

DISSERTATION



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LOW ALCOHOL OPAQUE BEER QUALITY: INFLUENCE OF MALT, MASHING CONDITIONS AND WORT DILUTION

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DECLARATION

I hereby confirm that this work is original, that it has not been submitted anywhere else by me for any other degree.

Signed

Date

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ABSTRACT

Research was carried out to determine the effect of high temperature mashing, use of short time germinated malt and low gravity fermentation on the quality of sorghum beer. The objective was to produce an acceptable low alcohol opaque beer. Mashing at high temperatures of 65-80°C resulted in lower generation of fermentable sugars with the least being obtained at 80°C leading to production of low alcohol. The main reason being that although beta- and alpha- amylase enzymes are inactivated at high temperatures beta-amylase is less temperature resistant than alpha-amylase. Thus the reduction in beta-amylase activity leads to reduction in the amount of fermentable sugar in the wort. The best low alcohol product was produced at 75°C. At 80°C although low alcohol was achieved than at 75°C there was the problem of poor body of the beer. On the other hand malt germinated for shorter period of time produced beers almost as good as those of the control brew. Only malt germinated for one day gave alcohol slightly lower than control. This shows that malt irrespective of having been germinated for 1, 2 or 3 days can produce an excellent product as long as the germination reached required levels during malting process so as to have a malt with sufficient diastatic power. Low gravity fermentation revealed that very low alcohol could be achieved by this method but dilution of wort meant also dilution of other beer characteristics resulting in a product which was watery and had no flavour. The major effect of diluting wort was that the content of fermentable sugars was reduced proportionally. However, the product of a 30% dilution was still acceptable since although alcohol was low, texture and flavour of the product were still reasonable. Thus mashing at 75°C, use of malt germinated for one day and method of 30% dilution can be recommended for the production of low alcohol opaque beers.

Key words: Sorghum beer, low alcohol, mashing, sorghum malting, low gravity fermentation, sensory analyses

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1.INTRODUCTION

Sorghum beer, also known as opaque beer, is unique to Sub-Saharan Africa. It differs in many respects from the conventional beer. For example, it is brewed with malted sorghum and not barley malt, is sour in taste as it is flavoured with lactic acid produced by fermentation during brewing, and is not hopped. The colour of the beer is pinkish-brown and it is opaque due mainly to the presence of gelatinised starch (Taylor, 1992). The yeast is not removed from the beer after fermentation, so that the presence of starch and yeast and its moderate alcohol content, approximately 3%(w/w), make sorghum beer a most nutritious beverage (Taylor, 1991). Sorghum beer is sold and consumed whilst fermenting. Because it is a microbiologically active product, it has a short shelf life of a maximum of 5 - 7 days.

Opaque beer is mainly consumed by the low income to middle income group and is a very popular drink in Zimbabwe as people refer to it as food and drink. The average alcohol content of sorghum beer consumed in Zimbabwe is 4% (w/w). Although approximately 600 million hectolitres of this product are produced and consumed each year in Zimbabwe not much research has been done with regard to low alcohol sorghum beer production.

As new technology develops, the art of brewing is also changing to a more practical, economic and versatile process. This results in new products with more consumer appeal and thus a better future for the brewing industry. Along with stricter traffic laws concerning

the use of alcohol and driving, greater consciousness of alcoholism and the need for lower caloric products, the viability of an alcohol-free product has to be evaluated and the technology to produce such a product developed (Lewis and Young, 1995).

In commercial applications, two process routes for alcohol reduction can be distinguished (Muller, 1990). These are;

1) Physical processes to remove alcohol after fermentation

2) Limited fermentations.

This project entails the production of low alcohol sorghum beer using the method of limited fermentations, which are; low gravity fermentation, high temperature mashing and use of short germinated malt

2. OBJECTIVES

The objectives of this research project were:

- *To determine the effect of high temperature mashing on the characteristics of opaque beer..

- *To determine the effect of low gravity fermentation on the characteristics of opaque beer.

- *To determine the effect of use of short time germinated malt on the characteristics of opaque beer.



3. LITERATURE REVIEW

3.1 The Science and Technology of Sorghum Malting

3.1.1 Objectives of sorghum malting

Malting can be defined as the germination of grain in moist air under controlled conditions (Dewar, Joustra and Taylor, 1995). There are three main objectives of sorghum malting for sorghum beer brewing which are:

- To mobilise the endogenous hydrolytic enzymes of the grain
- By means of these enzymes to modify the constituents of the grain during malting so that they are readily solubilised during the souring and mashing process of sorghum beer brewing in order to produce a fermentable medium
- By means of these enzymes to solubilise the unmalted cereal grain during the mashing process of sorghum beer brewing.

3.1.2 Grain Structure and modification during malting

The sorghum grain is composed of a number of parts which includes the germ or living part which on germination in the soil would develop into a sorghum plant and the endosperm or storage part (Dewar, Joustra and Taylor, 1995). The endosperm can be subdivided into two components: the floury endosperm, which contains mainly starch, which is stored in the starch granules; and the horny endosperm, which is rich in protein. The protein is stored mainly in organelles called protein bodies.

When sorghum germinates, certain hydrolytic enzymes migrate from the germ to the endosperm. Other hydrolytic enzymes already present in the endosperm become active during germination. During germination, the hydrolytic enzymes progressively degrade the protein and starch in the endosperm into amino acids and sugars, respectively. There is a wave of modification of the grain structure as the protein and starch is progressively hydrolysed. This process of modification renders the malt more easily solubilised during conversion and souring.

3.1.3 Starch degradation and amylase action during malting

Starch is composed of long chains of glucose molecules linked together by glucosidic bonds and it consist of two different types of molecules which are amylose and amylopectin. Sorghum mainly contains approximately 75% amylopectin and 25% amylose (Dewar, Joustra and Taylor,1995).

At the biochemical level, the degradation of starch during malting involves the action of a number of different hydrolytic enzymes in the malt of which the two most important of these enzymes are alpha-amylase and beta-amylase. Alpha-amylase and beta-amylase work together to bring about the almost complete degradation of starch into simple sugars.

Alpha-amylase attacks the alpha (1-> 4) glucosidic bonds within starch molecules to produce dextrans and a variety of sugars including maltotriose, maltose and glucose.

Beta-amylase cannot attack starch granules on its own, but attacks the dextrins produced by the alpha-amylase at the non-reducing end of the molecules, hydrolysing penultimate alpha (1-> 4) glucosidic bond to release maltose. The amount of joint alpha- and beta-amylase activity in sorghum malt is measured by means of the Diastatic Power (DP) assay and is expressed in Sorghum Diastatic Units (SDU) per gram of malt (Daiber and Taylor, 1995).

3.1.4 Protein degradation and protease action

Proteins are macromolecules consisting of chains of units called amino acids, which are linked together by peptid bonds. The protein bodies of sorghum comprises an aqueous alcohol-soluble protein called kafirin and it is surrounded by an acid - or base-soluble matrix of protein called glutelin (Taylor, Novellie and Liebenberg, 1984).

Protein hydrolysis is brought about by protease enzymes. There are two types of proteases: proteinases and peptidases. Proteinases catalyse the hydrolysis of internal peptide bonds within protein molecules to release peptides. Peptidases hydrolyse peptides into amino acids. The most important peptidases in malting and brewing are the carboxypeptidases which hydrolyse the terminal peptide bond at the "c" end of the peptides (Evans and Taylor, 1990a).

Both proteinases and peptidases are required to degrade proteins completely into amino acids. The amino acids and short peptides produced are collectively known as Free Amino Nitrogen (FAN).

FAN in sorghum malt is determined by the Ninhydrin assay and expressed as mg FAN per 100 gram malt (Taylor, Boyd and Pickerell, 1985).

In addition to starch and protein, a number of other components of the grain are metabolised and or solubilised during malting. They include non-starch carbohydrates, lipids, pigments, vitamins and minerals. These substances in malt can influence wort fermentability and the final character of the beer (Daiber and Taylor, 1995).

3.1.5 Effects of tannins during malting process

Sorghum cultivars can be divided into two groups: Bird resistant sorghums, those, which contain tannins and non-bird resistant sorghums, those that do not contain tannins. Tannins are located in the testa layer of the sorghum grain (Dewar, Joustra and Taylor, 1995).

The property of tannins of interest to maltsters and brewers is that they react with and precipitate proteins, particularly the malt enzymes rendering the malt enzyme inactive. The diastatic power of a water extract of an untreated malt made from bird resistant sorghum will be very low. The practical significance of this is that if the malt is used for brewing, starch will only be poorly hydrolysed into sugars. To use high tannin sorghums for malting, it is necessary to steep the grain in a very dilute solution of formaldehyde. The formaldehyde polymerises the tannins and prevents them from reacting with the malt enzymes (Daiber and Taylor, 1995).

3.1.6 Sorghum Malting Technology

Malting consist of three stages: steeping, germination and drying. In all these three stages control of temperature, humidity and air flow is required (Daiber and Taylor,1995).

Steeping: The objective of steeping is to hydrate the dry, resting grain sufficiently to initiate the metabolic processes of germination and also to clean the grain. During steeping the grain absorbs water and the germ becomes active and makes use of the oxygen dissolved in the steeping water. It is therefore essential that adequate levels of oxygen are maintained otherwise the grain will begin to respire anaerobically. Prolonged periods of anaerobiosis impair germination and may eventually kill the grain. Steep aeration is used to maintain the supply of oxygen and to ensure grain viability.

The rate at which grain germinates and grows increases with increasing temperature over a certain range. Therefore control of temperature is very important and this should be kept within reasonable range of 24-28°C. A study carried out at CSIR Food Science and Technology, South Africa revealed that steeping conditions had an effect on sorghum malt quality (Dewar, Taylor and Berjak,1997). The moisture content of the grain was found to increase with an increase in the length of steep and the rate at which water was absorbed into the grain was enhanced by increasing the temperature of the steep. The resulting malt's diastatic power increased significantly with an increase in steeping

time. The temperature at which the grain was steeped was found also to be affecting the quality of the malt produced. The diastatic power was enhanced by an increase in the temperature of the steeping water and with respect to FAN and the extract content a steeping temperature of 25°C was found to optimise the quality of the resulting malt. However, malting losses also increased with an increase in the temperature of steep.

Aeration during steeping also affects the malting quality of the grain by improving the extract content of the malt. It also has a significant effect on malting losses. Steeping the grain for increasing periods of time in non-aerated, stagnant water significantly reduces the losses incurred during malting (Dewar, Taylor and Joustra , 1995)

Germination: This is the process of seedling growth. During germination amylases, proteases and other endogenous hydrolytic enzymes of the grain mobilise from the germ into the endosperm. In the endosperm starch and protein are hydrolysed to sugars and amino acids respectively. Sugars are then respired and amino acids synthesised into new enzymic and structural proteins.

As germination continues the respiration rate increases, the main rise being attributed to the growing germ. Germination results in decline in dry matter therefore a balance must be struck where enough growth must be allowed to produce well modified malt, without allowing malt losses to become excessive. The intensity of grain respiration is greatly influenced by the conditions used in malting. Generally, within limits, grain respire faster at higher

temperatures and elevated oxygen levels . Oxygen levels increase grain respiration and accelerates enzyme production (Dewar, Taylor and Joustra ,1995). It is therefore essential to water sorghum at intervals throughout the germination period as failure to do so will result in malt of very low quality. However, high watering can also result in excessive malt losses.

During sorghum malting there is vigorous root and shoot growth and the malt becomes matted and these clumps can become very hot. This encourages mould growth on the malt, which is most undesirable because of the danger of mycotoxin formation. To avoid matting the malt is turned but this has to be done carefully as excessive breakages of roots and shoots can easily take place. Leakage of nutrients from the broken vegetative parts also encourages mould growth.

Drying: When germination is over the grain is dried in a flow of hot air and growth is stopped resulting in the seedling dying. The objective of this drying is to produce a shelf-stable product whilst maintaining the level of amylase activity. The malt is dried at a temperature of not more than 50°C to preserve the malt enzymes (Daiber and Taylor,1995).

3.2 The Science of sorghum (opaque) beer brewing

Sorghum beer brewing process comprises a number of steps, normally: souring, cereal adjunct cooking, mashing, spent grain separation and alcoholic fermentation. Figure1illustrates the entire

opaque beer brewing process. Opaque beer can be produced either by home brewing or by industrial brewing. In Southern Africa, sorghum beer brewing has developed during the 20th century from a home industry into a large scale industry enterprise (Haggblade and Holzapfel, 1989).

