

Full Length Research Paper

Stability profile of flavour-active ester compounds in ale and lager beer during storage

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Currently, one of the main quality problems of beer is the change of its chemical composition during storage, which alters its sensory properties. In this study, ale and lager beers were produced and aged for three months at two storage temperatures. Concentration of volatile ester compounds (VECs) in the beers was regularly monitored by gas chromatographic analysis of the headspace samples to establish changes in ester flavour profile with time. Generally, VECs were more stable during storage at 4°C, compared to room temperature for both ale and lager beer produced. Of the VECs produced, ethyloctanoate was the least stable in ale beer, with 32.47% decrease in concentration observed at room temperature, while phenyl ethyl acetate was the most stable compound decreasing by only 9.82% after three months. In lager beer, VECs were relatively stable decreasing by only 7.93% after three months, while ethyl decanoate was the least stable, with 36.77% decrease in concentration observed at room temperature. Results obtained in this study can be helpful in developing appropriate technological process to control the stability of these important flavour esters in beer.

Key words: Esters, stability, storage temperature, storage duration.

INTRODUCTION

Beer aging is considered to be a major quality problem because of the production of unpleasant aging flavours. Furthermore, the type of flavour evolution during storage is uncontrollable, making it hard for brewers to guarantee a consistent product quality or to meet some of the consumers' expectations regarding flavour (Vanderhaegen et al., 2007). Through storage, flavour appears to deteriorate significantly with time at a rate depending on the composition of beer (pH, oxygen, antioxidants, precursor concentrations, etc.) and storage conditions (packaging, temperature, light, etc.) (Callemien et al., 2006). The point where maturation ends and deterioration begins is undoubtedly different for different beers and probably different for different consumers (Briggs et al., 2004). The literature on beer staling exposes the inadequacies in dealing with the actual sensory changes during the stor-

age of beer. Dalglish (1977) detailed these changes and shows broad view of the sensory evolution and deterioration during beer storage and is therefore not applicable to every beer.

Oxidation is considered to be a chief source of stale flavour development in beer (Depraetere et al., 2008). Dissolved oxygen in bottled beer is primarily of concern to the brewing industry since the oxidation of various beer ingredients not only generate off flavours, but it can also have adverse effects on foam stability (Selles, 1997). The excess oxygen may cause a very rapid collapse of the foam through a chain of reactions (Engelhard and Kumke, 2006). The activated forms of oxygen (free radicals) have been specified to contribute in the ageing progression of beer (Uchida and Ono, 1996). Absorption of oxygen in the mash, throughout filtration, during boiling, in wort and beer, leads to oxidation, which can impair the flavour (Depraetere et al., 2008). Oxidation post fermentation is destructive to the taste and colloidal stability of the beer (Anastasova et al., 2008). Storage temperature affects the aging characteristics of beer, by affecting many

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chemical reactions involved. The reaction rate increase for a certain temperature depending on the reaction activation energy. This activation energy varies with the reaction type, which implies that the rate of different reactions do not increase with increasing temperature in a similar manner. Consequently, beer storage at different temperatures does not produce the same level of increase of the staling compounds. Few sensory studies confirm this prediction on flavour active ester compounds in beer (Vanderhaegen et al., 2007).

Volatile esters are responsible for the fruity character of fermented beverages and constitute an important group of aromatic compounds in beer. Although, volatile esters are only trace compounds in fermented beverages such as beer, they are extremely important for the flavour profile. The most important flavour-active esters in beer are ethyl acetate (solvent-like aroma), isoamyl acetate (fruity, bananaaroma), ethyl hexanoate and ethyl octanoate (sour apple), and phenyl ethyl acetate (flowery, roses, honey) (Verstrepen et al., 2003). However, the presence of different esters can have a synergistic effect on the individual flavours, which means that esters can also affect beer flavour well below their individual threshold concentrations. Moreover, the fact that most esters are present in concentrations around the threshold value implies that minor changes in concentration may have dramatic effects on beer flavour. Therefore, monitoring the concentration of volatile esters in beer during storage is important. In this study, the volatile ester composition and concentration in ale and lager beer was analyzed during storage at 4°C and room temperature to ascertain the effects of storage temperature on the stability of these important esters in beer (Verstrepen et al., 2003).

