1041.0	1001.5	1030.0	1045.0	1043.5	1001.5	1047.0	1041.0	1048.5
	5	1006.0	1009.5	1002.0	996.0	1005.5	1010.0	1008.5	995.0	999.0	1017.0	1012.5	1012.0
	6	997.5	1004.0	1007.5	989.0	998.0	1011.0	1018.0	1022.0	1015.0	1017.0	1018.5	1022.0
	7	1184.0	1199.0	1198.0	1218.5	1199.0	1210.0	1218.0	1235.0	1202.5	1224.0	1228.5	1236.0
	8	1020.5	1019.0	993.0	1021.0	991.5	1031.0	1037.5	1038.0	1009.0	1040.0	1050.0	1050.5
	9	1032.5	1041.0	1055.0	1065.0	1059.5	1061.0	1052.5	1063.5	1057.5	1060.5	1067.5	1071.5
	Average	**1020.3**	**1036.2**	**1041.2**	**1048.6**	**1019.8**	**1041.5**	**1051.6**	**1055.9**	**1025.5**	**1049.6**	**1059.9**	**1063.8**
	SD	**64.3**	**65.1**	**62.1**	**68.6**	**73.5**	**67.9**	**64.6**	**70.3**	**71.1**	**69.9**	**65.9**	**67.5**

~~*123.45*~~ = statistically excluded value

Table E3. Beer colour (°EBC) during storage over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	13.96	13.15	13.29	13.03	14.30	13.56	13.05	12.45	14.16	13.74	13.47	12.41
	2	14.06	13.76	13.63	13.12	14.39	14.27	13.54	13.57	14.97	14.66	14.05	14.05
	3	14.76	14.05	13.95	13.64	14.65	14.57	14.27	13.33	14.98	15.13	14.33	13.65
	4	14.77	14.01	13.63	13.38	14.92	14.48	13.62	13.11	14.98	14.53	14.29	14.02
	5	14.34	13.75	13.55	13.32	14.34	13.94	13.57	13.15	14.51	14.27	13.44	13.54
	6	14.11	12.73	12.77	11.97	13.92	13.02	12.83	11.75	14.28	14.21	13.12	13.18
	Average	**14.33**	**13.57**	**13.47**	**13.07**	**14.42**	**13.97**	**13.48**	**12.89**	**14.65**	**14.42**	**13.78**	**13.48**
	SD	**0.36**	**0.52**	**0.40**	**0.58**	**0.34**	**0.60**	**0.50**	**0.67**	**0.38**	**0.47**	**0.50**	**0.61**
16 °C	1	15.23	14.60	14.23	13.89	16.91	15.54	13.68	13.43	15.43	14.86	14.58	13.96
	2	15.14	14.95	14.47	14.55	14.94	14.95	14.60	14.12	16.45	14.91	14.57	14.39
	3	14.64	14.11	14.22	13.86	14.74	14.94	14.44	13.81	15.87	15.31	15.36	14.28
	4	13.48	13.96	13.04	12.59	14.00	13.42	13.85	13.74	14.33	14.55	13.85	13.94
	5	14.38	14.51	13.52	12.52	13.66	13.41	12.77	12.88	14.30	13.85	13.34	13.09
	6	14.33	13.62	13.71	12.93	14.32	14.14	13.97	13.42	14.51	14.09	13.97	13.68
	7	15.84	15.27	14.41	14.29	15.44	14.79	14.39	13.96	15.51	15.30	14.43	14.16
	8	15.03	14.99	14.23	13.58	15.20	14.79	14.27	14.24	15.25	15.17	15.02	14.38
	9	15.54	15.03	14.73	14.69	15.34	15.25	15.07	14.43	16.38	15.37	14.74	14.11
	Average	**14.84**	**14.56**	**14.06**	**13.65**	**14.95**	**14.58**	**14.11**	**13.78**	**15.34**	**14.82**	**14.43**	**14.00**
	SD	**0.72**	**0.56**	**0.53**	**0.82**	**0.96**	**0.76**	**0.66**	**0.48**	**0.82**	**0.55**	**0.62**	**0.41**
18 °C	1	14.10	13.26	13.18	12.14	14.25	13.64	13.20	12.86	14.82	13.99	14.11	13.27
	2	13.59	13.21	12.82	12.23	13.40	13.21	13.47	12.88	14.63	13.82	13.46	13.24
	3	14.43	14.32	14.05	13.77	15.93	14.49	14.46	13.69	16.15	14.69	14.56	14.23
	4	14.52	14.57	13.84	13.45	14.87	14.53	13.34	14.00	15.01	14.84	14.69	14.33
	5	14.12	12.94	11.99	12.74	13.97	13.27	13.17	12.44	14.21	14.00	13.48	12.20
	6	14.01	13.04	12.44	11.87	14.03	12.92	12.21	12.03	14.06	13.39	13.53	13.03
	7	16.54	15.40	14.24	13.74	16.10	16.08	16.18	14.30	17.74	17.09	16.17	15.11
	8	13.68	13.66	13.48	12.79	13.87	13.71	13.04	13.18	14.65	14.11	13.35	12.90
	9	15.31	15.18	14.74	14.11	15.58	14.94	14.77	13.88	15.61	15.43	15.19	14.64
	Average	**14.48**	**13.95**	**13.42**	**12.98**	**14.67**	**14.09**	**13.76**	**13.25**	**15.21**	**14.59**	**14.28**	**13.66**
	SD	**0.925**	**0.941**	**0.894**	**0.812**	**0.990**	**1.013**	**1.183**	**0.765**	**1.153**	**1.117**	**0.963**	**0.952**

~~*123.45*~~ = statistically excluded value

Table E4. Reducing Sugar (g/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	5.690	4.415	2.525	1.788	5.655	4.028	2.374	2.012	4.218	3.364	2.580	1.877
	2	5.232	3.715	2.677	2.352	5.107	4.148	2.533	1.495	5.156	3.021	2.283	1.914
	3	6.317	3.192	2.538	2.400	3.882	3.623	2.452	1.822	3.514	3.179	2.489	1.945
	4	4.584	2.779	2.213	2.030	5.820	4.370	2.507	1.996	3.820	3.287	~~3.189~~	~~2.150~~
	5	~~17.930~~	~~15.600~~	~~11.790~~	~~4.440~~	~~16.826~~	~~15.017~~	~~10.987~~	~~3.248~~	~~15.907~~	~~12.773~~	~~3.119~~	~~2.553~~
	6	~~15.599~~	~~13.411~~	~~4.910~~	~~2.755~~	~~15.138~~	~~12.524~~	~~3.909~~	~~1.474~~	~~12.551~~	~~4.214~~	1.696	0.998
	Average	**5.456**	**3.525**	**2.488**	**2.143**	**5.116**	**4.042**	**2.467**	**1.831**	**4.177**	**3.213**	**2.262**	**1.684**
	SD	**0.732**	**0.706**	**0.196**	**0.288**	**0.877**	**0.313**	**0.070**	**0.240**	**0.713**	**0.149**	**0.397**	**0.458**
16 °C	1	4.030	2.859	2.592	1.441	4.007	2.951	1.792	1.625	2.418	2.237	2.079	1.644
	2	4.316	2.970	2.488	1.993	3.719	2.661	2.330	2.079	2.532	2.453	2.317	2.296
	3	4.293	3.270	2.007	1.888	4.072	2.938	1.851	1.691	3.095	2.843	1.752	1.619
	4	3.985	2.739	2.430	2.030	3.530	2.515	2.417	2.304	4.637	3.378	2.546	1.670
	5	4.866	3.689	3.094	1.890	3.502	2.868	2.577	1.748	3.842	3.596	3.030	1.827
	6	4.541	3.515	2.654	2.185	5.245	3.465	3.277	2.058	4.473	3.089	2.627	1.782
	7	~~7.651~~	~~4.733~~	~~3.527~~	~~4.338~~	4.592	4.115	3.826	2.135	~~5.973~~	3.858	2.840	~~2.361~~
	8	4.422	3.167	2.933	2.284	~~7.468~~	~~5.160~~	~~4.853~~	~~4.146~~	~~6.266~~	~~4.637~~	~~4.506~~	~~3.290~~
	9	~~8.419~~	~~5.050~~	~~5.006~~	~~4.829~~	~~9.674~~	~~5.351~~	~~4.735~~	~~3.579~~	~~5.058~~	~~4.876~~	~~3.141~~	1.888
	Average	**4.350**	**3.173**	**2.600**	**1.959**	**4.095**	**3.073**	**2.581**	**1.949**	**3.722**	**3.065**	**2.456**	**1.818**
	SD	**0.302**	**0.347**	**0.354**	**0.271**	**0.631**	**0.547**	**0.739**	**0.259**	**1.058**	**0.594**	**0.442**	**0.233**
18 °C	1	3.035	2.699	2.586	2.010	3.050	2.629	2.251	1.969	2.883	2.860	2.753	2.117
	2	3.906	3.249	2.614	2.343	3.709	1.733	1.733	1.720	3.311	2.108	1.837	1.789
	3	3.687	3.058	2.836	2.192	3.398	2.804	2.685	2.399	2.611	2.518	2.439	2.368
	4	3.786	2.748	2.676	2.157	3.236	1.984	1.915	1.911	3.455	2.850	1.963	1.894
	5	2.914	2.722	2.642	1.391	4.046	3.635	2.136	1.866	2.760	2.507	2.322	2.287
	6	3.708	3.295	1.317	1.200	3.583	3.247	2.038	1.790	3.133	1.960	1.087	0.545
	7	1.867	1.832	1.550	1.165	2.867	1.850	1.582	1.129	2.538	1.532	1.444	1.171
	8	2.612	2.213	1.954	1.876	3.543	2.310	1.906	1.648	1.937	1.767	1.705	1.610
	9	3.811	3.109	2.916	2.120	3.819	3.260	3.087	1.842	4.113	3.580	3.128	2.874
	Average	**3.258**	**2.769**	**2.343**	**1.828**	**3.472**	**2.606**	**2.148**	**1.808**	**2.971**	**2.409**	**2.075**	**1.851**
	SD	**0.699**	**0.487**	**0.585**	**0.454**	**0.376**	**0.684**	**0.474**	**0.333**	**0.624**	**0.639**	**0.646**	**0.690**

~~123.45~~ = statistically excluded value

Table E5. Free amino nitrogen (mg/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	186.91	186.07	172.70	166.51	176.78	172.45	154.63	152.98	190.82	167.61	167.40	158.75
	2	188.74	180.34	159.98	136.39	180.13	173.81	163.92	161.29	177.12	156.84	153.11	145.47
	3	170.63	169.31	155.95	152.60	183.86	179.32	172.32	157.73	170.97	167.19	165.49	160.78
	4	179.45	159.85	150.31	146.11	177.24	168.38	159.17	148.57	155.95	151.83	151.71	138.05
	5	178.77	157.09	157.43	133.64	192.22	169.23	170.63	154.55	195.27	177.03	178.64	152.89
	6	196.25	187.93	175.76	161.34	190.56	186.58	181.36	169.86	185.26	177.79	177.29	170.29
	Average	**183.46**	**173.43**	**162.02**	**149.43**	**183.47**	**174.96**	**167.01**	**157.50**	**179.23**	**166.38**	**165.61**	**154.37**
	SD	**9.01**	**13.31**	**10.02**	**13.22**	**6.66**	**6.90**	**9.71**	**7.43**	**14.44**	**10.47**	**11.48**	**11.50**
16 °C	1	227.09	190.18	188.06	172.75	208.46	202.14	200.49	193.07	232.98	188.91	185.85	184.20
	2	200.23	197.73	167.83	162.01	195.02	184.67	176.82	166.13	193.28	182.59	180.55	179.67
	3	200.40	189.88	177.79	175.84	203.97	190.14	189.33	188.91	222.21	202.78	187.17	177.67
	4	202.48	181.15	174.49	174.40	213.09	190.35	174.23	164.94	192.73	190.56	185.64	166.13
	5	245.35	209.82	195.40	193.19	241.81	177.96	161.46	153.66	215.80	197.99	185.18	171.43
	6	215.34	199.00	193.11	172.24	223.95	218.86	208.42	176.90	193.41	180.76	177.20	159.17
	7	180.13	177.07	163.71	156.08	198.03	182.25	158.24	154.55	172.70	167.15	157.05	150.94
	8	189.16	173.13	168.04	160.66	193.66	187.34	172.36	162.23	184.67	177.62	177.96	160.74
	9	193.79	187.68	175.84	144.37	194.85	187.64	185.81	163.37	186.07	185.52	171.98	147.21
	Average	**206.00**	**189.52**	**178.25**	**167.95**	**208.09**	**191.26**	**180.80**	**169.31**	**199.32**	**185.99**	**178.73**	**166.35**
	SD	**20.18**	**11.55**	**11.49**	**14.00**	**16.15**	**12.31**	**16.81**	**14.07**	**19.82**	**10.72**	**9.57**	**12.93**
18 °C	1	178.64	148.36	149.54	136.86	167.02	164.60	159.21	151.96	164.05	159.81	156.41	141.06
	2	169.14	165.83	163.29	161.97	201.76	179.07	159.51	152.47	178.73	175.38	171.94	153.78
	3	186.32	172.87	172.24	165.37	188.02	186.02	178.69	153.53	180.42	177.20	176.52	152.22
	4	171.64	168.72	161.12	159.34	196.12	188.02	175.16	167.06	221.15	198.58	179.07	167.36
	5	183.48	153.53	148.48	139.57	174.53	172.36	140.68	133.04	194.21	163.03	152.94	127.27
	6	194.51	179.32	172.03	162.01	202.86	188.19	182.89	176.39	179.62	175.38	164.64	160.70
	7	194.64	194.55	191.28	182.55	196.76	195.27	191.92	183.39	189.42	189.42	182.46	180.64
	8	193.28	187.47	187.47	182.84	201.17	196.16	178.52	176.98	204.43	202.40	175.93	160.91
	9	246.39	175.29	174.02	171.64	193.02	190.82	182.38	176.56	189.54	188.06	176.95	174.15
	Average	**190.89**	**171.77**	**168.83**	**162.46**	**191.25**	**184.50**	**172.11**	**163.49**	**189.06**	**181.03**	**170.76**	**157.57**
	SD	**22.87**	**14.84**	**14.90**	**16.21**	**12.64**	**10.57**	**15.89**	**16.62**	**16.54**	**14.74**	**10.41**	**16.42**

~~*123.45*~~ = statistically excluded value

Table E6. Fructose (g/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	1.89	1.83	1.27	0.95	1.75	1.60	1.16	1.01	1.84	1.44	1.32	1.08
	2	2.01	1.90	1.60	1.10	1.99	1.62	1.60	1.40	1.87	1.84	1.72	1.65
	3	1.40	1.20	1.19	1.01	1.41	1.37	1.10	1.09	1.47	1.23	1.14	1.10
	4	1.48	1.35	1.18	1.03	1.46	1.27	1.20	1.13	1.51	1.31	1.29	1.08
	5	1.63	1.09	0.98	0.91	1.72	1.16	1.00	0.93	1.51	1.34	1.16	0.90
	6	1.30	1.18	1.14	0.99	1.21	0.96	0.94	0.78	2.15	1.42	1.19	0.88
	Average	**1.62**	**1.43**	**1.23**	**1.00**	**1.59**	**1.33**	**1.17**	**1.06**	**1.73**	**1.43**	**1.30**	**1.12**
	SD	**0.28**	**0.35**	**0.21**	**0.07**	**0.28**	**0.26**	**0.23**	**0.21**	**0.27**	**0.21**	**0.22**	**0.28**
16 °C	1	0.90	0.80	0.74	0.70	1.69	1.19	0.87	0.77	1.04	0.76	0.71	0.60
	2	0.97	0.91	0.90	0.89	1.13	1.00	0.98	0.95	0.98	0.98	0.97	0.93
	3	0.89	0.83	0.83	0.72	0.88	0.84	0.84	0.73	0.89	0.82	0.75	0.72
	4	1.06	1.05	0.82	0.76	0.96	0.84	0.78	0.75	0.84	0.84	0.80	0.80
	5	0.76	0.76	0.62	0.62	0.76	0.73	0.71	0.66	~~*0.62*~~	~~*0.60*~~	~~*0.60*~~	~~*0.60*~~
	6	0.90	0.80	0.75	0.74	0.92	0.84	0.75	0.73	0.75	0.71	0.70	0.68
	7	1.17	1.02	0.97	0.96	1.11	1.07	0.99	0.94	~~*1.62*~~	~~*1.29*~~	~~*1.28*~~	~~*1.23*~~
	8	1.28	1.14	1.04	1.00	1.08	1.06	1.03	0.91	1.11	1.06	1.06	1.05
	9	1.22	1.00	1.00	0.96	1.36	1.04	1.04	0.94	1.07	1.07	0.98	0.87
	Average	**1.02**	**0.92**	**0.85**	**0.82**	**1.10**	**0.96**	**0.89**	**0.82**	**0.95**	**0.89**	**0.85**	**0.81**
	SD	**0.18**	**0.13**	**0.14**	**0.14**	**0.28**	**0.15**	**0.13**	**0.11**	**0.13**	**0.14**	**0.15**	**0.16**
18 °C	1	1.72	1.62	1.52	1.37	1.72	1.61	1.50	1.48	1.98	1.67	1.58	1.02
	2	1.76	1.73	1.48	1.38	1.69	1.66	1.60	1.53	1.90	1.78	1.77	1.63
	3	1.43	1.40	1.35	1.27	1.49	1.38	1.34	1.28	1.33	1.31	1.29	1.19
	4	1.70	1.55	1.49	1.31	1.57	1.53	1.48	1.43	2.10	1.56	1.44	~~*1.38*~~
	5	~~*2.49*~~	~~*2.32*~~	~~*1.93*~~	~~*1.91*~~	~~*2.49*~~	~~*2.32*~~	~~*1.93*~~	~~*1.91*~~	~~*2.83*~~	~~*2.70*~~	~~*2.68*~~	1.34
	6	1.17	1.12	1.01	0.88	1.28	1.16	1.08	0.98	1.10	1.09	0.95	~~*0.54*~~
	7	~~*0.99*~~	~~*0.86*~~	~~*0.83*~~	~~*0.71*~~	~~*0.94*~~	~~*0.93*~~	~~*0.80*~~	~~*0.76*~~	~~*0.94*~~	~~*0.81*~~	~~*0.75*~~	0.71
	8	1.05	1.02	0.91	0.89	0.98	0.93	0.91	0.85	1.96	0.89	0.89	0.87
	9	1.16	1.14	1.06	1.04	1.13	1.11	1.08	1.05	1.18	1.06	1.05	0.98
	Average	**1.43**	**1.37**	**1.26**	**1.16**	**1.41**	**1.34**	**1.28**	**1.23**	**1.65**	**1.34**	**1.28**	**1.11**
	SD	**0.30**	**0.28**	**0.26**	**0.22**	**0.28**	**0.28**	**0.26**	**0.27**	**0.43**	**0.34**	**0.33**	**0.31**

