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Effects of fermentation temperature on the composition of beer volatile compounds, organoleptic quality and spent yeast density

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Abstract Production of good quality beer is dependent largely on the fermentation temperature and yeast strains employed during the brewing process, among others. In this study, effects of fermentation temperatures and yeast strain type on beer quality and spent yeast density produced after wort fermentation by two commercial yeast strains were investigated. Beer samples were assessed for colour, clarity and foam head stability using standard methods, whilst the compositions and concentration of Beer Volatile Compounds (BVCs) produced were assessed using GC-MS. The spent yeast density, measured as dry cell weight, ranged between 1.84 - 3.157 mg/ml for both yeast strains with the highest yield obtained at room temperature fermentation. A peak viable population of 2.56 x 10^7 cfu/ml was obtained for strain A, also during fermentation at room temperature. The foam head of the beers produced at 22.5ºC was most stable, with foam head ratings of 2.66 and 2.50 for yeast strain A and B, respectively. However, there was no significant (p = 0.242) difference in colour intensity between the beers produced at the different fermentation temperatures. Eight different BVCs were detected in all beer samples and were found to affect the organoleptic properties of the beer produced. Further optimizations are required to determine the effects of other parameters on beer quality.

Keywords: beer volatile compounds, fermentation temperature, organoleptic quality, spent yeast density

INTRODUCTION

Beer brewing is an established ancient art from as far back as 6000 B.C., during the building of the ancient cities of Mesopotamia (Cortacero-Ramirez et al. 2003) and has been practised for thousands of years. The practice of producing beer in small micro-breweries has been replaced by magnificent industrial production plants that push out volumes of beer that early brew-masters could only dream about (Rojas and Peterson, 2008). To facilitate effective fermentation process, the yeast is often pitched at a specific population size and allowed to grow via an aerobic step in the fermentation process (Tanguler and Erten, 2008). Fermentation temperature is known to influence beer aroma composition (Bekatorou et al. 2002). Low temperature brewing; in particular, has been reported to result in the production of beer with improved taste and aroma as well as high ethanol and beer productivities (Bardi et al. 1996a; Bardi et al. 1997). Immobilized cell technology processes have been shown to shorten the production time of beer from 12-15 days to 1-3 days, however, the major difficulty is to achieve the correct balance of sensory compounds to create an acceptable flavour profile within the time frame (Willaert and Nedovic, 2006). Beer produced by fermentation of wort by cells immobilized on glutten pellets have been reported to have reduced higher alcohols and higher ethyl acetate (Bardi et al. 1996b).

After the fermentation process, there is often a much greater amount of spent yeast present in the fermenter than that present at pitching (Shotipruk et al. 2005). The spent yeast generated during the fermentative process is often used as an inoculum for subsequent fermentations (Blieck et al. 2007). In

addition, yeast cell wall fractions contain a large percentage of β-glucans, which is highly advantageous in improving the physical and functional properties of foods, as a thickening and water-holding agent (Thammakiti et al. 2004) and for the gelatinization and retrogradation of starch (Satrapai and Suphantharika, 2007). β-glucans isolated from the cell wall fractions of spent brewer's yeast are good emulsifying stabilizer and are finding application as a form of fat replacement in the production of low-fat mayonnaise (Burkus and Temelli, 2000). The partial replacement of vegetable oil in mayonnaise using β-glucans derived from spent yeast extract has two distinct advantages; firstly, it decreases the calorie content of the emulsification and secondly, it results in the utilisation of industrial by-products (Worrasinchai et al. 2006). In addition, β-glucans have been reported to have been used as a form of immunomodulator in livestock (Eicher et al. 2006).

