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Research Paper

Determination of volatile compounds during deterioration of African opaque beer using a stir bar sorptive extraction technique and gas chromatography-high resolution mass spectrometry



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ABSTRACT

Opaque beer traditional to African communities undergoes quick deterioration and is consumed within 7 days of its production. The current study has utilized a stir bar sorptive extraction technique followed by GC-HRT determination to trace variations of 84 volatile compounds in four opaque beers commonly brewed in South Africa over the 7-day shelf life period. The major fruity esters were observed to increase up to Day 4 and eventually decreasing until Day 7 where their levels were finally lower than Day 1. Aldehydes reduced drastically and were less than 50% on Day 2 and becoming almost undetectable at Day 7. The common beer alcohols (phenylethyl alcohol and 3-methyl-1-butanol) decreased during beer shelf life while phenolics with undesirable medicinal tastes (creosol and *p*-cresol) increased up to 24-fold by Day 7. This study might open future research perspectives around opaque beer traditional to African rural communities.

1. Introduction

The presence of volatile compounds in beer especially ketones, aldehydes, esters and fatty acids is of importance as they are responsible for organoleptic experiences of beer. The characteristic flavour that defines the smell and taste of a specific beer is as a result of a mixture of these compounds existing at varying concentrations (Andrés-Iglesias et al., 2015; Alvim et al., 2017). Determination of the variations of these volatile compounds over time as the beer matures before consumption becomes an essential step in beer production in order to understand flavour stability (Andrés-Iglesias et al., 2016; Kobayashi et al., 2008). In this regard, studies on volatile compounds responsible for flavour and how their variations over time affect flavour stability have been mentioned recently in literature especially on commercial bottled beer including craft/draft/lager beer (Andrés-Iglesias et al., 2016; Gonzalez Viejo et al., 2019; Rossi et al., 2014), wines (Moyano et al., 2019; Zhang et al., 2011; Ubeda et al., 2016; Pérez-Jiménez and Pozo-Bayón, 2019; Ríos-Reina et al., 2019; Morales et al., 2017; Tang et al., 2019; Azzi-Achkouty et al., 2017) and whiskey/brandy/spirits (Biernacka and Wardencki, 2012; Johnson et al., 2017; Rodriguez Madrera et al., 2013).

Sensory perception due to variations in flavour stability maybe viewed in two ways with some types of beer becoming better with age while in others it leads to deterioration (Andrés-Iglesias et al., 2015, 2016). Deterioration is common with opaque beer traditional to African folk where the traditionally brewed beer among rural communities becomes unpalatable within 5 days while those brewed on a commercial scale may remain palatable up to 7 days. Opaque beer brewed among African communities is not preserved in any way and once packed in containers ready for consumption it remains exposed to ambient conditions and fermentation continues under non-controlled conditions. Various sorghum-brewed opaque beers traditional to African communities and their preparation processes are mentioned in two reviews where the authors also observe that production procedures may vary according to geographic location (Lyumugabe et al., 2012; Adebisi et al., 2018). Generally, most of the recent studies on African traditional beers have focused on nutritional value, microbial composition and hygiene (Lyumugabe et al., 2013; Matumba et al., 2011, 2014; Adekoya et al., 2018). Data on volatile compound variations responsible for fast deterioration kinetics of opaque beer traditional to African folk remains limited. A study by Lyumugabe et al., (2013) profiled volatile compounds in *ikigage*, a traditional sorghum beer in Rwanda for the purposes of

Abbreviations: CIS, cooled injection system; GC-HRT, gas chromatography-high resolution time-of-flight mass spectrometry; (HS)-SPME, (headspace)-solid phase microextraction; PDMS, polydimethylsiloxane; SBSE, stir bar sorptive extraction; TDU, thermal desorption unit.

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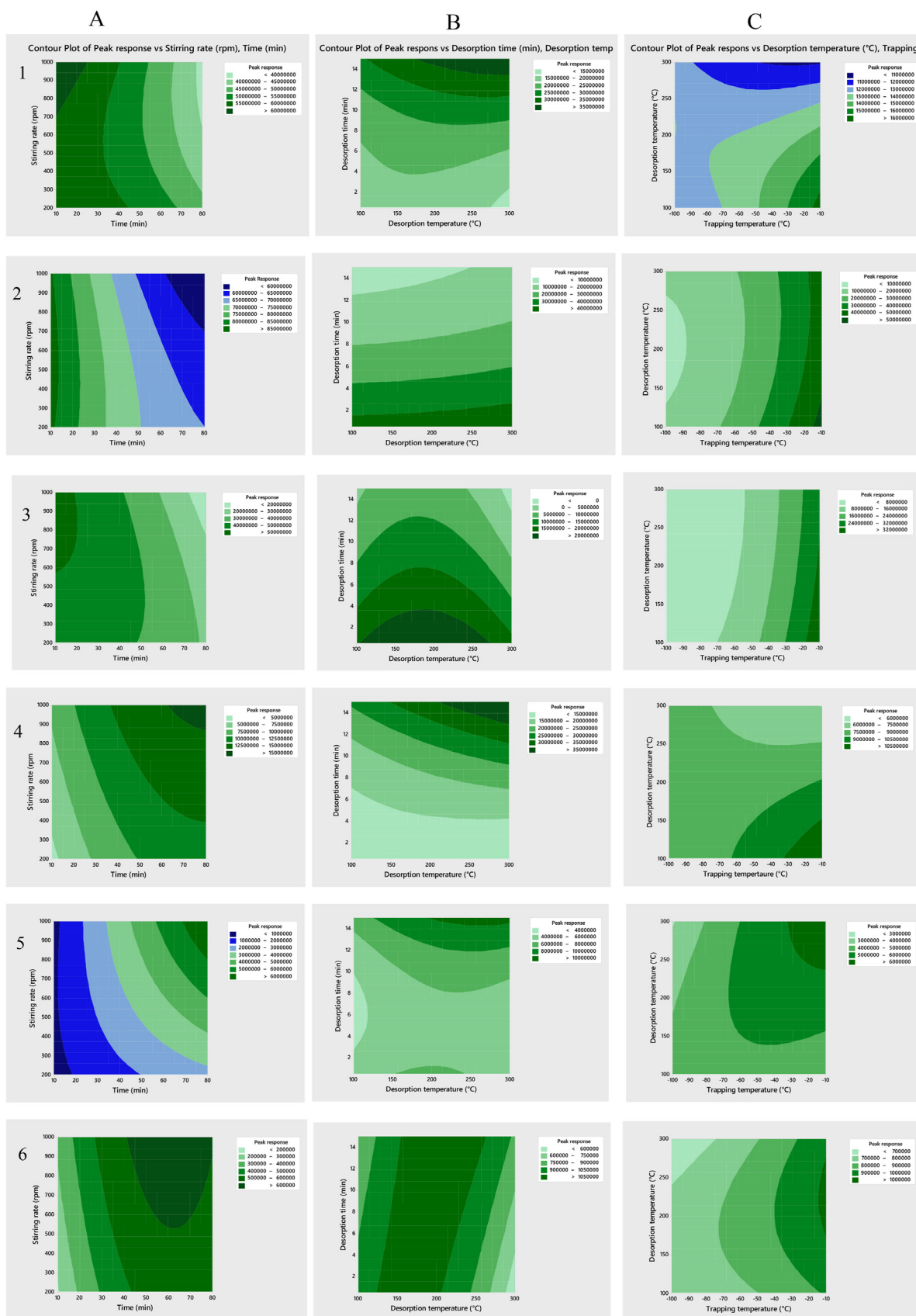








Fig. 1. Contour plots for paired optimization of (A) twister, (B) thermal desorption and (C) cooled injection parameters for (1) phenylethyl alcohol, (2) 1-butanol, 3-methyl-, (3) isopentyl acetate, (4) ethyl octanoate, (5) ethyl linoleate, (6) ethyl isopentyl succinate.

Table 1
Optimum conditions for analysis of model volatile compounds in beer samples.

| Model compound | Structure | Log Kow | Twister optimum values | | TDU optimum values | | CIS optimum values | |
|---------------------------|---|---------|------------------------|---------------------|-----------------------------|-----------------------|---------------------------|-----------------------------|
| | | | Stirring rate (rpm) | Stirring time (min) | Desorption temperature (°C) | Desorption time (min) | Trapping temperature (°C) | Desorption temperature (°C) |
| Phenylethyl alcohol |  | 1.36 | 1000 | 10 | 300 | 15 | -10 | 100 |
| 3-methyl-1-butanol |  | 1.16 | 570 | 10 | 300 | 0.5 | -10 | 100 |
| Isopentyl acetate |  | 2.25 | 970 | 10 | 188 | 15 | -10 | 100 |
| Ethyl octanoate |  | 3.81 | 1000 | 80 | 300 | 15 | -10 | 100 |
| Ethyl lenoleate |  | – | 1000 | 80 | 265 | 15 | -10 | 300 |
| Ethyl isopentyl succinate |  | 2.79 | 1000 | 60 | 209 | 15 | -10 | 225 |
| Universal conditions | | | 1000 | 40 | 300 | 15 | -10 | 150 |
| Compound desirability | | | 0.5636 | | 0.5423 | | 0.7593 | |

TDU – Thermal desorption unit, CIS – Cooled injection system.