~~*123.45*~~ = statistically excluded value

Table E7. Sucrose (g/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	0.30	0.28	0.28	0.28	0.29	0.29	0.28	0.27	0.30	0.28	0.28	0.28
	2	0.30	0.30	0.28	0.28	0.30	0.28	0.28	0.28	0.30	0.29	0.28	0.28
	3	0.29	0.29	0.29	0.28	0.32	0.30	0.29	0.28	0.32	0.30	0.29	0.29
	4	0.31	0.31	0.28	0.28	0.31	0.30	0.30	0.30	0.30	0.29	0.28	0.28
	5	0.29	0.28	0.28	0.28	0.31	0.29	0.28	0.28	0.33	0.31	0.29	0.28
	6	0.32	0.30	0.29	0.28	0.34	0.31	0.29	0.28	0.41	0.30	0.29	0.29
	Average	**0.30**	**0.29**	**0.28**	**0.28**	**0.31**	**0.30**	**0.29**	**0.28**	**0.33**	**0.30**	**0.29**	**0.28**
	SD	**0.01**	**0.01**	**0.01**	**0.00**	**0.02**	**0.01**	**0.01**	**0.01**	**0.04**	**0.01**	**0.01**	**0.01**
16 °C	1	0.33	0.32	0.32	0.29	0.36	0.35	0.32	0.32	0.34	0.34	0.33	0.33
	2	0.34	0.33	0.33	0.33	0.35	0.33	0.33	0.33	0.34	0.33	0.33	0.33
	3	0.35	0.33	0.33	0.33	0.34	0.33	0.33	0.33	0.33	0.33	0.33	0.33
	4	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.34	0.34	0.33	0.33
	5	0.33	0.33	0.33	0.33	0.34	0.33	0.33	0.33	0.36	0.34	0.34	0.33
	6	0.34	0.33	0.33	0.33	0.33	0.33	0.33	0.33	0.34	0.34	0.33	0.33
	7	0.34	0.34	0.33	0.33	0.34	0.33	0.33	0.33	0.48	0.35	0.34	0.34
	8	0.35	0.35	0.33	0.33	0.35	0.34	0.33	0.33	0.34	0.34	0.34	0.33
	9	0.34	0.33	0.33	0.33	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.33
	Average	**0.34**	**0.33**	**0.33**	**0.33**	**0.34**	**0.33**	**0.33**	**0.33**	**0.36**	**0.34**	**0.33**	**0.33**
	SD	**0.01**	**0.01**	**0.00**	**0.01**	**0.01**	**0.01**	**0.01**	**0.01**	**0.05**	**0.01**	**0.01**	**0.00**
18 °C	1	0.31	0.30	0.28	0.28	0.30	0.29	0.29	0.29	0.29	0.29	0.28	0.28
	2	0.30	0.29	0.28	0.28	0.29	0.29	0.29	0.27	0.32	0.30	0.30	0.28
	3	0.33	0.33	0.29	0.29	0.30	0.29	0.29	0.28	0.30	0.30	0.29	0.28
	4	0.32	0.32	0.31	0.31	0.34	0.29	0.29	0.29	0.32	0.29	0.28	0.28
	5	0.31	0.30	0.28	0.28	0.59	0.35	0.32	0.31	0.77	0.60	0.62	0.28
	6	0.39	0.31	0.30	0.27	0.29	0.29	0.28	0.28	0.30	0.29	0.29	0.29
	7	0.32	0.31	0.29	0.28	0.33	0.30	0.29	0.28	0.34	0.30	0.29	0.28
	8	0.33	0.33	0.33	0.33	0.34	0.33	0.33	0.33	0.34	0.33	0.33	0.33
	9	0.35	0.34	0.33	0.33	0.34	0.33	0.33	0.33	0.34	0.34	0.33	0.33
	Average	**0.33**	**0.31**	**0.30**	**0.29**	**0.35**	**0.31**	**0.30**	**0.30**	**0.37**	**0.34**	**0.33**	**0.29**	
	SD	**0.03**	**0.02**	**0.02**	**0.02**	**0.09**	**0.02**	**0.02**	**0.02**	**0.15**	**0.10**	**0.11**	**0.02**

~~*123.45*~~ = statistically excluded value

Table E8. Maltose (g/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	15.45	10.28	5.91	4.86	11.82	8.87	4.95	4.90	6.03	4.86	4.28	3.86
	2	10.66	9.49	4.43	3.87	10.52	6.40	3.94	3.90	9.64	4.07	3.93	3.89
	3	11.77	7.41	4.86	3.85	7.78	6.42	4.85	4.70	8.11	3.93	3.84	3.83
	4	12.04	7.69	4.87	4.85	8.52	6.07	3.87	3.84	7.83	3.88	3.87	3.85
	5	~~*35.47*~~	~~*34.19*~~	~~*29.67*~~	~~*11.99*~~	~~*35.62*~~	~~*32.03*~~	~~*31.18*~~	~~*6.71*~~	~~*29.70*~~	~~*27.65*~~	~~*4.44*~~	~~*3.92*~~
	6	~~*29.73*~~	~~*24.14*~~	~~*9.85*~~	~~*3.90*~~	~~*26.70*~~	~~*25.35*~~	~~*9.08*~~	~~*3.85*~~	~~*23.08*~~	~~*5.59*~~	~~*4.01*~~	~~*3.85*~~
	Average	**12.48**	**8.72**	**5.02**	**4.36**	**9.66**	**6.94**	**4.40**	**4.34**	**7.90**	**4.19**	**3.98**	**3.86**
	SD	**2.07**	**1.39**	**0.63**	**0.57**	**1.85**	**1.30**	**0.58**	**0.54**	**1.48**	**0.46**	**0.20**	**0.03**
16 °C	1	10.99	6.96	3.95	3.78	7.12	5.98	4.95	3.89	4.15	4.10	4.07	3.98
	2	10.07	8.17	4.07	4.06	8.10	5.08	4.05	4.03	4.34	4.09	4.07	3.05
	3	11.52	7.08	4.05	4.02	8.12	6.08	4.07	4.03	4.17	4.02	4.01	3.01
	4	11.02	9.90	5.94	4.31	10.03	8.09	4.17	4.10	7.41	7.09	6.26	4.02
	5	13.15	8.87	5.46	4.39	12.71	8.34	5.36	4.78	9.28	9.16	8.37	4.06
	6	11.35	9.64	4.66	3.98	9.01	7.60	6.37	3.87	9.59	9.08	6.37	4.15
	7	13.43	8.17	5.25	4.14	9.27	8.51	4.76	4.17	11.42	9.85	4.14	4.14
	Average	**11.65**	**8.40**	**4.77**	**4.10**	**9.19**	**7.10**	**4.82**	**4.12**	**7.19**	**6.77**	**5.33**	**3.77**
	SD	**1.21**	**1.15**	**0.79**	**0.21**	**1.81**	**1.36**	**0.85**	**0.31**	**3.02**	**2.66**	**1.71**	**0.51**
18 °C	1	9.08	5.05	4.91	3.90	8.49	4.91	4.01	3.91	6.18	4.93	3.97	2.90
	2	8.91	6.91	3.86	3.83	9.03	4.91	3.89	3.87	4.89	3.88	3.86	3.81
	3	7.52	5.06	3.89	3.89	8.23	4.06	3.92	3.84	6.72	4.24	3.86	3.84
	4	9.73	7.12	4.53	3.90	9.99	6.25	4.77	3.89	7.36	3.90	3.88	3.86
	5	8.96	6.95	3.93	3.89	7.95	5.93	3.91	3.89	4.09	3.98	3.94	3.88
	6	8.30	6.21	4.04	4.04	7.08	6.63	4.04	3.83	5.33	4.26	4.19	3.00
	7	7.84	4.41	4.20	4.04	8.21	4.48	4.30	3.88	4.77	4.25	4.09	4.05
	Average	**8.62**	**5.96**	**4.19**	**3.93**	**8.43**	**5.31**	**4.12**	**3.87**	**5.62**	**4.21**	**3.97**	**3.62**
	SD	**0.77**	**1.11**	**0.39**	**0.08**	**0.91**	**0.96**	**0.32**	**0.03**	**1.17**	**0.36**	**0.13**	**0.47**

~~*123.45*~~ = statistically excluded value

Table E9. Total simple sugars (g/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	17.64	12.39	7.46	6.09	13.86	10.76	6.39	6.18	8.17	6.58	5.88	~~*5.22*~~
	2	12.97	11.69	6.31	5.25	12.81	8.30	5.82	5.58	11.81	6.20	5.93	~~*5.82*~~
	3	13.46	8.90	6.34	5.14	9.51	8.09	6.24	6.07	9.90	5.46	5.27	5.22
	4	13.83	9.35	6.33	6.16	10.29	7.64	5.37	5.27	9.64	5.48	5.44	5.21
	5	~~*37.39*~~	~~*35.56*~~	~~*30.93*~~	~~*13.18*~~	~~*37.65*~~	~~*33.48*~~	~~*32.46*~~	~~*7.92*~~	~~*31.54*~~	~~*29.30*~~	~~*5.89*~~	5.10
	6	~~*31.37*~~	~~*25.62*~~	~~*11.28*~~	~~*5.17*~~	~~*28.26*~~	~~*26.62*~~	~~*10.31*~~	~~*4.91*~~	~~*25.70*~~	~~*7.35*~~	~~*5.49*~~	5.02
	Average	**14.48**	**10.58**	**6.61**	**5.66**	**11.62**	**8.70**	**5.96**	**5.78**	**9.88**	**5.93**	**5.63**	**5.14**
	SD	**2.14**	**1.72**	**0.57**	**0.54**	**2.05**	**1.40**	**0.46**	**0.43**	**1.50**	**0.55**	**0.33**	**0.10**
16 °C	1	12.22	8.08	5.01	4.77	9.21	7.56	6.15	4.98	5.53	5.20	5.11	4.91
	2	11.38	9.41	5.30	5.28	9.58	6.41	5.36	5.31	5.66	5.40	5.37	4.31
	3	12.76	8.24	5.21	5.07	9.34	7.25	5.24	5.09	5.39	5.17	5.09	4.06
	4	12.41	11.28	7.09	5.40	11.32	9.26	5.28	5.18	8.59	8.27	7.39	5.15
	5	14.24	9.96	6.41	5.34	13.81	9.40	6.40	5.77	10.26	10.10	9.31	4.99
	6	12.86	11.00	5.96	5.27	10.26	8.77	7.45	4.93	11.69	10.72	7.99	5.09
	7	15.06	9.66	6.62	5.47	10.72	9.91	6.08	5.44	12.83	11.26	5.46	5.34
	Average	**12.99**	**9.66**	**5.94**	**5.23**	**10.61**	**8.37**	**5.99**	**5.24**	**8.56**	**8.02**	**6.53**	**4.84**
	SD	**1.25**	**1.23**	**0.80**	**0.24**	**1.61**	**1.30**	**0.80**	**0.29**	**3.12**	**2.74**	**1.69**	**0.47**
18 °C	1	11.13	6.98	6.72	5.55	10.53	6.82	5.80	5.72	8.48	6.91	5.83	4.20
	2	10.67	8.64	5.50	5.39	12.55	11.38	5.75	5.43	9.61	6.31	5.96	5.28
	3	9.54	6.93	5.69	5.51	10.82	6.58	5.52	5.56	6.52	5.49	5.44	5.46
	4	12.53	9.74	6.74	5.00	10.14	5.88	5.69	5.28	8.76	5.28	5.12	4.69
	5	10.27	8.12	5.05	4.88	9.22	7.16	5.00	4.93	5.37	5.09	4.98	4.87
	6	9.68	7.56	5.28	5.26	8.40	7.89	5.28	5.01	7.63	5.48	5.41	4.20
	7	9.35	5.89	5.59	5.41	9.68	5.92	5.71	5.26	6.29	5.65	5.47	5.36
	Average	**10.45**	**7.69**	**5.80**	**5.29**	**10.19**	**7.38**	**5.54**	**5.31**	**7.52**	**5.74**	**5.46**	**4.87**
	SD	**1.12**	**1.27**	**0.67**	**0.26**	**1.32**	**1.90**	**0.30**	**0.28**	**1.53**	**0.64**	**0.35**	**0.53**

~~*123.45*~~ = statistically excluded value

Table E10. Ethanol (% v/v) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	4.47	4.64	5.29	5.35	4.60	4.82	5.16	5.17	4.69	5.05	5.46	5.66
	2	4.36	4.50	5.00	5.11	4.49	4.82	4.94	5.11	4.70	4.89	~~4.95~~	5.25
	3	~~5.11~~	5.26	~~5.54~~	~~5.59~~	~~5.27~~	~~5.43~~	~~5.53~~	~~5.67~~	~~5.39~~	~~5.42~~	5.53	~~5.71~~
	4	5.09	~~5.28~~	5.32	5.34	5.18	5.24	5.44	5.59	5.19	5.26	5.48	5.63
	5	~~3.92~~	~~4.00~~	~~4.38~~	~~4.65~~	~~4.18~~	~~4.37~~	~~4.54~~	~~4.75~~	~~4.21~~	~~4.74~~	5.31	5.48
	6	4.05	4.44	5.02	5.44	4.36	4.46	5.12	5.60	4.67	5.28	~~5.57~~	~~5.79~~
	Average	**4.49**	**4.71**	**5.16**	**5.31**	**4.66**	**4.84**	**5.17**	**5.37**	**4.81**	**5.12**	**5.45**	**5.51**
	SD	**0.44**	**0.38**	**0.17**	**0.14**	**0.36**	**0.32**	**0.21**	**0.26**	**0.25**	**0.19**	**0.09**	**0.19**
16 °C	1	5.18	5.33	5.43	5.47	5.23	5.40	5.42	5.51	5.29	5.33	5.38	5.65
	2	5.01	5.23	5.37	5.51	5.11	5.13	5.26	5.60	5.28	5.35	5.53	5.67
	3	4.45	4.77	5.30	5.37	4.68	4.89	4.92	5.15	5.08	5.14	5.27	5.35
	4	4.35	4.39	4.77	4.86	4.35	4.53	4.66	5.13	4.30	4.62	4.84	5.16
	5	4.33	4.36	4.38	5.32	4.64	4.65	5.28	5.40	4.83	4.83	5.18	5.48
	6	4.59	4.60	4.62	5.03	4.68	4.73	4.89	5.19	4.66	4.73	5.01	5.21
	7	4.09	4.16	4.28	4.31	4.21	4.28	4.38	4.73	4.27	4.48	5.04	5.19
	Average	**4.57**	**4.69**	**4.88**	**5.12**	**4.70**	**4.80**	**4.97**	**5.24**	**4.82**	**4.93**	**5.18**	**5.39**
	SD	**0.39**	**0.45**	**0.48**	**0.43**	**0.37**	**0.38**	**0.37**	**0.29**	**0.43**	**0.35**	**0.24**	**0.22**
18 °C	1	4.61	5.02	5.29	5.31	5.04	5.19	5.22	5.31	5.10	5.15	5.31	5.36
	2	4.72	5.34	5.27	5.34	4.82	4.96	5.17	5.29	5.38	5.10	5.15	5.40
	3	5.22	5.39	5.38	5.44	5.21	5.30	5.32	5.34	5.34	5.43	5.64	5.66
	4	5.10	4.97	5.11	5.30	5.20	5.04	5.13	5.31	5.08	5.32	5.65	5.71
	5	4.73	4.86	4.97	5.05	5.01	4.96	5.36	5.47	5.06	5.42	5.60	5.73
	6	5.13	5.32	5.36	5.40	5.31	5.40	5.43	5.54	5.33	5.37	5.39	5.65
	7	5.18	5.23	5.32	5.38	5.24	5.27	5.40	5.47	5.29	5.55	5.57	5.51
	Average	**4.96**	**5.16**	**5.24**	**5.32**	**5.12**	**5.16**	**5.29**	**5.39**	**5.23**	**5.33**	**5.47**	**5.57**
	SD	**0.26**	**0.21**	**0.15**	**0.13**	**0.17**	**0.18**	**0.12**	**0.10**	**0.14**	**0.16**	**0.19**	**0.15**