The fermentation step in beer production is facilitated through the metabolic activities of yeast, resulting in the conversion of fermentable sugars to carbondioxide (CO_2) and ethanol (Piškur et al. 2006). Whilst these metabolic activities produce the required ethanol from the fermentation, they also result in the production of large amounts of metabolic by-products, beer volatile compounds (BVCs), such as esters, ketones and higher alcohols which if present in high concentrations can influence the final aroma and flavour profile of the beer (Hansen, 1999; Šmogrovičoví and Dömény, 1999; Brown and Hammond, 2003; Vanbeneden et al. 2008). These compounds are derived from precursors of yeast metabolic pathways and some of them are essential for growth of the yeast (Brown and Hammond, 2003). Whilst the presence of these compounds may be considered as detrimental to many (especially those in industry), there are a select few that regard these compounds as important flavour enhancers, especially those with an acquired taste for speciality beers. It is therefore important to determine the effects of these BVCs on beer quality as well as the mechanisms involved in their generation in order to develop methods to facilitate their control.

Over the past three decades, research in brewing has focussed on the application of immobilized cells, mainly to facilitate continuous processing, shorten maturation time and consequently reduce production costs (Kopsahelis et al. 2007). However, there appears to be limited studies on the effects of fermentation parameters on the production of BVCs and the consequences on the organoleptic quality of the final product as well as on the spent yeast density produced. This study is therefore aimed at investigating the effects of fermentation temperatures and yeast strain type on the production of spent yeast and BVCs as well as on the overall quality of beer produced. This would have significant repercussions on the South African economy, especially because the beer industry is a considerable player in the country's economy, and the continuous increase in demand by the consumer.

MATERIALS AND METHODS

Wort preparation and fermentation

The wort used for the fermentation was made using canned-hopped malt extract purchased from National Food Products (Johannesburg, South Africa) and was prepared according to the manufacturer's instructions. Fermentations were set-up to determine the effects of different fermentation temperatures and commercial yeast strains on beer quality using mini-fermenters (3.5 L) designed to facilitate the fermentation process on a small scale. Two litres of wort was dispensed into each sterile fermenter vessels after being allowed to cool and sterile standard rubber tubing (5 mm inner diameter) was attached to the outlets for sampling. The free end of the tubing was placed into a 2 l flask containing sterile distilled water to form the air-lock. Two commercial yeast strains, National Food Product yeast and Anchor yeast (designated as "strain A" and "strain B", respectively) were used to pitch the fermentation. The yeast strains were grown in malt extract broth for 24 hrs at 30ºC with shaking at 120 rpm and then pitched at an optical density of 0.4 at an absorbance of 600 nm, which corresponds to a cell density of 5×10^6 Colony forming units per millilitre (cfu/ml) according to the McFarland standard. The fermenters containing each type of yeast were then incubated at one of three fermentation temperatures *viz*., room temperature (RT) (± 18ºC), 22.5ºC and 30ºC for a period of one week. These temperatures were chosen to check the effects of the varying temperature ranges on the composition and concentrations of volatile compounds in the final beer. Gas evolution was monitored from the air-lock mechanism to ensure that fermentations were not stuck.

Bottling and bottle conditioning

After a period of one week, the beer from each fermenter was aseptically transferred into sterile 500 ml sample bottles. To each bottle, a teaspoon of sugar was added prior to the addition of beer for bottle conditioning and the bottles were sealed and allowed to condition for a period of one week. After bottle conditioning, all bottles were stored at 4ºC to facilitate yeast settlement and the maturation process.

Measurement of spent yeast density and viability

Spent yeast density was measured by the method of Soley et al. (2005). Ten millilitre samples were removed from each fermenter after fermentation and centrifuged (6000 rpm for 10 min at 4ºC). The pellet was washed and resuspended in a normal saline solution (0.9% w/v NaCl), filtered through a previously dried and pre-weighed Whatman grade GF/A (Ø 47 mm) glass microfiber filter, and dried to a constant weight at 105ºC. Thereafter, weight of the filter was subtracted from the weight of the filter containing the dried cellular material to acquire the mass of spent yeast produced. Viable yeast cell population was determined by the method of Nagodawithana et al. (1974). Yeast cells present in fermentation reactor were first thoroughly dispersed to ensure equal distribution of cells. Thereafter, a 1-ml sample was serially diluted before spread plating 0.1 ml of appropriate dilutions onto malt extract agar. The plates were incubated at 30ºC for 48 hrs, and the number of colonies on plates for the dilution containing 30 to 300 colonies were counted and expressed as colony forming units per millilitre (cfu/ml).