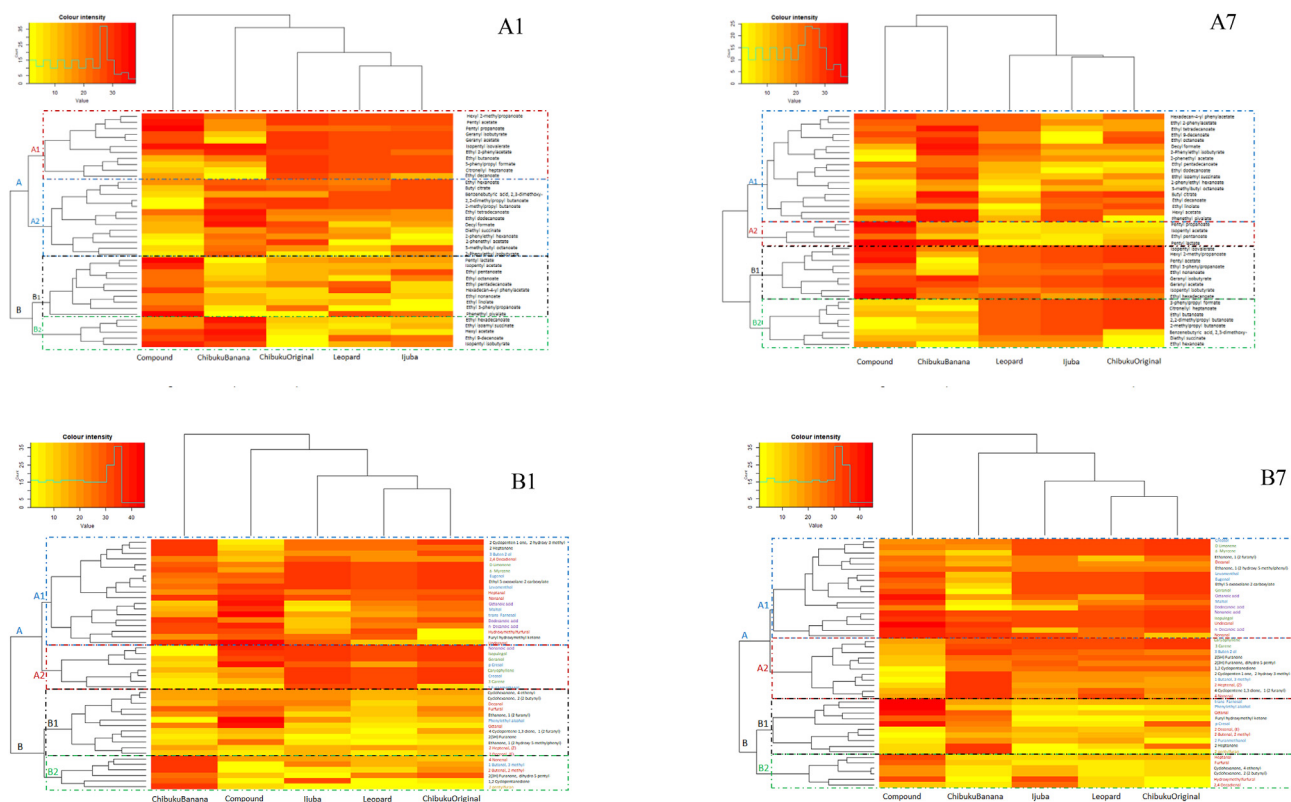


Fig. 2. Hierarchically clustered heatmaps for (A) esters and (B) other volatiles at Day 1 and 7 for Chibuku Original, Chibuku Banana, Ijuba and Leopard opaque beers.

understanding the impact of using *V. amygdalina* leaves during brewing (Lyumugabe et al., 2013) while Bvochora et al., 2005 studied some phenolic volatile compounds during preparation of Zimbabwean opaque beer (Bvochora et al., 2005). It is therefore imperative that more studies are done to understand the volatile compound variations during deterioration of the opaque beer.

The effective identification of volatile compounds that really define the flavour of food is somehow dependant on the extraction procedure and identification technique used. In this regard, solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE) are the most preferred extraction techniques followed by identification and quantitation using gas chromatography-mass spectrometry (GC-MS) (Andrés-Iglesias et al.,

2015). The application of SBSE on alcoholic beverage samples is currently on the rise with almost 50% of those studies reported in 2019 (Pérez-Jiménez and Pozo-Bayón, 2019; Ríos-Reina et al., 2019; Tang et al., 2019; Ruvalcaba et al., 2019; Marsili and Laskonis, 2019). SBSE was introduced in 1999 by Baltussen and co-workers (Baltussen et al., 1999). The SBSE twisters consist of a polymer embedded on a magnetic stirrer with polydimethylsiloxane (PDMS) as the most common polymer sorbent. Extraction occurs by sorption, a partitioning process in which each analyte reaches its own partitioning equilibrium based on its octanol-water distribution coefficient (Baltussen et al., 1999; David et al., 2019). In this regard, the SBSE technique can be a more relevant technique for comprehensive profiling of food flavour compounds.

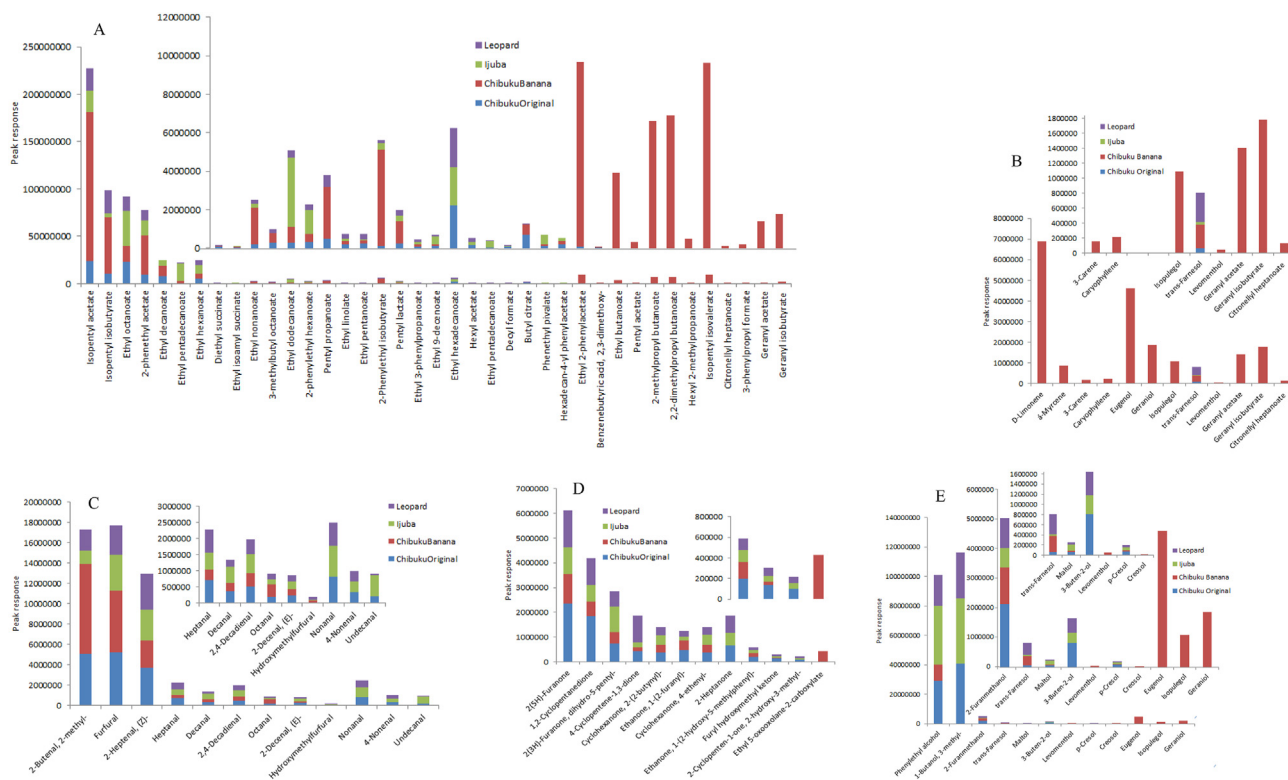


Fig. 3. Relative composition of volatiles in the beer samples on Day 1. (A) esters, (B), terpenes, (C) aldehydes, (D) ketones and (E) alcohols.

The objective of the present study was therefore to identify and trace the variations of volatile compounds during the deterioration process of four common opaque beers traditional to South Africa. The beers are prepared commercially and have a shelf life of 7 days. We hypothesized that there are various volatile compounds involved in opaque beer flavour stability and their variations are related to the deterioration process of the opaque beer. A stir bar sorptive extraction technique in combination with GC-HRT fitted with a thermal desorption unit (TDU) was then used for isolation and identification of the volatile compounds. The changes in the content of each volatile compound was traced over a 7-day period relative to the day the beer is distributed to retailers (taken as Day 1).

2. Materials and methods

2.1. Instrumentation and apparatus

The SBSE twistlers (GERSTEL GmbH & Co. KG, Germany) used during extraction consisted of a 0.5 mm PDMS sorbent thickness supported on a 10 mm magnetic stir bar. Ultrapure water (UHP) was prepared using a Milli-Q® Reference Water Purification System (Merck Millipore, Bedford, MA, USA).