~~123.45~~ = statistically excluded value

Table E11. Propanol (mg/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	14.00	15.68	15.81	16.89	13.34	14.44	17.26	17.30	15.68	16.46	16.87	16.98
	2	13.84	16.76	19.06	19.14	16.52	17.19	18.08	18.07	16.58	16.78	17.29	18.26
	3	19.43	20.12	22.35	22.98	15.41	18.13	19.52	21.59	18.17	19.63	20.03	22.32
	4	18.01	18.76	19.56	21.25	18.05	18.48	19.97	21.23	18.97	20.94	21.33	21.02
	5	11.88	12.74	14.08	15.14	13.13	13.34	13.87	13.92	11.94	12.80	14.88	15.02
	6	13.27	14.57	16.43	16.59	14.72	14.75	14.93	17.55	15.75	16.19	16.40	17.04
	Average	**15.07**	**16.44**	**17.88**	**18.67**	**15.20**	**16.06**	**17.27**	**18.28**	**16.18**	**17.13**	**17.80**	**18.44**
	SD	**2.96**	**2.71**	**3.00**	**3.01**	**1.89**	**2.15**	**2.45**	**2.84**	**2.46**	**2.86**	**2.41**	**2.74**
16 °C	1	15.44	15.76	16.38	16.80	15.30	15.68	15.71	16.32	15.17	15.72	16.27	16.76
	2	16.21	17.38	17.73	17.76	14.93	16.98	18.54	21.63	16.29	16.53	17.06	17.34
	3	15.93	16.73	18.92	19.53	16.96	17.27	17.24	17.56	16.97	18.32	18.95	19.29
	4	13.10	13.31	15.86	16.02	12.98	15.01	15.69	17.76	15.88	15.99	16.45	16.52
	5	11.47	11.92	12.02	12.27	11.91	12.13	12.18	12.97	10.60	12.43	12.84	14.36
	6	12.32	12.36	13.42	14.21	12.35	12.41	14.05	15.17	11.57	12.13	13.59	15.24
	7	14.03	16.02	16.19	17.03	15.21	15.27	15.60	17.10	14.78	15.76	16.50	17.63
	8	13.74	15.04	15.13	15.47	14.37	14.50	14.64	14.80	14.93	15.04	15.57	15.84
	9	15.25	15.87	16.21	16.96	15.16	15.76	17.40	19.23	16.13	17.24	17.36	17.82
	Average	**14.17**	**14.93**	**15.76**	**16.23**	**14.35**	**15.00**	**15.67**	**16.95**	**14.70**	**15.46**	**16.07**	**16.76**
	SD	**1.66**	**1.95**	**2.08**	**2.10**	**1.63**	**1.78**	**1.92**	**2.55**	**2.18**	**2.04**	**1.88**	**1.48**
18 °C	1	17.66	18.44	18.61	18.92	17.03	19.15	19.31	19.62	16.64	17.10	18.76	18.82
	2	15.46	16.80	18.06	18.32	18.64	19.77	19.96	20.91	18.16	18.72	18.83	18.84
	3	24.01	26.82	29.04	37.49	26.80	31.79	31.40	36.24	25.22	28.38	29.68	29.93
	4	25.94	27.00	28.44	31.34	26.28	26.76	28.90	29.32	23.08	28.11	28.45	29.59
	5	19.99	23.53	20.38	20.37	18.85	19.46	19.88	22.34	19.82	20.51	20.58	21.66
	6	14.35	15.23	17.28	17.42	16.00	16.54	16.86	17.92	17.75	18.77	18.33	22.18
	7	18.29	18.41	18.62	19.72	16.58	17.61	17.85	19.94	14.08	16.70	17.32	23.53
	8	16.72	16.98	17.54	17.79	16.14	16.43	17.16	18.62	16.14	16.74	17.16	17.61
	9	18.78	19.24	20.24	21.61	18.53	18.82	19.66	19.97	20.12	20.34	20.63	21.16
	Average	**19.02**	**20.27**	**20.91**	**22.55**	**19.43**	**20.70**	**21.22**	**22.76**	**19.00**	**20.60**	**21.08**	**22.59**
	SD	**3.81**	**4.40**	**4.57**	**7.02**	**4.18**	**5.16**	**5.23**	**6.07**	**3.49**	**4.56**	**4.69**	**4.47**

~~*123.45*~~ = statistically excluded value

Table E12. Ethyl acetate (mg/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	35.28	24.57	23.34	21.14	33.62	25.55	~~21.23~~	21.21	35.11	26.68	24.93	23.35
	2	~~57.16~~	32.99	23.77	21.66	40.90	34.14	33.00	32.59	~~59.46~~	41.62	38.81	38.13
	3	40.74	39.99	39.27	34.93	38.03	35.29	33.92	33.31	38.96	38.80	38.06	37.03
	4	35.70	35.57	35.13	31.82	39.72	39.19	38.27	37.27	40.33	38.07	37.61	34.77
	5	~~21.12~~	~~15.07~~	~~13.51~~	~~13.07~~	~~19.14~~	~~16.30~~	~~16.15~~	~~15.85~~	~~22.57~~	~~21.05~~	~~19.10~~	~~14.85~~
	6	25.69	~~22.67~~	~~17.30~~	~~16.83~~	~~24.20~~	~~24.08~~	21.48	~~19.40~~	24.46	~~22.55~~	~~22.11~~	~~20.53~~
	Average	**34.35**	**33.28**	**30.38**	**27.39**	**38.07**	**33.54**	**31.67**	**31.10**	**34.72**	**36.29**	**34.85**	**33.32**
	SD	**6.29**	**6.49**	**8.06**	**7.03**	**3.19**	**5.75**	**7.17**	**6.90**	**7.18**	**6.59**	**6.63**	**6.79**
16 °C	1	28.47	26.24	25.65	25.31	27.32	25.85	25.62	23.92	25.37	25.20	24.96	22.00
	2	35.12	33.17	32.63	28.49	31.03	31.12	21.22	25.76	32.62	30.69	29.14	27.07
	3	31.65	29.93	27.71	26.90	28.53	27.64	27.96	19.69	29.93	30.84	26.78	26.23
	4	27.61	24.54	23.95	20.61	21.88	28.18	27.09	19.49	27.07	24.39	22.95	22.90
	5	22.24	22.14	20.77	19.95	25.49	21.77	23.23	22.93	26.86	26.04	22.88	22.36
	6	24.27	23.60	21.21	20.42	24.23	24.57	20.47	19.96	26.67	26.11	22.57	20.83
	7	27.79	21.38	24.25	19.29	31.38	29.74	24.64	21.94	30.46	29.47	27.67	24.19
	Average	**28.16**	**26.56**	**25.17**	**23.59**	**27.12**	**26.98**	**24.32**	**21.96**	**28.43**	**27.53**	**25.28**	**23.65**
	SD	**4.31**	**3.82**	**4.08**	**3.42**	**3.51**	**3.18**	**2.84**	**2.40**	**2.60**	**2.72**	**2.63**	**2.29**
18 °C	1	34.30	31.87	30.68	29.90	35.82	34.60	33.46	28.66	37.11	36.16	33.98	29.94
	2	31.30	29.88	29.15	31.32	34.37	32.58	32.16	31.87	35.38	33.38	33.08	32.48
	3	35.91	32.34	31.52	32.82	40.78	36.23	35.16	34.65	36.14	34.19	34.08	31.89
	4	27.01	25.99	33.61	25.65	40.13	~~39.97~~	~~39.31~~	~~38.66~~	30.66	30.32	26.12	33.15
	5	29.53	27.66	27.45	26.59	30.86	28.10	28.05	27.34	33.07	32.43	30.31	27.35
	6	33.05	32.58	31.94	26.11	37.05	28.70	26.71	24.51	33.81	38.04	27.98	29.15
	7	30.85	29.94	29.48	25.27	31.95	30.03	28.84	27.49	31.05	30.51	29.86	26.71
	Average	**31.71**	**30.04**	**30.55**	**28.24**	**35.85**	**31.71**	**30.73**	**29.09**	**33.89**	**33.58**	**30.77**	**30.10**
	SD	**3.00**	**2.49**	**2.04**	**3.06**	**3.79**	**3.30**	**3.35**	**3.62**	**2.48**	**2.84**	**3.08**	**2.52**

~~123.45~~ = statistically excluded value

Table E13. Isoamyl alcohol (mg/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	74.03	75.98	81.56	90.53	~~49.63~~	~~76.42~~	~~85.28~~	98.35	~~75.17~~	~~80.76~~	~~85.12~~	101.53
	2	71.02	84.52	93.17	102.09	~~88.24~~	89.60	98.08	102.91	89.09	91.94	96.21	102.58
	3	~~95.20~~	~~112.83~~	~~118.19~~	~~128.27~~	80.06	89.02	~~109.03~~	~~114.15~~	~~106.36~~	~~117.14~~	~~118.13~~	~~119.55~~
	4	~~88.37~~	~~99.61~~	~~100.70~~	~~114.30~~	86.41	87.33	90.77	111.14	88.79	90.32	93.68	~~121.01~~
	5	74.90	80.18	81.10	90.34	83.49	83.78	88.71	~~89.23~~	78.64	85.65	99.94	103.59
	6	77.05	80.98	83.93	96.24	82.19	~~83.50~~	93.40	96.75	77.71	88.66	94.45	107.39
	Average	**74.25**	**80.42**	**84.94**	**94.80**	**83.04**	**87.43**	**92.74**	**102.29**	**83.56**	**89.14**	**96.07**	**103.77**
	SD	**2.50**	**3.51**	**5.63**	**5.58**	**2.66**	**2.62**	**4.04**	**6.45**	**6.23**	**2.69**	**2.79**	**2.55**
16 °C	1	74.68	80.86	87.37	94.77	78.32	86.68	87.51	94.36	79.83	89.24	89.57	89.92
	2	75.46	97.93	98.22	104.19	76.40	79.88	97.70	109.54	75.69	96.25	100.31	103.47
	3	76.10	81.80	84.45	103.08	68.66	87.70	86.66	100.57	69.30	88.27	93.06	95.91
	4	76.07	80.19	81.22	86.83	74.90	76.15	86.78	81.83	77.69	77.84	81.32	83.72
	5	73.33	79.68	82.01	83.40	76.38	78.06	86.79	94.82	71.61	80.12	83.74	89.43
	6	75.88	80.65	82.94	85.28	76.08	77.15	77.85	89.46	78.57	80.83	84.74	84.95
	7	79.13	85.47	94.84	97.84	82.44	88.21	93.05	94.70	76.84	89.44	91.23	95.51
	Average	**75.81**	**83.80**	**87.29**	**93.63**	**76.17**	**81.98**	**88.05**	**95.04**	**75.65**	**86.00**	**89.14**	**91.84**
	SD	**1.77**	**6.52**	**6.69**	**8.57**	**4.12**	**5.33**	**6.16**	**8.63**	**3.83**	**6.59**	**6.51**	**6.93**
18 °C	1	70.67	88.30	97.05	100.54	92.47	96.59	97.27	98.84	75.37	80.69	98.98	106.66
	2	78.58	81.96	82.04	93.28	85.82	91.58	92.84	145.68	74.14	91.13	125.92	130.93
	3	78.61	103.42	95.52	96.08	109.85	126.36	133.61	99.80	89.17	110.57	99.19	100.76
	4	75.45	88.81	83.95	95.15	72.43	91.17	99.72	103.58	79.91	93.78	101.01	118.37
	5	87.59	83.62	97.62	100.58	72.74	86.99	93.58	101.21	92.53	99.67	90.45	91.78
	6	79.82	86.64	96.56	106.81	78.77	97.14	101.56	106.52	83.60	93.91	99.02	102.96
	7	75.39	92.12	94.14	100.34	66.34	71.52	84.54	97.20	70.02	78.06	87.40	96.85
	Average	**78.02**	**89.27**	**92.41**	**98.97**	**82.63**	**94.48**	**100.45**	**107.55**	**80.68**	**92.54**	**100.28**	**106.90**
	SD	**5.21**	**7.09**	**6.55**	**4.54**	**14.89**	**16.48**	**15.65**	**17.10**	**8.23**	**11.04**	**12.42**	**13.49**

~~123.45~~ = statistically excluded value

Table E14. Isoamyl acetate (mg/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	0.37	~~0.32~~	0.33	0.32	0.38	~~0.34~~	~~0.33~~	~~0.31~~	0.39	~~0.38~~	~~0.39~~	0.35
	2	0.45	0.45	0.39	0.37	0.64	0.57	0.43	0.38	0.57	~~0.60~~	~~0.36~~	~~0.32~~
	3	~~1.05~~	0.49	~~0.55~~	~~0.43~~	~~0.77~~	0.46	0.45	0.42	~~0.88~~	0.49	0.40	0.39
	4	~~0.86~~	~~0.56~~	0.49	0.41	~~0.88~~	~~0.53~~	~~0.50~~	0.47	~~0.80~~	0.52	0.41	0.38
	5	0.46	0.33	~~0.31~~	~~0.31~~	0.34	0.35	0.45	~~0.31~~	0.56	0.46	0.39	~~0.30~~
	6	0.53	0.50	0.44	0.42	0.57	0.52	0.49	0.41	0.57	0.50	0.46	0.44
	Average	**0.45**	**0.44**	**0.41**	**0.38**	**0.48**	**0.48**	**0.46**	**0.42**	**0.52**	**0.49**	**0.42**	**0.39**
	SD	**0.07**	**0.08**	**0.07**	**0.05**	**0.15**	**0.09**	**0.03**	**0.04**	**0.09**	**0.02**	**0.03**	**0.04**
16 °C	1	0.40	0.42	0.34	0.34	0.39	0.41	0.38	0.38	0.40	0.36	0.34	0.32
	2	0.40	0.39	0.34	0.32	0.50	0.43	0.35	0.34	0.37	0.36	0.32	0.29
	3	0.41	~~0.96~~	0.34	~~0.90~~	0.39	0.40	~~0.99~~	~~0.81~~	~~0.92~~	~~0.82~~	~~0.77~~	~~0.70~~
	4	~~1.00~~	0.69	~~0.93~~	0.61	0.67	~~1.06~~	0.62	0.60	0.67	0.66	0.65	0.64
	5	0.63	0.66	0.61	0.59	~~0.98~~	0.57	0.53	0.52	0.60	0.59	0.56	0.50
	6	0.65	0.61	0.57	0.56	0.59	0.59	0.56	0.44	0.59	0.61	0.58	0.52
	7	0.64	0.69	0.63	0.63	0.61	0.69	0.62	0.59	0.65	0.60	0.55	0.34
	Average	**0.52**	**0.58**	**0.47**	**0.51**	**0.53**	**0.52**	**0.51**	**0.48**	**0.55**	**0.53**	**0.50**	**0.44**
	SD	**0.13**	**0.14**	**0.15**	**0.14**	**0.12**	**0.12**	**0.12**	**0.11**	**0.13**	**0.13**	**0.14**	**0.14**
18 °C	1	0.54	0.51	0.37	0.33	0.61	0.58	0.33	0.31	0.46	0.44	0.31	0.31
	2	0.48	~~0.41~~	0.38	0.36	0.52	0.43	0.43	0.36	0.48	0.37	0.34	0.32
	3	~~1.06~~	0.51	0.43	0.37	~~0.97~~	0.55	0.45	0.44	~~1.09~~	0.49	0.40	0.37
	4	~~1.14~~	0.60	0.57	0.44	~~1.27~~	~~0.89~~	0.64	0.60	~~1.22~~	0.56	0.50	0.45
	5	0.64	0.63	~~0.61~~	0.53	0.55	0.52	0.51	0.50	0.52	0.50	0.46	0.37
	6	0.68	0.59	0.56	0.54	0.58	0.56	0.52	0.52	0.64	0.63	0.61	0.59
	7	0.36	0.36	0.33	~~0.32~~	0.35	0.34	~~0.32~~	~~0.29~~	0.31	~~0.28~~	~~0.27~~	~~0.26~~
	Average	**0.54**	**0.53**	**0.44**	**0.43**	**0.54**	**0.53**	**0.44**	**0.43**	**0.48**	**0.50**	**0.44**	**0.40**
	SD	**0.13**	**0.10**	**0.10**	**0.09**	**0.13**	**0.10**	**0.10**	**0.09**	**0.12**	**0.09**	**0.11**	**0.10**