Analysis of BVCs

Analysis of BVCs present in the beer samples was measured using dynamic headspace extraction methods and analyzed by gas chromatography and mass spectrometry (GC-MS). The volatiles from 100 ml of each sample was assessed by enclosing the sample bottle in a polyacetate bag and pumping air from the bag through a small cartridge filled with 1 mg of tenax® and 1 mg of carbotrap® activated charcoal at a flow rate of 50 ml/min for 30 min. A control was taken from an empty polyacetate bag sampled for the same duration. GC-MS analysis of the samples was carried out using a Varian CP-3800 GC (Varian, Palo Alto, California) with a 30 m x 0.25 mm internal diameter (film thickness 0.25 µm) Alltech EC-WAX column coupled to a Varian 1200 quadruple mass spectrometer in electron-impact ionization mode. Cartridges were placed in a Varian 1079 injector equipped with a "Chromatoprobe" thermal desorbtion device. Helium was used as a carrier gas at a flow rate of 1 ml min^{-1}. The injector was held at 40ºC for 2 min with a 20:1 split and then increased to 200ºC at 200ºC min^{-1} in splitless mode for thermal desorbtion. After a 3 min hold at 40ºC, the GC oven was ramped up to 240ºC at 10ºC min^{-1} and held there for 12 min. Compounds were identified using the Varian workstation software with the NIST05 mass spectral library and verified, where possible, using retention times of authentic standards and published Kovats indices. Compounds present at similar abundance in the control were considered to be contaminants and excluded from analysis. To ensure accuracy with quantification of emission rates, standards were injected into cartridges and thermally desorbed under identical conditions to the samples.

Measurement of foam head stability

The foam head stability was assessed according to the modified mini foam shake test developed by Van Nierop et al. (2004). A 20 ml sample of each beer (in triplicate) was dispensed into 50 ml glass measuring cylinders and all of the cylinders were sealed with parafilm. Each set of cylinders were shaken at the same time, vigorously up and down 10 times, after which the cylinders were set down on the counter, the parafilm pierced, and a timer set for 15 min. The foam was evaluated visually and the cylinders were arranged from best to worst. Ratings of 1 through 3 were given, where 3 was the greatest stability and 1 the worst.

Analysis of beer clarity and colour

The clarity of the beer was determined using a Hach P2100 Turbidimeter, while beer colour was measured spectrophotometrically at a wavelength of 430 nm as described elsewhere (Seaton and Cantrell, 1993). In both cases, distilled water served as a blank and a commercial beer was included in the analysis as positive control.

Organoleptic quality assessment

The taste profile of beer produced was assessed by a survey conducted with 10 independent samplers, with no previous beer quality assessment skills. The survey consisted of questionnaires asking the samplers to rate beer from 1 to 10 (1 being very bad and 10 being excellent), for the presence of 10 different characteristics such as; banana aromas, sour apple taste, sweet "butterscotch" aroma, etc. Samplers were asked to give a rating of 0 if they felt a certain trait was absent. These values were assigned categories such that a rating from 0 - 3 was regarded as "low", 4 - 6 as "medium" and 7 - 10 "high". The data obtained was then used to determine the percentage of samplers which felt that the presence of the compounds was "low", "medium" or "high". A commercial beer was also sampled to serve as the control.