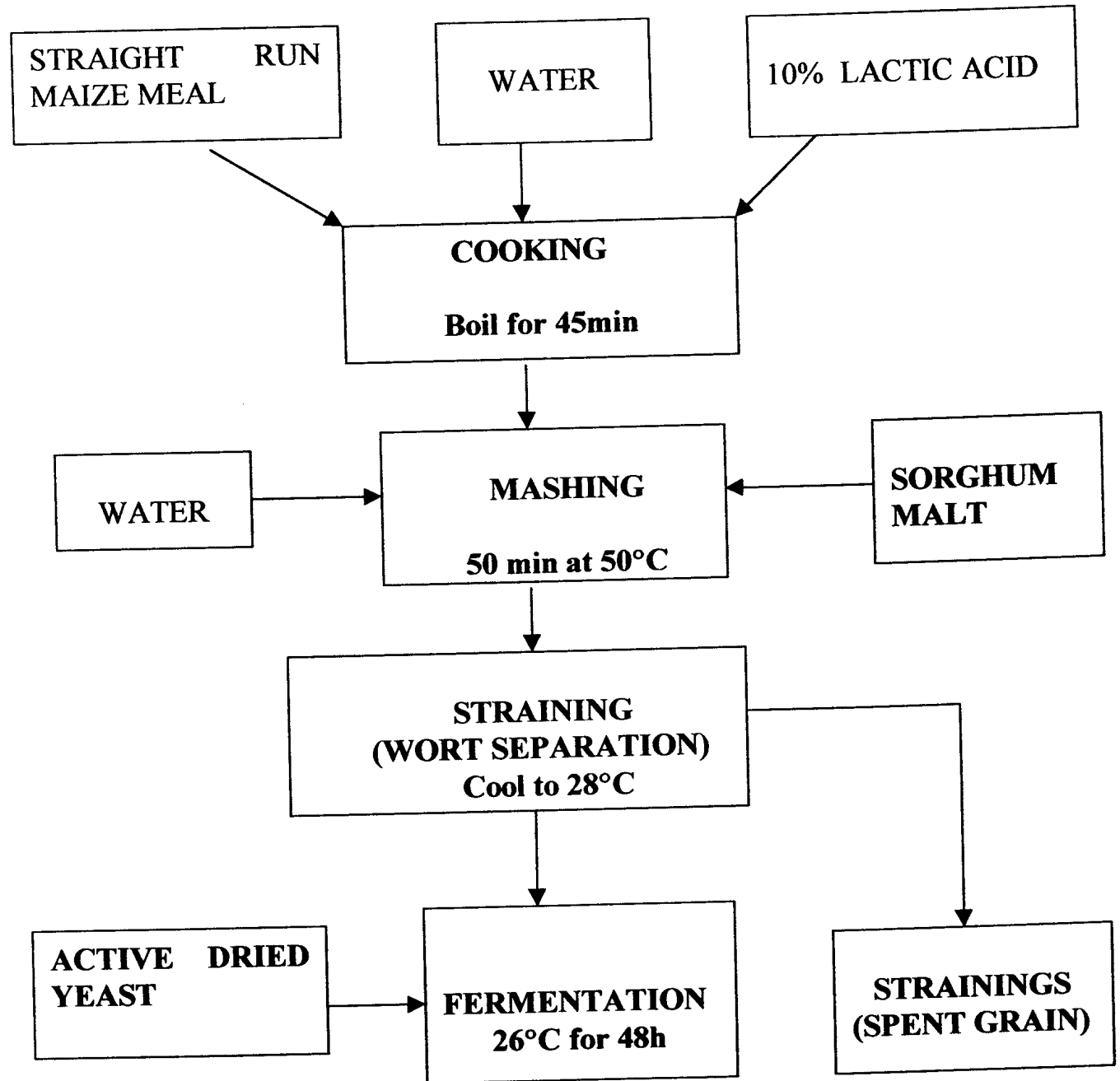


Fig.1 Flow chart for opaque beer brewing process

3.2.1 Souring

Souring is a process of lactic acid production by lactobacillus fermentation (Daiber and Taylor,1995). Despite souring being an extended period of all malt mashing, sugar formation is negligible because the low souring temperature limits availability of the starch substrate and the low pH (3.0-3.2) of the sour restricts enzyme actions.

Souring performs three important functions in sorghum beer brewing: the lactic acid impacts the beer with its characteristic taste, lowers the pH of the beer which slows down the rate of microbial spoilage and inhibits the growth of pathogenic microorganisms. The resulting residual starch in the beer contributes to its opaque, viscous character.

There are two types of souring: spontaneous and inoculated souring. Spontaneous souring is rather a hit and miss process, whose success is dependant on the presence of the lactic acid bacteria which form part of the natural microflora of the sorghum malt. Lactic acid production varies greatly between different batches of malt. The exact reason for this is not known, but obviously it is related to the number and type of bacteria on the malt and nutrient and buffering status of the malt.

Inoculated souring means that a portion of the previous sour, normally 10% by volume, which contains a high concentration of viable lactic acid bacteria, is used to inoculate the new sour. The conditions of temperature and time of souring are highly variable

but temperature needs to be strictly controlled. If the temperature is not strictly controlled this can result in the growth of mesophilic, heterofermentative lactic acid bacteria. These organisms produce acetic acid, ethanol and carbon dioxide in addition to lactic acid, plus minor by-products such as formic acid and glycerine. In home brewed beer these compounds which are other than lactic acid impart a flavour which may or may not be acceptable according to the local taste (Daiber and Taylor,1995).

In industrial sorghum beer brewing the temperature of souring should be strictly controlled, in the range of 48-50°C. This temperature prevents the growth of mesophilic organisms and favours the growth of thermophilic, homofermentative *Lactobacillus-delbrueckii* which produces solely lactic acid. Where souring is carried out at the brewery, the duration is normally up to two days and the final concentration of the lactic acid is normally 1-2% and the pH 3.0-3.2. If the lactic acid is produced separately and supplied to the brewery, the concentration is normally considerably higher up to 10%.

Malt enzymes also play a part during souring. Hydrolysis of malt starch by the diastatic enzymes appears to be limited since the temperature of souring is below the gelatinisation temperature of the starch and the low pH for most of souring inactivates the enzymes. Production of amino acids and peptides during souring by the action of the malt protease enzymes on malt protein is, however, considerable. Further, the amount of FAN taken up by the bacteria is negligible in comparison with that produced (Daiber and Taylor,1995).

3.2.2. Cereal Adjunct Cooking

The purpose of cooking is to gelatinise the starch in the unmalted cereal adjunct, thus rendering it readily hydrolysable during mashing by the malt amylase enzymes (Daiber and Taylor, 1995). Ungelatinised starch is only very slowly attacked by the malt alpha-amylase enzymes and not at all by the beta-amylase. The starch used as adjunct in opaque beer brewing i.e. from maize, sorghum or millet, has a high gelatinisation temperature in the range of 62-75°C. Above 70°C the malt amylase enzymes are normally rapidly inactivated. Alpha-amylase at this temperature can be prolonged by the addition of calcium ions (Taylor and Daiber, 1988)

3.2.3. Mashing

Mashing in sorghum beer brewing is performed with a large quantity of cooked starchy adjunct and some of this adjunct starch is not saccharified and contributes to the opaque character of the beer (Daiber and Taylor, 1995).

During mashing, the sorghum malt starch is not substantially hydrolysed because the normal mashing temperature, 55-60°C, is below its gelatinization temperature. Sorghum malt has good alpha-amylase activity but low beta-amylase and its starch has a high gelatinization temperature of 65-68°C (Daiber and Taylor, 1995). Research has shown that these particular characteristics of sorghum malt could possibly be used to produce novel clear beers rich in nutritionally desirable complex carbohydrates and low in

alcohol (Winstanley, 1990).

The mashing process involves the incubation, at optimal temperatures, of milled sorghum malt with the cooked starchy adjunct in water with the objective of physically and enzymically solubilizing components of the malt adjunct, to produce a fermentable wort (Daiber and Taylor,1995). In mashing many factors control the rate and extent of the enzymic degradation of starch and protein e.g. the enzymic activity of the malt, the ratio of malt to adjunct, mashing temperature, time and pH and the nature and form of the substrates.

Two major processes occur during mashing i.e. Starch hydrolysis and protein hydrolysis. Starch hydrolysis during mashing is mainly dependant on the joint action of the malt alpha- and beta-amylase enzymes. When malt is added to adjunct, at the start of mashing, the alpha-amylase enzymes hydrolyse glycosidic bonds within the adjunct starch molecules to produce dextrans. If alpha-amylase acts in the absence of beta-amylase a wort containing little fermentable sugar is produced. Alpha-amylase is therefore responsible for solubilisation of the mash i.e. hydrolysis of the starch to dextrans, and beta-amylase is responsible for hydrolysis of the dextrans to fermentable sugars (maltose) (Daiber and Taylor,1995).

Mash solubilisation is affected by mashing temperature. Up to 60°C there is only a small increase in solubilisation with temperature, but from 60 - 70°C there is a dramatic increase in solubilisation (Daiber and Taylor,1995). Above 75°C there is a reduction in solubilisation

due to thermal inactivation of the alpha-amylase. As for pH this only strongly affects mash solubilisation at high mashing temperatures. At 75°C mash solubilisation increases significantly with increasing pH (Taylor,1992).

Beta-amylase on the other hand, hydrolyses the terminal glucosidic bonds at the non-reducing ends of starch and dextrin chains to release maltose. Unlike barley malts, sorghum malts have a lower level of beta-amylase, which normally contributes only 20-30% of the total diastatic activity (Taylor and Robbins,1993).

Protein hydrolysis: There is no equivalent physical process to starch gelatinisation, which will render protein readily hydrolysable by proteolytic enzymes during mashing. During mashing the contribution of proteolysis to the FAN content of wort is relatively small, between 15 and 30% of the total (Taylor and Boyd,1986). The largest source of wort FAN is from the malt, as a result of protein hydrolysis during mashing. The remainder of the FAN in wort is from the sour and the starchy adjunct (Taylor and Boyd, 1986).

3.2.4 Fermentation

Fermentation is anaerobic respiration by yeast, where sugar is converted to ethanol and carbon dioxide. There are, however, a number of metabolic processes involved in fermentation which include nitrogen metabolism, as well as carbohydrate metabolism and fusel oil formation (Daiber and Taylor,1995).

Carbohydrate metabolism involves glycolysis and biosynthetic reactions. By means of the glycolysis pathway high concentration of fermentable sugars cause yeast to respire anaerobically. Through permeases the fermentable sugars in wort are taken up into the yeast cell with glucose being absorbed most rapidly, then maltose and finally maltotriose. The role of glycolysis is to harness the chemical energy present in glucose by making the high energy compound ATP (Fig. 1) (Daiber and Taylor, 1995).

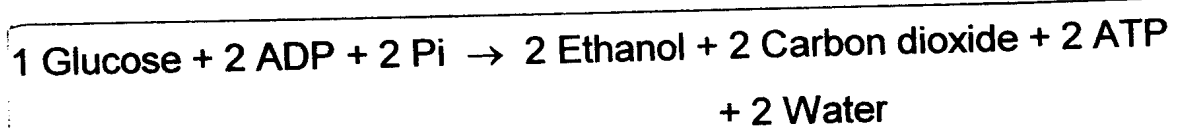


Fig.2 Overall reaction of glycolysis

Energy in ATP can then be used to drive energy requiring reactions such as the biosynthetic reactions of the yeast. The product of the glycolysis, pyruvate, a three carbon compound, is the same in lactobacilli and yeast but however, in yeast the pyruvate is subsequently degraded to ethanol.

Nitrogen metabolism also takes place during fermentation. In beer fermentation the bulk of the yeast's nitrogen requirement is met by amino acids and peptides (FAN). The FAN is taken up by yeast in a specific order during fermentation. The nitrogen assimilated by yeast is used for the synthesis of amino acids which are then incorporated into yeast proteins (Daiber and Taylor, 1995).

Fusel oils are higher alcohols, remaining after distillation of ethanol from fermented liquid. They are potentially aromatic and exert a considerable influence on the flavour and aroma of alcoholic drinks.

They are also the components responsible for giving one a hangover. Fusel oils are, however, found in low concentration in sorghum or barley beer. The actual levels of fusel oils in sorghum beer is rather higher than in barley beer and this may be due to the higher fermentation temperature for sorghum beer 25-30°C compared with 10-20°C (Daiber and Taylor, 1995).

Fusel oils are formed by yeast from the oxoacids. Thus, fusel oil formation is linked to amino acid metabolism. High levels of FAN in wort, in excess of that required by yeast for normal growth, can lead to elevated levels of fusel oils in the beer (Daiber and Taylor, 1995).

3.3 The Science and Technology of producing low alcohol beer

Alcohol reduction can be achieved through two process routes (Muller, 1990). The processes described concern conventional barley beer but could possibly be applied to opaque beer. The process routes are:

1) Physical processes to remove alcohol after fermentation

- a) Technical dialysis with or without vacuum distillation
- b) Reverse osmosis
- c) Vacuum evaporation in a thin film evaporator

2) Limited fermentations

- a) High gravity brewing
- b) High temperature mashing
- c) Cold contact process
- d) Use of special yeast
- e) Low gravity fermentation

3.3.1 Technical Dialysis

Technical dialysis is generally applied to beer, which has fermented to completion, meaning that all taste relevant substances will have developed fully (Saier, 1995; Lewis and Young, 1995). This process of dialysis operates according to the diffusion principle based on difference in concentration.

Beer containing alcohol is separated by a membrane from the alcohol-free medium (dialyzate). Only very low molecular weight substances such as alcohol can pass (permeate) through the membrane, while higher molecular components of the mixture are retained. Concentration goes to equilibrium due to difference in concentration of the permeable substances. The diffusion flow (volume per unit time) of e.g. alcohol as the permeable component passing through the membrane is a function of its mobility (coefficient of diffusion), the difference in concentration and the diffusion path. The latter is equivalent to the membrane thickness. The thinner the membrane, the higher will be the diffusion flow. The membrane used in dialysis has a larger pore diameter than the one used for reverse osmosis (Regan, 1989).

As the media flow in countercurrent path, the concentration gradient is maintained and the alcohol is continuously removed from the medium. Compared to the other higher molecular substances in the barley beer, alcohol diffuses considerably faster through the membrane. This leads to a strong selection also among the other permeable components of the medium e.g. the aroma substances

(Saier, 1995)

The degree of alcohol removal can be easily controlled by adjusting the flow velocities of the media. In the case of beer, alcohol removal is carried out at cellar temperatures (Saier, 1995), although there is no restriction on the temperature that the dialyzate can be exposed to (Regan, 1989). The flow in the unit is low and results in equally low mechanical stress. The natural carbon dioxide content of the beverage is maintained provided that the dialyzate contains the same quantity of carbon dioxide as the beer. The degree of alcohol removal can be regulated by adjusting only the flow velocities of beer and dialyzate (Saier, 1995)

3.3.2 Reverse Osmosis

So as to understand reverse osmosis, the concept of osmosis has to be understood first. In fluids concentration gradients equalise by diffusion. Should the concentration equalisation take place through a semi-permeable membrane, this diffusion process is referred to as osmosis. This pressure gradient between solutions on a semi-permeable membrane is referred to as osmotic pressure (Von Hodenberg, 1991).