~~123.45~~ = statistically excluded value

Table E15. Ethyl hexanoate (mg/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	0.50	~~0.46~~	0.46	~~0.45~~	0.51	~~0.48~~	~~0.48~~	~~0.47~~	0.52	~~0.49~~	0.50	0.49
	2	0.55	~~0.55~~	0.53	~~0.51~~	0.61	0.59	0.55	0.54	0.59	0.55	0.51	0.52
	3	~~0.65~~	0.52	~~0.56~~	0.50	~~0.62~~	0.53	0.54	0.51	~~0.63~~	0.53	~~0.53~~	~~0.54~~
	4	~~0.60~~	0.52	~~0.55~~	0.50	0.59	0.53	0.53	0.49	~~0.60~~	0.52	0.50	0.51
	5	0.50	0.48	0.45	0.45	~~0.47~~	~~0.47~~	~~0.48~~	~~0.44~~	0.51	~~0.48~~	~~0.47~~	~~0.44~~
	6	0.52	0.51	0.51	0.48	0.53	0.52	0.51	0.48	0.53	0.55	0.51	0.46
	Average	**0.52**	**0.51**	**0.49**	**0.48**	**0.56**	**0.54**	**0.53**	**0.51**	**0.54**	**0.54**	**0.51**	**0.50**
	SD	**0.02**	**0.02**	**0.04**	**0.02**	**0.05**	**0.03**	**0.02**	**0.03**	**0.04**	**0.02**	**0.01**	**0.03**
16 °C	1	0.51	0.50	0.48	0.48	0.51	0.53	0.49	0.48	0.51	0.51	0.50	0.45
	2	0.49	0.49	0.48	0.46	0.48	0.48	0.47	0.45	0.48	0.47	0.49	0.46
	3	0.49	0.49	0.49	0.48	0.50	0.50	0.49	0.47	0.51	0.50	0.47	0.47
	4	0.50	0.49	0.51	0.51	0.48	0.48	0.51	0.49	0.53	0.53	0.52	0.46
	5	0.50	0.50	0.49	0.49	0.56	0.56	0.50	0.49	0.53	0.51	0.50	0.50
	6	0.51	0.49	0.48	0.47	0.48	0.47	0.47	0.47	0.52	0.48	0.48	0.46
	7	0.50	0.50	0.49	0.48	0.50	0.49	0.49	0.48	0.49	0.49	0.49	0.46
	Average	**0.50**	**0.49**	**0.49**	**0.48**	**0.50**	**0.50**	**0.49**	**0.48**	**0.51**	**0.50**	**0.49**	**0.47**
	SD	**0.01**	**0.01**	**0.01**	**0.02**	**0.03**	**0.03**	**0.01**	**0.01**	**0.02**	**0.02**	**0.02**	**0.02**
18 °C	1	0.51	0.52	0.55	0.48	0.54	0.53	0.48	0.47	0.52	0.51	0.51	0.47
	2	0.53	0.52	0.51	0.49	0.57	0.57	0.52	0.50	0.60	0.54	0.52	0.49
	3	0.59	0.51	0.54	0.47	0.57	0.55	0.54	0.54	0.61	0.54	0.55	0.52
	4	0.53	0.52	0.50	0.48	0.51	0.51	0.56	0.58	0.50	0.51	0.49	0.46
	5	0.58	0.52	0.50	0.47	0.54	0.51	0.49	0.48	0.64	0.56	0.53	0.46
	6	0.56	0.54	0.54	0.50	0.55	0.53	0.51	0.49	0.52	0.50	0.50	0.49
	7	0.58	0.55	0.50	0.51	0.55	0.52	0.52	0.49	0.53	0.55	0.51	0.49
	Average	**0.55**	**0.53**	**0.52**	**0.49**	**0.55**	**0.53**	**0.52**	**0.51**	**0.56**	**0.53**	**0.52**	**0.49**
	SD	**0.03**	**0.01**	**0.02**	**0.02**	**0.02**	**0.02**	**0.03**	**0.04**	**0.06**	**0.02**	**0.02**	**0.02**

~~*123.45*~~ = statistically excluded value

Table E16. Ethyl octanoate (mg/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	~~0.19~~	~~0.15~~	~~0.11~~	~~0.07~~	~~0.26~~	~~0.25~~	~~0.24~~	~~0.23~~	0.39	0.36	0.34	0.30
	2	0.45	0.44	~~0.28~~	~~0.28~~	0.52	0.48	0.43	0.40	0.47	0.46	0.40	0.34
	3	~~0.87~~	0.59	0.49	0.35	~~0.78~~	~~0.62~~	~~0.62~~	0.52	~~1.07~~	~~0.67~~	~~0.68~~	0.44
	4	~~0.96~~	~~0.81~~	0.53	0.34	0.56	0.47	0.42	0.32	0.63	0.45	0.31	0.36
	5	0.44	0.37	0.35	0.34	0.42	0.45	0.38	~~0.14~~	~~0.28~~	~~0.26~~	~~0.20~~	~~0.12~~
	6	0.52	0.46	0.45	0.27	0.59	0.51	0.49	~~0.24~~	0.59	0.54	0.34	~~0.19~~
	Average	**0.47**	**0.47**	**0.46**	**0.33**	**0.52**	**0.48**	**0.43**	**0.41**	**0.52**	**0.45**	**0.35**	**0.36**
	SD	**0.04**	**0.09**	**0.08**	**0.04**	**0.07**	**0.03**	**0.05**	**0.10**	**0.11**	**0.07**	**0.04**	**0.06**
16 °C	1	0.26	0.23	0.19	0.17	0.32	0.37	0.24	0.25	0.34	0.34	0.23	0.16
	2	0.31	0.27	~~0.11~~	~~0.07~~	~~0.79~~	~~0.85~~	~~0.10~~	~~0.09~~	0.30	0.27	0.17	~~0.62~~
	3	0.24	0.21	0.35	0.33	0.46	0.43	~~0.68~~	0.58	~~0.81~~	~~0.74~~	~~0.64~~	0.28
	4	~~0.77~~	0.46	~~0.62~~	0.54	~~0.73~~	~~0.67~~	0.34	0.25	0.44	0.44	0.30	0.43
	5	0.48	~~0.80~~	0.35	0.32	0.41	0.38	0.46	0.44	0.39	0.37	0.30	0.25
	6	0.32	0.27	0.21	0.19	0.29	0.17	0.34	0.27	0.36	0.25	0.25	0.20
	7	0.46	0.44	0.44	0.33	0.46	0.44	0.33	0.24	0.49	0.49	0.31	0.28
	Average	**0.35**	**0.31**	**0.31**	**0.31**	**0.39**	**0.36**	**0.34**	**0.34**	**0.39**	**0.36**	**0.26**	**0.27**
	SD	**0.10**	**0.11**	**0.11**	**0.13**	**0.08**	**0.11**	**0.08**	**0.14**	**0.07**	**0.09**	**0.05**	**0.09**
18 °C	1	0.20	0.18	0.17	~~0.12~~	0.24	0.34	0.58	~~0.19~~	0.51	~~0.17~~	0.27	~~0.13~~
	2	0.49	0.44	0.43	0.39	0.40	0.63	~~0.75~~	0.67	0.47	0.39	0.32	0.21
	3	0.29	0.19	0.17	0.19	~~0.96~~	~~0.84~~	0.40	0.37	~~1.00~~	0.43	~~0.80~~	0.26
	4	0.60	0.53	0.38	0.32	0.56	0.43	0.46	0.37	0.54	~~0.83~~	0.42	~~0.77~~
	5	0.21	~~0.13~~	0.54	0.51	0.59	0.51	~~0.12~~	~~0.08~~	~~0.82~~	0.49	0.50	0.26
	6	0.54	0.49	~~0.08~~	0.40	0.65	0.56	0.55	0.41	0.20	~~0.17~~	~~0.12~~	~~0.09~~
	7	~~0.75~~	~~0.67~~	0.47	0.40	0.58	0.45	0.43	0.39	0.53	0.47	0.53	0.21
	Average	**0.39**	**0.37**	**0.36**	**0.37**	**0.50**	**0.49**	**0.48**	**0.44**	**0.45**	**0.45**	**0.41**	**0.24**
	SD	**0.18**	**0.17**	**0.16**	**0.11**	**0.15**	**0.10**	**0.08**	**0.13**	**0.14**	**0.04**	**0.11**	**0.03**

~~123.45~~ = statistically excluded value

Table E17. Total ester (mg/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	36.34	25.50	24.24	21.98	34.77	26.62	22.28	22.22	36.41	27.91	26.16	24.49
	2	~~58.61~~	34.43	24.97	22.82	~~42.67~~	35.78	34.41	33.91	61.09	43.23	40.08	39.31
	3	~~43.31~~	~~41.59~~	~~40.87~~	~~36.21~~	40.20	36.90	35.53	34.76	41.54	40.49	39.67	38.40
	4	38.12	37.46	36.70	33.07	41.75	~~40.72~~	39.72	38.55	42.36	39.56	38.83	36.02
	5	22.52	~~16.25~~	~~14.62~~	~~14.17~~	~~20.37~~	~~17.57~~	~~17.46~~	~~16.74~~	23.92	22.25	20.16	15.71
	6	27.26	24.14	18.70	18.00	25.89	25.63	22.97	20.53	26.15	24.14	23.42	21.62
	Average	**31.06**	**30.38**	**26.15**	**23.97**	**35.65**	**31.23**	**30.98**	**29.99**	**36.62**	**33.03**	**32.02**	**30.13**
	SD	**7.42**	**6.56**	**7.57**	**6.42**	**7.16**	**5.93**	**7.89**	**8.08**	**7.46**	**8.24**	**8.43**	**8.31**
16 °C	1	29.64	27.39	26.66	26.27	28.51	27.16	26.69	24.95	26.56	26.39	26.01	22.87
	2	~~36.32~~	~~34.30~~	~~33.61~~	~~29.38~~	~~39.68~~	~~32.16~~	22.17	26.68	33.66	31.65	30.01	27.92
	3	32.76	31.00	28.64	27.75	32.17	28.71	28.95	27.40	~~43.91~~	31.97	27.76	27.15
	4	29.35	26.20	25.54	~~20.47~~	31.06	30.67	29.27	21.57	32.21	26.48	24.89	24.74
	5	24.07	23.78	~~22.22~~	22.01	23.49	23.41	22.34	~~20.79~~	28.65	27.61	24.30	23.74
	6	~~22.71~~	25.83	22.35	21.76	27.76	26.69	25.03	24.60	28.76	27.93	24.27	22.45
	7	23.29	22.96	22.38	21.82	25.70	22.50	~~21.81~~	21.22	~~23.53~~	~~23.27~~	~~22.71~~	~~19.62~~
	8	25.72	~~22.75~~	22.47	20.51	23.05	~~21.75~~	~~21.22~~	~~12.85~~	28.14	~~26.45~~	~~23.82~~	~~21.77~~
	9	29.39	27.95	25.81	24.88	~~33.06~~	31.36	26.08	23.25	32.09	31.05	29.02	25.27
	Average	**27.75**	**26.44**	**24.84**	**23.57**	**27.39**	**27.21**	**25.79**	**24.24**	**30.01**	**29.01**	**26.61**	**24.88**
	SD	**3.45**	**2.70**	**2.49**	**2.73**	**3.53**	**3.37**	**2.85**	**2.37**	**2.62**	**2.46**	**2.34**	**2.07**
18 °C	1	35.55	33.08	31.77	30.83	37.21	35.91	34.46	29.63	~~98.71~~	~~67.22~~	39.14	30.85
	2	32.80	31.25	30.47	~~25.60~~	35.86	33.92	33.41	33.02	38.70	37.46	35.11	33.50
	3	~~38.30~~	34.01	33.05	32.35	~~42.99~~	37.96	36.73	35.91	37.55	34.84	34.35	33.04
	4	~~40.19~~	~~37.88~~	34.85	33.88	~~43.06~~	~~42.36~~	~~41.26~~	40.51	39.08	36.18	35.95	34.94
	5	28.11	~~27.67~~	27.43	26.97	29.77	29.56	29.45	28.69	32.22	31.82	27.49	28.44
	6	28.87	27.73	~~24.98~~	~~23.29~~	32.57	~~28.58~~	28.17	25.87	35.17	34.38	31.95	30.66
	7	30.60	28.63	28.32	27.43	38.08	29.75	~~25.74~~	~~24.90~~	~~63.15~~	38.96	~~26.93~~	~~22.54~~
	8	35.61	34.98	34.23	28.21	34.46	32.28	31.05	29.40	36.02	31.67	29.50	28.11
	9	33.13	32.10	31.44	27.01	31.19	30.53	28.75	~~26.36~~	32.95	32.53	31.63	28.39
	Average	**32.10**	**31.68**	**31.45**	**29.53**	**34.16**	**32.84**	**31.72**	**31.86**	**35.96**	**34.73**	**33.14**	**30.99**
	SD	**3.01**	**2.69**	**2.64**	**2.82**	**3.12**	**3.23**	**3.23**	**4.99**	**2.69**	**2.68**	**3.74**	**2.61**

~~123.45~~ = statistically excluded value

Table E18. Total fusel alcohol (mg/l) content in beer stored over a period of 12 weeks.