RESULTS AND DISCUSSION

Spent yeast density and viable yeast population recovery after fermentation

Effect of the different fermentation temperatures on the spent yeast density and viable yeast population was investigated. Spent yeast density decreases with increasing fermentation temperature (Figure 1a). Fermentation at room temperature produced the most spent yeast density with a yield of 2.47 mg/ml and 3.15 mg/ml obtained for strain A and strain B, respectively. Spent density of strain A produced at a fermentation temperature of 22.5ºC was almost equal to that produced at room temperature, with only 1.215% reduction in the spent yeast density whilst only 8.25% less of strain B spent density was produced at 22.5ºC compared to that produced at the room temperature. It is possible that the available fermentable sugars present at these temperatures were converted into biomass at a similar rate as these temperatures are relatively close to 18ºC, which is the upper temperature limit that is commonly used for lager beer fermentations (Brown and Hammond, 2003). The 30ºC fermentation resulted in the lowest spent yeast density production of both strains with a 25.50% and 32.06% reduction in spent density produced compared to the room temperature fermentations for strain A and strain B, respectively. The reduction of spent yeast density and viable yeast population after fermentation at 30ºC could be attributed to increased metabolic rate at this higher temperature which could have led to faster utilization of sugars, and resulting in cell starvation, cell death and autolysis (Blieck et al. 2007).

Similarly, an increase in fermentation temperature led to a steady decrease in the viability of yeast cells (Figure 1b). A peak density of 2.56 x 10^7 cfu/ml was obtained for strain A at room temperature, which is about 2-fold higher than those obtained for strain B at 22.5ºC fermentation. The least viable population of both strains was observed at 30ºC fermentation, with about 11-fold and 3-fold reduction in population of yeast strain A and B, respectively, obtained compared to the peak population. In this study, fermentations was conducted for a period of 7 days disregarding the specific gravity of the wort, which is generally used to determine the remaining fermentable sugar concentrations in the wort solution in order to know when to terminate the fermentation process. Thus, it is possible that all fermentable sugars have been utilised before termination of the fermentation. Previous studies have shown a decrease in cell density as a result of decrease in fermentable sugars present in the wort. The decrease in cell viability with time has also been attributed to nutrient depletion and early entry of the organisms into the death phase (Blieck et al. 2007).

Beer colour, clarity and foam head stability

There was no significant (p = 0.242) difference in colour developed between the experimental beers produced by the yeast strains under the different fermentation temperatures; however, the colour intensity of all experimental beers was significantly ($p < 0.05$) lower than that of the control beer. The control beer had the deepest colour intensity with an absorbance of 0.198, while the maximum absorbance for beer produced with strain A and B at room-temperature was 0.149 and 0.143, respectively, with a maximum absorbance of 0.144 obtained for beer produced with the two strains at 22.5ºC (Figure 2a). Also, the absorbance of beer produced with strain A and B at 30ºC was 0.143 and 0.135, respectively (Figure 2a). Colour development in beer has been mostly attributed to the malt extract used in the respective beers instead of the fermentation parameters (Kopsahelis et al. 2007). Generally, the malt extract used has been reported to have the greatest effect on beer colour as the degree of colour intensity of the malt extract depends on the degree of kilning or roasting of the malted

barley (Seaton and Cantrell, 1993; Kopsahelis et al. 2007). Thus, it is possible that the control beer may have been produced from a malt extract which was differentially roasted compared to the malt extract used in this study.