The osmotic pressure on the side of the membrane with the higher concentration of soluble molecules has the higher osmotic pressure, so water or another solvent may move from the lower osmotic pressure to the higher to equalise the concentration gradient (Bailey and Ollis, 1986). Reverse osmosis is the

movement of solvent from the high osmotic pressure to the lower pressure by means of a pressure exerted on the high osmotic pressure side, greater than the osmotic pressure, so that the solvent move to the lower osmotic pressure side of the membrane.

The membranes used in the process of removal of alcohol in the beer by reverse osmosis have to have a high flux capacity for alcohol, low permeability for other substances in the beer, be able to withstand a wide range of pH (pH 2-11) and be temperature resistant (Bailey and Ollis,1986). A cellulose-acetate membrane is commonly used and is made up of modules installed between spacers and support plates. During alcohol removal, the beer (concentrate) runs between the membrane and the spacer. The alcohol-water mixture (permeate) goes through the membrane in the direction of the support plate and from there into the void area of the plate, and is taken away in a hose (Von Hodenberg, 1991).

3.3.3 Vacuum Distillation

Alcohol can also be removed from the beer by means of vacuum distillation due to the higher volatility of the ethanol than the majority of the other components present in the beer. According to Regan (1989), distillation columns were originally used for this kind of application, but nowadays an evaporation plant usually consist of single or multiplestage plate evaporators.

These units are similar in appearance to large plate heat exchangers and achieve evaporation by the transfer of latent heat

through a plate in the beer stream. Vacuum steam of 50-60°C is normally used as the heating medium. The beer is conventionally preheated to the evaporation temperature to prevent wasted heat. The two phase beer/vapour mixture is then passed through to the separator. The alcoholic vapour is removed from the top and the dealcoholised product from the bottom of the vessel. This type of equipment has been used for this type of application since 1980 and can be operated with a single unit where the beer is recirculated or as a multi-stage unit (Regan,1989).

The maximum temperature the beer reaches is below 40°C and with a retention time of 3-5 minutes. The alcohol by-product has a consistent composition at about 8-9% (v/v) ethanol. This by-product is normally sold for acetification to produce vinegar (Regan,1989). The carbon dioxide in the product is also removed by the vacuum evaporation and has to be replaced together with the fraction of water evaporated. Vacuum evaporation can reduce the alcohol content lower than 0.3% (v/v) ethanol.

3.3.4 High Gravity Fermentation

High gravity is used for brewing standard lager beer. It can, however, be adapted to brew low alcohol beer by mashing the grits in the normal way and fermented by using an appropriate yeast (Muller,1990). The beer can then be diluted back to a standard flavour level rather than a standard alcohol content. The brewer can manipulate the process to produce a higher quantity of esters rather than a high amount of alcohol, especially at higher gravities.

When this product is diluted it still contains a high amount of flavour compounds, but with lower amount of alcohol. For lager beers bitterness and colour can be adjusted by using hop extracts and crystal malt. The total nitrogen in the product is also low and results in a poor head retention and a low amount of foam.

The low alcohol beers produced by high gravity brewing are very similar to normal beers. In addition, they have a low gravity, a low attenuation limit, and they do not contain usually high levels of fermentable sugars or dextrans. A major drawback of this process in producing non alcoholic beer is that it would have no mouthfeel and would be experienced as very thin (Muller, 1990).

3.3.5 Use of Special Yeasts

According to Muller (1990) the use of special yeasts can produce beers with ethanol values of 0.5% (v/v) or less. These yeasts are not genetically engineered but have been carefully selected from the natural environment. Some of these yeasts have been used for many years in wine fermentation. A standard grist is mashed in the usual way and the wort is boiled and pitched with this special yeast. Conditioning, filtration and dilution are as standard.

The principle of this process relies on the fact that the major fermentable sugar in wort is maltose (75%) which some yeasts are unable to ferment this sugar. Such yeasts ferment any glucose, sucrose and fructose available and are thus able to convert the wort to beer. The result is a beer with approximately 0.5% (v/v) ethanol.

An important consideration is that maltose is much less sweet than sucrose (30% relative sweetness) or glucose, so careful use of bittering substances should mask any effect on flavour. The major disadvantage of this beer is the difficulty in controlling the microbial activity both during fermentation and in the final product, due to the low rate of yeast activity (Muller, 1990).

3.3.6 Cold Contact Process

The cold contact process is of considerable interest since it is the only form of limited fermentation that yields an alcohol-free beer, that is with less than 0.05% (v/v) ethanol (Muller, 1990). A normal grist is mashed in the usual way with standard wort boiling. The wort is cooled to approximately -1°C to 0°C and is pitched with yeast. The wort is held at this temperature for several days, then the yeast is removed and the brewing process continues as normal.

One might consider that very little could happen during this fermentation process, but it is in fact not the case. Certainly little or no yeast metabolism can occur, however various biochemical reactions do take place. Carbonyl compounds are reduced which remove warty flavours, and esters can be formed, by biochemical and not by enzymatic reactions, to give beer flavours (Muller, 1990).

Adsorption processes also occur by the hop and wort which adsorbs to the yeast surface, so that wort is converted to beer at these low temperatures. The major advantage of this process is the low amount of alcohol produced (Muller, 1990).

3.3.7 High temperature mashing

This method is based on the principle of inhibiting diastatic enzyme activity. The mashing of the malt takes place at high temperatures, which allow only the alpha-amylase to produce fermentable sugars and a considerable quantity of dextrans, which is not fermentable.

The beta-amylase, which produces only maltose, a fermentable sugar, cannot survive the high temperatures and is denatured before it can be active (Muller,1990). Beta-amylase is more temperature sensitive than alpha-amylase and as a result the temperature differences between them and the intrinsically low ratio of beta-amylase to alpha-amylase in sorghum malt apparently facilitates the production of beers of low alcohol content, but rich in complex carbohydrates (Taylor , 1992).

Mashing at high temperatures (80°C) leaves wort that is 25-27% fermentable and free of starch (Muller,1990). It inactivates beta-amylase almost instantly while still allowing hydrolysis of all the starch present. At 85°C the denaturing effect on amylases is more acute and starch breakthrough could be a problem (Muller,1990). This method of production leaves high levels of dextrans in the beer and there has been concern regarding the stability of the product. The high levels of dextrans may be a point of concern, because it leaves a gap for some spoilage of the fermented beer to occur. Pasteurisation may however prevent this from occurring.

The temperature differences between alpha- and beta-amylase and

the intrinsically low ratio of beta-amylase to alpha-amylase in sorghum malt apparently facilitate the production of beers of low alcohol content but rich in complex carbohydrate. Also the solids content of the mash influences starch solubilization and fermentable sugar production (Muller,1991)

A major drawback of this method is the control of the mashing temperature because a lower temperature of less than 80°C may result in more fermentability and higher temperatures may result in gelatinised starch breakthrough (Muller,1990). The biochemistry of which this relies is shown in Figure 3.

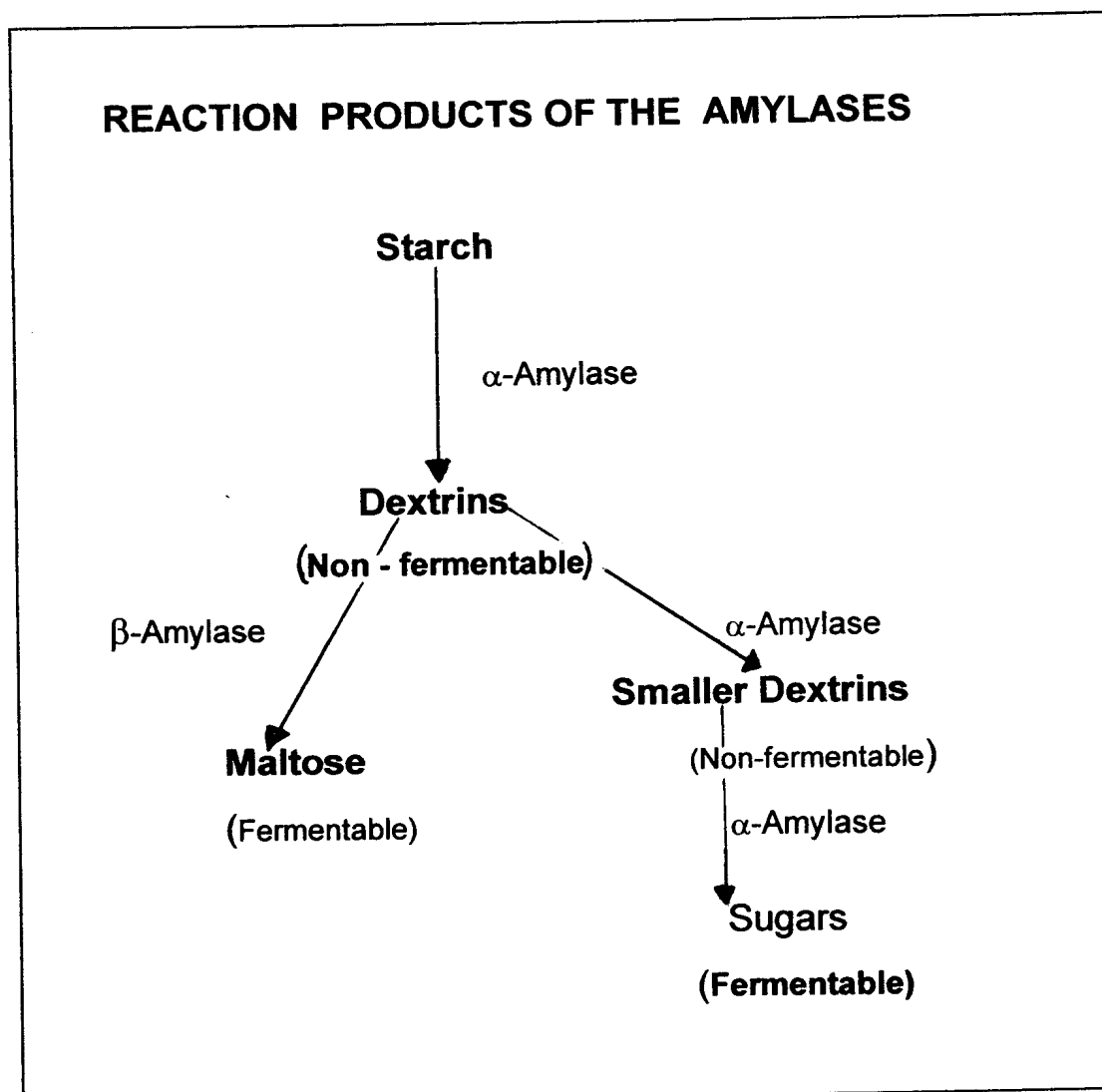


Fig.3 Reaction products of the amylases (Muller,1990)

According to Chandrasekhar (1994) low alcohol beer and alcohol-free beer can also be produced using a combination of high temperature mashing and cold contact fermentation.

Sorghum beer is brewed with malted sorghum and not with barley malt and one of the major problems in sorghum beer brewing is that of the production of sugar. According to a study carried out by Taylor and Daiber (1988) to determine the effect of calcium ions on sugar production and other parameters of mashing in an industrial - type sorghum beer brewing process it was observed that calcium improves liquefaction, yields and accelerates mash run-off.

Added Calcium ions during high temperature mashing protect alpha-amylase against inactivation at extremes of temperature and pH. About 200 ppm is required but a typical level of calcium in municipal tap water used for sorghum beer brewing is approximately 30 ppm, which is very suboptimal (below the required level) for alpha-amylase survival. During this study it was observed that supplementation with calcium enables the same amount of sugars to be produced with a considerable lower ratio of malt to adjunct i.e. in the presence of 200 ppm calcium, 10% malt produced as much sugar as 20% malt with straight tap water (Taylor and Daiber, 1988).

3.3.8 Low gravity fermentation

Low gravity fermentations simply involve diluting the wort to a low gravity (approximately S.G. 1.010) and fermenting and processing as usual. Alternatively beer produced at normal gravity could be

diluted after fermentation. Low levels of alcohol can certainly be reached by dilution but dilution of alcohol is paralleled by dilution of flavour compounds. Some additional measures can be employed to increase the flavours with the dilution in mind, but nevertheless leaves a product with lack of flavour and “thinness” of the beer being the major disadvantage (Muller, 1990).

Both alcohol removal systems and limited fermentations have some advantages and disadvantages with regard to the production of the low alcohol beer (Muller, 1990).

Advantages and disadvantages of alcohol removal systems are:

Advantages

- Can achieve < 0.05%(v/v) ethanol
- Employ normal brewing processes
- Some methods yield food grade ethanol

Disadvantages

- Extra plant is required
- Extra processing is required
- Some flavour loss
- Some methods can leave undesirable taints.

Advantages and disadvantages of limited fermentations are:

Advantages

- No extra specialised equipment is needed.
- No extra process is required
- No flavour loss

Disadvantages

- Requires fine control of existing equipment
- Generally can not achieve less than 0.5% Ethanol
- Some methods leave undesirable flavours

In a speech during a Seminar held at the Brewing Research Foundation, Murray (1989) indicated that much more could be gained from high gravity and high temperature mashing and from new removal techniques (dialysis, reverse osmosis). They offered better product for the future but more work on them was deemed necessary.