MATERIALS AND METHODS

Yeast strain and cultivation conditions

All experiments were carried out using either a lager or an ale strain of *Saccharomyces pastorianus* or *Saccharomyces cerevisiae*, obtained from the culture collection of the Department of Microbiology at the University of KwaZulu-Natal (Pietermaritzburg). The cultures were grown in malt extract broth for 24 h at 30°C with shaking at 120 rpm. Two milliliter pre-culture was inoculated into 200 ml malt extract broth for 6 h at 30°C with shaking at 120 rpm until an OD₆₀₀ of 1.120 for the lager strain and 0.451 for the ale strain was reached. Samples were centrifuged at 4000 rpm for 15 min at 4°C and the pellet was resuspended in 200 ml wort. Twenty milliliter of inoculum was used to pitch 2 L wort at a pitching rate of 20×10^6 cfu/ml for lager and 6×10^6 cfu/ml for ale (Saerens et al., 2008).

Wort preparation

Malt wort was prepared by adding 3.080 kg of crushed pale malt to 9.2 L of water. Mashing was carried out at the following temperatures: 63.5°C for 60 min to allow for β -amylase activity, 71°C for 30 min allowing for α -amylase activity and 74°C for 10 min to inactivate all enzymes. Mash was then centrifuged to separate spent grain

and wort. The residual mash was washed with approximately 4 L of water. Mash was then transferred into four 5 L beakers and washed with 9 L of warm water to remove residual sugars. The wort was then brought to a boil; 5 g of Southern hop pellets was added and allowed to boil for 1 h, followed by the addition of 2.5 g Saaz hop pellets and allowed to boil for a further 10 min.

Wort fermentation

Fermentations were set up to determine the effect of fermentation temperature, pH, zinc sulphate and L-leucine on fermentation performance and ester production using mini-fermenters (3.5 L) to facilitate the fermentation process on a small scale. All fermentations were carried out in triplicate in fermentation vessels containing 2 L of wort which were performed at 14°C for lager fermentation and 18°C for ale fermentation at pH 5. Fermentations were monitored by an air lock mechanism to ensure that the fermentations were not incomplete. During fermentation, samples were withdrawn from the fermentation vessel and analysed as described below. Once fermentation was complete fermenter vessels were incubated at 4°C for 5 days to allow for yeast to settle.

Bottling and conditioning

Settled yeast was removed from each fermenter and beer was transferred into sterile 750 ml sample bottles. The bottles containing the beer were allowed to stand for 30 min before capping. Eight millilitres of a brown sugar solution (1 g/ml) was added to each bottle to allow for carbonation. The bottles were capped and incubated at 14°C for five days for conditioning. Thereafter, beer was stored at 4°C and room temperature (22.5°C) until required for further analysis.

Fermentation analysis

Samples were collected daily from the fermentation vessel; this was performed by opening the tap at the bottom of the fermentation vessel and removing 5 ml of wort to carry out analyses. Thereafter, the tap was immediately closed and incubation continued to allow fermentation to continue. Samples were analysed immediately to determine total yeast cell density, free amino nitrogen, reducing sugar and ethanol concentration.

Reducing sugar content

Three millilitres of the DNS reagent was added to 3 ml wort that was diluted 10 times, in a test tube and the tube contents were heated in boiling water for 5 min. While the contents of the test tube was still warm, 1 ml of 40% Rochelle salt solution was added. This was then cooled and the intensity of the dark red colour that had developed was read at 510 nm. Standards were run using varying concentrations of glucose to generate a standard curve from which the amount of reducing sugars present in the sample was estimated (Sadasivam and Manickum, 1996).