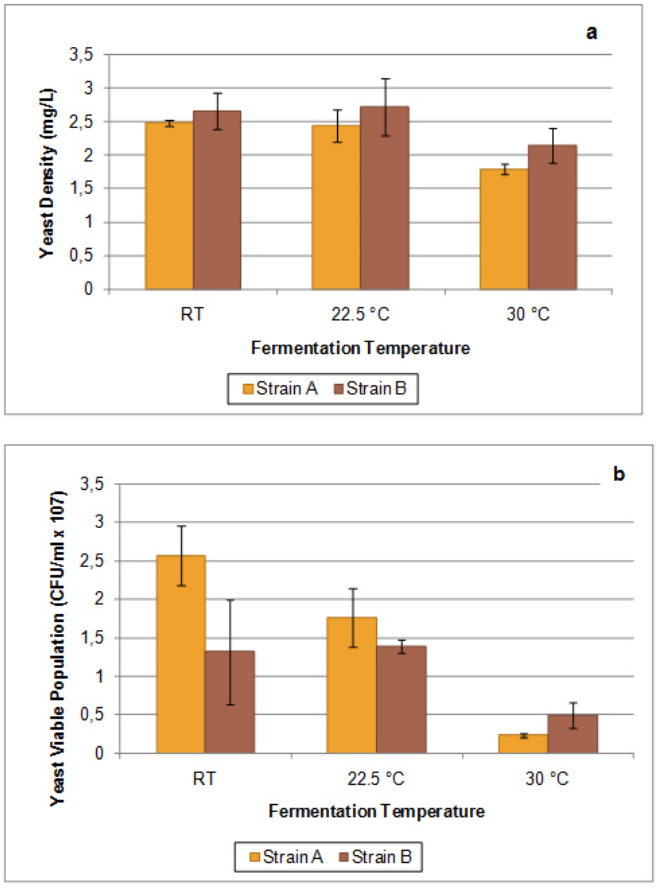

Fig. 1 Spent yeast density (a) and Total yeast viable population (b) produced by different yeast strains at the different fermentation temperatures. Values are average from six values ± standard deviation

There was no direct correlation between fermentation temperature and the clarity of beer produced with the two yeast strains. However, beers produced with strain B were generally clearer compared to those produced using strain A, with 56.13%, 21.46% and 57.58% reduction in turbidity at room temperature, 22.5ºC and 30ºC fermentation temperatures, respectively (Figure 2b). All the experimental beers produced were relatively turbid compared to the control. The extremely good clarity found in the control beer may be attributed to additional processing steps, such as centrifugation and microfiltration, that are used in the production of commercial beers (such as the control) to increase clarity (Seaton and Cantrell, 1993; Kuiper et al. 2002; Shotipruk et al. 2005). The beers produced in this experiment were bottle conditioned and were not subjected to further processing as the control beer. Also, it has been generally observed that bottle conditioned beers are more turbid than their commercial counterparts due to the presence of the residual yeast used for conditioning (Kuiper et al. 2002). It was also noted that yeast strain A produced beer with higher turbidity than strain B and this could be that yeast strain A produced and released higher concentrations of haze active proteins since the presence of these proteins has been shown to increase turbidity in beer (Seaton and Cantrell, 1993).

The control beer used had the best foam head stability compared to the experimental beers. The foam head of the experimental beers produced at 22.5ºC was most stable, retaining as high as 88.67% foam head stability compared to the control beer, while those prepared at room temperature had the least foam head stability rating (Figure 3). This could be due to variations in climatic temperature and light

intensity at room temperature which could have stressed the yeast cells and hence led to alterations in the yeast cell membranes, resulting in the release of free fatty acids into the beer samples (Rodriguez-Vargas et al. 2007). Also, at 30ºC fermentations, yeast cell density may have been lost due to autolysis and could have resulted in an increase in free fatty acid concentrations in the beer because of solubilisation of membrane lipids, thus resulting in lower foam head stability. It has been previously reported that the presence of lipids or free fatty acids in beer could lead to a decrease in beer foam head stability (Dickie et al. 2001; Van Nierop et al. 2004).