According to Sfat and Doncheck (1990) non-alcoholic beers with flavour profiles ranging from estery and winery to grainy and malty could be prepared by dilution without using reverse osmosis, evaporation, or separation equipment to remove alcohol.

During his speech at the Institute of Brewing Seminar held at the Brewing Research Foundation in November, Winstanley (1990) suggested that for further market growth of low alcohol beers in the USA markets, there had to be improvements in the taste characteristics and less negative marketing. The product had to be sold in its own right rather than by emphasising that it had little or no alcohol.

According to work published by Taylor,(1992) investigations of a wide range of mashing conditions during the production of opaque beer revealed that a large increase in extract took place over the

temperature range of 65-70°C because gelatinisation and saccharification of the sorghum malt starch. Fermentable sugar formation was, however, maximal at only 65°C. When mashing was performed with all-sorghum malt grist, high extract but low fermentable sugars were obtained at a constant mashing temperature of 75°C. Best all round results were achieved with a triple-decoction mashing process that facilitated gelatinization and saccharification of starch and fermentable sugar formation. It was observed that these particular properties of sorghum malt apparently enable mashing at elevated temperatures to produce worts rich in nutritionally desirable complex carbohydrates (dextrins) and low in fermentable sugars.

The low ratio of beta-amylase to alpha-amylase in sorghum malt and the temperature differential between the enzymes appear to facilitate mashing at an elevated temperature, which produces worts rich in complex carbohydrates and low in fermentable sugars, from which novel low-alcohol beers could be made (Taylor, 1992).

Although a lot of work has been done with regard to production of low alcohol barley beers, not much research has been done on opaque low alcohol beer. This work was carried out using the methods of low gravity fermentation (diluting the wort), high temperature mashing (to inactivate the beta-amylase) and the use of malt germinated for short period of time (low amylase malt).

4. EXPERIMENTAL

4.1 Materials

Raw materials used for this project were sorghum malt, maize meal, lactic acid and yeast. Industrial malted sorghum grain cultivar DC75 was used for all the brews. Straight run maize meal was used as an adjunct. Commercially prepared lactic acid (10%) and yeast were also used for the production of these low alcohol beers.

4.2. Characterisation of the raw materials

4.2.1 Sorghum malt

Sorghum grain was malted by the maltsters as follows: steeping was carried out for 10 h and then the grain was air-rested for 8 h. This was followed by germination for 4 d and wilting for 24 h. Kilning was done for 5 h at 50°C after which the temperature was raised to 60°C and left there until moisture of 15% was achieved. To complete the drying process the temperature was raised to 65°C and left for 3 h. When it was dry the grain was then milled and was ready for use. Before it was used it was however analysed for sorghum diastatic power (DP), moisture, gradient extract, Free Amino Nitrogen (FAN) and Soluble Nitrogen.

4.2.2. Straight run meal

Maize was milled using the hammer-mill with screen size 1.0 mm nominal aperture size. The meal obtained was then analysed first

before it was used. Moisture analysis was done using an Infra-red moisture analyser. Sieve analysis was carried out using Wire Mesh 12 (Nominal Aperture Size 1.40 mm), Mesh 16 (Nominal Aperture Size 1.00 mm) and Mesh 30 (Nominal Aperture Size 500 microns).

Fat analysis was done using the Soxhlet Extractor Apparatus. Diethyl ether/petroleum ether 1:1 mixture with boiling point of 40 - 60°C was used.

Percentage extractable starch content of the milled maize was determined using the standard gradient regime (mashing bath method).

4.2.3 Yeast

Commercially prepared active dried yeast was used. This yeast was obtained from Anchor Yeast, Gweru, Zimbabwe. Yeast was analysed for the following parameters first before being used :

- a) Physio-Chemical parameters at 28°C i.e. Foam-head and ethanol production at 20 h
- b) Microbiological analyses i.e. *Escherichia coli*, live yeast count, contaminating bacteria, wild yeast count, lactobacilli, viable yeast count and coliform bacteria.

4.2.4 Lactic Acid

Commercially prepared lactic acid of 10% strength was used. It was only analysed for its strength by titration with 1 Molar sodium hydroxide. Titration was carried out to a pH of 2.25.

4.3 Brewing process

The brewing processes were carried out at a lab-scale using 18 litre vessels, hot plates and laboratory stirrers. The same raw materials (i.e. maize, sorghum malt, yeast and lactic acid) were used for the normal (control) brews as well as for the test brews and the brews only differed in the way they were processed. All experiments were carried out in duplicate and a control run parallel to them. The brewing process of the standard sorghum beer is illustrated in Figure 4.

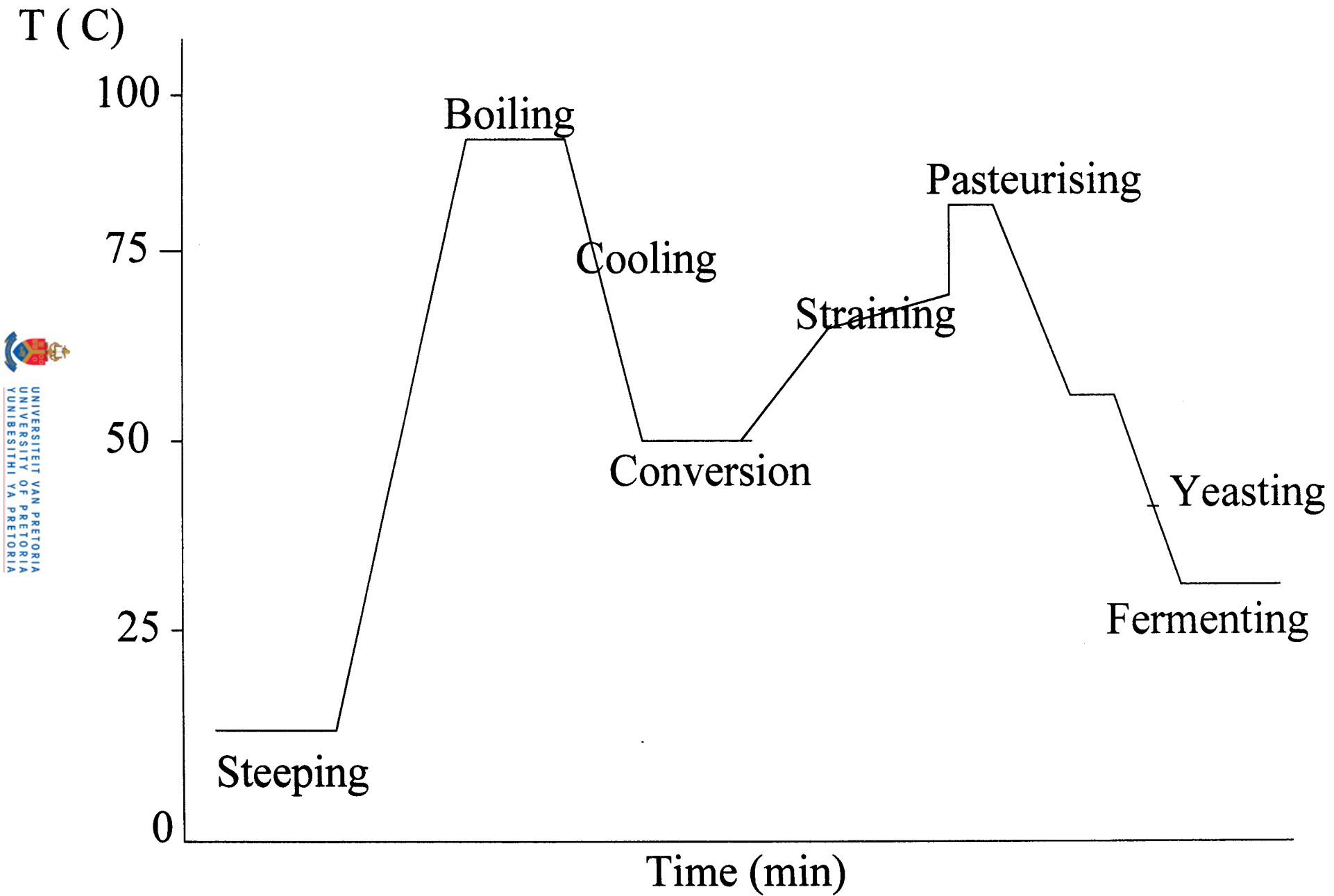


Fig.4 Standard opaque beer brewing process

4.3.1 Cereal Adjunct Cooking

The standard raw material recipe used for the 18 litre brew was 2160 g straight run maize, 82 ml lactic acid and 15 L of water. These were steeped together and cooked for 45 min at boiling temperature. With the addition of lactic acid a pH of 3.5-4.0 was aimed at and this addition of lactic acid has the effect of softening the protein which tightly encloses the starch granules thus allowing more rapid water uptake by the starch granules and speeding up gelatinisation..The purpose of this cooking was to gelatinise the starch in the unmalted cereal adjunct, thus rendering it readily hydrolysable during mashing by the malt amylase enzymes.

4.3.2 Mashing (Conversion)

After the cooking process of 45 min approx. 3 L of water was added to cool down the brew to 53°C. At 53°C, 528 g sorghum malt was added and the brew further cooled to 50°C where it was held for 50 min (standard conversion time). This mashing process involved the incubation of milled sorghum malt with the cooked starchy adjunct in water. The objective of this process was to physically and enzymically solubilise components of the malt and adjunct to produce a fermentable wort.

4.3.3. Straining (Spent Grain Separation)

At the end of conversion, the temperature was raised to 70°C and the wort was strained. Straining was carried out using a 1 mm wire mesh sieve. The objective of this straining was to remove coarse

particles of cereal such as pericarp from the mash.

4.3.4 Pasteurisation

After straining, the wort temperature was raised to 80°C and pasteurisation took place for 15 min. After the 15 min the wort was cooled down to 65°C through addition of water to make up the volume to 18 L.

4.3.5 Second Conversion

At 65°C, 48 g sorghum malt was added for the second conversion and temperature held at 65°C for 10 min. The wort was left to cool down to 35°C where it was then pitched using 6 g yeast. The pitched wort was cooled down to 28°C and left to ferment.

4.4 Treatment

4.4.1 Method of low gravity fermentation

A set of brews was conducted using the method of low gravity fermentation. These brews were carried out as the standard brew up to the wort stage and then the wort was diluted to low gravity. A range of dilutions was used i.e. 30, 40 and 50%. These brews were then yeasted and left to ferment as usual.

4.4.2 Method of high temperature mashing

Another set of brews was carried out using the method of high

temperature mashing at different temperatures of 65, 70, 75 and 80 °C. Calcium chloride 200 ppm was added during mashing so as to conserve alpha-amylase activity (Taylor and Daiber, 1988).

4.4.3 Method of short malting time

The final set of brews was put up using malt germinated for shorter periods of 1 day, 2 days and 3 days and compared with the brews which were carried out using malt of standard germination of 4 days.

4.5 Process Analyses

Several analyses were carried out on the raw materials, during the process and on the final products. The final products were then analysed up to 120 h. Analyses carried out were Diastatic Power (DP), Gradient-Extract, Soluble Nitrogen, Free Amino Nitrogen (FAN), Specific gravity (SG), pH, Soluble solids(refractometry), Total Reducing Sugars (TRS), Viscosity, Total solids (TS), Total acids (TA), Ethanol content, Settling and Foam-head.

4.5.1 Diastatic Power (DP)

Diastatic Power is expressed in sorghum diastatic units (SDU) where 1 SDU equals approximately 2 Lintner. Diastatic Power is a measure of amylase enzyme activity. The enzymes alpha- and beta-amylase are required during brewing to hydrolyse starch into fermentable sugars. A typical DP specification for sorghum malt purchased by a sorghum beer brewery would be a minimum of 28

SDU/ g malt sample.

The malt was ground to a standard fineness and an aqueous peptone extract was made thereafter and filtered. This extract was allowed to act on a standard buffered starch for 30 min at 30°C. At the end of this period the action was stopped by the addition of sodium hydroxide solution. The sugars that were produced by the malt extract were estimated by boiling the aliquot of the solution with ferricyanide and the unchanged ferricyanide was determined by titration. A blank was carried out in order to make allowance for sugar, i.e. that already in the malt extract. Diastatic power was then calculated according to a given formula (South African Bureau of Standards, 1970.)

4.5.2 Solubility

Solubility is a measure of how effectively the high-tannin sorghum grain has been treated to inactivate the tannins. This was determined by extracting the malt with water and measuring the DP, then expressing it as a percentage of peptone extract DP.

4.5.3 Free Amino Nitrogen (FAN)

FAN is a source of nitrogen for yeast growth during fermentation. A typical FAN specification for sorghum malt would be a minimum of 110 mg FAN/100 g dry mass of malt sample. FAN was determined by the European Brewery Convention (EBC)-Ninhydrin method. It measures amino acids, ammonia and to some extent, end group alpha-amino nitrogen in peptides and proteins. The diluted sample

was heated with ninhydrin and the colour produced was measured at 570 nm absorption (Morrall, Boyd, Taylor and Van der Walt, 1986).

4.5.4 Hot water extract

This is a measure of how much of the malt will be solubilised during the mashing process. In other words it is the percentage of dry substance in the malt, which is soluble in water when extracted over a standard gradient regime. It was measured using the EBC method and determined by specific gravity and calculated using Plato table. It was expressed as a percentage (%w/w). (Morall, Boyd, Taylor and Van der Walt, 1986).