Free amino nitrogen content

Free alpha amino nitrogen (FAN) levels in the wort were determined by the standard ninhydrin method using glycine as the reference amino acid. One ml of wort was diluted with 9 ml of distilled water and 2 ml transferred into test tubes. Ninhydrin colour reagent (1 ml) was added before heating the tubes in boiling water for 16 min.

Samples were allowed to cool and the absorbance was recorded at 570 nm against a blank containing water in place of the sample (Sadasivam and Manickum, 1996).

Viable yeast population determination

A 1 ml sample taken from the fermentation vessel was serially diluted and 0.1 ml of the appropriate dilution was spread plated onto Malt Extract agar. The plates were incubated at 30°C for 48 h, and the number of colonies on the plates for the dilution containing 30 to 300 colonies were counted and expressed as colony forming units per milliliter (cfu/ml) of the sample.

Ethanol concentration

Beer sample solution (1 mL) was dispensed into a 1.5 mL Eppendorf tube. 0.1 mL of the sample solution was injected directly into a GC with a syringe to determine the ethanol concentration. Ethanol was detected at a column temperature of 120°C with nitrogen used as the carrier gas. Standards were run using varying concentrations of ethanol to generate standard curves from which the concentration of ethanol present in the sample was estimated.

Measurement of foam head stability

Foam head stability on the beer samples were assessed according to the modified mini foam shake tests developed by Van Nierop et al. (2004). Twenty milliliters of beer was dispensed into 50 ml glass measuring cylinders, in triplicate 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 and the parafilm pieced, and a timer set for 15 min. After 15 min, the foam was evaluated visually and the cylinders were arranged from best to worst. Ratings of 1 through 5 were given, where 5 is the greatest foam stability and 1 is the worst.

Analysis of beer colour

Beer colour was measured spectrophotometrically at a wavelength of 430 nm based on the method of Seaton and Cantrell (1993) using distilled water as a blank.

Measurement of spent yeast density

Spent yeast density was measured by the method of Soley et al. (2005). Ten millilitre samples were centrifuged (6000 rpm for 10 min at 4°C). The pellet was then resuspended in a NaCl solution (0.9%, w/v), filtered through a previously dried and weighted Whatman grade GF/A (Ø 47 mm) glass microfibre filter, and dried at 105°C to a constant mass. Thereafter, weight of the filter was subtracted from the weight of the filter containing the dried cellular material to obtain the mass of spent yeast produced. In order to reduce the experimental error, measurements were performed in triplicate.

Analysis of beer volatile esters

The composition and concentrations of esters in beer was measured by headspace analysis of the sample in a gas chromatograph coupled with a flame ionisation detector (GC-FID). The volatiles from 100 ml of each sample were assessed for acetate esters and ethyl esters in the beer. Beer samples were collected in 250 ml se-

rum bottles (Wheaton) and were immediately closed. Samples were heated for 25 min at 70°C in a water bath before injecting 1 ml of the headspace into the GC. The oven temperature was held at 50°C for 5 min, then increased to 200°C at a rate of 5°C per min and finally held at 200°C for 3 min. The FID temperature was kept constant at 250°C and nitrogen was used as the carrier gas. Standards were run using varying concentrations of ethyl acetate, isoamyl acetate, phenyl ethyl acetate, ethyl decanoate, ethylhexanoate and ethyl octanoate to generate standard curves from which the concentration of esters present in the sample was estimated.

RESULTS

Fermentation analysis

The profile of sugar utilization and ethanol production during the fermentation period is shown in Figure 1a, while the FAN content and yeast density at the same period is shown in Figure 1b by both an ale and a lager yeast strain. Initial concentration of reducing sugars and FAN in the wort was 2355 and 2449.25 mg/l, respectively for the ale strain and 2348 and 2394 mg/l, respectively, for the lager strain. In ale beer, 33.70% of FAN was utilized by day six of fermentation, whereas 49.25% FAN was utilised in lager beer. The ale yeast strain utilized 17.43% more reducing sugars than the lager yeast strain, resulting in higher ethanol production. Yeast cell population in ale and lager strains increased up to day 4 of fermentation thereafter slowly decreasing.