A Pegasus LECO GCxGC-HRT 4D (LECO Corporation, St. Joseph, MI, USA) fitted with a GERSTEL MPS autosampler controlled by a GERSTEL MAESTRO software, a TDU and a cooled injection system (CIS) all supplied by GERSTEL (GmbH & Co. KG, Germany) was used in this study. This instrument uses quadrupole time-of-flight mass spectrometer with high resolution deconvolution. Separation was achieved in one-dimensional mode on a 30 m × 0.32 mm × 25 mm RXI-5SIL MS capillary column (Restek Corp., Bellefonte, PA, USA). The temperature program was initially set at 50 °C and ramped to 280 °C over a total runtime of 43 min. The autosampler method was set to the GC runtime method followed by a 5 min cool down time. The back-inlet temperature and the transfer line were kept at 250 and 300 °C respectively. Peak detection and mass spectral deconvolution were achieved on ChromaTOF HRT

Version 5.10 software. The spectral match threshold was set at 800 with over a thousand peaks detected in each chromatogram. The identified peaks were eventually narrowed down to 84 compounds used in understanding the sensory defects of deteriorating beer.

2.2. Beer samples

Four traditional beers (*Chibuku Original*, *Chibuku Banana*, *Ijuba* and *Leopard*) all brewed using traditional methods by the United National Breweries (Pty) Ltd, Midrand, South Africa and sold in either plastic or canvas containers were collected from Devland depot, Johannesburg, South Africa. The ingredients are mainly the sorghum malt, maize, water and yeast. The finished product has 3 %v/v alcohol content. These traditional beers are distributed every Monday to retailers and are consumed within 7 days. The beers were therefore collected from the depot on a Monday to ensure that the study traced the volatile compounds from the time the beers are delivered until they become unpalatable. At the retailers, the traditional beer is kept in open shelves under ambient conditions with the fermentation process remaining active.

2.3. Stir bar sorptive extraction

The twister bar was placed in a 20 mL Gerstel headspace vial. The vial was filled with 20 mL of the beer sample and sealed with silicone/PTFE caps. This was done in triplicate for each of the four beer samples. The beer packs were picked randomly and thoroughly shaken by hand for at least 1 min as advised by the brewer. New packs were opened every day. The sealed vials were then placed on magnetic stirrers and extraction performed under optimized conditions specified in Section 3.1.1.

2.4. Analytical procedure

After performing the SBSE procedure, the twistlers were removed from the vials, rinsed with UHP water and wiped dry using paper towel. The twistlers were then placed in TDU desorption tubes and set on the GC

instrument for analysis. An autosampler was used to transfer the vials into the TDU system. The desorbed volatile compounds were trapped in a cooled injection system (CIS) at sub-zero temperatures and finally released into the GC-HRT system for separation and identification.

2.5. Optimization of SBSE twister and gas chromatography injection parameters

The extraction and GC-injection parameters were optimized via a central composite design created on Minitab 18.1 software (Minitab Inc., Pennsylvania, USA) using six model compounds identified in *Chibuku* beer samples during preliminary runs. The interactive effects of stirring rate (200–1000 rpm) and time (10–80 min) were optimized for adsorption of volatile compounds on to the twister. Desorption of volatile compounds into the GC was done using a TDU mounted on the GC back injection system. In this regard, the interactive effects of desorption temperature and time were investigated in the 100–300 °C and 0.5–15 min range, respectively. The volatile compounds were desorbed and trapped into a CIS before being released into the GC column. The interactive effects of trapping temperature (–100 to –10 °C) and the release temperature (100–300 °C) were also optimized. All temperature ramps were done at a standard mode ramp of 10 °C s^{–1}. The trapping temperature was equilibrated for 0.05 s while the release temperature was held for 0.5 s. The interactive effects were visualized using contour plots while the optimum values were summarized as optimization plots of interactive effects.

The acceptability of parameter values at an optimum point that maximizes the response due to interactive effects was estimated as a desirability value and given as part of optimization plots. With the view that all the target compounds had to be extracted simultaneously, universal values for each parameter were predicted by altering the optimum values identified in optimization plots. The optimal universal parameter values for an effective analysis of various volatile compounds were identified as a geometric mean of the new individual desirability values using a compound desirability function (Eq. (1)). Compound desirability values range from zero (non-desirable) to one (most desirable). The universal optimal conditions were then applied in the analysis of volatile compounds in beer samples.

$$D(Y) = (d_1y_1 \cdot d_2y_2 \dots d_my_m)^{1/m} \quad (1)$$

where $D(Y)$ is the compound desirability, d_iy_i is the individual desirability of a single interactive effect on an i^{th} response after altering the original optimum values, and m is the number of responses.

Since there were no standards used for the model compounds, effort was made to ensure that optimization of the SBSE twister parameters was done at the same time using the same opened *Chibuku* beer packs followed by GC-HRT analysis. In each case, triplicate extractions were done for three randomly selected beer packs. The same approach was adopted for TDU parameters but on a different day using a new set of *Chibuku* beer packs. The CIS parameters were also done on a different day using new beer packs. For analysis of samples, a set of three packs for each beer type was selected randomly on each day and subjected to SBSE-TD-GC/HRT technique. The other beer packs were kept under ambient conditions in the laboratory to resemble the shelf conditions in retailer shops. A new set of beer packs was selected and opened every day over a 7-day period.

3. Results and discussion

3.1. Optimization results

3.1.1. SBSE twister parameters

The interactive effects of stirring rate and time on the ability of the twister to adsorb model volatile compounds are summarized as contour plots in Fig. 1. A difference was observed in the optimum conditions for adsorption of alkyl alcohols and esters with the extent of adsorption of

alkyl alcohols affected mainly by time while esters were affected by both extraction rate and time. Generally, fast adsorption kinetics for alkyl alcohols were observed with 10 min as the optimum extraction time for all model alkyl alcohols (Table 1). Increasing extraction time at any extraction rate resulted in a steady decrease in the amount adsorbed (Fig. 1). On the other hand, the contour plots for esters show a positive rising ridge pattern implying that the interactive effects of extraction rate and time correlated linearly resulting in an increase in adsorption of esters. Maximum extraction occurred at 1000 rpm for 60–80 min. With the esters taking longer to adsorb on the twister, this might result in displacement of the sterically smaller alkyl alcohols from the twister pores which might explain the observed behaviour of alcohols when extraction is done for longer periods. Other twister studies for volatile compounds in alcoholic beverages have mentioned stirring at 200 rpm for 60 min (Ubeda et al., 2016), 500 rpm for 90 min (Elpa et al., 2014), 1000 rpm for 2 h (Marsili and Laskonis, 2019), 750 rpm for 90 min (Richter et al., 2017).

3.1.2. Thermal desorption parameters

The contour plots in Fig. 1 show that desorption time was the most important factor in desorption of model compounds from the twister. The aliphatic alkyl alcohol (3-methyl-1-butanol) was desorbed within 0.5 min regardless of desorption temperature, while all the other compounds needed longer desorption times of up to 15 min and desorption temperatures between 200 and 300 °C (Table 1). Adsorption into the twister occurs inside pores and therefore higher desorption temperatures and times are needed to transfer the compounds to the surface of the twister. Other studies where the conditions were not optimized have reported desorption of volatile compounds from twisters at 210 °C (Ubeda et al., 2016), 240 °C (Pérez-Jiménez and Pozo-Bayón, 2019) and 250 °C (Ríos-Reina et al., 2019) all for 5 min as well as 280 °C for 10 min (Elpa et al., 2014), 280 °C for 4 min (Marsili and Laskonis, 2019) and 240 °C for 10 min (Richter et al., 2017).

3.1.3. Cooled injection system parameters

The trapping and release temperatures of the CIS were not so important with only 3-methyl-1-butanol mainly affected by trapping temperature (Fig. 1). Generally, less extreme (higher) trapping temperatures were more effective with –10 °C being optimal for all the model compounds (Table 1). The increase of temperature from its trapping value to its release temperature was done at 10 °C s^{–1} (CIS is designed for maximum of 12 °C s^{–1}) and this might not have been long enough to fully defrost the compounds when trapping was done at extremely low temperatures. The contour plots have shown that the release temperature from the CIS into the GC column was not an important factor regardless of the trapping temperature (Fig. 1). However, the CIS release temperature might still be related to the molecular weight of the trapped compound with results showing that release of low molecular weight compounds was maximized at 100 °C while larger molecular weight compounds (MW > 200 g mol^{–1}) were better released at higher temperatures of 200–300 °C. Elsewhere, other studies where the CIS conditions were not optimized have mentioned trapping at –35 °C and releasing at 260 °C (Ubeda et al., 2016; Ríos-Reina et al., 2019), –100 °C and releasing at 240 °C (Pérez-Jiménez and Pozo-Bayón, 2019), –140 to 300 °C (Elpa et al., 2014), –100 to 280 °C (Marsili and Laskonis, 2019) and –60 to 250 °C (Richter et al., 2017). While the release temperatures are similar, our study has shown through multivariate analysis that extreme trapping temperatures may affect the amount of volatile compounds released into the GC.

3.1.4. Universal parameters

Optimum values for the twister, TDU and the CIS for extraction of individual compounds are summarized in Table 1. For an effective simultaneous extraction of both alcohols and esters from beer samples, a maximum compound desirability value of 0.5636 was obtained when the universal interactive effects of twister stirring rate and time were set at

100 rpm for 40 min, respectively. The optimal universal TDU parameters values were found to be 300 °C desorption temperature and 15 min desorption time with a compound desirability value of 0.5423. The CIS universal conditions were trapping at –10 °C and releasing into the GC column at 150 °C. The compound desirability was 0.7593. Overall, the twister-TDU-CIS method gave an acceptable compound desirability value of 0.6145 indicating that the SBSE-TD-GC-HRT approach can be potentially used as an alternative sample preparation technique for the simultaneous analysis of volatile compounds in beer samples. In this regard, the optimized technique was finally applied in understanding the variations of volatile compounds as traditional beer matures on the counter.