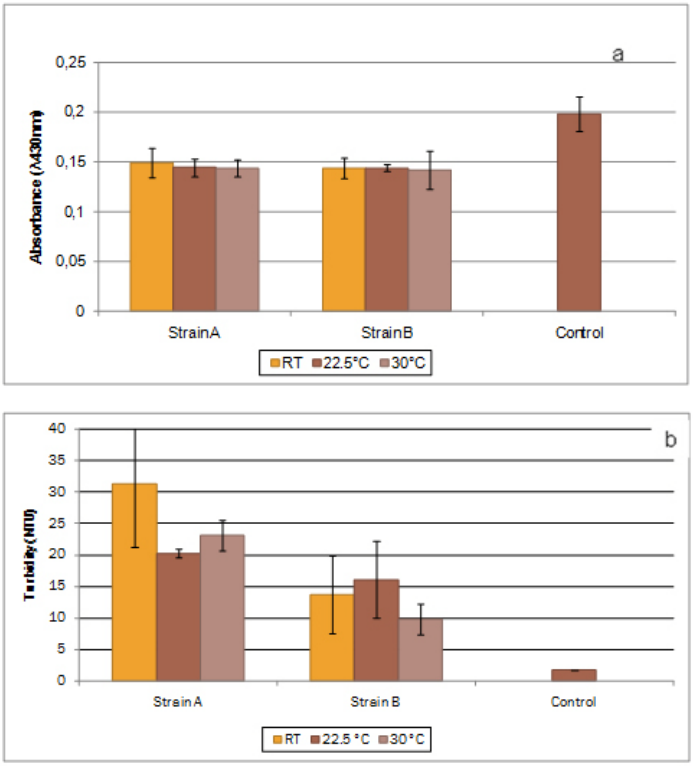

Fig. 2 Colour profiles (a) and clarities (b) of beer produced by different yeast strains under varying fermentation conditions. Values are average from six values ± standard deviation.

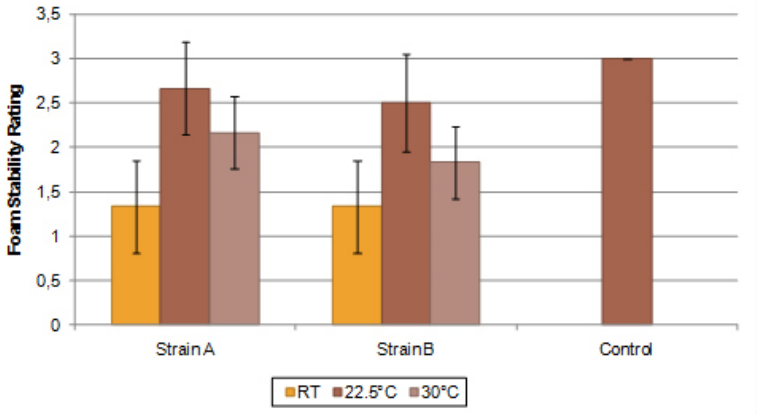

Fig. 3 Foam head stability of beer produced by the different strains at varying fermentation temperatures. Values are average from six values ± standard deviation.

Beer volatile compounds and organoleptic quality assessment

The relative percentages of the important volatile compounds detected in the beer samples are indicated in Table 1. Higher alcohol, isoamyl alcohol, constituted a large percentage of the BVCs in most samples, constituting up to 49% of the total BVCs in beer samples produced with strain B at room temperature fermentation. Most of the other compounds constituted below 15% of the total BVCs, however, isoamyl acetate constituted approximately 30% of the BVCs in the control sample and between 8.345-17.712% and 8.382-10.247% of the total BVCs in beer produced with strain A and B, respectively, at the different temperatures. Furthermore, ethyl caproate constituted roughly 10% or more of the volatiles found in all samples, while 2-phenylethyl acetate constituted between 4% and 7% in most samples except for the control, and beer samples produced at 22.5ºC where it constituted greater than 10% of the total BVCs (Table 1). GC-MS chromatogram of beer samples showing the different peaks representing the BVCs detected is shown in Figure 4. The seven volatile compounds detected in the beer samples produced in this study have also been found in beer produced from a previous study (Kopsahelis et al. 2007).

Table 1. Relative percentages of volatile compounds detected in beers produced.