Beer analysis

Beer colour was measured after bottle conditioning to determine the colour intensities in the beers produced. Beer colour for both ale and lager beer were similar, with the spent yeast density ranging from 2.175 to 2.500 mg/ml for the two yeast strains used (Table 1). A commercial beer which served as a control had the deepest colour intensity with an absorbance value of 0.862. The commercial beer also had the best foam head stability compared to ale and lager beers produced in this study. Ale beer produced a better foam head stability than lager beer, exhibiting 17% greater foam head stability (Table 1).

Stability of volatile ester compounds in lager beer over time

Stability profile of aroma-active esters in lager beer during storage at 4°C and room temperature over a three month period is shown in Table 2. Generally, beer (ale and lager) produced in this study were more stable at 4°C compared to room temperature during storage. At 4°C, there was a decrease in total ester concentration by 7.92% with ethyl decanoate being the least stable compound decreasing by 14.04% after three months compared to the fresh beer. Phenyl ethyl acetate was the most stable ester compound decreasing by only 3.79% after

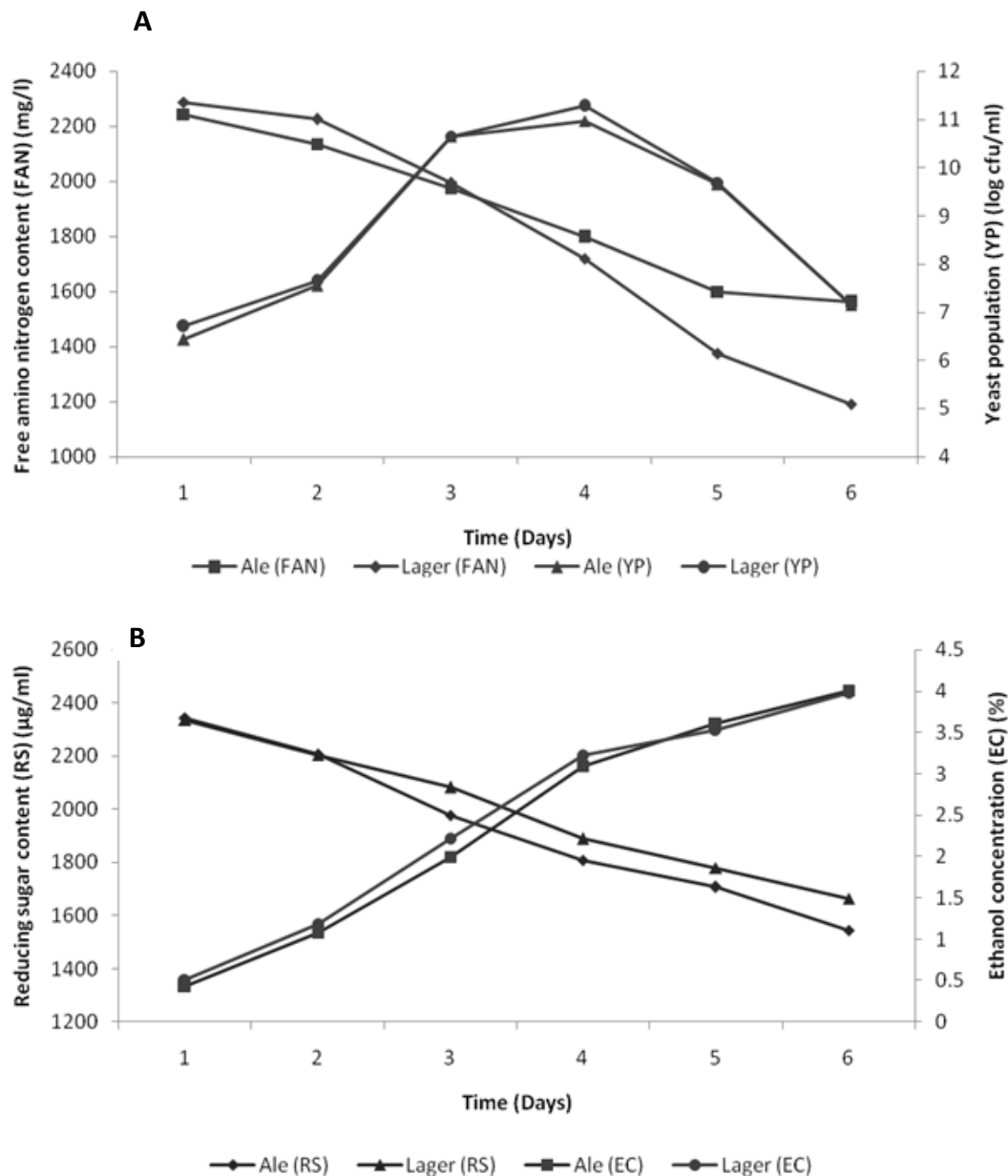


Figure 1. Profiles of (a) reducing sugar and ethanol content and (b) free amino nitrogen concentration and yeast population in wort during fermentation.

Table 1. Colour profiles, foam head stability and spent yeast density of ale and lager beer produced.

| Sample | Beer colour (430 nm) | Foam head stability rating | Spent yeast density (mg/ml) |
|-----------------|----------------------|----------------------------|-----------------------------|
| Lager | 0.642 ± 0.03 | 2.00 ± 0.00 | 2.500 ± 0.01 |
| Ale | 0.656 ± 0.01 | 2.33 ± 0.00 | 2.175 ± 0.03 |
| Commercial beer | 0.862 ± 0.00 | 5.00 ± 0.00 | n/a |

three months. Acetate esters were generally more stable than ethyl esters decreasing by 7.48% compared to 13.19% observed for ethyl esters. By week eight, the

ethyl acetate concentration decrease below the flavour threshold level. After 12 weeks of storage at 4°C, phenyl ethyl acetate and ethyl decanoate concentrations

Table 2. Stability profile of aroma-active esters in lager beer during storage at 4 °C and room temperature ($\pm 22.5^\circ\text{C}$).