	Storage Temperature	0 °C				4 °C				18 °C			
	Weeks	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**	**3**	**6**	**9**	**12**
Fermentation	**Batch**												
14 °C	1	88.03	91.66	97.37	107.42	62.97	90.86	102.54	115.65	90.85	97.22	101.99	118.51
	2	84.86	101.28	112.23	121.23	104.76	106.79	116.16	120.98	105.67	108.72	113.50	120.84
	3	114.63	132.95	140.54	151.25	95.47	107.15	128.55	135.74	124.53	136.77	138.16	141.87
	4	106.38	118.37	120.26	135.55	104.46	105.81	110.74	132.37	107.76	111.26	115.01	142.03
	5	86.78	92.92	95.18	105.48	96.62	97.12	102.58	103.15	90.58	98.45	114.82	118.61
	6	90.32	95.55	100.36	112.83	96.91	98.25	108.33	114.30	93.46	104.85	110.85	124.43
	Average	**95.17**	**105.46**	**110.99**	**122.29**	**93.53**	**101.00**	**111.48**	**120.37**	**102.14**	**109.55**	**115.72**	**127.72**
	SD	**12.29**	**16.65**	**17.37**	**17.94**	**15.52**	**6.63**	**9.83**	**12.13**	**13.28**	**14.44**	**12.02**	**11.23**
16 °C	1	90.12	96.62	103.75	111.57	82.77	102.36	103.22	110.68	95.00	104.96	105.84	106.68
	2	91.67	112.96	119.20	121.95	93.25	96.86	116.24	132.76	111.36	112.78	117.37	120.81
	3	112.91	114.66	117.14	124.95	113.61	115.47	119.23	127.10	92.66	117.99	123.10	136.84
	4	89.20	95.11	100.31	119.10	89.38	102.71	102.35	118.33	85.18	104.26	109.51	112.43
	5	87.54	92.11	93.24	94.02	80.57	86.90	89.55	94.80	62.68	90.27	92.51	96.98
	6	85.65	86.05	92.41	101.04	87.25	88.56	100.83	109.99	89.26	92.25	94.91	98.96
	7	89.91	95.70	98.20	100.43	91.59	93.33	102.39	106.56	86.39	87.82	100.24	107.06
	8	92.87	95.69	98.07	100.75	90.45	91.65	92.49	92.70	93.50	95.87	100.31	100.79
	9	99.18	101.34	111.05	114.80	97.60	103.97	110.45	113.93	92.97	106.68	108.59	113.33
	Average	**93.23**	**98.92**	**103.71**	**109.85**	**91.83**	**97.98**	**104.08**	**111.87**	**89.89**	**101.43**	**105.82**	**110.43**
	SD	**8.31**	**9.38**	**9.91**	**11.11**	**9.67**	**9.07**	**9.88**	**13.26**	**12.71**	**10.46**	**10.07**	**12.49**
18 °C	1	~~*88.33*~~	106.74	115.66	~~*119.46*~~	109.50	115.74	116.58	118.46	92.01	97.79	~~*105.57*~~	~~*107.81*~~
	2	94.04	~~*98.76*~~	100.10	111.60	104.46	111.35	112.80	~~*116.30*~~	105.67	108.72	113.50	120.84
	3	118.47	130.24	~~*158.92*~~	~~*183.84*~~	136.65	~~*158.76*~~	~~*165.01*~~	~~*181.92*~~	~~*144.62*~~	~~*162.82*~~	~~*178.53*~~	~~*181.36*~~
	4	~~*127.79*~~	~~*151.24*~~	154.78	159.05	~~*151.46*~~	153.12	170.42	174.64	112.25	138.68	154.37	160.52
	5	98.60	112.34	115.90	116.45	91.28	110.63	119.60	122.14	99.73	114.29	119.77	122.42
	6	89.80	98.85	~~*101.23*~~	112.57	~~*80.45*~~	~~*83.85*~~	~~*98.66*~~	121.50	110.28	118.44	119.34	140.55
	7	105.88	115.25	116.24	120.30	89.32	104.60	111.43	121.15	~~*68.74*~~	~~*95.88*~~	107.77	115.31
	8	96.54	103.62	114.10	124.60	94.91	113.57	118.72	125.14	99.74	110.65	116.18	120.57
	9	94.17	111.36	114.38	121.95	84.87	90.34	104.20	117.17	90.14	98.40	108.03	118.01
	Average	**99.64**	**111.20**	**118.74**	**123.79**	**101.57**	**114.19**	**121.96**	**128.60**	**101.40**	**112.42**	**119.85**	**128.32**
	SD	**9.68**	**10.08**	**16.89**	**16.27**	**17.71**	**19.15**	**22.00**	**20.46**	**8.52**	**13.88**	**15.98**	**16.40**

~~*123.45*~~ = statistically excluded value

APPENDIX F: STORED BEER QUALITY PARAMETER CORRELATIONS

Table F1. Correlation coefficient (r^2) values for quality parameters of beer fermented at 14 °C and stored at 0 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.6278														
Salinity	-0.9344	-0.8631													
TDS	-0.9813	-0.7640	0.9835												
Reducing Sugars	0.9370	0.6517	-0.8947	-0.9457											
FAN	0.9403	0.5750	-0.8618	-0.9297	0.9949										
Fructose	0.9465	0.5785	-0.8679	-0.9351	0.9946	0.9998									
Sucrose	0.9399	0.7433	-0.9401	-0.9685	0.9915	0.9747	0.9755								
Maltose	0.9433	0.7936	-0.9672	-0.9813	0.9756	0.9526	0.9546	0.9956							
Ethanol	-0.9042	-0.7029	0.8953	0.9326	-0.9939	-0.9794	-0.9780	-0.9929	-0.9791						
Propanol	-0.9522	-0.7018	0.9293	0.9684	-0.9964	-0.9861	-0.9870	-0.9979	-0.9899	0.9914					
Ethyl acetate	0.8825	0.5048	-0.7887	-0.8686	0.9800	0.9894	0.9867	0.9470	0.9131	-0.9688	-0.9599				
Isoamyl alcohol	-0.9390	-0.4763	0.8168	0.9036	-0.9738	-0.9917	-0.9921	-0.9409	-0.9130	0.9454	0.9603	-0.9831			
Isoamyl acetate	0.8750	0.4862	-0.7750	-0.8582	0.9754	0.9865	0.9835	0.9396	0.9037	-0.9633	-0.9534	0.9997	-0.9821		
Ethyl hexanoate	0.9021	0.7323	-0.9078	-0.9376	0.9901	0.9715	0.9704	0.9945	0.9842	-0.9991	-0.9905	0.9575	-0.9330	0.9511	
Ethyl octanoate	0.7333	0.0627	-0.4908	-0.6366	0.7979	0.8520	0.8489	0.7138	0.6529	-0.7494	-0.7533	0.8886	-0.9027	0.8972	0.7209

Table F2. Corresponding p-values for the correlated quality parameters of beer fermented at 14 °C and stored at 0 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.3722														
Salinity	0.0656	0.1369													
TDS	0.0187	0.2360	0.0165												
Reducing Sugars	0.0630	0.3483	0.1053	0.0543											
FAN	0.0597	0.4250	0.1382	0.0703	0.0051										
Fructose	0.0535	0.4215	0.1321	0.0649	0.0054	0.0002									
Sucrose	0.0601	0.2567	0.0599	0.0315	0.0085	0.0253	0.0245								
Maltose	0.0567	0.2064	0.0328	0.0187	0.0244	0.0474	0.0454	0.0044							
Ethanol	0.0958	0.2971	0.1047	0.0674	0.0061	0.0206	0.0220	0.0071	0.0209						
Propanol	0.0478	0.2982	0.0707	0.0316	0.0036	0.0139	0.0130	0.0021	0.0101	0.0086					
Ethyl acetate	0.1175	0.4952	0.2113	0.1314	0.0200	0.0106	0.0133	0.0530	0.0869	0.0312	0.0401				
Isoamyl alcohol	0.0610	0.5237	0.1832	0.0964	0.0262	0.0083	0.0079	0.0591	0.0870	0.0546	0.0397	0.0169			
Isoamyl acetate	0.1250	0.5138	0.2250	0.1418	0.0246	0.0135	0.0165	0.0604	0.0963	0.0367	0.0466	0.0003	0.0179		
Ethyl hexanoate	0.0979	0.2677	0.0922	0.0624	0.0099	0.0285	0.0296	0.0055	0.0158	0.0009	0.0095	0.0425	0.0670	0.0489	
Ethyl octanoate	0.2667	0.9373	0.5092	0.3634	0.2021	0.1480	0.1511	0.2862	0.3471	0.2506	0.2467	0.1114	0.0973	0.1028	0.2791

Table F3. Correlation coefficient (r^2) values for quality parameters of beer fermented at 14 °C and stored at 4 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.7391														
Salinity	-0.9367	-0.9046													
TDS	-0.9725	-0.8686	0.9916												
Reducing Sugars	0.9803	0.8554	-0.9740	-0.9941											
FAN	0.9993	0.7532	-0.9477	-0.9788	0.9827										
Fructose	0.9689	0.8515	-0.9929	-0.9969	0.9837	0.9772									
Sucrose	0.9482	0.8660	-0.9963	-0.9907	0.9699	0.9593	0.9971								
Maltose	0.9243	0.9337	-0.9961	-0.9875	0.9752	0.9344	0.9826	0.9849							
Ethanol	-0.9918	-0.7987	0.9471	0.9804	-0.9942	-0.9905	-0.9686	-0.9476	-0.9459						
Propanol	-0.9975	-0.7773	0.9475	0.9807	-0.9908	-0.9969	-0.9731	-0.9529	-0.9409	0.9982					
Ethyl acetate	0.9075	0.8921	-0.9935	-0.9737	0.9440	0.9223	0.9830	0.9939	0.9834	-0.9104	-0.9150				
Isoamyl alcohol	-0.9920	-0.6501	0.8863	0.9354	-0.9495	-0.9877	-0.9325	-0.9050	-0.8687	0.9740	0.9832	-0.8526			
Isoamyl acetate	0.9744	0.5911	-0.8345	-0.8969	0.9222	0.9658	0.8888	0.8533	0.8193	-0.9574	-0.9650	0.7906	-0.9934		
Ethyl hexanoate	0.9892	0.6471	-0.8990	-0.9395	0.9427	0.9882	0.9445	0.9229	0.8745	-0.9625	-0.9765	0.8767	-0.9946	0.9789	
Ethyl octanoate	0.9694	0.8798	-0.9904	-0.9993	0.9960	0.9752	0.9936	0.9865	0.9895	-0.9818	-0.9799	0.9695	-0.9307	0.8936	0.9316

Table F4. Corresponding p-values for the correlated quality parameters of beer fermented at 14 °C and stored at 4 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.2609														
Salinity	0.0633	0.0954													
TDS	0.0275	0.1314	0.0084												
Reducing Sugars	0.0197	0.1446	0.0260	0.0059											
FAN	0.0007	0.2468	0.0523	0.0212	0.0173										
Fructose	0.0311	0.1485	0.0071	0.0031	0.0163	0.0228									
Sucrose	0.0518	0.1340	0.0037	0.0093	0.0301	0.0407	0.0029								
Maltose	0.0757	0.0663	0.0039	0.0125	0.0248	0.0656	0.0174	0.0151							
Ethanol	0.0082	0.2013	0.0529	0.0196	0.0058	0.0095	0.0314	0.0524	0.0541						
Propanol	0.0025	0.2227	0.0525	0.0193	0.0092	0.0031	0.0269	0.0471	0.0591	0.0018					
Ethyl acetate	0.0925	0.1079	0.0065	0.0263	0.0560	0.0777	0.0170	0.0061	0.0166	0.0896	0.0850				
Isoamyl alcohol	0.0080	0.3499	0.1137	0.0646	0.0505	0.0123	0.0675	0.0950	0.1313	0.0260	0.0168	0.1474			
Isoamyl acetate	0.0256	0.4089	0.1655	0.1031	0.0778	0.0342	0.1112	0.1467	0.1807	0.0426	0.0350	0.2094	0.0066		
Ethyl hexanoate	0.0108	0.3529	0.1010	0.0605	0.0573	0.0118	0.0555	0.0771	0.1255	0.0375	0.0235	0.1233	0.0054	0.0211	
Ethyl octanoate	0.0306	0.1202	0.0096	0.0007	0.0040	0.0248	0.0064	0.0135	0.0105	0.0182	0.0201	0.0305	0.0693	0.1064	0.0684

Table F5. Correlation coefficient (r^2) values for quality parameters of beer fermented at 14 °C and stored at 18 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.3970														
Salinity	-0.9297	-0.7004													
TDS	-0.9286	-0.6976	0.9997												
Reducing Sugars	0.9616	0.6275	-0.9951	-0.9947											
FAN	0.8878	0.4824	-0.9084	-0.9173	0.9179										
Fructose	0.9432	0.5558	-0.9677	-0.9724	0.9759	0.9823									
Sucrose	0.8487	0.7921	-0.9814	-0.9835	0.9599	0.9042	0.9476								
Maltose	0.7379	0.8500	-0.9267	-0.9312	0.8898	0.8703	0.8948	0.9815							
Ethanol	0.3739	-0.5412	-0.1161	-0.1303	0.1911	0.4637	0.3495	0.0447	-0.0195						
Propanol	-0.9678	-0.5330	0.9723	0.9753	-0.9856	-0.9665	-0.9963	-0.9371	-0.8694	-0.3448					
Ethyl acetate	0.6761	-0.4004	-0.3576	-0.3562	0.4485	0.4492	0.4645	0.1923	0.0244	0.7408	-0.5152				
Isoamyl alcohol	-0.9852	-0.3871	0.9269	0.9298	-0.9565	-0.9491	-0.9741	-0.8655	-0.7750	-0.4648	0.9858	-0.6505			
Isoamyl acetate	0.9973	0.4545	-0.9476	-0.9454	0.9739	0.8784	0.9435	0.8713	0.7639	0.3050	-0.9685	0.6318	-0.9760		
Ethyl hexanoate	0.9848	0.2947	-0.8677	-0.8636	0.9107	0.7990	0.8713	0.7584	0.6223	0.3720	-0.9096	0.7574	-0.9471	0.9805	
Ethyl octanoate	0.9422	0.6623	-0.9809	-0.9763	0.9831	0.8299	0.9196	0.9339	0.8515	0.0529	-0.9403	0.4210	-0.9069	0.9640	0.9111

Table F6. Corresponding p-values for the correlated quality parameters of beer fermented at 14 °C and stored at 18 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.6030														
Salinity	0.0703	0.2996													
TDS	0.0714	0.3024	0.0003												
Reducing Sugars	0.0384	0.3725	0.0049	0.0053											
FAN	0.1122	0.5176	0.0916	0.0827	0.0821										
Fructose	0.0568	0.4442	0.0323	0.0276	0.0241	0.0177									
Sucrose	0.1513	0.2079	0.0186	0.0165	0.0401	0.0958	0.0524								
Maltose	0.2621	0.1500	0.0733	0.0688	0.1102	0.1297	0.1052	0.0185							
Ethanol	0.6261	0.4588	0.8839	0.8697	0.8089	0.5363	0.6505	0.9553	0.9805						
Propanol	0.0322	0.4670	0.0277	0.0247	0.0144	0.0335	0.0037	0.0629	0.1306	0.6552					
Ethyl acetate	0.3239	0.5996	0.6424	0.6438	0.5515	0.5508	0.5355	0.8077	0.9756	0.2592	0.4848				
Isoamyl alcohol	0.0148	0.6129	0.0731	0.0702	0.0435	0.0509	0.0259	0.1345	0.2250	0.5352	0.0142	0.3495			
Isoamyl acetate	0.0027	0.5455	0.0524	0.0546	0.0261	0.1216	0.0565	0.1287	0.2361	0.6950	0.0315	0.3682	0.0240		
Ethyl hexanoate	0.0152	0.7053	0.1323	0.1364	0.0893	0.2010	0.1287	0.2416	0.3777	0.6280	0.0904	0.2426	0.0529	0.0195	
Ethyl octanoate	0.0578	0.3377	0.0191	0.0237	0.0169	0.1701	0.0804	0.0661	0.1485	0.9471	0.0597	0.5790	0.0931	0.0360	0.0889

Table F7. Correlation coefficient (r^2) values for quality parameters of beer fermented at 16 °C and stored at 0 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.1112														
Salinity	-0.5295	0.5654													
TDS	-0.9667	0.0779	0.5293												
Reducing Sugars	0.9628	-0.0277	-0.4618	-0.9970											
FAN	0.9784	0.0200	-0.4679	-0.9951	0.9977										
Fructose	0.9654	0.0801	-0.3806	-0.9838	0.9938	0.9953									
Sucrose	0.9606	-0.0164	-0.4445	-0.9953	0.9998	0.9973	0.9953								
Maltose	0.9593	0.1849	-0.3038	-0.9601	0.9763	0.9821	0.9943	0.9792							
Ethanol	-0.9941	-0.0327	0.6187	0.9623	-0.9500	-0.9655	-0.9422	-0.9457	-0.9269						
Propanol	-0.9866	-0.1124	0.4277	0.9818	-0.9877	-0.9955	-0.9947	-0.9880	-0.9909	0.9682					
Ethyl acetate	0.9924	0.0180	-0.5377	-0.9909	0.9870	0.9943	0.9816	0.9849	0.9666	-0.9876	-0.9920				
Isoamyl alcohol	-0.9708	0.0777	0.5454	0.9997	-0.9952	-0.9946	-0.9814	-0.9932	-0.9574	0.9682	0.9818	-0.9929			
Isoamyl acetate	0.5032	0.8964	0.1636	-0.2977	0.3284	0.3837	0.4166	0.3344	0.5012	-0.4475	-0.4699	0.4065	-0.3038		
Ethyl hexanoate	0.9950	0.0150	-0.5727	-0.9844	0.9771	0.9869	0.9699	0.9741	0.9539	-0.9947	-0.9856	0.9985	-0.9878	0.4148	
Ethyl octanoate	0.7104	-0.0248	-0.0449	-0.8280	0.8607	0.8341	0.8691	0.8685	0.8620	-0.6616	-0.8141	0.7709	-0.8140	0.1456	0.7347

Table F8. Corresponding p-values for the correlated quality parameters of beer fermented at 16 °C and stored at 0 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.4048														
Salinity	0.3501	0.0249													
TDS	0.0464	0.4417	0.4502												
Reducing Sugars	0.0169	0.4913	0.4627	0.0144											
FAN	0.0092	0.4695	0.4331	0.0202	0.0012										
Fructose	0.0219	0.4627	0.4461	0.0063	0.0018	0.0040									
Sucrose	0.0714	0.3450	0.3745	0.0111	0.0448	0.0499	0.0290								
Maltose	0.0131	0.3294	0.3080	0.0229	0.0203	0.0158	0.0157	0.0273							
Ethanol	0.0286	0.5486	0.4479	0.1251	0.0577	0.0459	0.0766	0.1799	0.0795						
Propanol	0.0248	0.5702	0.4795	0.1030	0.0417	0.0331	0.0591	0.1591	0.0714	0.0023					
Ethyl acetate	0.0449	0.4549	0.3403	0.1776	0.1043	0.0849	0.1233	0.2196	0.0968	0.0153	0.0286				
Isoamyl alcohol	0.0042	0.4768	0.4099	0.0585	0.0175	0.0104	0.0272	0.0969	0.0299	0.0137	0.0091	0.0375			
Isoamyl acetate	0.0908	0.7540	0.6380	0.1876	0.1018	0.0942	0.1294	0.2708	0.1649	0.0241	0.0215	0.0631	0.0569		
Ethyl hexanoate	0.0566	0.4845	0.3621	0.1978	0.1180	0.0979	0.1394	0.2451	0.1151	0.0166	0.0309	0.0010	0.0457	0.0572	
Ethyl octanoate	0.0534	0.3780	0.3956	0.0037	0.0273	0.0319	0.0153	0.0022	0.0195	0.1477	0.1273	0.1907	0.0732	0.2277	0.2137