Volatile Compounds		Control	Relative percentage in samples					
			Strain A			Strain B		
			*RT	22.5ºC	30ºC	RT	22.5ºC	30ºC
Sulphur Compounds	Methionol	0.115	0.222	0.318	0.158	0.216	0.229	0.167
Esters	Ethyl acetate	5.516	7.335	12.252	5.572	7.705	5.361	5.796
	Isoamyl acetate	30.017	8.345	15.469	17.712	8.382	9.162	10.247
	Ethyl caproate	16.044	10.768	12.813	11.178	12.497	15.211	11.211
	Ethyl caprylate	8.799	7.956	9.877	5.945	5.905	6.582	3.651
	2-phenylethyl acetate	15.040	5.306	13.244	6.211	5.442	10.857	5.102
Higher Alcohols	Isoamyl alcohol	14.098	44.716	13.903	43.215	49.028	44.502	40.307
	Ethanol	7.354	13.6199	11.274	4.875	6.506	3.592	9.049

*Room Temperature.

Assessment of the experimental and control beers by a group of samplers revealed the organoleptic quality of the different beer samples. As represented in Figure 5, about 35% of the samplers felt that the beers produced had a moderate sour apple taste. This taste profile is usually characteristic of the flavour, volatile esters; ethyl caprylate and ethyl caproate (Verstrepen et al. 2003). Also, roughly 65% of the samplers felt that all beers produced had a moderate warm mouth-feel and this characteristic is generally attributed to the presence of ethanol produced from fermentation as well as the presence of fusel alcohols (Ter Schure et al. 1998). Roughly 40% of samplers felt that a moderate medicinal aroma was present in beers produced at the 22.5ºC and 30ºC fermentations using strain A as well as the room-temperature fermentation for strain B. Generally, these characteristics are attributed to the presence of volatile phenolic compounds in beers (Vanbeneden et al. 2008), while the moderate solvent aroma felt by the samplers in some of the beers is usually attributed to the presence of ethyl acetate (Verstrepen et al. 2003). The non-detection of phenolic compounds could explain the general moderate medicinal smell feelings by most of the samplers. Perhaps, some of these compounds were not present in these beer samples since their generation depends on the activities of the yeast (Peddie, 1990; Brown and Hammond, 2003) as well as the composition of the wort (Kobayashi et al. 2008). Alternatively, the lack of detection could be attributed to limitation of the methods used for the analysis. Previous studies by Saison et al. (2008) and Pinho et al. (2006) have shown that fibres used for headspace analysis are efficient for detection of different classes of volatiles in beer. Thus, it is possible that the Tenax and Carbotrap fibres used in this analysis lacked the affinity required to detect some of the volatiles in the beer. This is a subject of further investigation in our laboratory.