| Storage temperature | Storage time (week) | Acetate ester (mg/l) | | | Ethyl ester (mg/l) | | |
|---|---------------------|----------------------|-----------------|----------------------|--------------------|------------------|------------------|
| | | Ethyl acetate | Isoamyl acetate | Phenyl ethyl acetate | Ethyl decanoate | Ethyl octanoate | Ethyl hexanoate |
| 4°C | 0 | 30.69 \pm 0.11 | 1.06 \pm 0.01 | 4.17 \pm 0.02 | 2.42 \pm 0.03 | 0.455 \pm 0.01 | 0.163 \pm 0.01 |
| | 2 | 30.66 \pm 0.03 | 1.06 \pm 0.00 | 4.14 \pm 0.02 | 2.39 \pm 0.04 | 0.451 \pm 0.01 | 0.163 \pm 0.00 |
| | 4 | 30.66 \pm 0.03 | 1.05 \pm 0.01 | 4.12 \pm 0.02 | 2.37 \pm 0.00 | 0.453 \pm 0.01 | 0.162 \pm 0.00 |
| | 6 | 30.51 \pm 0.03 | 1.06 \pm 0.00 | 4.11 \pm 0.01 | 2.32 \pm 0.15 | 0.447 \pm 0.01 | 0.157 \pm 0.00 |
| | 8 | 29.31 \pm 0.43 | 1.04 \pm 0.01 | 4.07 \pm 0.01 | 2.11 \pm 0.01 | 0.422 \pm 0.00 | 0.151 \pm 0.00 |
| | 10 | 28.54 \pm 0.08 | 1.02 \pm 0.01 | 4.04 \pm 0.00 | 2.11 \pm 0.01 | 0.415 \pm 0.00 | 0.148 \pm 0.00 |
| Room temperature ($\pm 22.5^\circ\text{C}$) | 12 | 28.21 \pm 0.42 | 1.01 \pm 0.01 | 4.01 \pm 0.01 | 2.08 \pm 0.02 | 0.410 \pm 0.00 | 0.147 \pm 0.00 |
| | 0 | 30.70 \pm 0.01 | 1.06 \pm 0.00 | 4.17 \pm 0.01 | 2.42 \pm 0.04 | 0.457 \pm 0.00 | 0.166 \pm 0.00 |
| | 2 | 30.27 \pm 0.02 | 1.06 \pm 0.00 | 4.13 \pm 0.01 | 2.33 \pm 0.03 | 0.441 \pm 0.01 | 0.157 \pm 0.00 |
| | 4 | 30.16 \pm 0.05 | 1.05 \pm 0.00 | 4.08 \pm 0.02 | 2.31 \pm 0.03 | 0.429 \pm 0.01 | 0.156 \pm 0.00 |
| | 6 | 29.95 \pm 0.07 | 1.05 \pm 0.00 | 4.02 \pm 0.01 | 2.30 \pm 0.02 | 0.413 \pm 0.01 | 0.153 \pm 0.00 |
| | 8 | 28.39 \pm 0.10 | 1.03 \pm 0.00 | 4.00 \pm 0.01 | 2.12 \pm 0.01 | 0.421 \pm 0.01 | 0.134 \pm 0.00 |
| | 10 | 28.21 \pm 0.01 | 1.02 \pm 0.01 | 3.90 \pm 0.05 | 2.05 \pm 0.01 | 0.406 \pm 0.00 | 0.127 \pm 0.00 |
| | 12 | 27.10 \pm 0.01 | 0.95 \pm 0.04 | 3.70 \pm 0.04 | 1.53 \pm 0.51 | 0.389 \pm 0.01 | 0.111 \pm 0.01 |

remained above the threshold level while isoamyl acetate, ethyl octanoate and ethyl hexanoate remained below the flavour threshold. At room temperature, there was a 13.32% decrease in total ester concentration after three months of storage. Ethyl decanoate was the least stable compound decreasing by 36.53% after three months storage, while isoamyl acetate was the most stable compound at room temperature decreasing by 10.65%. Acetate esters were more stable at room temperature than ethyl esters, with only 11.65% decrease observed after three months compared to 33.08% decrease in ethyl esters. Ethyl acetate and phenyl ethyl acetate decreased below the threshold level by weeks six and 12, respectively, while ethyl decanoate remained above the threshold level after 12 weeks of storage at room tem-

perature.

Stability of volatile ester compounds in ale beer over time

Stability profile of aroma-active esters in beer during storage at 4°C and room temperature over a three month period is shown in Table 3. Generally, beer produced in this study was more stable at 4°C compared to room temperature during storage. At 4°C, there was a decrease in total ester concentration by 6.93% with ethyl octanoate being the least stable compound decreasing by 18.83% after three months. Phenyl ethyl acetate was the most stable ester compound decreasing by only 2.23% after three months. Acetate esters were more stable than ethyl esters decreasing by

6.88% compared to 7.46% observed for ethyl esters after three months. By week 10, ethyl acetate concentration decreases below the flavour threshold level. After 12 weeks storage at 4°C, phenyl ethyl acetate and ethyl decanoate concentrations remained above the threshold level while, ethyl octanoate and ethyl hexanoate remained below the flavour threshold.