3.2. Identification of essential volatile compounds in beer samples

There were 84 volatile compounds with a potential to affect opaque beer flavour experiences positively identified using fragmentation patterns, exact mass and retention times. These included 39 esters, 12 aldehydes, 12 ketones, 12 alcohols, 4 terpenes (8 other terpenes are classified under esters and/or alcohols) and 4 fatty acids. 2-pentyl furan was identified in addition to other furan derivatives classified under aldehydes, ketones and alcohols. The volatile compounds identified in each beer sample on Day 1 and how they varied over the 7-day period are given in Tables 2 and 3. The results of the current study are comparable with the study done on another sorghum-derived traditional opaque beer in Rwanda in which 75 volatile compounds were identified of which 32 were esters, 12 alcohols, 9 carbonyls (ketones and aldehydes), 7 fatty acids, 7 terpenes and 8 other compounds classified as sulphurs (Lyumugabe et al., 2013). Notable volatile esters identified only in the current study include geranyl acetate, geranyl isobutyrate, citronellyl heptanoate, ethyl linolate, phenethyl pivalate and 2,3-dimethoxy-benzenebutyric acid. The main alcohols identified in this study were also reported in Rwandan traditional beer (Lyumugabe et al., 2013). Most of the volatile compounds detected in the current study have also been reported in commercial beers (Rendall et al., 2015), wines (Ubeda et al., 2016) and whiskeys/spirits (Biernacka and Wardencki, 2012; Zhao et al., 2013).

Tables 2 and 3 show that most of the volatile compounds in this study were identified in all beer samples except for terpenes which existed exclusively in the *Chibuku Banana* beer. About 50% of the esters and alcohols existed in all the beer samples whereas for fatty acids, aldehydes and ketones the composition that existed in all the beer samples was between 75 and 77%. In addition to the 12 terpenes, there were other volatile compounds that existed exclusively in the *Chibuku Banana* beer consisting of 7 esters, a ketone and a fatty acid constituting 25% of the total volatile compounds. Furthermore, 19% of the volatile compounds (8 esters, 3 aldehydes, 2 ketones and 2 alcohols) existed in two or three of the four beer samples. All the 3 aldehydes in this category existed exclusively in the *Chibuku Original*, *Ijuba* and *Leopard*. The implications of these observations, their variations throughout the study and how they contributed to beer deterioration are discussed in Section 3.4.

3.3. Hierarchical cluster analysis

Hierarchical cluster analysis prepared in RStudio version 3.6.1 using the gplots program was used to identify any correlational behaviour of volatile compounds on Day 1 when the beer is first distributed and made available to the consumers. This was also done on Day 7 when the beer is no longer palatable. The results were visualized using dendrograms and heatmaps as shown in Fig. 2. A hierarchical cluster for esters on Day 1 shows two separate groups, A (24 esters) and B (15 esters) which were further divided into various sub-clusters. Cluster A further divides into cluster A1 consisting of 11 esters mainly those that existed in the *Chibuku Banana* beer only. This sub-cluster is related to Cluster A2 which had 13 esters. Cluster A2 is further divided into 2 other clusters, one with esters existing in 2 or more opaque beer samples regardless of amounts and the other with alkylalkyl esters that exist in all the beer samples notably phenylethyl esters. Cluster B was different from Cluster A in that the

amount of volatile compounds in this group was similar in 3 or all of the beer samples. Thus, cluster B1 had ethyl- and pentyl-esters that existed in related amounts in all the four beer samples while B2 had esters whose amounts were similar in 3 of the 4 beer samples. For beer samples, there was a strong correlation between *Chibuku Original*, *Ijuba* and *Leopard*. *Ijuba* and *Leopard* form a further cluster indicating that there were esters with characteristics unique to these beer samples. At Day 7, the esters were clustered into two groups, A with 22 esters whose amounts were comparable in 2 or more beer samples and B consisting 17 esters mainly detected in the *Chibuku Banana* beer only. Cluster A1 consists of 18 esters whose amounts were similar in at least 2 beer samples. These were further divided into various clusters notably those where the beer samples seem to form pairs in terms of amount of the ester.

For other volatile compounds, the hierarchically clustered heatmap for Day 1 (Fig. 2) shows that Cluster A was further divided into A1 (19 volatile compounds) and A2 (8 volatile compounds). There is no clear correlation between volatile compounds in sub-cluster A1. However, sub-cluster A2 had volatile compounds that mainly existed in the *Chibuku Banana* beer. In Cluster B, there were 18 volatile compounds mainly those that existed in similar amounts in at least 3 beer samples. As for beer samples, *Chibuku Original* and *Leopard* had a very strong correlation and also clustered with *Ijuba*. The *Chibuku Banana* beer showed a strong dependence on the volatile compounds rather than showing a close correlation with other beer samples. On Day 7, there was Cluster A consisting of 18 volatile compounds mainly those existing in some beer samples but not in others. Cluster B had 16 volatile compounds that existed in all the beer samples with most of them existing in similar quantities in at least 3 beer samples. On Day 7, the *Chibuku Banana* beer had a correlation with a cluster consisting of the other three beers.

3.4. Variations in beer volatile compounds

Since the volatile compounds in this study were not quantified, the flavour stability was therefore discussed in terms of the amounts of volatile compounds that existed throughout the study relative to the peak areas obtained at Day 1. In this regard, the authors rather discuss the potential impact on beer deterioration based on the observed increase or decrease in the volatile compound amounts without considerations of flavour threshold values. A general observation was that most important volatile compounds increased in amount up to Day 4 and eventually decreased gradually. Thus, at Day 7 some values had reduced to levels below the initial amounts observed at Day 1 while others remained higher than at Day 1. The specific behaviours and how they compare with literature are discussed in the following sub-sections.

3.4.1. Statistical analysis

The relative standard deviation of compound peak responses for triplicate extraction for each beer sample per day was $\leq 24.7\%$. A comparison of the total peak responses for esters on Day 1 is given in Table 2. Notably, the total peak responses due to esters from the *Chibuku Original* and *Leopard* beer samples were not significantly different at 95% confidence interval and contributed 13.0 and 13.6% of the total ester peak responses, respectively. The behaviour of the esters between different beer samples over the 7-day shelf life period relative to Day 1 was not significantly different at 95% confidence interval for most esters regardless of the beer sample and the relative initial peak responses. Those showing variance are marked in Table 2. As for alcohols (Table 3), the total alcohol peak responses were significantly different for all beer samples with *Ijuba* beer samples contributing 36.7%, *Chibuku Original* at 31.5%, *Leopard* 23.2% and *Chibuku Banana* contributing 8.7% of the total alcohol peak responses. Consequently, the relative amount of the alcohols was inversely dependent on the initial peak response on Day 1 with the exception of phenyl alcohol and 3-methyl-1-butanol in different beer samples which showed no significant difference between beer samples at 95% confidence interval. On the other hand, the different total

Table 2
Esters identified in opaque beer samples and their relative amounts over a seven-day shelf life.