4.5.5 Soluble Nitrogen

This was determined by the Kjeldahl method (European Brewery Convention, 1987). The organic material was digested with concentrated sulphuric acid in the presence of a catalyst and sodium or potassium sulphate. The nitrogen evolved in the form of ammonia combined with the sulphate ions to form ammonium sulphate which, in the presence of excess alkali and zinc catalyst, further dissociated to liberate ammonia. As the ammonia distilled over into the receiver, it was trapped in a solution of boric acid, in which form it was determined titrimetrically.

This method was used for wort, beer and malt extract. For malt it was then expressed as % Total nitrogen (TN) on dry malt and for wort or beer as mg Total Soluble Nitrogen/100 ml wort/beer and expressed as % Total Soluble Nitrogen (TSN)/ dry gram of malt.

4.5.6 Total Reducing Sugars

This was determined by the potassium ferricyanide method. The diluted sample was mixed with ferricyanide solution and boiled in a water bath for 20 min. A portion of the ferricyanide was reduced by the sugar to ferrocyanide. The remaining ferricyanide reacted with the potassium iodide to form iodine. The liberated iodine was then titrated with standard sodium thiosulphate in the presence of a starch indicator. Maltose was used as standard. Total reducing sugars were expressed as g/100 g sample but then expressed as percentage (Chibuku Breweries Limited Analytical Manual, 1985).

4.5.7 Ethanol

Ethanol was determined by steam distillation (Chibuku Breweries Limited Analytical Manual, 1985). By distillation of beer, alcohol and other volatile substances (the distillate) were separated from the other constituents (the residue). With this method, the assumption is made that the distillate contains only alcohol and water. The % ethanol was then measured in a volume of water/alcohol equal to the volume of the beer from which it was distilled and calculated using specific gravity. Ethanol was expressed as %(v/v).

4.5.8 Specific Gravity

Specific gravity of a liquid, is the density of that liquid, relative to the density of distilled water, at a particular temperature (in this case 20°C). The Plato value is the gram of pure sucrose in 100 g of solution, or the grams of extract in 100 g of wort, equivalent to the relative density of that solution to 20°C The Plato value P is also

referred to as degrees Plato °P or %P. Plato tables for % extract equivalent to the SG at 20°C were then used to obtain the specific gravity of wort (Chibuku Breweries Limited Analytical Manual, 1985).

4.5.9 Total Solids

Total solids in a beer means suspended solids or particles plus any substances in solution i.e. sugars and other soluble substances. The solids determination was carried out by evaporating off all the water from the sample, cooling it in a desiccator and then calculated using mass difference and expressed as %(w/w) (Chibuku Breweries Limited Analytical Manual, 1985).

4.5.10 Total Acidity

The measurement of total acidity is important because too little acid gives a bland beer and too much results in a sour beer which is undesirable and can be considered as an off-flavour. Acidity is one of the main factors determining the shelf-life of beer.

Total acidity was determined by direct titration with 0.1 M sodium hydroxide and using phenolphthalein as an indicator. Phenolphthalein was chosen as the indicator because of the weak organic acids present in the beer, the predominant one being lactic acid and both have an end point pH range of pH 8.2 to 9.8. Total acidity was expressed as %(v/v) (Chibuku Breweries Limited Analytical Manual, 1985).

4.5.11 Viscosity

Viscosity is an important parameter since it affects the rate of settling in the beer and is important for mouth feel. Viscosity is affected by the amount of conversion which takes place as under-conversion will give a viscous product but the alcohol levels will be less and over-conversion will give more alcohol but the beer will be thin and will settle more easily.

Viscosity was measured using the Brookfield Synchro-Lectric Viscometer (Brookfield Engineering Laboratories. Inc. Stoughton, Massachusetts). This viscometer measures viscosity by measuring the force required to rotate a spindle in a fluid. The force to which the spindle responds are extremely small, and the optimum performance of the instrument depends on the elimination of any extraneous friction which might disturb the sensitivity of their measurement. The viscosity will also depend on the temperature of the liquid. Viscosity was measured at a temperature of 25°C and spindle no.2 was used at speed no.30 and was expressed in centipoise per second (1cPs=1mPas) .

4.5.12 Settling

Beer (100 ml) was poured into a 250 ml measuring cylinder and left to stand for 30 min. At the end of the stand duration the height of the watery layer was measured and expressed as a percentage of the height of the liquid. This was expressed as %(v/v).

4.5.13 Foam-head

Beer (100 ml) was poured into a 250 ml measuring cylinder and left to stand for 5 min. At the end of 5 min the height of the foam was measured and expressed as a percentage of the height of the liquid (%v/v).

4.4.10 Sensory Analyses

An experienced test panel consisting five men aged between 31 - 40 years was used for this project. All beers were tested at 48 h and evaluated for colour, head, bite, settling, texture, smell and taste. The responses were categorised as follows;

Like very much

Like

Neither like nor dislike

Dislike

Dislike very much

The highest score of 5 was given for like very much while the least score of 1 was given for dislike very much. From this the mean of each parameter was calculated followed by mean acceptability of the product as a whole.

5.0 RESULTS

5.1 Raw materials quality

5.1.1. Malt

Malt, which was used for all the investigations, was analysed first before being used. Results of the malt germinated for the standard 4 days were all within specifications as shown in Table 1. Malt germinated for shorter times gave values generally lower than specifications (Table 2).

Malt germinated for only one day had the least diastatic power of 13 SDU/g (std 22-33) and FAN 106 while standard is > 150 ppm. All the other parameters were also lower than specifications. Malt quality parameters increased with germination days.

Table 1. Quality of sorghum malts used in the investigation

Parameter	Batch A 025	Batch A 040	Specifi- cations
Moisture (%)	6.8	8.0	5 -10
Diastatic Power (SDU/g)	29	26	22 - 33
Solubility (%)	97	92	> 90
Hot water extract (%w/w)	40.6	48.0	> 40
Soluble Nitrogen (%)	0.059	0.052	> 0.050
Free Amino Nitrogen (ppm)	169	189	> 150

Table 2. Quality of sorghum malt germinated for short time

Parameter	1 Day	2 Days	3 Days	Specifications
Moisture (%)	8.0	9.3	8.4	5 - 10
Diastatic Power (SDU/g)	13	19	26	22 - 33
Solubility (%)	65	83	90	> 90
Hot water extract (%w/w)	39.9	40.4	44.1	> 40
Soluble Nitrogen (%)	0.028	0.032	0.043	>0.050
Free Amino Nitrogen (ppm)	106	121	139	> 150

5.1.2 Straight run maize meal and Yeast

Analytical results for the straight run maize meal, which was used for the investigations, are given in Table 3. All parameters were within specifications. Table 4 shows yeast results and again here parameters were within specifications.

Table 3. Quality of straight run maize meal used for the investigations

Parameter	Results	Specifications
Moisture (%)	11.0	< 12
Fat (%w/w)	3	< 4
Hot water extract (%w/w)	76.9	> 70
<u>Sieve Analysis</u>		
% retention 12 Mesh	0	0
% retention 16 Mesh	2.9	< 4.0
% retention 30 Mesh	33.2	25 - 40



Table 4. Quality of yeast used for the investigations (Batch 704)

Parameter	Results	Specifications
<u>PHYSICO-CHEMICAL</u>		
Foreign matter	0	0
Moisture (%)	8.5	< 10
Foam head at 16 h (%)	12.9	> 10
Ethanol at 18 h (%v/v)	3.0	> 2.0
<u>MICROBIOLOGICAL</u>		
Viable yeast count (%)	98	> 90
<i>E. Coli</i>	0	0
Live yeast count (c/g)	9×10^{11}	$> 1 \times 10^{10}$
Total contaminating bacteria (c/g)	4×10^7	$< 5 \times 10^7$
Wild yeast count (c/g)	0	$< 1 \times 10^5$
Lactobacilli (c/g)	3×10^5	$< 5 \times 10^5$

5.2. Product profile results of all brews

Three sets of investigations were carried out. Each treatment had its on control run parallel to it. Duplicate brews were done and the mean of the representative experiments is presented. All brews carried out were allowed to ferment up to 120h while being analytically and organoleptically analysed.

Table 5 shows specifications up to 72h for a standard opaque beer while Tables 6 to 10 show results of the first treatment of brews converted at different temperatures .

Tables 11 to 13 show results of brews, which used malt, germinated different times while Tables 14 to 16 show results of brews carried out using low gravity fermentation method.

Table 5. Product specifications for standard opaque beer

Parameters	Fermentation time (h)			
	0	24	48	72
pH	5.0 - 5.3	3.9 - 4.1	3.6 - 3.7	3.5 - 3.6
Soluble solids (%)	9.0 - 9.5	5.5 - 6.0	4.5 - 5.0	4.0 - 4.5
Total Reducing sugars (%)	7.0 - 7.5	2.5 - 3.0	1.0 - 1.5	0.3 - 0.4
Ethanol (%v/v)	0	1.8 - 2.2	3.4 - 3.7	3.7 - 4.0
Total acids (%v/v)	0.09 - 0.11	0.15 - 0.20	0.28 - 0.30	0.30 - 0.34
Viscosity (cP)	60 - 70	50 - 60	50 - 60	50 - 60
Total solids (%w/w)	12	ND	ND	ND
Foam head (%)	0	ND	> 3.0	ND
Settling (%)	0	0	0	ND
Original gravity (°P)	1.040	ND	ND	ND

ND - Not determined

5.2.1 Effect of fermentation time on the quality of opaque beer converted at different temperatures

Table 6. Effect of fermentation time on the quality of the control opaque beer for brews converted at different temperatures

Parameters	Fermentation time (h)					
	0	24	48	72	96	120
pH	5.18	3.98	3.83	3.68	3.58	3.47
Soluble solids (%)	9.1	5.7	4.6	4.2	3.9	3.6
TRS (%)	7.5	2.9	1.3	0.6	0	0
Ethanol (%v/v)	ND	2.8	3.8	4.2	4.5	4.3
Total acids (%v/v)	0.12	0.18	0.26	0.34	0.40	0.42
Viscosity (cP)	58	56	55	55	53	53
Total solids (%w/w)	11.5	7.4	5.9	4.8	4.4	3.8
Foam head (%)	ND	4.3	5.0	2.4	0.4	0
Settling (%)	ND	0	0	0	0.28	0.41
Original gravity (°P)	1.045	ND	ND	ND	ND	ND

ND, not determined

TRS, Total reducing sugars



Tables 6 to 10 show that while all other parameters decreased with fermentation time, ethanol, settling and total acidity increased.

The major difference however, noted was that with increase in conversion temperature there was a decrease in initial sugars in wort. High wort sugar was however, being taken up faster than low wort sugar. Initial sugars in wort in the control brew was 7.5% while converting at 80°C resulted in an initial wort sugar as low as 4.6% (Fig. 5 and Tables 6 to 10)

Ethanol production was lower with higher conversion temperatures as opposed to lower conversion temperatures of 50-65°C which are optimal conversion temperatures for opaque beer. Conversion at 50°C achieved the highest ethanol of 4.5% at 96 h while the least ethanol of 3.3% was achieved from converting at 80°C (Fig. 6 and Tables 6 to 10).

Viscosities of the brews mashed at higher temperatures were lower than those of the control brew but were however, still within acceptable levels for opaque beer.

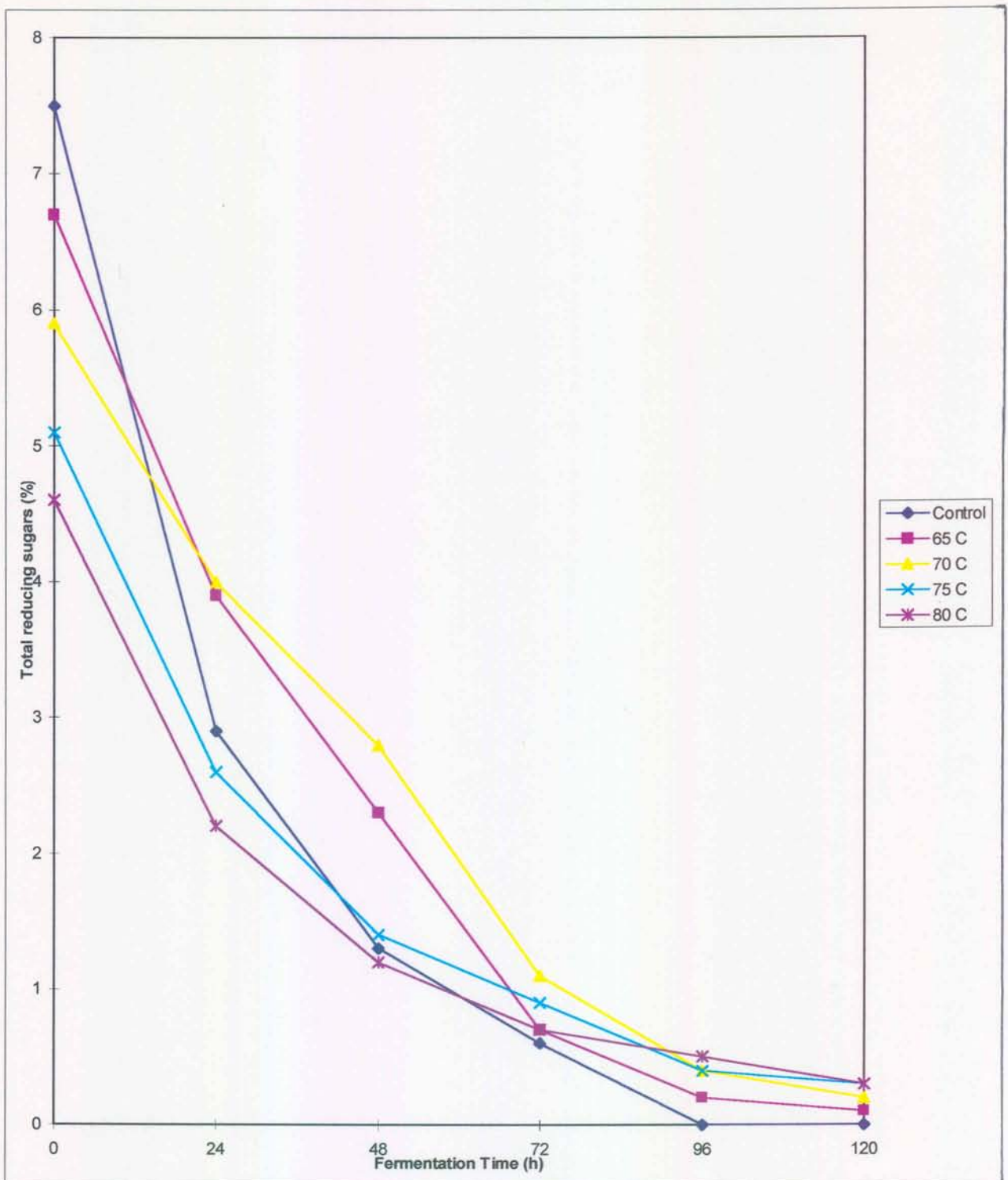


Fig.5 Effect of conversion temperature on total reducing sugar uptake during opaque beer fermentation