At room temperature, there was a 16.90% decrease in total ester concentration after three months. The least stable compound was ethyl octanoate decreasing by 32.47% after three months, while the most stable compound at room temperature was phenyl ethyl acetate decreasing by 9.82%. Ethyl esters were more stable at room temperature than acetate esters, decreasing by 15.64% compared to acetate esters that

Table 3. Stability profile of aroma-active esters in ale beer during storage at 4 °C and room temperature (± 22.5 °C).

| Storage temperature | Storage time (week) | Acetate ester (mg/l) | | | Ethyl ester (mg/l) | | |
|-----------------------------------|---------------------|----------------------|-----------------|----------------------|--------------------|------------------|------------------|
| | | Ethyl acetate | Isoamyl acetate | Phenyl ethyl acetate | Ethyl decanoate | Ethyl octanoate | Ethyl hexanoate |
| 4°C | 0 | 31.88 \pm 0.72 | 1.24 \pm 0.02 | 4.48 \pm 0.03 | 2.55 \pm 0.03 | 0.154 \pm 0.00 | 0.262 \pm 0.01 |
| | 2 | 31.85 \pm 0.00 | 1.22 \pm 0.01 | 4.44 \pm 0.01 | 2.54 \pm 0.01 | 0.150 \pm 0.00 | 0.261 \pm 0.00 |
| | 4 | 31.27 \pm 0.01 | 1.22 \pm 0.01 | 4.43 \pm 0.01 | 2.54 \pm 0.01 | 0.147 \pm 0.00 | 0.261 \pm 0.00 |
| | 6 | 31.24 \pm 0.09 | 1.22 \pm 0.01 | 4.40 \pm 0.01 | 2.51 \pm 0.01 | 0.142 \pm 0.00 | 0.260 \pm 0.00 |
| | 8 | 30.29 \pm 0.06 | 1.21 \pm 0.01 | 4.44 \pm 0.03 | 2.43 \pm 0.00 | 0.138 \pm 0.00 | 0.257 \pm 0.00 |
| | 10 | 29.54 \pm 0.06 | 1.15 \pm 0.01 | 4.41 \pm 0.07 | 2.40 \pm 0.01 | 0.131 \pm 0.00 | 0.255 \pm 0.00 |
| | 12 | 29.51 \pm 0.01 | 1.12 \pm 0.01 | 4.38 \pm 0.00 | 2.37 \pm 0.00 | 0.126 \pm 0.00 | 0.249 \pm 0.00 |
| Room temperature (± 22.5 °C) | 0 | 31.88 \pm 0.10 | 1.24 \pm 0.01 | 4.48 \pm 0.01 | 2.55 \pm 0.00 | 0.154 \pm 0.00 | 0.262 \pm 0.00 |
| | 2 | 30.45 \pm 0.01 | 1.21 \pm 0.01 | 4.46 \pm 0.00 | 2.50 \pm 0.00 | 0.153 \pm 0.00 | 0.260 \pm 0.00 |
| | 4 | 30.21 \pm 0.01 | 1.19 \pm 0.01 | 4.40 \pm 0.01 | 2.47 \pm 0.04 | 0.149 \pm 0.00 | 0.254 \pm 0.00 |
| | 6 | 29.45 \pm 0.00 | 1.14 \pm 0.01 | 4.35 \pm 0.02 | 2.42 \pm 0.01 | 0.137 \pm 0.00 | 0.254 \pm 0.00 |
| | 8 | 28.39 \pm 0.03 | 1.09 \pm 0.01 | 4.28 \pm 0.01 | 2.36 \pm 0.01 | 0.131 \pm 0.00 | 0.251 \pm 0.00 |
| | 10 | 27.21 \pm 0.01 | 1.04 \pm 0.00 | 4.21 \pm 0.01 | 2.33 \pm 0.00 | 0.125 \pm 0.00 | 0.235 \pm 0.00 |
| | 12 | 26.28 \pm 0.52 | 0.89 \pm 0.00 | 4.04 \pm 0.01 | 2.18 \pm 0.00 | 0.104 \pm 0.00 | 0.218 \pm 0.00 |

decreased by 16.99%. Ethyl acetate and phenyl ethyl acetate decreased below the threshold level by week six and four, respectively while ethyl decanoate remained above the threshold level after 12 weeks of storage at room temperature.