| Compound | Peak area only | | | | Relative to Day 1 | | | | | | | | | |
|-------------------------------------|----------------|-----------|-----------|-----------|-------------------|-----|-----|-----|-------|-----|-----|-----|-------|--|
| | Day 1 | | | | Day 2 | | | | Day 3 | | | | Day 4 | |
| | OG | BN | JB | LP | OG | BN | JB | LP | OG | BN | JB | LP | OG | |
| Isopentyl acetate* | 23423119 | 158014891 | 22070010 | 23784677 | 1.6 | 0.5 | 2.3 | 1.7 | 1.8 | 0.3 | 2.3 | 0.9 | 1.2 | |
| Ethyl exanoate | 5555205 | 5034815 | 9094110 | 4731141 | 0.9 | 0.8 | 1.0 | 0.9 | 0.6 | 0.5 | 0.7 | 0.3 | 0.4 | |
| Isopentyl isobutyrate | 10566099 | 59696145 | 3808519 | 24207876 | 1.0 | 1.4 | 0.7 | 1.1 | 0.7 | 1.1 | 0.9 | 0.6 | 0.4 | |
| Diethyl succinate* | 72961 | 34095 | 18769 (2) | 25153 | 0.6 | 1.8 | 1 | 1.6 | 1.5 | 6.3 | 2.2 | 1.3 | 4.1 | |
| Ethyl isoamyl succinate* | 17709 | 68776 | 23378 | 17232 (3) | 2.2 | 1.7 | 1.0 | ND | 3.3 | 3.4 | 1.3 | 1.0 | 7.5 | |
| Ethyl octanoate | 22782808 | 16950956 | 37248488 | 14744377 | 0.9 | 0.8 | 0.8 | 1 | 0.7 | 0.9 | 0.7 | 0.8 | 0.6 | |
| 2-phenethyl acetate | 9438105 | 41391785 | 15649447 | 10770658 | 0.8 | 0.8 | 0.7 | 1.3 | 0.8 | 0.7 | 0.6 | 1.4 | 0.7 | |
| Ethyl nonanoate* | 193713 | 1915238 | 219753 | 203921 | 0.6 | 1.1 | 0.7 | 0.8 | 0.8 | 1.1 | 0.7 | 0.9 | 0.4 | |
| Ethyl decanoate | 8022348 | 10870911 | 23197991 | 6533017 | 0.8 | 1.1 | 0.8 | 1.0 | 0.8 | 1.0 | 0.7 | 0.7 | 0.8 | |
| 3-methylbutyl octanoate | 293069 | 481451 | 599556,5 | 206401 | 0.8 | 0.9 | 0.8 | 1.1 | 0.9 | 0.8 | 0.8 | 1.2 | 1.0 | |
| Ethyl dodecanoate | 289122 | 810778 | 3617628 | 367113 | 0.9 | 0.9 | 0.7 | 0.9 | 0.7 | 0.9 | 0.7 | 1.0 | 0.8 | |
| Ethyl pentadecanoate | 348098 | 2119519 | 19017978 | 658503 | 1.3 | 1.0 | 0.9 | 0.7 | 0.3 | 0.1 | 0.7 | 1.0 | 0.4 | |
| 2-phenylethyl hexanoate | 333774 | 417862 | 1214523 | 309643 | 0.9 | 1.2 | 0.8 | 1.3 | 1.0 | 1.1 | 0.9 | 1.4 | 1.0 | |
| Pentyl propanoate* | 473704 | 2719409 | 583329,5 | 617507 | 1.3 | 0.9 | 1.0 | 1.5 | 0.9 | 0.4 | 0.8 | 1.2 | 0.6 | |
| Ethyl linolate | 199413 | 188476 | 116732 | 232884 | 1.3 | 1.1 | 1.0 | 0.9 | 1.7 | 1.0 | 3.4 | 1.3 | 1.3 | |
| Ethyl pentanoate* | 240793 | 153839 | 75723 | 264897 | 2.4 | 1.4 | 1.7 | 0.8 | 0.7 | 1.1 | 1.0 | 0.6 | 0.5 | |
| 2-Phenylethyl isobutyrate | 140724 | 4996183 | 340278 | 138906 | 1.1 | 0.7 | 0.7 | 1.2 | 1.4 | 0.6 | 0.7 | 1.6 | 1.9 | |
| Pentyl lactate* | 247101 | 1155562 | 289100 | 278830 | 1.2 | 3.7 | 1.0 | 0.9 | 1.0 | 2.1 | 0.9 | 0.7 | 0.9 | |
| Ethyl 3-phenylpropanoate | 65359 | 131959 | 116937 | 141158 | 0.9 | 1.1 | 1.0 | 1.2 | 0.9 | 1.2 | 0.7 | 1.1 | 0.9 | |
| Ethyl 9-decenoate | 104595 | 93070 | 410765 | 76206 | 0.9 | 0.9 | 1.0 | 1.0 | 1.0 | 0.9 | 1.0 | 1.0 | 1.0 | |
| Ethyl hexadecanoate* | 2250799 | ND | 1946861 | 2043126 | 0.5 | ND | 0.8 | 1.1 | 0.9 | ND | 1.6 | 1.2 | 1.5 | |
| Hexyl acetate* | 159607 | ND | 157177 | 206742 | 0.7 | ND | 1.4 | 0.6 | 0.6 | ND | 0.8 | 0.2 | 1.6 | |
| Ethyl pentadecanoate | 38982 | ND | 328835 | 41452 | 0.8 | ND | 1.0 | ND | 1.3 | ND | 1.1 | ND | 0.8 | |
| Decyl formate* | 74017 | ND | 31335 | 19498 | 0.9 | ND | 2.5 | 0.8 | 0.5 | ND | 2.7 | ND | 0.6 | |
| Butyl citrate* | 710548 | 526249 | ND | 48333 | 0.1 | 0.2 | ND | 0.7 | 0.1 | 0.1 | ND | 1.0 | 0.1 | |
| Phenethyl pivalate | 120775 | 102189 | 461076 | ND | 1.0 | 1.3 | 1.0 | ND | 1.2 | 1.2 | 1.2 | ND | 1.5 | |
| Hexadecan-4-yl phenylacetate | 197062 | 166079 | 170546,5 | ND | 0.5 | 0.5 | 0.6 | ND | 0.3 | 0.4 | 0.4 | ND | 0.3 | |
| Ethyl 2-phenylacetate | 73215 | 9595820 | 46948 (3) | ND | 0.8 | 0.8 | ND | ND | 1.0 | 0.8 | ND | ND | 1.4 | |
| Benzenebutyric acid, 2,3-dimethoxy- | 26843 | 23657 | ND | ND | 1.0 | 1.2 | ND | ND | 1.2 | 1.2 | ND | ND | 2.4 | |
| Ethyl butanoate | ND | 3931116 | ND | ND | ND | 2.3 | ND | ND | ND | 3.1 | ND | ND | ND | |
| Pentyl acetate | ND | 309700 | ND | ND | ND | 1.1 | ND | ND | ND | 0.8 | ND | ND | ND | |
| 2-methylpropyl butanoate | ND | 6604642 | ND | ND | ND | 0.8 | ND | ND | ND | 0.6 | ND | ND | ND | |
| Dimethylpropyl butanoate | ND | 6924924 | ND | ND | ND | 0.8 | ND | ND | ND | 0.5 | ND | ND | ND | |
| Hexyl 2-methylpropanoate | ND | 492702 | ND | ND | ND | 0.7 | ND | ND | ND | 0.7 | ND | ND | ND | |
| Isopentyl isovalerate | ND | 9627585 | ND | ND | ND | 1.3 | ND | ND | ND | 1.2 | ND | ND | ND | |
| Citronellyl heptanoate | ND | 131959 | ND | ND | ND | 1.1 | ND | ND | ND | 1.2 | ND | ND | ND | |
| 3-phenylpropyl formate | ND | 196201 | ND | ND | ND | 0.9 | ND | ND | ND | 1.0 | ND | ND | ND | |
| Geranyl acetate | ND | 1406001 | ND | ND | ND | 1.1 | ND | ND | ND | 0.9 | ND | ND | ND | |
| Geranyl isobutyrate | ND | 1781833 | ND | ND | ND | 1.1 | ND | ND | ND | 1.0 | ND | ND | ND | |
| Total peak response | 86459667 | 349066377 | 139672908 | 90669251 | | | | | | | | | | |
| %Total | 13.0 | 52.4 | 21.0 | 13.6 | | | | | | | | | | |

OG – Chibuku Original, BN – Chibuku Banana, JB – Ijuba, LP – Leopard, ND – Not detected. *The variation of this ester over the 7-day period is significantly different between beer samples at 95% confidence interval.

peak responses for fatty acids and for aldehydes did not affect their relative amounts with a few exceptions. For example, only hydroxymethylfurfural and 2-methyl-2-butenal were affected by their initial peak responses. The total ketone peak response was significantly different with the behaviour of 5 of its compounds related to the initial concentrations (Table 3). The behaviour of the specific groups of volatile compounds during the 7-day shelf life period is discussed in the further sub-sections.

3.4.2. Esters

The fruity flavour in any food source is due to esters. During fermentation, enzymatic processes within yeast combine fatty acids and alcohols to form esters (Kobayashi et al., 2008; Vanderhaegen et al., 2007). Fig. 3 and Table 2 show that the peak response for isopentyl acetate (banana flavour) on Day 1 was highest in all the four beer samples with more than 50% total peak response than any other ester. This was followed by isopentyl isobutyrate (apricot flavour), ethyl octanoate (sour apple) and 2-phenethyl acetate (rose honey flavour) while peak responses for ethyl decanoate, ethyl pentadecanoate and ethyl hexanoate were also notably high compared to other esters. A previous study has

also reported that ethyl acetate, ethyl octanoate, ethyl hexanoate and ethyl decanoate were the most abundant in Rwandese traditional beer (Lyumugabe et al., 2013). These esters have also been considered the most important flavour active esters among western beers synthesized from malt (Kobayashi et al., 2008; Horák et al., 2007; Vera et al., 2011; Wei et al., 2001).

Isopentyl acetate with its banana flavour note was expectedly high in the Chibuku Banana beer and its concentrations were between 6.6 and 7.2 times the concentrations in the other beer samples. Isopentyl isobutyrate (apricot flavour) and 2-phenethyl acetate (rose honey flavour) were also up to 15 and 4 times respectively in the Chibuku Banana beer than the concentrations observed in the other beer samples. The sweetness of the Chibuku Banana beer was also enhanced by the fruity flavours due to presence of 10 other esters notably 4 butanoates and 3 terpenyl esters that were unique to it (Fig. 3). At Day 7, it was observed that isopentyl acetate reduced by about 80% while in the other beer samples it had reduced by an average 20%. Generally, an overall 60% reduction in the 7 prominent esters based on peak responses ranging from 10% (isopentyl acetate in Ijuba beer sample) to 90% (2-phenethyl acetate, ethyl pentadecanoate and ethyl hexanoate in Chibuku Banana, Ijuba and Leopard