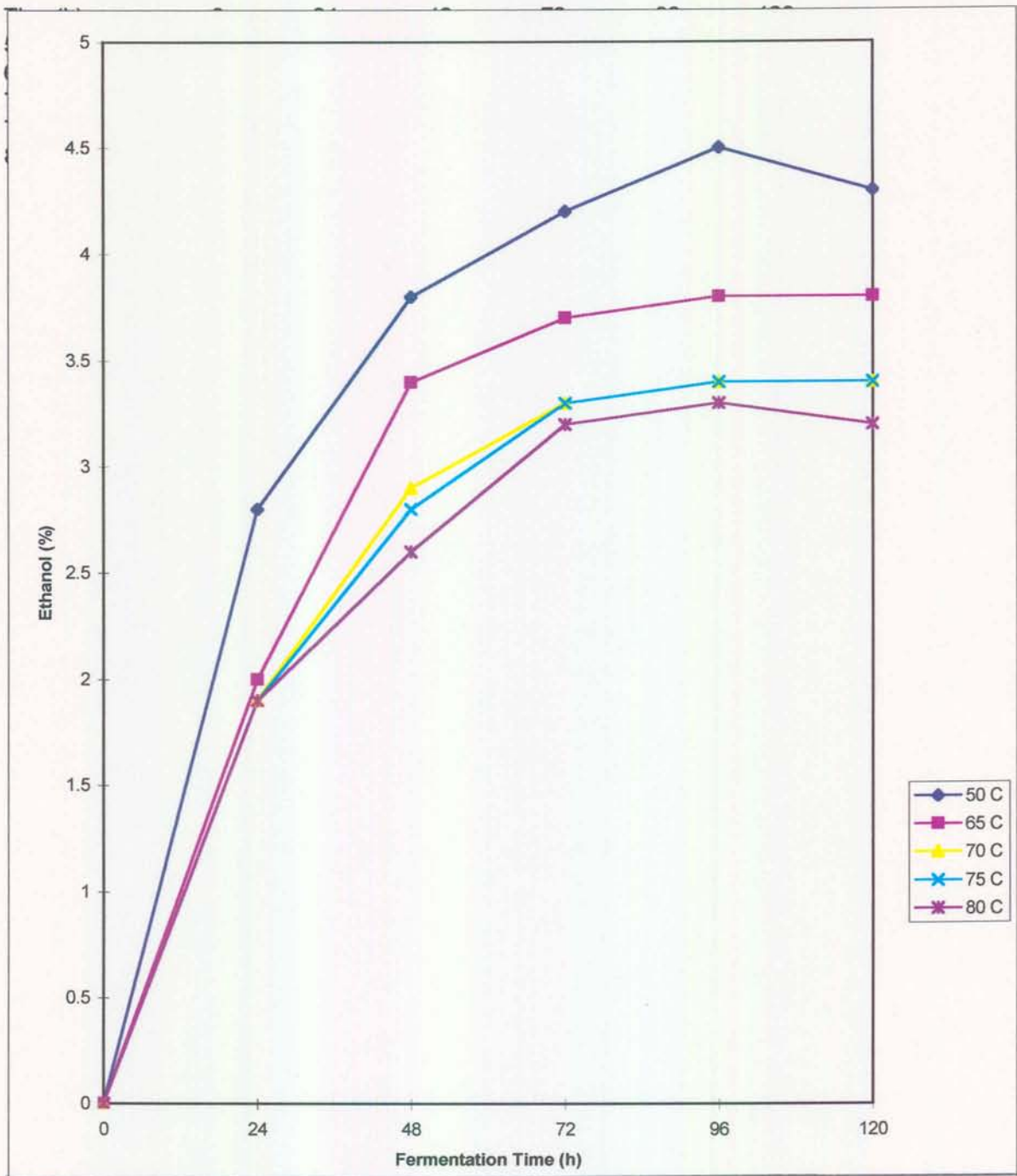


Fig.6 Effect of conversion temperature on ethanol production during opaque beer fermentation

5.2.2 Effect of fermentation time on the quality of opaque beer made with malt germinated for short period.

Table 11. Effect of fermentation time on the quality of opaque beer made with malt germinated for one day.

Parameters	Fermentation time (h)					
	0	24	48	72	96	120
pH	5.35	4.24	3.89	3.76	3.63	3.51
Soluble solids (%)	10.1	7.5	6.4	5.3	5.0	4.6
TRS (%)	5.9	2.8	1.3	0.9	0.6	0.2
Ethanol (%v/v)	ND	2.3	3.1	3.3	3.4	3.3
Total acids (%v/v)	0.10	0.16	0.33	0.39	0.41	0.43
Viscosity (Cp)	53	50	50	50	49	48
Total solids (%w/w)	13.2	9.3	6.4	5.2	5.0	4.7
Foam head (%)	ND	3.9	5.6	2.6	0	0
Settling (%)	ND	0	0	0	0.29	0.36
Original gravity (°P)	1.045	ND	ND	ND	ND	ND

ND - Not determined TRS - total reducing sugars

Table 12. Effect of fermentation time on the quality of opaque beer made with malt germinated for two days.

Parameters	Fermentation time (h)					
	0	24	48	72	96	120
pH	5.28	3.98	3.85	3.70	3.64	3.56
Soluble solids (%)	10.5	8.1	5.7	5.3	4.8	4.5
TRS (%)	6.2	3.6	1.3	0.9	0.6	0.2
Ethanol (%v/v)	ND	2.3	3.6	4.2	4.3	3.9
Total acids (%v/v)	0.10	0.22	0.31	0.39	0.41	0.44
Viscosity (Cp)	58	55	53	50	51	52
Total solids (%w/w)	13.0	8.5	7.3	6.0	5.6	4.9
Foam head (%)	ND	2.6	7.5	4.7	2.7	0
Settling (%)	ND	0	0	0.13	0.25	0.34
Original gravity (°P)	1.045	ND	ND	ND	ND	ND

Table 13. Effect of fermentation time on the quality of opaque beer made with malt germinated for three days

Parameters	Fermentation time (h)					
	0	24	48	72	96	120
pH	5.35	4.27	3.84	3.67	3.60	3.53
Soluble solids (%)	9.7	7.5	6.1	5.2	4.9	4.2
TRS (%)	6.7	4.2	2.9	0.8	0.4	0.2
Ethanol (%v/v)	ND	2.4	3.7	4.3	4.4	4.2
Total acids (%v/v)	0.13	0.26	0.32	0.40	0.41	0.43
Viscosity (Cp)	68	62	62	60	58	58
Total solids (%w/w)	13.5	11.1	8.4	5.8	5.2	4.0
Foam head (%)	ND	4.8	8.0	4.6	2.4	0.5
Settling (%)	ND	0	0	0	0.20	0.28
Original gravity (°P)	1.045	ND	ND	ND	ND	ND

ND - Not determined

TRS - total reducing sugars

Tables 11 - 13 show that malt germinated for short periods can be used for opaque beer brewing but product quality will be slightly different from the one that uses malt germinated for the standard period of 4 days.

The major difference noted here again were mainly on total reducing sugars depletion and on ethanol production. Malt germinated for only one day had low wort sugars and depletion of the sugars was slower as compared to malt germinated for four days. Although soluble solids in wort for the brews carried out using malt germinated for short periods were higher than those of the control the actual reducing sugars were lower than those of the control. Ethanol production was also lower than that of opaque beer brewed using malt germinated for four days. Ethanol production and

initial TRS in wort increased with germination days (Fig 7 - 8 & Tables 11-13).

However, product quality results of brews carried out using malt germinated for 2 and 3 days were similar to the control brew. Although initial sugars in wort were lower than specification, ethanol production was still within acceptable levels.

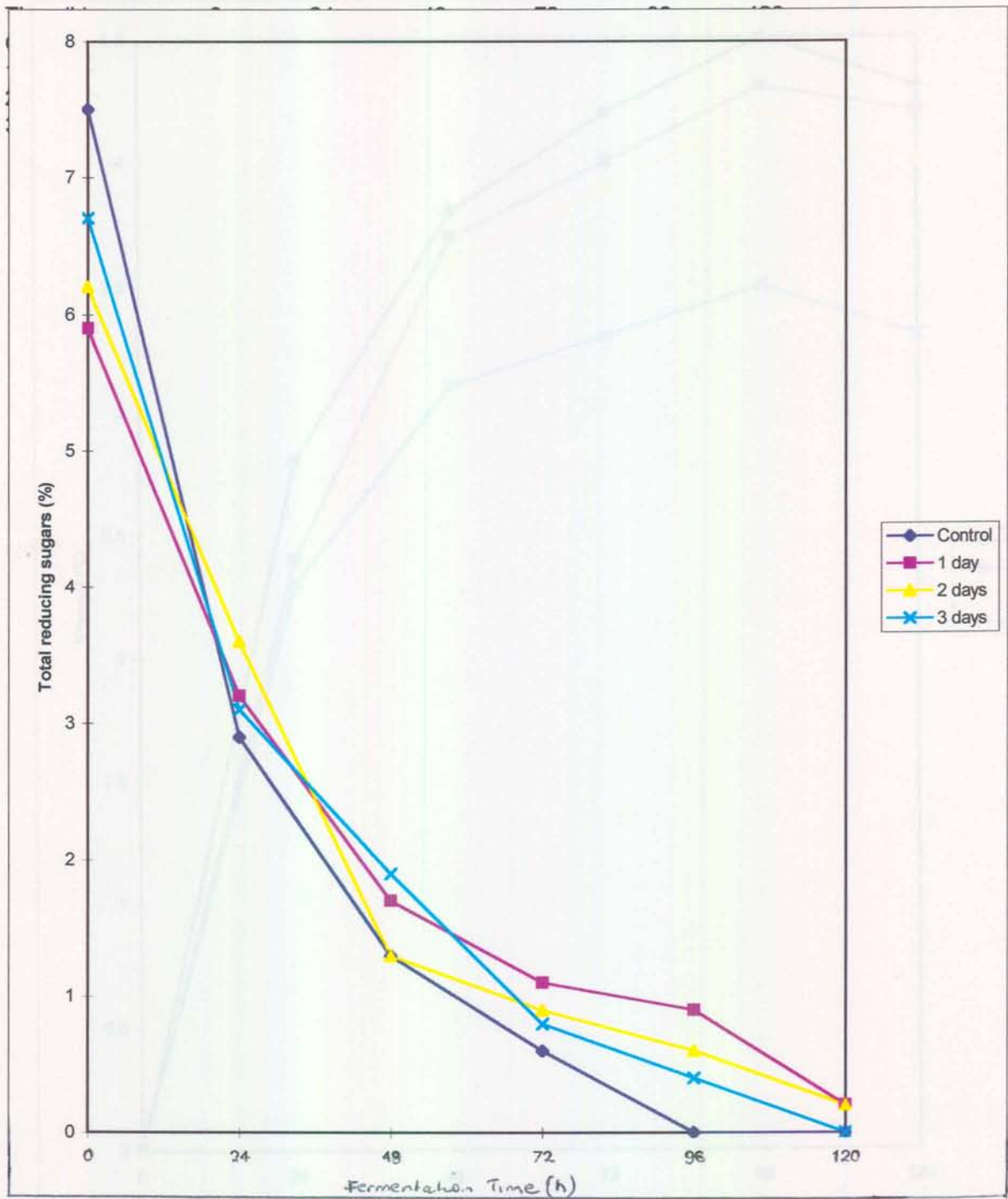


Fig.7 Effect of brewing with malt germinated for different times on total reducing sugar uptake during opaque beer fermentation