Table F9. Correlation coefficient (r^2) values for quality parameters of beer fermented at 16 °C and stored at 4 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.5952														
Salinity	0.6499	0.9751													
TDS	-0.9536	-0.5583	-0.5498												
Reducing Sugars	0.9831	0.5087	0.5373	-0.9856											
FAN	0.9908	0.5305	0.5669	-0.9798	0.9988										
Fructose	0.9781	0.5373	0.5539	-0.9937	0.9982	0.9960									
Sucrose	0.9286	0.6550	0.6255	-0.9889	0.9552	0.9501	0.9710								
Maltose	0.9869	0.6706	0.6920	-0.9771	0.9797	0.9842	0.9843	0.9727							
Ethanol	-0.9714	-0.4514	-0.5521	0.8749	-0.9423	-0.9541	-0.9234	-0.8201	-0.9205						
Propanol	-0.9752	-0.4298	-0.5205	0.8970	-0.9583	-0.9669	-0.9409	-0.8409	-0.9286	0.9977					
Ethyl acetate	0.9551	0.5451	0.6597	-0.8224	0.8957	0.9151	0.8767	0.7804	0.9032	-0.9847	-0.9714				
Isoamyl alcohol	-0.9958	-0.5232	-0.5901	0.9415	-0.9825	-0.9896	-0.9728	-0.9031	-0.9701	0.9863	0.9909	-0.9625			
Isoamyl acetate	0.9092	0.2460	0.3620	-0.8124	0.8982	0.9058	0.8706	0.7292	0.8351	-0.9759	-0.9785	0.9369	-0.9431		
Ethyl hexanoate	0.9434	0.5155	0.6379	-0.8022	0.8820	0.9021	0.8606	0.7549	0.8849	-0.9834	-0.9691	0.9990	-0.9543	0.9428	
Ethyl octanoate	0.9466	0.6220	0.6044	-0.9963	0.9727	0.9681	0.9847	0.9978	0.9805	-0.8523	-0.8727	0.8093	-0.9268	0.7723	0.7863

Table F10. Corresponding p-values for the correlated quality parameters of beer fermented at 16 °C and stored at 4 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.9857														
Salinity	0.5225	0.6401													
TDS	0.0430	0.6965	0.6595												
Reducing Sugars	0.0002	0.9956	0.5133	0.0486											
FAN	0.0027	0.9679	0.5868	0.0395	0.0034										
Fructose	0.0001	0.9895	0.5095	0.0444	0.0002	0.0039									
Sucrose	0.0927	0.5737	0.6566	0.0129	0.1004	0.0932	0.0933								
Maltose	0.0658	0.6731	0.5332	0.0172	0.0713	0.0747	0.0646	0.0096							
Ethanol	0.0290	0.7777	0.4209	0.1396	0.0246	0.0383	0.0277	0.2139	0.1608						
Propanol	0.0137	0.9976	0.6560	0.0574	0.0141	0.0049	0.0161	0.1227	0.1102	0.0454					
Ethyl acetate	0.0317	0.7363	0.6309	0.0009	0.0366	0.0297	0.0328	0.0184	0.0174	0.1189	0.0476				
Isoamyl alcohol	0.0050	0.8856	0.5577	0.0189	0.0071	0.0062	0.0055	0.0555	0.0382	0.0573	0.0206	0.0116			
Isoamyl acetate	0.0017	0.9965	0.5649	0.0470	0.0018	0.0005	0.0026	0.1031	0.0804	0.0301	0.0058	0.0359	0.0081		
Ethyl hexanoate	0.0029	0.9735	0.4679	0.0432	0.0031	0.0107	0.0020	0.0845	0.0518	0.0320	0.0292	0.0317	0.0064	0.0091	
Ethyl octanoate	0.0811	0.8695	0.8019	0.1576	0.0797	0.0602	0.0861	0.2542	0.2491	0.0871	0.0316	0.1455	0.1020	0.0602	0.1134

Table F11. Correlation coefficient (r^2) values for quality parameters of beer fermented at 16 °C and stored at 18 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.0143														
Salinity	0.4775	-0.3599													
TDS	-0.9570	-0.3035	-0.3405												
Reducing Sugars	0.9998	-0.0044	0.4867	-0.9514											
FAN	0.9973	0.0321	0.4132	-0.9605	0.9966										
Fructose	0.9999	0.0105	0.4905	-0.9556	0.9998	0.9961									
Sucrose	0.9073	0.4263	0.3434	-0.9871	0.8996	0.9068	0.9067								
Maltose	0.9342	0.3269	0.4668	-0.9828	0.9287	0.9253	0.9354	0.9904							
Ethanol	-0.9710	0.2223	-0.5791	0.8604	-0.9754	-0.9617	-0.9723	-0.7861	-0.8392						
Propanol	-0.9863	-0.0024	-0.3440	0.9426	-0.9859	-0.9951	-0.9839	-0.8773	-0.8898	0.9546					
Ethyl acetate	0.9683	0.2637	0.3691	-0.9991	0.9634	0.9703	0.9672	0.9816	0.9826	-0.8811	-0.9524				
Isoamyl alcohol	-0.9950	-0.1144	-0.4423	0.9811	-0.9929	-0.9938	-0.9945	-0.9445	-0.9618	0.9427	0.9794	-0.9884			
Isoamyl acetate	0.9983	0.0035	0.4351	-0.9530	0.9982	0.9995	0.9974	0.8969	0.9196	-0.9699	-0.9942	0.9641	-0.9919		
Ethyl hexanoate	0.9971	0.0265	0.5321	-0.9568	0.9969	0.9893	0.9980	0.9155	0.9482	-0.9680	-0.9708	0.9683	-0.9936	0.9909	
Ethyl octanoate	0.9189	-0.1305	0.1981	-0.8424	0.9203	0.9398	0.9139	0.7458	0.7509	-0.9129	-0.9684	0.8545	-0.8980	0.9398	0.8866

Table F12. Corresponding p-values for the correlated quality parameters of beer fermented at 16 °C and stored at 18 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.9857														
Salinity	0.5225	0.6401													
TDS	0.0430	0.6965	0.6595												
Reducing Sugars	0.0002	0.9956	0.5133	0.0486											
FAN	0.0027	0.9679	0.5868	0.0395	0.0034										
Fructose	0.0001	0.9895	0.5095	0.0444	0.0002	0.0039									
Sucrose	0.0927	0.5737	0.6566	0.0129	0.1004	0.0932	0.0933								
Maltose	0.0658	0.6731	0.5332	0.0172	0.0713	0.0747	0.0646	0.0096							
Ethanol	0.0290	0.7777	0.4209	0.1396	0.0246	0.0383	0.0277	0.2139	0.1608						
Propanol	0.0137	0.9976	0.6560	0.0574	0.0141	0.0049	0.0161	0.1227	0.1102	0.0454					
Ethyl acetate	0.0317	0.7363	0.6309	0.0009	0.0366	0.0297	0.0328	0.0184	0.0174	0.1189	0.0476				
Isoamyl alcohol	0.0050	0.8856	0.5577	0.0189	0.0071	0.0062	0.0055	0.0555	0.0382	0.0573	0.0206	0.0116			
Isoamyl acetate	0.0017	0.9965	0.5649	0.0470	0.0018	0.0005	0.0026	0.1031	0.0804	0.0301	0.0058	0.0359	0.0081		
Ethyl hexanoate	0.0029	0.9735	0.4679	0.0432	0.0031	0.0107	0.0020	0.0845	0.0518	0.0320	0.0292	0.0317	0.0064	0.0091	
Ethyl octanoate	0.0811	0.8695	0.8019	0.1576	0.0797	0.0602	0.0861	0.2542	0.2491	0.0871	0.0316	0.1455	0.1020	0.0602	0.1134

Table F13. Correlation coefficient (r^2) values for quality parameters of beer fermented at 18 °C and stored at 0 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	-0.9683														
Salinity	-0.9904	0.9449													
TDS	-0.9596	0.8702	0.9830												
Reducing Sugars	0.9974	-0.9811	-0.9890	-0.9483											
FAN	0.9374	-0.8580	-0.9758	-0.9902	0.9333										
Fructose	0.9900	-0.9874	-0.9637	-0.9105	0.9916	0.8820									
Sucrose	0.9857	-0.9137	-0.9802	-0.9782	0.9717	0.9448	0.9604								
Maltose	0.9586	-0.8584	-0.9722	-0.9944	0.9411	0.9702	0.9123	0.9882							
Ethanol	-0.9715	0.8981	0.9926	0.9977	-0.9646	-0.9917	-0.9288	-0.9787	-0.9881						
Propanol	-0.9805	0.9872	0.9784	0.9244	-0.9919	-0.9250	-0.9779	-0.9367	-0.9047	0.9475					
Ethyl acetate	0.8712	-0.9123	-0.8987	-0.8332	0.9008	0.8781	0.8611	0.7971	0.7812	-0.8666	-0.9468				
Isoamyl alcohol	-0.9749	0.9276	0.9961	0.9836	-0.9762	-0.9877	-0.9404	-0.9630	-0.9648	0.9934	0.9727	-0.9177			
Isoamyl acetate	0.9357	-0.8979	-0.8840	-0.8520	0.9184	0.7832	0.9511	0.9421	0.8862	-0.8566	-0.8684	0.6633	-0.8397		
Ethyl hexanoate	0.9625	-0.9712	-0.9723	-0.9203	0.9778	0.9342	0.9530	0.9140	0.8911	-0.9448	-0.9952	0.9717	-0.9750	0.8177	
Ethyl octanoate	0.9682	-0.9758	-0.9752	-0.9231	0.9824	0.9336	0.9601	0.9212	0.8964	-0.9471	-0.9973	0.9660	-0.9759	0.8315	0.9997

Table F14. Corresponding p-values for the correlated quality parameters of beer fermented at 18 °C and stored at 0 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.0317														
Salinity	0.0096	0.0551													
TDS	0.0404	0.1298	0.0170												
Reducing Sugars	0.0026	0.0189	0.0110	0.0517											
FAN	0.0626	0.1420	0.0242	0.0098	0.0667										
Fructose	0.0100	0.0126	0.0363	0.0895	0.0084	0.1180									
Sucrose	0.0143	0.0863	0.0198	0.0218	0.0283	0.0552	0.0396								
Maltose	0.0414	0.1416	0.0278	0.0056	0.0589	0.0298	0.0877	0.0118							
Ethanol	0.0285	0.1019	0.0074	0.0023	0.0354	0.0083	0.0712	0.0213	0.0119						
Propanol	0.0195	0.0128	0.0216	0.0756	0.0081	0.0750	0.0221	0.0633	0.0953	0.0525					
Ethyl acetate	0.1288	0.0877	0.1013	0.1668	0.0992	0.1219	0.1389	0.2029	0.2188	0.1334	0.0532				
Isoamyl alcohol	0.0251	0.0724	0.0039	0.0164	0.0238	0.0123	0.0596	0.0370	0.0352	0.0066	0.0273	0.0823			
Isoamyl acetate	0.0643	0.1021	0.1160	0.1480	0.0816	0.2168	0.0489	0.0579	0.1138	0.1434	0.1316	0.3367	0.1603		
Ethyl hexanoate	0.0375	0.0288	0.0277	0.0797	0.0222	0.0658	0.0470	0.0860	0.1089	0.0552	0.0048	0.0283	0.0250	0.1823	
Ethyl octanoate	0.0318	0.0242	0.0248	0.0769	0.0176	0.0664	0.0399	0.0788	0.1036	0.0529	0.0027	0.0340	0.0241	0.1685	0.0003

Table F15. Correlation coefficient (r^2) values for quality parameters of beer fermented at 18 °C and stored at 4 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	-0.9613														
Salinity	-0.9455	0.8699													
TDS	-0.9426	0.8901	0.9959												
Reducing Sugars	0.9834	-0.9306	-0.9882	-0.9871											
FAN	0.9788	-0.9969	-0.9043	-0.9194	0.9562										
Fructose	0.9974	-0.9739	-0.9490	-0.9524	0.9860	0.9887									
Sucrose	0.9113	-0.7963	-0.9885	-0.9721	0.9624	0.8408	0.9067								
Maltose	0.9310	-0.8517	-0.9991	-0.9950	0.9811	0.8879	0.9354	0.9904							
Ethanol	-0.9620	0.9991	0.8589	0.8771	-0.9243	-0.9953	-0.9723	-0.7861	-0.8392						
Propanol	-0.9931	0.9488	0.9081	0.8993	-0.9577	-0.9648	-0.9839	-0.8773	-0.8898	0.9546					
Ethyl acetate	0.9726	-0.8857	-0.9886	-0.9761	0.9918	0.9196	0.9672	0.9816	0.9826	-0.8811	-0.9524				
Isoamyl alcohol	-0.9954	0.9454	0.9724	0.9691	-0.9961	-0.9681	-0.9945	-0.9445	-0.9618	0.9427	0.9794	-0.9884			
Isoamyl acetate	0.9994	-0.9684	-0.9352	-0.9340	0.9779	0.9835	0.9974	0.8969	0.9196	-0.9699	-0.9942	0.9641	-0.9919		
Ethyl hexanoate	0.9917	-0.9723	-0.9597	-0.9665	0.9904	0.9875	0.9980	0.9155	0.9482	-0.9680	-0.9708	0.9683	-0.9936	0.9909	
Ethyl octanoate	0.9334	-0.8968	-0.7775	-0.7618	0.8557	0.9041	0.9139	0.7458	0.7509	-0.9129	-0.9684	0.8545	-0.8980	0.9398	0.8866

Table F16. Corresponding p-values for the correlated quality parameters of beer fermented at 18 °C and stored at 4 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.0387														
Salinity	0.0545	0.1301													
TDS	0.0574	0.1099	0.0041												
Reducing Sugars	0.0166	0.0694	0.0118	0.0129											
FAN	0.0212	0.0031	0.0957	0.0806	0.0438										
Fructose	0.0026	0.0261	0.0510	0.0476	0.0140	0.0113									
Sucrose	0.0887	0.2037	0.0115	0.0279	0.0376	0.1592	0.0933								
Maltose	0.0690	0.1483	0.0009	0.0050	0.0189	0.1121	0.0646	0.0096							
Ethanol	0.0380	0.0009	0.1411	0.1229	0.0757	0.0047	0.0277	0.2139	0.1608						
Propanol	0.0069	0.0512	0.0919	0.1007	0.0423	0.0352	0.0161	0.1227	0.1102	0.0454					
Ethyl acetate	0.0274	0.1143	0.0114	0.0239	0.0082	0.0804	0.0328	0.0184	0.0174	0.1189	0.0476				
Isoamyl alcohol	0.0046	0.0546	0.0276	0.0309	0.0039	0.0319	0.0055	0.0555	0.0382	0.0573	0.0206	0.0116			
Isoamyl acetate	0.0006	0.0316	0.0648	0.0660	0.0221	0.0165	0.0026	0.1031	0.0804	0.0301	0.0058	0.0359	0.0081		
Ethyl hexanoate	0.0083	0.0277	0.0403	0.0335	0.0096	0.0125	0.0020	0.0845	0.0518	0.0320	0.0292	0.0317	0.0064	0.0091	
Ethyl octanoate	0.0666	0.1032	0.2225	0.2382	0.1443	0.0959	0.0861	0.2542	0.2491	0.0871	0.0316	0.1455	0.1020	0.0602	0.1134

Table F17. Correlation coefficient (r^2) values for quality parameters of beer fermented at 18 °C and stored at 18 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	-0.9516														
Salinity	-0.9286	0.8153													
TDS	-0.9378	0.8611	0.9921												
Reducing Sugars	0.9751	-0.9236	-0.9743	-0.9893											
FAN	0.9876	-0.9864	-0.8711	-0.8970	0.9517										
Fructose	0.9928	-0.9180	-0.9170	-0.9128	0.9511	0.9706									
Sucrose	0.9832	-0.9077	-0.8864	-0.8790	0.9250	0.9637	0.9971								
Maltose	0.9325	-0.8169	-0.9998	-0.9905	0.9742	0.8746	0.9229	0.8937							
Ethanol	-0.9837	0.9826	0.9086	0.9393	-0.9786	-0.9904	-0.9555	-0.9381	-0.9096						
Propanol	-0.9978	0.9292	0.9378	0.9387	-0.9713	-0.9758	-0.9973	-0.9888	-0.9424	0.9704					
Ethyl acetate	0.9044	-0.9835	-0.7950	-0.8566	0.9092	0.9477	0.8508	0.8293	0.7930	-0.9646	-0.8743				
Isoamyl alcohol	-0.9866	0.9433	0.9612	0.9777	-0.9978	-0.9698	-0.9649	-0.9430	-0.9620	0.9885	0.9816	-0.9223			
Isoamyl acetate	0.8461	-0.9657	-0.6379	-0.7048	0.7941	0.9192	0.8033	0.8013	0.6394	-0.9010	-0.8110	0.9582	-0.8251		
Ethyl hexanoate	0.9990	-0.9450	-0.9442	-0.9527	0.9836	0.9820	0.9899	0.9772	0.9474	-0.9840	-0.9974	0.9024	-0.9921	0.8312	
Ethyl octanoate	0.8795	-0.8973	-0.6443	-0.6610	0.7588	0.9162	0.8916	0.9153	0.6541	-0.8528	-0.8695	0.8095	-0.8001	0.8930	0.8568

Table F18. Corresponding p-values for the correlated quality parameters of beer fermented at 18 °C and stored at 18 °C.