| Relative to Day 1 | | | | | | | | | | | | | | | |
|-------------------|-----|-----|-------|-----|-----|-----|-------|-----|-----|-----|-------|-----|-----|-----|--|
| Day 4 | | | Day 5 | | | | Day 6 | | | | Day 7 | | | | |
| BN | JB | LP | OG | BN | JB | LP | OG | BN | JB | LP | OG | BN | JB | LP | |
| 0.3 | 1.2 | 1.2 | 1.1 | 0.3 | 1.6 | 0.7 | 0.4 | 0.2 | 0.8 | 0.6 | 0.7 | 0.2 | 0.9 | 0.8 | |
| 0.4 | 0.6 | 0.4 | 0.3 | 0.3 | 0.5 | 0.2 | 0.2 | 0.2 | 0.3 | 0.2 | 0.2 | 0.2 | 0.4 | 0.1 | |
| 1.0 | 0.4 | 0.4 | 0.2 | 0.9 | 0.2 | 0.3 | 0.1 | 0.8 | 0.2 | 0.3 | 0.5 | 0.8 | 0.2 | 0.2 | |
| 32 | 6.3 | 2.7 | 8.9 | 57 | 14 | 4.9 | 9.2 | 52 | 18 | 11 | 14 | 61 | 34 | 24 | |
| 6.8 | 4.1 | 2.2 | 12 | 8.5 | 5.0 | 3.6 | 13 | 9.1 | 6.5 | 7.0 | 15 | 10 | 11 | 14 | |
| 0.9 | 0.4 | 0.7 | 0.6 | 0.8 | 0.5 | 0.6 | 0.4 | 0.5 | 0.3 | 0.5 | 0.3 | 0.5 | 0.3 | 0.4 | |
| 0.5 | 0.5 | 1.2 | 0.6 | 0.3 | 0.5 | 1.0 | 0.4 | 0.2 | 0.3 | 1.0 | 0.3 | 0.1 | 0.3 | 0.7 | |
| 1.1 | 0.4 | 0.4 | 0.4 | 0.9 | 0.4 | 0.4 | 0.3 | 0.9 | 0.2 | 0.4 | 0.4 | 0.7 | 0.3 | 0.4 | |
| 1.0 | 0.6 | 1.0 | 0.7 | 0.8 | 0.5 | 0.8 | 0.5 | 0.6 | 0.4 | 0.7 | 0.5 | 0.6 | 0.4 | 0.6 | |
| 0.9 | 0.6 | 1.2 | 0.8 | 0.7 | 0.7 | 1.0 | 0.6 | 0.6 | 0.5 | 0.8 | 0.6 | 0.6 | 0.5 | 0.7 | |
| 1.1 | 0.6 | 1.0 | 0.7 | 0.9 | 0.5 | 0.9 | 0.6 | 0.7 | 0.4 | 0.6 | 0.5 | 0.8 | 0.4 | 0.7 | |
| 0.4 | 0.6 | 0.6 | 0.6 | 0.1 | 0.1 | 0.4 | 0.4 | 0.2 | 0.2 | 0.4 | 0.4 | 0.2 | 0.1 | 0.4 | |
| 1.0 | 1.0 | 1.6 | 0.8 | 0.8 | 0.8 | 1.2 | 0.6 | 0.7 | 0.5 | 0.9 | 0.5 | 0.8 | 0.5 | 0.7 | |
| 0.3 | 0.5 | 0.6 | 0.5 | 0.3 | 0.5 | 0.3 | 0.3 | 0.2 | 0.2 | 0.4 | 0.3 | 0.1 | 0.4 | 0.3 | |
| 1.0 | 4.0 | 0.8 | 1.1 | 0.5 | 1.6 | 0.5 | 0.4 | 0.3 | 0.7 | 0.8 | 0.4 | 0.2 | 0.7 | 0.6 | |
| 1.0 | 2.0 | 0.6 | 0.4 | 1.4 | 1.5 | 0.5 | 1.0 | 1.4 | 1.6 | 0.4 | 0.7 | 1.3 | 2.3 | 0.8 | |
| 0.4 | 0.8 | 1.7 | 1.6 | 0.3 | 0.8 | 1.6 | 1.4 | 0.2 | 0.7 | 1.8 | 1.3 | 0.2 | 0.8 | 0.7 | |
| 0.0 | 0.9 | 0.7 | 1.1 | 0.0 | 0.9 | 0.6 | 0.7 | ND | 0.6 | 0.6 | 0.7 | ND | 0.6 | 0.5 | |
| 1.7 | 0.8 | 0.9 | 0.8 | 1.1 | 0.7 | 0.6 | 0.8 | 1.0 | 0.6 | 0.7 | 0.7 | 1.0 | 0.7 | 0.6 | |
| 0.8 | 0.9 | 1.2 | 0.9 | 0.8 | 0.8 | 0.8 | 0.8 | 0.6 | 0.5 | 0.7 | 0.6 | 0.6 | 0.4 | 0.7 | |
| ND | 1.4 | 1.7 | 0.6 | ND | 0.8 | 1.0 | 0.8 | ND | 0.7 | 1.5 | 0.5 | ND | 0.5 | 1.2 | |
| ND | 1.0 | 0.3 | 0.4 | ND | 0.7 | 0.2 | 0.5 | ND | 0.3 | 0.1 | 0.2 | ND | 0.6 | 0.1 | |
| ND | 1.0 | ND | 0.8 | ND | 0.9 | ND | 0.7 | ND | 0.9 | ND | 0.6 | ND | 0.8 | ND | |
| ND | 2.5 | ND | 0.5 | ND | 2.5 | ND | 0.4 | ND | 1.7 | ND | 0.4 | ND | 1.9 | ND | |
| 0.2 | ND | 0.6 | 0.1 | 0.2 | ND | 0.8 | 0.1 | 0.1 | ND | 0.5 | ND | ND | ND | 0.5 | |
| 1.7 | 1.1 | ND | 0.5 | 1.7 | 0.4 | ND | 0.3 | 0.4 | 0.2 | ND | 0.3 | 0.4 | 0.1 | ND | |
| 0.4 | 0.3 | ND | 0.3 | 0.3 | 0.3 | ND | 0.3 | 0.3 | 0.2 | ND | 0.2 | 0.3 | 0.2 | ND | |
| 0.7 | 1.1 | ND | 1.4 | 0.6 | 1.0 | ND | 1.2 | 0.5 | 1.0 | ND | 2.5 | 0.5 | 1.2 | ND | |
| 1.4 | ND | ND | 0.8 | 1.0 | ND | ND | 0.5 | 1.1 | ND | ND | 0.6 | 0.8 | ND | ND | |
| 3.3 | ND | ND | ND | 3.1 | ND | ND | ND | 4.3 | ND | ND | ND | 6.8 | ND | ND | |
| 0.5 | ND | ND | ND | 0.4 | ND | ND | ND | 0.4 | ND | ND | ND | 0.4 | ND | ND | |
| 0.4 | ND | ND | ND | 0.2 | ND | ND | ND | 0.2 | ND | ND | ND | 0.2 | ND | ND | |
| 0.4 | ND | ND | ND | 0.2 | ND | ND | ND | 0.2 | ND | ND | ND | 0.3 | ND | ND | |
| 0.6 | ND | ND | ND | 0.9 | ND | ND | ND | 0.5 | ND | ND | ND | 0.5 | ND | ND | |
| 1.1 | ND | ND | ND | 0.9 | ND | ND | ND | 0.3 | ND | ND | ND | 0.4 | ND | ND | |
| 0.6 | ND | ND | ND | 0.4 | ND | ND | ND | 0.3 | ND | ND | ND | 0.1 | ND | ND | |
| 1.0 | ND | ND | ND | 0.9 | ND | ND | ND | 0.8 | ND | ND | ND | 0.8 | ND | ND | |
| 0.8 | ND | ND | ND | 0.6 | ND | ND | ND | 0.5 | ND | ND | ND | 0.4 | ND | ND | |
| 1.0 | ND | ND | ND | 0.8 | ND | ND | ND | 0.6 | ND | ND | ND | 0.5 | ND | ND | |

beers, respectively) was observed at Day 7. The only two succinate esters detected in the beer samples (diethyl- and ethyl isoamyl succinate) were observed to increase with beer maturation. These are butanedioic acid esters (fruity-apple flavour) and at Day 7 they had increased to between 10 and 61-fold the original amounts detected on Day 1. The concentrations of all the other esters were observed to either remain relatively constant or slightly reduce. This has also been observed on a 6-months aging craft beer in Italy (Mascia et al., 2016), a one year aging Belgian lager (Vanderhaegen et al., 2007) and some whiskey samples over a one year aging period (Rodriguez Madrera et al., 2013). In the current study, only ethyl butanoate showed an increase and was 6-fold at Day 7 in the *Chibuku Banana* beer. While esters produced during fermentation are slowly broken by esterase enzymes, the process is relatively slow and its impact on higher esters is minimal. In this regard, it was noted that all pentyl and phenylethyl esters initially increased up to Day 4 but eventually reduced and at Day 7 their levels were lower than at Day 1. The same was observed for acetate and formate esters. For example, the amount of isopentyl acetate increased by 2.3-fold on Day 4 in the *Chibuku Banana* beer but reduced immensely down to 0.2-fold at Day 7. Quite a number of ethyl esters were also less affected and increased up to Day 4

before reducing again including ethyl linolate, ethyl pentanoate, ethyl-phenyl propanoate, ethyl nonanoate, 3-methylbutyl octanoate as well as ethylpenta- and ethylhexa-decanoate (Table 2). A similar behaviour of acetates and pentyl- and phenylethyl esters has been noted in western beers where they either slightly increased or did not show any clear pattern during maturation of Portuguese lager beers (Rendall et al., 2015). Generally, the current study observes that Day 4 was the most relevant day during which the quantities of most esters in the beer samples maximized during storage. On the next day (Day 5), the quantities had decreased and continued to gradually decrease which might be an indication of the beginning of deterioration. Regular drinkers and retailers have confirmed that commercial opaque beer usually starts to lose its flavour at about Day 4.