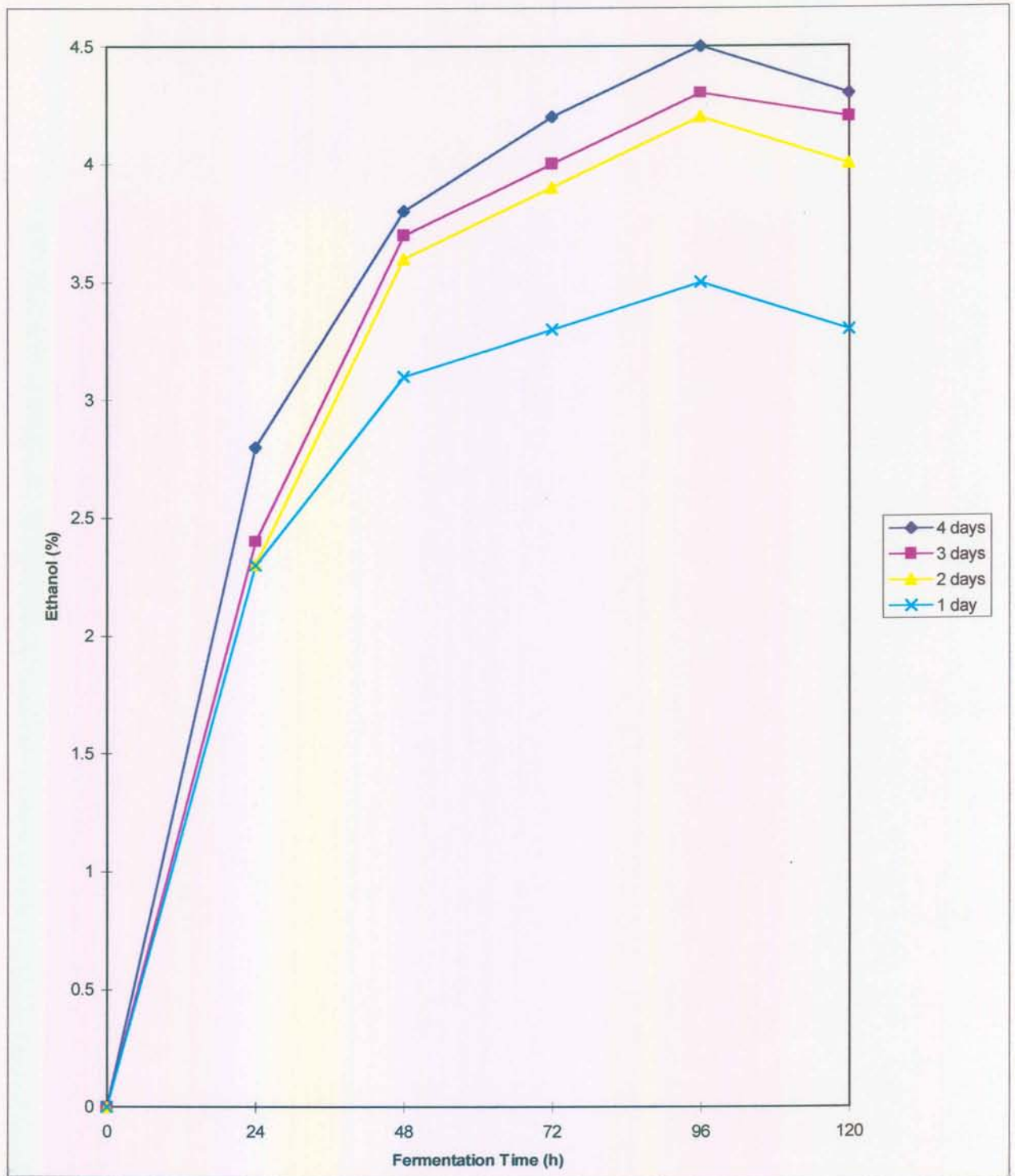


Fig.8 Effect of brewing with malt germinated for different times on ethanol production during opaque beer fermentation

5.2.3 Effect of fermentation time on the quality of opaque beer produced from low gravity fermentation

Table 14. Effect of fermentation time on the quality of opaque beer made from wort diluted by 30%.

Parameters	Fermentation time (h)				
	0	24	48	72	96
pH	5.35	3.91	3.70	3.60	3.53
Soluble solids (%)	7.3	5.8	4.0	3.7	3.3
TRS (%)	4.9	3.3	1.7	0.9	0.3
Ethanol (%v/v)	ND	2.0	2.9	3.2	3.1
Total acids (%v/v)	0.07	0.19	0.25	0.31	0.42
Viscosity (Cp)	40	43	41	39	40
Total solids (%w/w)	10.2	9.0	7.4	5.2	4.9
Foam head (%)	ND	1.3	2.0	1.8	0
Settling (%)	ND	0	0.3	2.7	3.5
Original gravity (°P)	1.030	ND	ND	ND	ND

ND - Not determined

TRS - total reducing sugars

Table 15. Effect of fermentation time on the quality of opaque beer made from wort diluted by 40%.

Parameters	Fermentation time (h)				
	0	24	48	72	96
pH	5.44	3.86	3.56	3.48	3.42
Soluble solids (%)	6.5	4.0	3.3	3.2	3.0
TRS (%)	4.3	3.1	2.2	1.1	0.5
Ethanol (%v/v)	ND	2.0	2.5	2.8	2.7
Total acids (%v/v)	0.08	0.19	0.32	0.36	0.41
Viscosity (Cp)	29	22	21	21	21
Total solids (%w/w)	8.5	7.1	5.0	4.6	4.4
Foam head (%)	ND	0	0	0	0
Settling (%)	ND	1.1	3.9	4.7	5.7
Original gravity (°P)	1.026	ND	ND	ND	ND

ND - Not determined

TRS - total reducing sugars



Table 16. Effect of fermentation time on the quality of opaque beer made from wort diluted by 50%.

Parameters	Fermentation time (h)				
	0	24	48	72	96
pH	5.54	3.97	3.68	3.54	3.42
Soluble solids (%)	5.4	3.3	3.0	2.8	2.5
TRS (%)	3.4	2.6	1.2	0.7	0.3
Ethanol (%v/v)	ND	1.4	2.1	2.4	2.2
Total acids (%v/v)	0.06	0.18	0.26	0.39	0.44
Viscosity (Cp)	11	10	10	10	11
Total solids (%w/w)	7.3	5.1	4.7	3.9	3.2
Foam head (%)	ND	0	0	0	0
Settling (%)	ND	2.2	5.1	5.6	6.2
Original gravity (°P)	1.021	ND	ND	ND	ND

ND - Not determined

TRS - total reducing sugars

Tables 14 - 16 shows the effect of fermentation time on opaque beer from low gravity fermentation. Dilution of wort resulted in dilution of most of the parameters. Soluble solids were less than those of the control (9.0%) with the least being that of 50% dilution (5.4%). Total reducing sugars in wort were very low with 30% dilution having 4.9%, 40% (4.3%) and 50% (3.4%) as compared to control with 7.5% (Fig 9 & Tables 14-16).

Ethanol production was lower with wort dilution.. At 72 h 30% dilution obtained only 3.2%, 40% dilution had 2.8% and 50% dilution had as little as 2.4% as compared to control with 4.2%. Ethanol levels for the test brews dropped very slightly after 72 h as opposed to control where ethanol increased between 72 and 96 h (Fig 10).



Foam-head was poor for these brews as compared to the control and it collapsed only after a few hours. Viscosities following 30% dilution were only slightly below spec but those for 40% and 50% dilution were far below specifications resulting in early settling of the product. Total solids were less and as a result the product was very thin and watery (Tables 14-16).



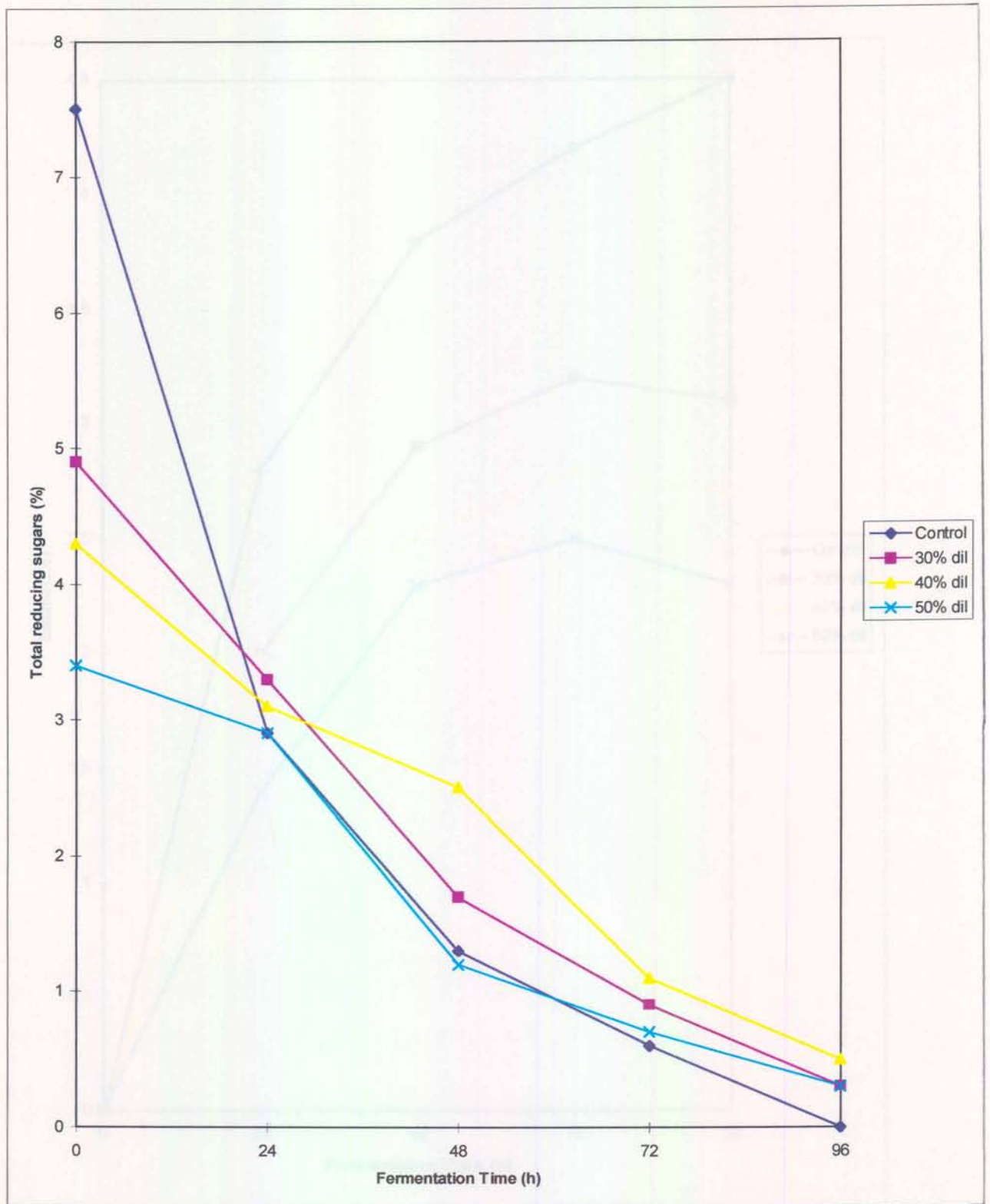


Fig.9 Effect of wort dilution on total reducing sugar uptake during opaque beer fermentation



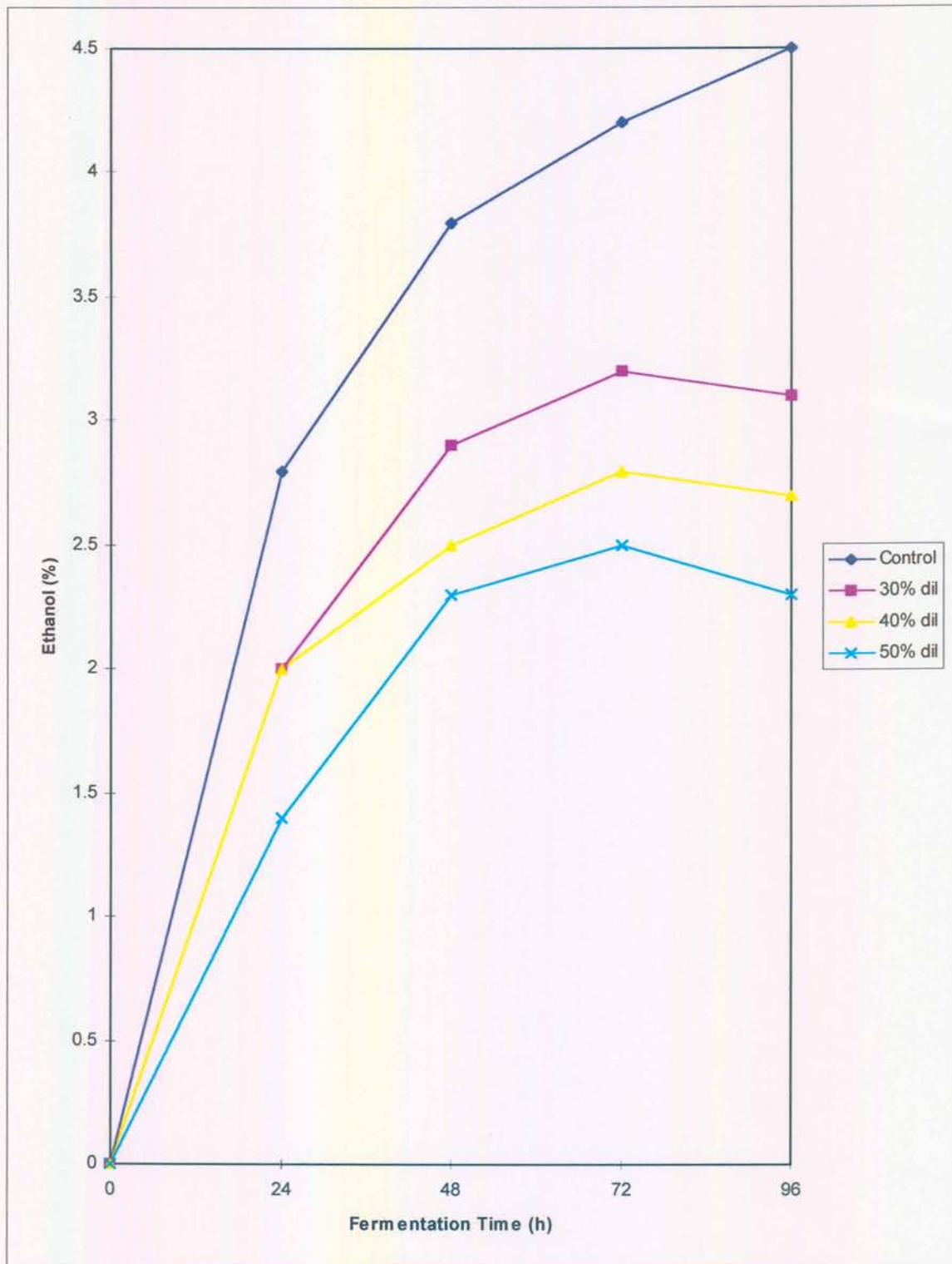


Fig.10 Effect of wort dilution on ethanol production during opaque beer fermentation

5.3 Product acceptance survey results

Results of product acceptance for opaque beer brewed using three different treatments are shown in Tables 17 to 19.