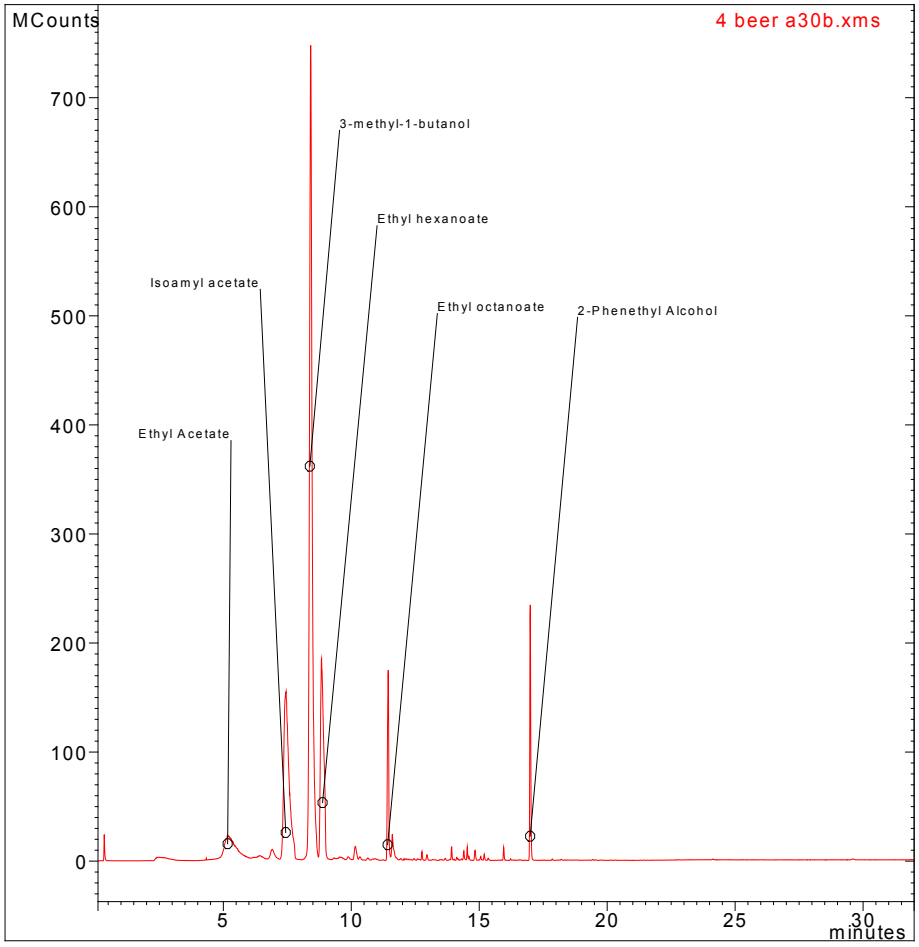

Fig. 4 GC-MS Chromatogram of beer samples showing the different peaks representing the beer volatile compounds.

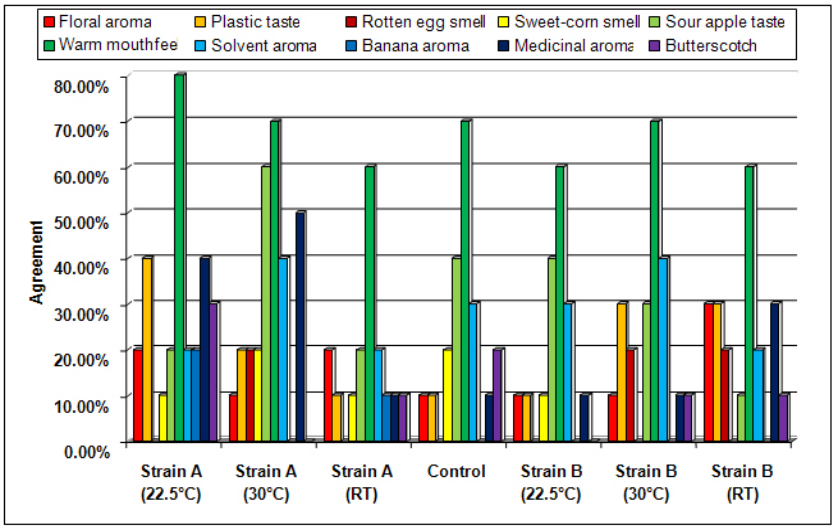

Fig. 5 The percentage of samplers that agreed that specfic flavour or aroma characteristics were present in moderate levels in beers produced by different yeast strains at different fermentation temperatures.

CONCLUDING REMARKS

Results from this study have shown that different yeast strains and fermentation temperatures affects beer quality, especially turbidity, foam head stability, spent yeast density and yeast viability. However, these parameters had not much effect on the colour profiles of the beers produced and no effect on the qualitative properties of volatiles produced but rather on the relative quantities of the BVCs as evident in the headspace GC-MS analysis. However, in order to increase accuracy of volatile detection, it would be prudent to investigate the effect of the different headspace trapping fibres on detectable BVCs profile. The presence of varying concentrations of BVCs appeared to affect the organoleptic properties of the beer; however, the employment of qualified beer samplers is required to provide a more accurate view. Further optimization is required to determine the effects of other fermentation parameters on overall beer quality as well as investigate gene expression profiles by the different yeast strains under the different fermentation conditions.

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