3.4.3. Alcohols

Phenylethyl alcohol and 3-methyl-1-butanol, were the two most prominent alcohols in the beer samples with high peak responses even though 3-methyl-1-butanol was not detected in the *Chibuku Banana* beer (Table 3 and Fig. 3). The two alcohols have also been detected in high concentrations in other African traditional beer remedies (Lyumugabe

Table 3

Alcohols, fatty acids, aldehydes, ketones and terpenes identified in opaque beer samples and their relative amounts over a seven day shelf life.

| Compound | Peak area only | | | | Relative to Day 1 | | | | | | | | | |
|---|----------------|----------|------------|----------|-------------------|-----|-----|-----|-------|-----|-----|-----|-------|--|
| | Day 1 | | | | Day 2 | | | | Day 3 | | | | Day 4 | |
| | OG | BN | JB | LP | OG | BN | JB | LP | OG | BN | JB | LP | OG | |
| Alcohols | | | | | | | | | | | | | | |
| Phenylethyl alcohol | 29242887 | 10929568 | 39891924 | 21015811 | 0.7 | 0.9 | 0.6 | 0.9 | 0.7 | 1.0 | 0.6 | 0.6 | 0.6 | |
| 3-Methyl-1-butanol | 40957158 | ND | 44261653 | 31016948 | 1.2 | ND | 1.2 | 1.5 | 1.2 | ND | 1.4 | 1.2 | 1.1 | |
| 2-Furanmethanol | 2129404 | 1251732 | 664157 | 992163 | 1.0 | 1.5 | 3.4 | 1.9 | 1.4 | 1.8 | 5.6 | 1.2 | 1.9 | |
| <i>trans</i> -Farnesol | 62967 | 315171 | 33230 | 397786 | 6.5 | 1.3 | 9.8 | 1.1 | 5.4 | 1.5 | 12 | 1.4 | 8.3 | |
| Maltol | 64010 | 23928 | 125053 | 48236 | 0.9 | 3.9 | 0.7 | 1.1 | 1.5 | 3.7 | 1.1 | 2.0 | 4.9 | |
| 3-Buten-2-ol | 809182 | ND | 365627.5 | 470584 | 1.2 | ND | 2.7 | 1.4 | 2.2 | ND | 6.8 | 2.1 | 5.3 | |
| Levomenthol | ND | 47785 | ND | ND | ND | 2.0 | ND | ND | ND | 2.4 | ND | ND | ND | |
| p-Cresol | 75884 | 17995 | 63611 | 43514 | 0.7 | 2.8 | 0.7 | 0.9 | 1.4 | 3.9 | 1.0 | 0.7 | 3.4 | |
| Creosol | ND | 17995 | ND | ND | ND | 2.8 | ND | ND | ND | 3.9 | ND | ND | ND | |
| Eugeno | ND | 4626285 | ND | ND | ND | 0.9 | ND | ND | ND | 0.9 | ND | ND | ND | |
| Isopulegol | ND | 1090830 | ND | ND | ND | 1.1 | ND | ND | ND | 0.9 | ND | ND | ND | |
| Geraniol | ND | 1867600 | ND | ND | ND | 0.9 | ND | ND | ND | 0.9 | ND | ND | ND | |
| Total peak response | 73341492 | 20188889 | 85405255.5 | 53985042 | | | | | | | | | | |
| %Total | 31.5 | 8.7 | 36.7 | 23.2 | | | | | | | | | | |
| Fatty acids | | | | | | | | | | | | | | |
| Octanoic acid | 691132 | 212186 | 13358201 | 524512 | 0.9 | 1.0 | 0.8 | 1.0 | 1.0 | 0.9 | 0.9 | 0.9 | 1.3 | |
| n-Decanoic acid | 602349 | 691233 | 1928969 | 576740 | 1.0 | 1.4 | 1.0 | 1.1 | 1.2 | 1.3 | 1.1 | 1.2 | 1.9 | |
| Dodecanoic acid | 76373 | 88103 | 227717 | 62948 | 1.1 | 1.5 | 1.2 | 1.2 | 1.4 | 1.3 | 1.2 | 1.4 | 1.0 | |
| Nonanoic acid | ND | 117939 | ND | ND | ND | 1.1 | ND | ND | ND | 1.1 | ND | ND | ND | |
| Total peak response | 1369854 | 1109461 | 15514887 | 1164200 | | | | | | | | | | |
| %Total | 7.1 | 5.8 | 81 | 6.1 | | | | | | | | | | |
| Aldehydes | | | | | | | | | | | | | | |
| 2-methyl-2-butenal | 5076154 | 8850335 | 1310998 | 2043126 | 0.5 | 0.6 | 2.4 | 1.1 | 0.8 | 0.4 | 6.1 | 1.2 | 2.8 | |
| Furfural | 5218300 | 6056978 | 3548961 | 2867137 | 0.4 | 0.4 | 0.6 | 0.8 | 0.4 | 0.5 | 1.0 | 0.3 | 1.2 | |
| Heptanal | 725949 | 321785 | 511556 | 727334 | 0.8 | | 0.8 | 0.8 | 0.7 | 0.2 | 0.9 | 0.5 | 0.6 | |
| 2-Heptenal, (Z)- | 3692383 | 2707106 | 3058727 | 3528092 | 0.4 | 0.2 | 0.3 | 0.4 | 0.3 | 0.1 | 0.3 | 0.2 | 0.1 | |
| Decanal | 369430 | 250971 | 516061 | 207613 | 0.3 | 0.4 | 0.2 | 0.3 | 0.2 | 0.3 | 0.1 | 0.3 | 0.2 | |
| 2,4-Decadiena | 514175 | 413978 | 600848 | 436059 | 0.5 | 0.5 | 0.3 | 0.6 | 0.4 | 0.5 | 0.4 | 0.5 | 0.4 | |
| Octanal | 186972 | 400515 | 145249 | 185221 | 0.5 | 0.4 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.2 | |
| 2-Decenal, (E)- | 248303 | 183327 | 240475 | 187534 | 0.3 | 0.3 | 0.3 | 0.4 | 0.3 | 0.3 | 0.3 | 0.3 | 0.2 | |
| Hydroxymethylfurfural | 14171 | 69712 | 25363 | 93112 | 10 | 4.3 | 5.9 | 1.7 | 21 | 2.8 | 18 | 2.5 | 77 | |
| Nonanal | 834160 | ND | 941375 | 728845 | 0.6 | ND | 0.4 | 0.4 | 0.4 | ND | 0.4 | 0.4 | 0.3 | |
| 4-Nonenal | 346583 | ND | 319972 | 342206 | 0.7 | ND | 0.8 | 0.6 | 5.0 | ND | 0.6 | 0.4 | 0.4 | |
| Undecanal | 222686 | ND | 651495 | 27879 | 0.4 | ND | 0.1 | ND | 0.2 | ND | ND | ND | 0.1 | |
| Total peak response | 17449266 | 19254707 | 11871080 | 11374158 | | | | | | | | | | |
| %Total | 29.1 | 32.1 | 19.8 | 19.0 | | | | | | | | | | |
| Ketones | | | | | | | | | | | | | | |
| 2(5H)-Furanone* | 2360898 | 1181294 | 1075098 | 1509645 | 1.2 | 1.8 | 2.4 | 1.4 | 1.4 | 1.3 | 4.0 | 1.0 | 2.9 | |
| 4-Cyclopentene-1,3-dione | 415020 | 164935 | 196697 | 1100572 | 0.7 | 1.3 | 2.7 | 1.4 | 1.0 | 1.1 | 2.7 | 1.5 | 1.6 | |
| 1,2-Cyclopentanedione | 1838453 | 584828 | 675699 | 1100572 | 1.4 | 4.8 | 2.7 | 1.4 | 1.3 | 3.6 | 6.3 | 1.5 | 4.3 | |
| 1-(2-hydroxy-5-methylphenyl)-ethanone | 196411 | 165407 | 117470 | 110991 | 0.6 | 0.8 | 0.8 | 1.0 | 0.7 | 0.6 | 1.1 | 1.0 | 1.1 | |
| Dihydro-5-pentyl-2(3H)-furanone | 724690 | 465820 | 1048993 | 596003 | 0.9 | 0.9 | 0.7 | 1.0 | 0.9 | 0.9 | 0.7 | 1.1 | 1.0 | |
| Furyl hydroxymethyl ketone* | 134399 | 33329 | 57957 | 78080 | 1.2 | 6.1 | 2.5 | 1.6 | 2.2 | 7.2 | 6.7 | 3.4 | 5.9 | |
| 2-(2-butynyl) cyclohexanon | 364108 | 310832 | 401162 | 321160 | 0.6 | 0.7 | 0.5 | 0.7 | 0.4 | 0.6 | 0.5 | 0.5 | 0.5 | |
| 1-(2-furanyl)-ethanone* | 480180 | 374702 | 172993 | 210651 | 1.0 | 0.7 | 2.7 | 1.5 | 1.3 | 1.2 | 4.5 | 1.5 | 2.1 | |
| 4-ethenyl- cyclohexanone | 373255 | 310832 | 401162 | 321160 | 1.0 | 0.7 | 0.5 | 0.7 | 0.7 | 0.6 | 0.5 | 0.5 | 0.5 | |
| 2-Heptanone | 666208 | ND | 519850 | 695986 | 0.9 | ND | 0.8 | 0.8 | 0.6 | ND | 0.7 | 0.5 | 0.4 | |
| 2-hydroxy-3-methyl-2-cyclopenten-1-one* | 98500 | ND | 55515 | 60886 | 1.0 | ND | 1.6 | 1.1 | 1.6 | ND | 3.4 | 1.0 | 3.7 | |
| Ethyl 5-oxoxolane-2-carboxylat | ND | 427166 | ND | ND | ND | 1.1 | ND | ND | ND | 0.7 | ND | ND | ND | |
| Total peak response | 7652122 | 4019145 | 4722596 | 6105706 | | | | | | | | | | |
| %Tota | 34.0 | 17.9 | 21.0 | 27.1 | | | | | | | | | | |
| Furan derivative | | | | | | | | | | | | | | |
| 2-Pentylfura | 1277939 | ND | 1023330 | 1013477 | 0.5 | ND | 0.6 | 0.5 | 0.3 | ND | 0.5 | 0.2 | 0.2 | |
| Terpenes | | | | | | | | | | | | | | |
| α -Myrcen | ND | 856291 | ND | ND | ND | 0.6 | ND | ND | ND | 0.4 | ND | ND | ND | |
| D-Limonen | ND | 6877375 | ND | ND | ND | 1.1 | ND | ND | ND | 0.8 | ND | ND | ND | |
| 3-Caren | ND | 154496 | ND | ND | ND | 1.3 | ND | ND | ND | 1.1 | ND | ND | ND | |
| Caryophyllene | ND | 216312 | ND | ND | ND | 0.8 | ND | ND | ND | 0.9 | ND | ND | ND | |