Table 17. Effect of conversion temperature on acceptability of opaque beer

Parameter	Conversion temperature (°C)				
	50 (control)	65	70	75	80
Colour	4.0	4.0	4.0	4.0	4.0
Foam-head	4.0	4.0	4.4	4.0	4.0
Bite	3.6	3.2	4.0	3.2	2.8
Settling	3.6	4.0	3.2	3.6	3.2
Texture	4.0	3.6	3.2	3.6	3.2
Smell	4.0	4.4	4.0	4.0	4.0
Taste	4.0	4.4	4.4	4.0	4.0
Mean	3.89	3.94	3.89	3.78	3.60

Table 18. Effect of different germination time on acceptability of opaque beer

Parameter	Germination time (days)			
	4 (control)	3	2	1
Colour	4.0	4.0	4.4	4.0
Foam-head	4.0	4.4	4.4	3.2
Bite	3.6	3.6	3.2	2.8
Settling	3.6	4.0	4.0	4.0
Texture	4.0	4.0	4.0	4.0
Smell	4.0	4.0	4.0	4.0
Taste	4.0	4.4	3.6	4.0
Mean	3.89	4.10	3.94	3.71

Table 19. Effect of different levels of wort dilution on acceptability of opaque beer

Parameter	Wort dilution (%)			
	0 (control)	30	40	50
Colour	4.0	4.0	4.0	2.8
Foam-head	4.0	4.0	2.8	2.2
Bite	3.6	3.6	3.2	1.4
Settling	3.6	2.8	2.4	1.6
Texture	4.0	2.8	2.0	2.0
Smell	4.0	4.0	4.0	3.6
Taste	4.0	4.0	3.2	1.6
Mean	3.89	3.60	3.09	2.17

Overall product acceptance for brews converted at different temperatures decreased with increasing temperature as from 65°C upwards (Table 17). The highest acceptance was of product converted at 65°C while the least acceptance was of product converted at 80°C. Product converted at 80°C scored low on bite, settling and texture.

Product acceptance for product of malt germinated different days was good except for the one that was germinated for only one day. This product scored low mainly on foam-head and on bite (Table 18).

Acceptability of the product following different levels of wort dilution was very poor (Table 19). When comparing with the control highest acceptance was at 30% dilution while the least acceptance was at 50% dilution. Product of 50% dilution scored very low in almost all parameters except smell while that of 40% dilution scored better only on smell and colour.

6. DISCUSSION

Effect of mashing temperature

Brews were carried out using different mashing temperatures. It was observed that mashing temperature played an important role during the brewing process. It was noted that with increase in mashing temperature there was a decrease in initial sugars in wort. High wort sugar was, however, being taken up by yeast faster than low wort sugar as has been revealed in previous research by Muller (1990). This is due to the effect of the osmotic pressure. High wort sugar has high concentration of soluble molecules and therefore exerts high osmotic pressure. It is therefore utilised by yeast faster as compared to low wort sugar with less concentration of soluble molecules.

Increasing mashing temperatures affected the beer characteristics and acceptability due to various reasons. Mashing at higher temperatures favours alpha-amylase activity rather than beta-amylase activity because beta-amylase is more temperature sensitive than alpha-amylase (Taylor,1992). Alpha-amylase although it produces some fermentable sugars also produces considerable quantities of dextrans which are not fermentable, thus leading to low levels of total reducing sugars in wort (Taylor,1992).

Figure 4 shows that with increase in mashing temperature there was a decrease in initial sugars in wort. Highest wort sugar was obtained from mashing at 50°C while the least was obtained from mashing at 80°C. Due to the low total reducing sugars in wort at higher temperatures, ethanol development was poor at higher mashing temperatures as opposed to lower mashing temperatures of 50-60°C which are optimal

conversion temperatures for opaque beer (Daiber and Taylor, 1995).

Changes in mash temperature have a great effect on the activity of malt amylase enzymes and on their starch substrate, hence, on the carbohydrate composition of wort. Mashing at lower temperatures of 60-70°C results in partial gelatinisation and saccharification of sorghum malt starch leading to an increase in mash solubilisation (Taylor, 1992). However, mashing at very high temperatures like 80°C results in total gelatinisation and saccharification of starch leading to poor body of the opaque product.

Starch solubilisation is brought about by alpha-amylase and it leads to the degradation of large starch molecules into dextrans and sugars. Mash solubilisation is however, affected by mashing temperature and mash pH. At high mashing temperature of 75-80°C total reducing sugars (trs) produced were low as compared to the total reducing sugars produced at lower mashing temperatures of 50-65°C. According to research work carried out by Botes, Joubert and Novellie (1967) whereby they were looking at the purification and properties of sorghum malt beta-amylase, it was observed that there was an increase in trs at lower mashing temperatures of 50-70°C probably due to gelatinisation of malt starch. There was a decrease in trs at higher temperatures presumably due to thermal inactivation of alpha-amylase.

It was also observed that with increase in mashing temperature wort pH decreased so that at very high temperatures of 75-80°C pH dropped to below 5.0. Related work carried out by Okon and Uwaifo

(1984) also revealed that sugar formation was affected by mash pH. At moderate mashing temperature of 50-70°C sugar formation was fairly constant over the pH range 4.0-6.0. However, it decreased sharply below 4.0 at higher temperatures and this is in general agreement with the pH optima for alpha- and beta-amylase, pH 4.5-5.0 and 5.0-5.5 respectively. At elevated mashing temperatures of 70-80°C sugar formation declined sharply with increasing mash acidity. This was probably because high temperature combined with low pH more rapidly inactivated the beta-amylase enzyme.

Effect of wort dilution

Wort dilution also affected beer characteristics and acceptability. The diluted product resulted in very low viscosities and due to these low viscosities settling started as early as 24 hours. All the dense particles quickly settled at the bottom while a watery layer formed at the top.

According to Taylor (1992) sorghum beer mashing is performed with a large quantity of cooked starchy adjunct. Some of this adjunct starch is not saccharified and contributes to the opaque character of the beer. Thus opaque beer is opaque due to the presence of suspended particles of sorghum malt, cereal adjunct and yeast and the cereal particles which are mainly gelatinised starch give beer a viscous consistency.

Since opaque beer is a suspension diluting the wort leads to rapid breakdown of the suspension resulting in early settling of the product. Diluting the wort meant diluting of everything else i.e. fermentable sugars, gelatinised starch, vitamins and minerals. According to research work carried out by Taylor (1992) it was observed that high gelatinisation temperature of sorghum malt starch 64-68°C makes simultaneous

gelatinisation and saccharification to fermentable sugars difficult. Thus in a Reef type (sour is cooked with the unmalted cereal adjunct) sorghum beer mashing process under moderate conditions of 50-60°C, some starch remains at the end of mashing, amounting to more than 25% of total carbohydrate. This starch occurs in two forms i.e. enzyme-susceptible (gelatinised) and raw.

Head retention for this product was poor as no foam-head was visible yet control brew produced significantly better foam-head. Viscosity of beer was too low to slow down the escape of CO₂. The quality of beer-foam is due to a complicated combination of factors some of which are intrinsic properties while others are extrinsic thus the thinning effect led to poor head formation.

According to Evans, Sheehan and Stewart (1999) the foam head on a beer is one of the first characteristics that a consumer uses to judge beer quality. Therefore as foam quality will impact upon customers purchase decisions, brewers are particularly interested in optimising it. The research work that they carried out using a multi-linear regression analysis of the beer foam revealed that there are a number of interrelated malt derived factors that impact on beer foam. However the foam and the settlement measurements give little indication of how the tasters rate the foam and the sedimentation. The measurement of the viscosity seem to predict well the findings of the tasters findings of the texture.

Bamforth (1985) divided foam quality into the following components; foam stability, quality, lacing (adhesion or cling), whiteness, creaminess, density, viscosity and strength. He observed that proteinaceous components, non starch polysaccharides, metal ions and other factors are foam promoting, while lipids and high concentrations of ethanol

inhibit foam stability. He also established that high molecular weight proteins are foam positive and enhance foam stability and the majority of these proteins are considered to have originated from malt.

As has been shown in earlier research (Muller,1990) low gravity fermentation proved not to be particularly successful with lack of flavour and thinness of the beer being the major disadvantages.

Effect of use of short germinated malt

Brewing with malt germinated for different times affected the beer characteristics and acceptability but only to a marginal extent. The slightly lower alcohol obtained while using malt germinated for only one day was as a result of low wort sugars due to the low amylase activity of the short germinated malt. Beta-amylase is more labile than alpha-amylase and is influenced by germination time and temperature (Owuama,1999). A rapid increase in beta-amylase activity occurs within the first 2 days of germination and subsequently declines in rate of increase up to 5 days. This explains why malt germinated for 2 to 3 days had just as good malt parameters as malt germinated for the standard 4 days. Malt germinated for only one day had however, low activity beta-amylase which led to low alcohol production.

On the other hand carboxypeptidases and proteinases are important in protein mobilisation during grain germination (Owuama, 1999). Carboxypeptidases specifically hydrolyse solubilised proteins to free alpha amino nitrogen (FAN) essential for anabolic functions of germinating seedling and as nutrients for yeast metabolism in wort. However germination conditions influence carboxypeptidase activity so that carboxypeptidase activity

increases with germination time up to 4 days. This means that for the malt, which was germinated for less than 4 days carboxypeptidase, activity was low, leading to low FAN in the malt. Low FAN in malt leads to poor yeast metabolism resulting in poor fermentation. Therefore alcohol production is reduced as was with the case when malt germinated only for 1 day was used.

Previous research has shown that in sorghum beer brewing, the formation by proteolysis during malting and mashing of sufficient free alpha-amino nitrogen (FAN) to support good yeast growth during fermentation is of considerable importance (Taylor and Boyd, 1986). This is because, according to Evans and Taylor (1990b) the opaque beer cereal grist contains a low proportion of malt and a high proportion of cereal grits.

The low diastatic power and FAN obtained after germination was carried out for only one day are in agreement with the work carried out by Morrall, Boyd, Taylor and Van der Walt (1986). This work revealed that germination time; temperature and moisture have a highly significant effect on malt diastatic power, free alpha-amino nitrogen and extract. In general diastatic power of the malts increased with germination time. For this project only germination time was varied while moisture and temperature were kept constant.

Instead of varying germination time to effect malt quality one could probably vary germination temperature or moisture. High temperature germinated malt or low moisture germinated malt

could be a way of producing low alcohol beer. As was revealed in the study of Morrall, Boyd, Taylor and Van der Walt higher germination temperatures of 35-38°C resulted in low FAN as opposed to low germination temperatures of 24-32°C. As regards germination moisture this had a strong effect on malt FAN. FAN was greatest in malts germinated at high moisture while medium and low moisture resulted in significantly and progressively less FAN.

7. CONCLUSIONS AND RECOMMENDATIONS

Low alcohol opaque beers can be achieved through limited fermentations which usually do not require extra capital. Limited fermentation is when the normal brewing process is modified so that the fermentation is in a way limited and alcohol is not produced. It would be, however, very interesting to note as Winstanley (1990) said in his speech, how other countries define their low alcohol products. In the USA low alcohol would be less than 2.5% (v/v), in New Zealand low alcohol would be less than 3% (v/v) and in Australia it is defined as 1.5-3.8% (v/v). All this however, relates to clear lager beers only. Despite an exhaustive literature search, the author was not able to obtain a definition or definitions used in any country relating to low alcohol opaque beers (%v/v). Thus, for purposes of this research work, a figure of 2.5% (v/v) was derived by using guidelines from clear beer lagers.

On mashing at high temperatures optimum results were obtained from mashing at 75°C. The only disadvantage noted with this method was that it requires very careful control of the brewing process as lower mashing temperatures would result in increased wort fermentability while higher temperature mashing would cause starch breakthrough. However, if temperatures are controlled well optimum results can be achieved.

Malt germinated for a short period of time produced beers with alcohol levels just as good as those of the control brew except for malt germinated for only one day which gave alcohols slightly lower than those of the control. Low alcohol was due to the low amylase activity of the short germinated malt.

Use of low gravity fermentation proved inferior to the other two methods due to the adverse effect it had on beer characteristics. Dilution of wort meant dilution not only of the alcohol produced but also of the flavour compounds and the body. Use of 30% dilution can, however, be recommended as this resulted not only in a low alcohol product but also in the acceptance of the product although it was perceived to be slightly thin. This product if sold in its own right could do very well. What only would need to be improved is the flavour and also sedimentation needs to be reduced.

In conclusion, mashing at 75°C, use of malt germinated for one day, and the method of 30% dilution of wort, can be recommended as methods and processes for the production of low alcohol opaque beers. The methods have the advantage that they do not require any extra plant or specialised equipment and process systems.

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