DISCUSSION

Yeast activity is important in achieving consistent fermentations that result in beers of acceptable quality (Verbelen et al., 2009). Both the ale and lager strains used in this study demonstrated good growth and efficient nutrient utilization as indicated by FAN utilization commonly used as index for potential yeast growth and efficiency (Lekkaset al., 2007). However, the lager strain utilized a higher concentration of FAN than the ale strain. In the brewing industry, surplus yeast (spent yeast) is recovered by natural sedimentation or centrifugation at the end of the fermentation and conditioning and is very high in protein and B vitamins, and may serve as a feeding supple-

ment to livestock (Goldammer, 2008) and humans. Thus, the high spent yeast density recovered after fermentation by the lager and ale yeast strain could be used for this purpose. Colour development in beer has been mostly attributed to the malt extract used in the respective beers instead of 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 germinated barley (Seaton and Cantrell, 1993; Kopsahelis et al., 2007). Thus, it is possible that the malt extract used in this study was roasted differently from those used for commercial beer, hence the lighter colour intensity as previously reported (Olaniran et al., 2011). Also, the difference in beer foam head stability has been attributed to the brewing process and the raw materials used as well as the presence of a number of compounds which affect foam formation and stability (Bamforth, 1985), hence the observed difference in the foam head stability of the ale and the lager beer produced in this study.

The intensity of ester aroma character in beer is regarded as an important component of its sensory quality. Generally, the intensity of ester aroma character is higher in ale beers compared to lager beers (Verstrepen et al., 2003). In both ale and lager beer, the concentration of the various ester compounds gradually decreased over the three month storage period, with beers stored at room temperature resulting in a much faster decrease in ester concentration than those stored at 4°C. This decrease in ester concentration may be due to esterase activity of the yeast, resulting in cell autolysis during fermentation and maturation (Neven et al., 1997). The optimal esterase activity in beer is between 15 and 20°C, hence, the higher decrease in ester concentration at room temperature. Also, esterase activity is strain dependent, with top-fermenting (ale) yeasts more active than bottom fermenting (lager) yeasts (Neven et al., 1997). Therefore, there is a higher decrease in ester concentration during storage in ale beer than in lager beer.

Vanderhaegen et al. (2003) reported that the concentration of all acetate esters decreased during aging and that larger molecular weight esters are hydrolyzed to a higher degree during storage. This is comparable to results obtained in this study, as the highest molecular weight esters (ethyl decanoate and ethyl octanoate) decreased the most in lager beer. However, in ale beer, this trend was not observed, probably due to the inverse variation of the reaction activation energy with the molecular weight of the ester, although reaction kinetics also depend on the initial ester, alcohol, and acid concentrations (Ramey and Ough, 1980). Optimization of the brewing process with respect to flavour stability requires a clear insight of the types of flavour changes during storage and the nature of the molecules involved. Since decreases in ester concentration during storage are largely attributed to esterase activity, pasteurization of beer is required to inactivate these esterases that hydrolyse esters, thus preventing decrease in ester concentration during storage.

Antioxidants are also known to be responsible for flavour staling in beer. For example, (E)-2-nonenal is majorly responsible for the cardboard flavour of beer. Additionally, methional, 3-methylbutanal, 2-furfuryl ethyl ether, b-damascenone and acetaldehyde are key contributors to the aged flavour and to a lesser extent (E,E)-2,4-decadienal, phenylacetaldehyde, 2-methylpropanal, diacetyl and 5-hydroxymethylfurfural (Saison et al., 2009). It is necessary to clarify the reaction pathways leading to the staling compounds in beer and understand the influence of the production process on the staling reactions. Knowledge of the aging phenomenon in a particular type of beer can be used to develop appropriate technological process to control its particular flavour stability. Besides their relevance for flavour stability, the investment costs for suggested process modifications must be evaluated and a balance should be made between better and longer

flavour stability and costs.

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