	EBC colour	pH	Salinity	TDS	Reducing Sugars	FAN	Fructose	Sucrose	Maltose	Ethanol	Propanol	Ethyl acetate	Isoamyl alcohol	Isoamyl acetate	Ethyl hexanoate
EBC colour															
pH	0.0484														
Salinity	0.0714	0.1847													
TDS	0.0622	0.1389	0.0079												
Reducing Sugars	0.0249	0.0764	0.0257	0.0107											
FAN	0.0124	0.0136	0.1289	0.1030	0.0483										
Fructose	0.0072	0.0820	0.0830	0.0872	0.0489	0.0294									
Sucrose	0.0168	0.0923	0.1136	0.1210	0.0750	0.0363	0.0029								
Maltose	0.0675	0.1831	0.0002	0.0095	0.0258	0.1254	0.0771	0.1063							
Ethanol	0.0163	0.0174	0.0914	0.0607	0.0214	0.0096	0.0445	0.0619	0.0904						
Propanol	0.0022	0.0708	0.0622	0.0613	0.0287	0.0242	0.0027	0.0112	0.0576	0.0296					
Ethyl acetate	0.0956	0.0165	0.2050	0.1434	0.0908	0.0523	0.1492	0.1707	0.2070	0.0354	0.1257				
Isoamyl alcohol	0.0134	0.0567	0.0388	0.0223	0.0022	0.0302	0.0351	0.0570	0.0380	0.0115	0.0184	0.0777			
Isoamyl acetate	0.1539	0.0343	0.3621	0.2952	0.2059	0.0808	0.1967	0.1987	0.3606	0.0990	0.1890	0.0418	0.1749		
Ethyl hexanoate	0.0010	0.0550	0.0558	0.0473	0.0164	0.0180	0.0101	0.0228	0.0526	0.0160	0.0026	0.0976	0.0079	0.1688	
Ethyl octanoate	0.1205	0.1027	0.3557	0.3390	0.2412	0.0838	0.1084	0.0847	0.3459	0.1472	0.1305	0.1905	0.1999	0.1070	0.1432

APPENDIX G: BREWING CALCULATIONS

Section G1. Mash extract yield efficiency calculation.

Considering the mash gravity in table D1;

Gravity = 14.06 °P = 14.06 % w/w

Now 14.06 % w/w implies 14.06 g extract in 100 g of water.

Considering the mash volume of 15.38 l and taking the density of water to be 1 kg/l;

14.06 g: 100 g

x: 15380 g

$$x \quad \frac{15380 \quad 14.06}{100}$$

x = 2162.43 g extract.

If the mash had 2162.43 g extract at liquor: grist ratio of 5.2, then at the standard working ratio of 3.4;

$C_1M_1 = C_2M_2$

$3.4x = 5.2 \times 2162.43$

$$x \quad \frac{5.2 \quad 2162.43}{3.4}$$

$$\therefore x \quad 3307.25 \text{ g}$$

The grist amount per batch was known to be 5795.10 g with approximately ≈ 8 % of the weight accounted for as moisture.

Compensated dry weight = 5331.40 g.

⇒

$$\text{Extract yield} \quad \frac{3307.25}{5331.40} \quad 100\ \%$$

$$\therefore \text{Mash extract yield} \quad 62.03\ \%$$

Section G2. Spent grain losses

A congress mash of 20 g spent grain and initial water of 86 ml was for estimation of spent grain losses and considering reducing sugars in table D1;

Reducing sugar losses = Spent grain mash + spent grain lauter runs

Reducing sugar losses (2.49 0.086) (1.66 0.028)

(1.09 0.028) (0.58 0.014)

Reducing sugar losses 0.214 0.047 0.031 0.008

Reducing sugar losses 0.2993 g

Considering an extraction yield of 62.03 % it implies that 37.97 % of the grist dry weight remained as spent grain and extract loss.

spent grain dry weight 0.3797 5331.40

∴spent grain 2024.18 g

With consideration that 0.2993 g of reducing sugars are lost in 20 g of spent grain;

⇒ 20 g: 0.2993 g

2024.18 g: x

$$x \quad \frac{2024.18 \quad 0.2993}{20}$$

$$x \quad 30.29 \text{ g}$$

∴ Spent grain losses $\frac{30.29}{2024.18}$ 100 % 1.496 %

Table D2 allows us to calculate the same losses by utilising total simple sugars found in the spent grain. Consider the following sugar amounts;

Total sugar losses = Spent grain mash + spent grain lauter runs

Total sugar loss (8.29 0.086) (6.68 0.028)

(6.29 0.028) (6.04 0.014)

Total sugar loss 0.713 0.187 0.176 0.085

Total sugar loss 1.161 g

1.161 g of total simple sugars were lost in 20 g of spent grain, and so in 2024.18 g;

⇒ 20 g: 1.161 g

2024.18 g: x

$$x \quad \frac{2024.18 \quad 1.161}{20}$$

$$x \quad 117.50 \text{ g}$$

∴ Spent grain losses $\frac{117.50}{2024.18}$ 100 % 5.805 %

Average losses (Reducing sugar losses Total sugar losses)/2

∴ Average losses $\frac{1.496 \quad 5.805}{2}$ 3.651 %

Section G3. Brew house kettle liquor losses

Consider the brew house reducing sugars in table D1;

Reducing sugar amount concentration volume

65.949 26.45

∴ Kettle reducing sugars 1744.35 g

Considering 78 % utilization of the 462 g maltose added in boil, i.e., (300 ml ≡ 462 g)

⇒ Malt-derived reducing sugars in kettle = 1744.35 – 360.36 = 1383.99 g

Now considering total malt derived reducing sugars from upstream;

Reducing sugars $_{upstream}$ = Mash + 1st run + 2nd run + 3rd run

(89.46 15.38) (33.59 7.67) (3.87 7.58)

(1.74 5.45)

∴ Reducing sugars 1672.53 g

The difference in the two reducing sugar amounts is attributed to losses due to trub discarding after boiling.

∴Trub losses 1672.53 1383.99 288.54 g

⇒ Wort volume lost as trub; 1672.53 g in 35.71 l

288.54 g in x l

$$x \quad \frac{288.54 \quad 35.71}{1672.53} \quad 6.16 \text{ l}$$

Volume difference in unboiled and boiled wort is = 35.71 – 26.45 = 9.26 l

Wort volume lost as vapour = 9.26 – 6.16 = 3.099 l

Consider total simple sugars in the kettle i.e., table D2;

Total simple sugars concentration volume

136.60 26.45

∴ Kettle total sugars 3613.07 g

Now excluding the maltose additions at wort boiling stage;

⇒ Malt-derived simple sugars in kettle = 3613.07 – 360.36 = 3252.71 g

Total malt-derived simple sugars from upstream;

Simple sugars $_{upstream}$ = Mash + 1st run + 2nd run + 3rd run

(161.53 15.38) (89.92 7.67) (63.23 7.58)

(35.18 5.45)

∴ Simple sugars 3845.25 g

The difference in upstream and downstream (kettle) simple sugar amounts is attributed to trub losses.

∴ Trub losses 3845.25 3252.71 592.54 g

⇒ Wort volume lost as trub; 3845.25 g in 35.71 l

592.54 g in x l

$$x \quad \frac{592.54 \quad 35.71}{3845.25} \quad 5.50 \text{ l}$$

Wort volume lost as vapour = 9.26 – 5.50 = 3.757 l

To give a good estimate of the two methods used for liquor losses consider the following averages;

Final trub losses (6.16 5.50)/2 5.83 l

Final vapour losses (3.099 3.757)/2 3.43 l

Section G4. Maltotriose compensation calculations

Consider the literature citing for major brewing sugars in table G1 below;

Table G1. A representation of five literature citing"s with respect to the three major brewing sugars mash content i.e., glucose, maltose and maltotriose.

	MacWilliam, 1968	**Tenhunen *et al*., 1994**	**Fix, 1999**	**Goode *et al*., 2005**	**Montanari *et al*., 2005**	**Average**
Sugar (% w/w)						
Glucose	14.19	13.35	9.00	12.79	10.18	11.90
Maltose	57.64	71.68	48.00	71.05	69.08	63.49
Maltotriose	18.57	15.01	15.00	14.46	16.71	15.95

With maltotriose averaging at 15.95 % w/w it implies that the total mash sugars in the experiment had a systematic error that resulted in their increase.

$$\text{Systematic error increase} \quad \frac{15.95}{(100\ 15.95)} \quad 100\ \% \quad 18.98\ \%$$

This implies that all experimental concentrations were more by 18.98 % w/w of their original values, when maltotriose was considered a contributor to the total simple sugars value.

Fructose 5.31 † 1.1898 4.46 g/l i.e., $5.31 \quad \frac{100}{(100\ 1\ 8.98)}$

Glucose 72.72 † 1.1898 61.12 g/l

Sucrose = 1.85 ÷ 1.1898 = 1.55 g/l

Maltose 75.53 † 1.1898 63.48 g/l

Table G2. Experimental and compensated mash concentrations of the four investigated simple sugars with respect to the literature maltotriose concentration.

		Fructose	Glucose	Sucrose	Maltose	Maltotriose	Total
Experimental concentrations	(% w/w)	3.29	45.02	1.15	50.54	-	100,00
	(g/l)	5.31	72.72	1.85	75.53	-	161.53
Compensated concentrations	(% w/w)	2.86	39.33	1.00	40.85	15.95	100,00
	(g/l)	4.46	61.12	1.55	63.48	24.79	155.41

Glucose was way above the 9 – 14 % w/w range depicted by literature;

Glucose increase 61.12 -(155.41 0.119) 42.63 g/l

Maltose was slightly below the 48 – 72 % w/w concentration range;

Maltose decrease (155.41 0.6349) - 63.48 35.18 g/l

Now, 1 Maltose g = 1.053 g of 2 glucose molecules, so the maltose decrease resulted in;

Maltose-hydrolysis glucose (35.18 1.053) 37.05 g/l

⇒ Potential α-amylase glucose = 42.63 – 37.05 = 5.59 g/l

Section G5. Fermentation and bottle conditioning calculations

Considering results in table D3, real degree of fermentation is governed by the following;

$RE \quad 0.18(OE) \quad 0.82(AE)$

$RA \quad 1 - {}^{RE}/_{OE}$

Where; RE Real extract ≡ Final gravity with ethanol compensation

OE Original extract ≡ Initial gravity

AE Apparent extract ≡ Final gravity

RA Real attenuation ≡ Real degree of fermentation

$\Longrightarrow RE_{14} = 0.18(12.301) + 0.82(5.240) = 6.511\ °P$

$RA_{14} \quad 1 - (6.511/12.301) \quad 100\ \% \quad 47.07\ \%$

$RE_{16} = 0.18(11.907) + 0.82(4.500) = 5.833\ °P$

$RA_{16} \quad 1 - (5.833/11.907) \quad 100\ \% \quad 51.01\ \%$

$RE_{18} = 0.18(12.106) + 0.82(3.828) = 5.318\ °P$

$RA_{18} \quad 1 - (5.318/12.106) \quad 100\ \% \quad 56.07\ \%$

In a 750 ml bottle, 743 ml of beer + 3 ml culture + 4 ml of 50 % maltose syrup were added.

Considering the solids content of the syrup together with the different sugars stipulated by the certificate of analysis;

$\Longrightarrow$ Mass of sugars Utilized syrup volume (maltose dextrose maltotriose)

4 0.5 0.785 (maltose dextrose maltotriose)

1.57 ml (maltose dextrose maltotriose)

Now; $\rho_{maltose}$ = 1.54 g/ml; maltose content = 55.20 %

$\rho_{dextrose}$ = 1.54 g/ml; dextrose content = 3.70 %

$\rho_{maltotriose}$ = 1.75 g/ml; maltotriose content = 17.10 %

Mass of sugars (1.57 0.552 1.54) (1.57 0.037 1.54) (1.57 0.171 1.75)

1.3346 0.0895 0.4698

$\therefore$ Mass of sugars 1.894 g

Using the general law of; M $\rho \times$ V

$\Longrightarrow$14 °C bottle mass = $\rho_{end\text{-}of\text{-}rack} \times$ Volume

1.019 743 757.12 g

16 °C bottle mass 1.017 743 755.63 g

18 °C bottle mass 1.014 743 753.42 g

To express the added conditioning sugars in % w/w i.e., °P units consider the following;

For the 14 °C bottles, 1.894 g in 757.12 g

$\therefore x$ g in 100.00 g

14 °C bottle gravity increase x 1.894 (100/757.12) 0.250 °P

16 °C bottle gravity increase x 1.894 (100/755.63) 0.251 °P

18 °C bottle gravity increase x 1.894 (100/753.42) 0.252 °P

Harris‟ law states that, 1 °P w/w sugars = 0.645 % v/v ethanol.

∴ All conditioned bottles according to Harris‟ law will have ≈ 0.162 % v/v ethanol increase.

APPENDIX H: BREWING PROCESS AND QUALITY ASSESSMENTS

Section H1. Brewing process flow

Figure H1. Brewing raw materials, (a) Pale malt and (b) a mixture of pale and black malt after milling.

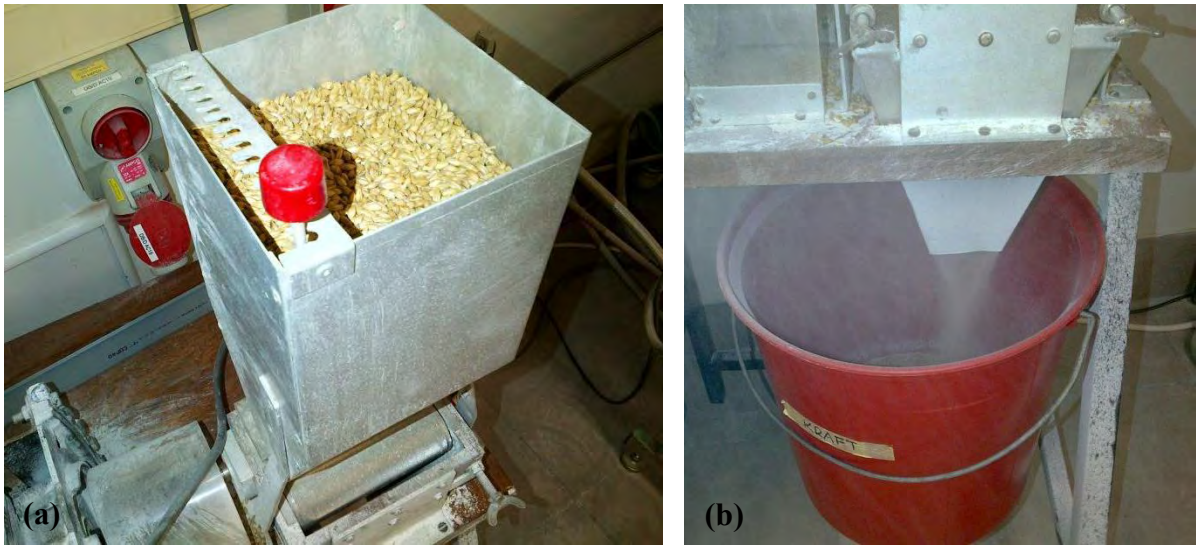


Figure H2. (a) Top view and (b) bottom-side view of the milling process.

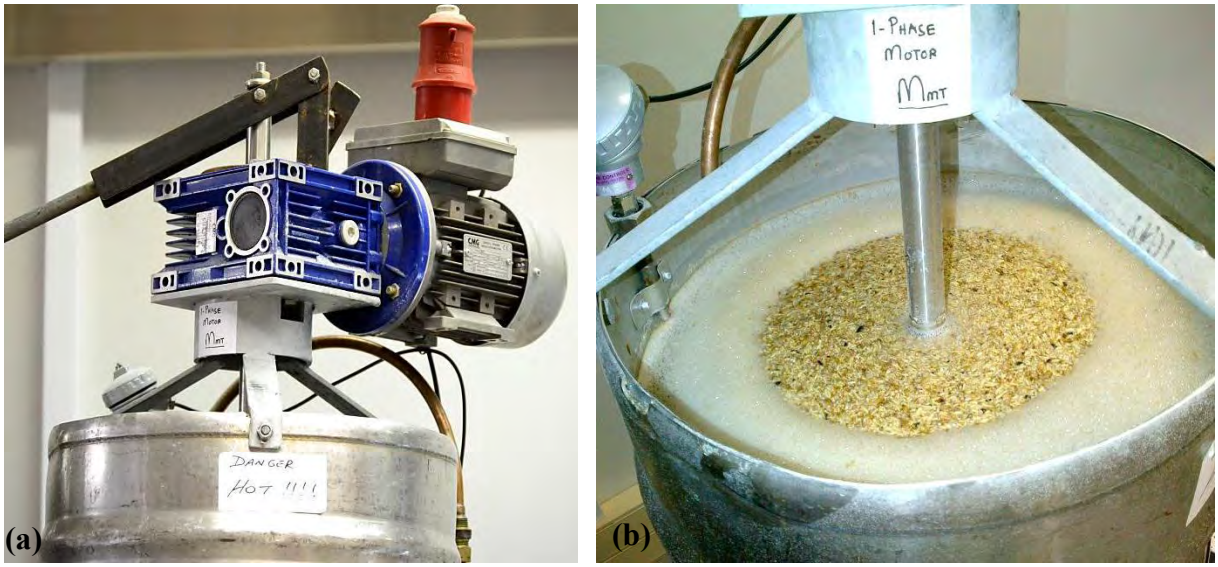


Figure H3. The external (a) and (b) internal views of the mashing process during the first stand at 64 °C.

Figure H4. (a) Mash pump over step and (b) wort recirculation before sparging.

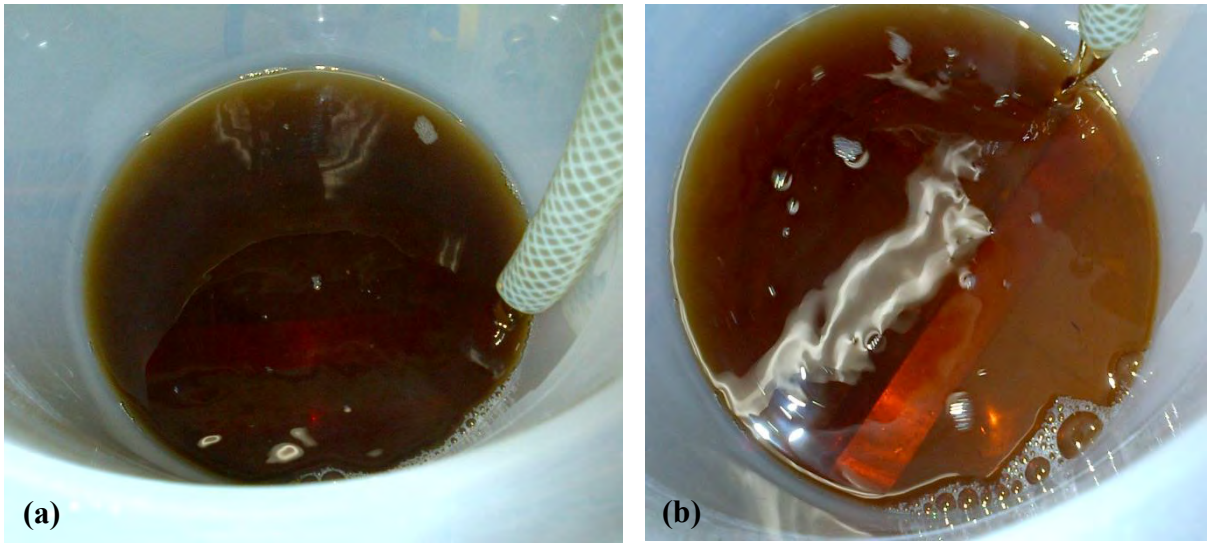


Figure H5. The clarifying effect of wort recirculation across the grain bed after (a) 15 min and (b) 40 min for first wort runnings.

Figure H6. (a) Hop addition to wort in the kettle (b) to achieve a vigorous fusion of the bittering hop α-acids during the 60 min duration.

Figure H7. (a) Cool wort transfer into the distributing keg for (b) partitioned fermentations at the desired experimental temperature.

Figure H8. Beer maturation vessel prepped for beer bottling.

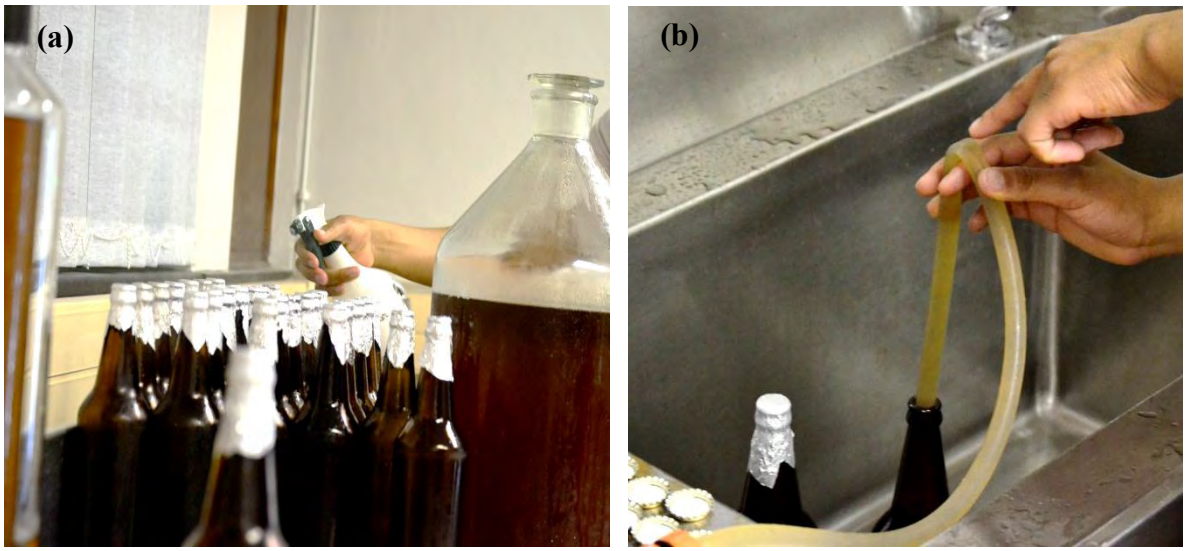


Figure H9. Bottle filling process i.e., (a) bottle and work space sanitation and (b) beer delivery into bottles by means of a sterile pipe.

Figure H10. (a) Bottle conditioning additive preparations and (b) spiked bottles lined up for capping and storage in the conditioning incubator.

Figure H11. Beer sample collection for physico chemical and chemical analyses.

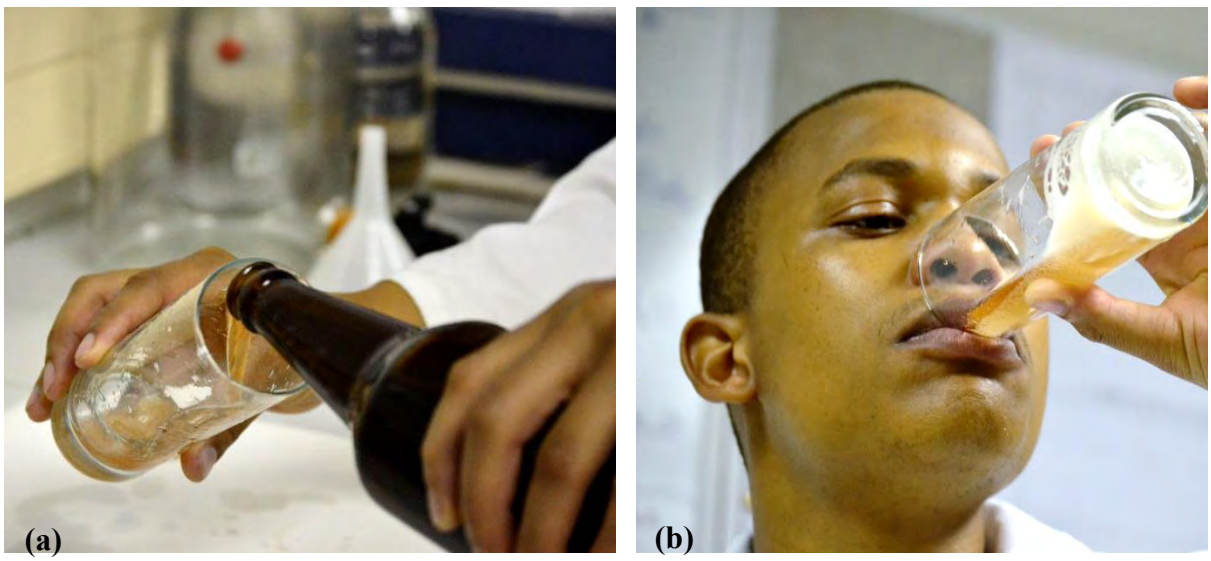


Figure H12. Beer tasting (a) by gently pouring for foam visual analysis and (b) sipping for flavour and mouth feel characteristics.

Section H2. Brewing quality checks

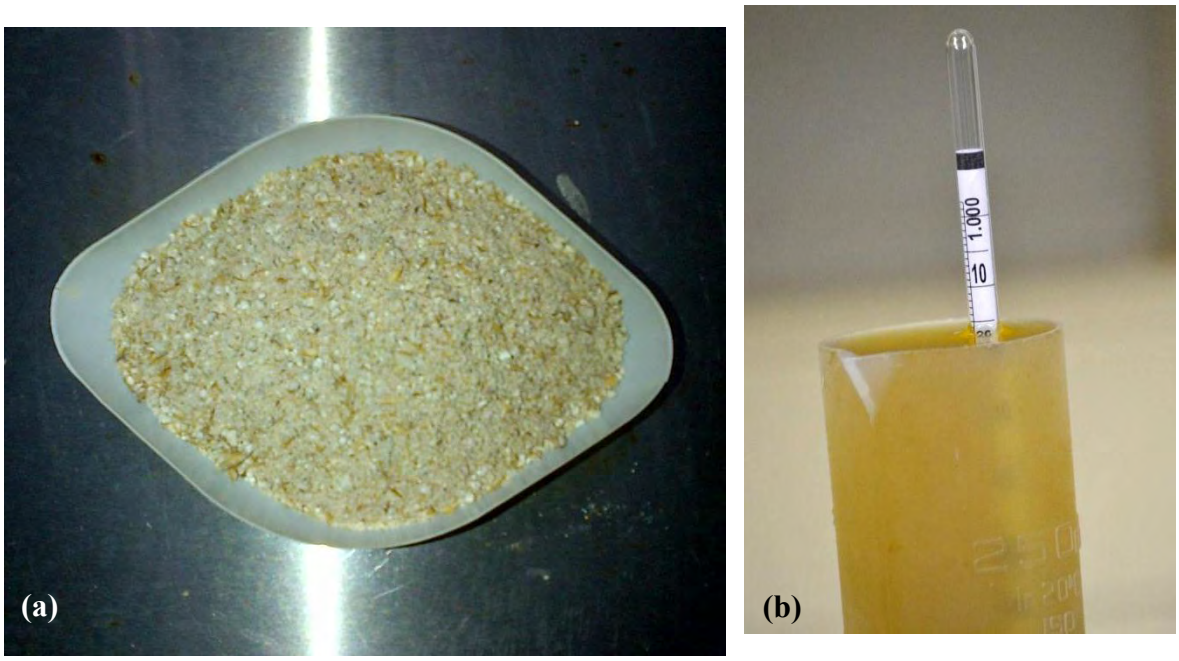


Figure H13. (a) Milled grist mass rectification before mashing and (b) second wort runnings gravity check.

Figure H14. Lautered wort volume measurement before transferring to the kettle.

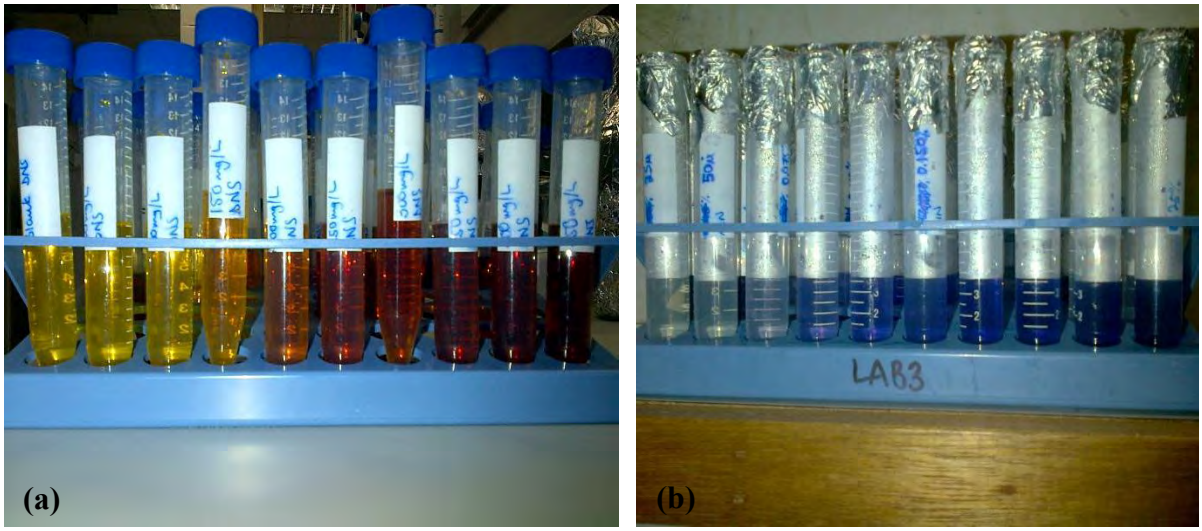


Figure H15. Reducing sugars calibration assay (a) and FAN calibration assay (b).

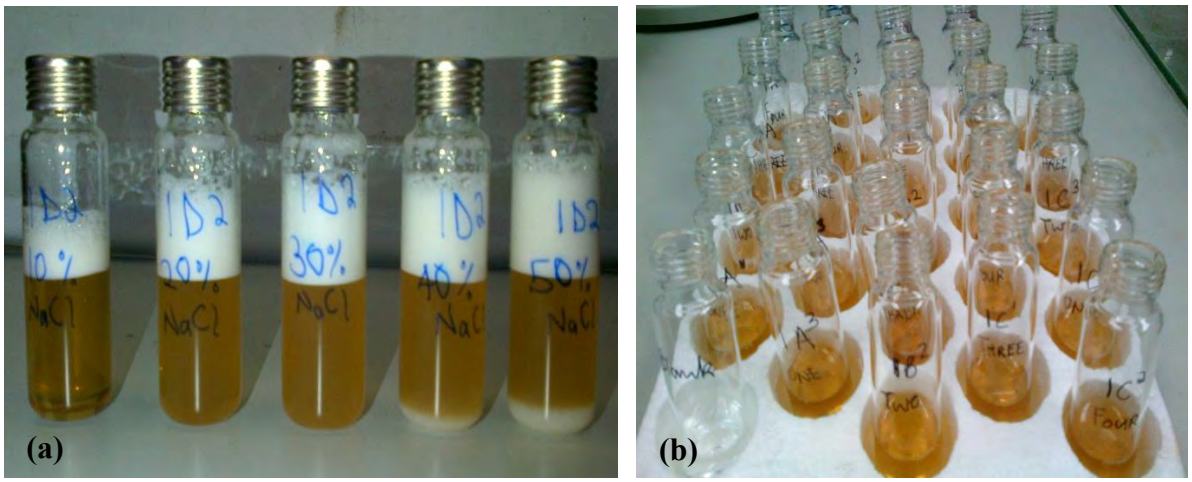


Figure H16. (a) Salting experiment for the optimisation of the head space GC assay and (b) GC sample trials for column sensitivity.