OG – Chibuku Original, BN – Chibuku Banana, JB – Ijuba, LP – Leopard, ND – Not detected. *volatile compounds with significant difference in peak responses between beer samples over the 7-day shelf life period.

et al., 2013). Phenylethyl alcohol is considered a marker for fermentation parameters and contributes an intense rosellike flavour to beers (Nešpor et al., 2018). On the other hand, 3-methyl-1-butanol is an amyl alcohol and contributes towards that repulsive alcoholic taste in the mouth. This amyl alcohol and other aldehydes discussed in Section 3.4.3 give the *Chibuku Original*, *Ijuba* and *Leopard* beers that vinous mouth sensation

absent from the rather sweetened *Chibuku Banana* beer. A general decrease in the amount of phenylethyl alcohol was observed from Day 1 and between 20 and 50% was found at Day 7. This behaviour has been observed in aging whiskey (Rodriguez Madrera et al., 2013) while some studies in lager beers have reported an increase of phenylethyl alcohol (Rendall et al., 2015). A similar reduction was observed for

| Relative to Day 1 | | | | | | | | | | | | | | |
|-------------------------|-----|-----|-------|-----|-----|-----|-------|-----|-----|-----|-------|-----|-----|-----|
| Day 4 | | | Day 5 | | | | Day 6 | | | | Day 7 | | | |
| BN | JB | LP | OG | BN | JB | LP | OG | BN | JB | LP | OG | BN | JB | LP |
| Alcohols | | | | | | | | | | | | | | |
| 0.8 | 0.4 | 0.8 | 0.6 | 0.4 | 0.5 | 0.9 | 0.4 | 0.3 | 0.3 | 0.6 | 0.4 | 0.2 | 0.3 | 0.5 |
| ND | 1.2 | 1.2 | 1.0 | ND | 1.1 | 1.0 | 0.9 | ND | 1.0 | 1.2 | 0.9 | ND | 1.0 | 1.2 |
| 2.3 | 3.3 | 1.1 | 0.5 | 0.5 | 1.3 | 0.3 | 0.5 | 0.6 | 0.4 | 0.7 | 0.5 | 0.3 | 0.3 | 0.3 |
| 1.7 | 13 | 1.4 | 5.3 | 1.0 | 11 | 0.9 | 3.2 | 0.7 | 5.9 | 0.9 | 4.1 | 0.9 | 5.6 | 0.7 |
| 4.9 | 1.5 | 2.1 | 1.1 | 1.1 | 0.7 | 0.9 | 1.0 | 0.8 | 0.5 | 0.8 | 1.1 | 0.5 | 0.3 | 0.9 |
| ND | 8.6 | 2.9 | 1.8 | ND | 4.1 | 1.4 | 1.7 | ND | 2.5 | 2.5 | 0.7 | ND | 2.3 | 1.4 |
| 5.9 | ND | ND | ND | 5.7 | ND | ND | ND | 3.9 | ND | ND | ND | 4.2 | ND | ND |
| 5.3 | 1.3 | 1.4 | 6.3 | 9.0 | 1.3 | 1.4 | 6.7 | 13 | 1.3 | 1.9 | 10 | 24 | 1.8 | 2.9 |
| 5.3 | ND | ND | ND | 9.0 | ND | ND | ND | 13 | ND | ND | ND | 24 | ND | ND |
| 0.9 | ND | ND | ND | 0.7 | ND | ND | ND | 0.6 | ND | ND | ND | 0.6 | ND | ND |
| 0.9 | ND | ND | ND | 0.8 | ND | ND | ND | 0.7 | ND | ND | ND | 0.7 | ND | ND |
| 0.8 | ND | ND | ND | 0.7 | ND | ND | ND | 0.6 | ND | ND | ND | 0.6 | ND | ND |
| Fatty acids | | | | | | | | | | | | | | |
| 1.3 | 0.9 | 1.0 | 1.0 | 1.8 | 0.8 | 0.8 | 0.9 | 1.7 | 0.6 | 0.8 | 1.4 | 1.4 | 0.5 | 0.7 |
| 1.8 | 1.4 | 1.4 | 1.4 | 1.6 | 1.2 | 1.2 | 1.4 | 1.5 | 1.0 | 1.4 | 1.3 | 1.4 | 0.9 | 1.3 |
| 1.5 | 1.7 | 1.3 | 1.0 | 1.4 | 1.0 | 1.5 | 0.8 | 1.1 | 0.9 | 1.3 | 1.0 | 1.2 | 0.7 | 1.4 |
| 1.3 | ND | ND | ND | 1.2 | ND | ND | ND | 1.0 | ND | ND | ND | 0.8 | ND | ND |
| Aldehydes | | | | | | | | | | | | | | |
| 0.8 | 6.7 | 1.7 | 0.5 | 0.3 | 3.0 | 1.0 | 0.6 | 0.4 | 1.8 | 1.5 | 0.4 | 0.3 | 1.5 | 1.2 |
| 0.2 | 0.5 | 0.4 | 0.2 | 0.2 | 0.4 | 0.2 | 0.2 | 0.5 | 0.4 | 0.7 | 0.3 | 0.3 | 0.3 | 0.4 |
| 0.1 | 0.6 | 0.3 | 0.4 | 0.3 | 0.5 | 0.3 | 0.3 | 0.6 | 0.3 | 0.3 | 0.3 | 0.4 | 0.3 | 0.4 |
| 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | ND | 0.1 | 0.1 | 0.1 | ND | 0.1 | 0.1 |
| 0.3 | 0.1 | 0.4 | 0.2 | 0.2 | 0.1 | 0.3 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 | 0.1 | 0.2 |
| 0.4 | 0.3 | 0.3 | 0.3 | 0.4 | 0.2 | 0.3 | 0.3 | 0.4 | 0.2 | 0.3 | 0.2 | 0.3 | 0.1 | 0.3 |
| 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.3 | 0.3 | 0.3 | 0.1 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 |
| 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.1 | 0.2 | 0.1 | 0.2 | 0.1 | 0.1 |
| 4.4 | 22 | 2.7 | 14 | 3.0 | 12 | 1.0 | 14 | 3.4 | 6.9 | 2.4 | 7.4 | 1.6 | 3.5 | 1.5 |
| ND | 0.2 | 0.3 | 0.3 | ND | 0.1 | 0.2 | 0.1 | ND | 0.2 | 0.2 | 0.2 | ND | 0.1 | 0.1 |
| ND | 0.4 | ND | 0.3 | ND | 0.3 | ND | 0.3 | ND | 0.1 | ND | 0.1 | ND | 0.1 | ND |
| ND | 0.1 | ND | 0.1 | ND | ND | ND | 0.1 | ND | ND | ND | ND | ND | ND | ND |
| Ketones | | | | | | | | | | | | | | |
| 2.3 | 4.1 | 1.2 | 0.8 | 1.1 | 1.7 | 0.5 | 0.5 | 1.0 | 0.7 | 0.9 | 0.3 | 0.5 | 0.5 | 0.5 |
| 1.2 | 2.5 | 1.7 | 0.6 | 0.6 | 1.4 | 0.6 | 0.2 | 0.7 | 0.3 | 1.3 | 0.1 | 0.6 | 0.2 | 0.7 |
| 6.2 | 1.1 | 1.7 | 1.1 | 1.5 | 2.8 | 0.6 | 0.8 | 1.5 | 1.0 | 1.3 | 0.4 | 0.7 | 0.8 | 0.7 |
| 0.7 | 1.2 | 0.7 | 0.6 | 0.6 | 0.6 | 0.5 | 0.5 | 0.5 | 0.5 | 0.6 | 0.3 | 0.4 | 0.4 | 0.4 |
| 1.0 | 0.7 | 1.0 | 1.0 | 1.0 | 0.7 | 1.2 | 0.9 | 1.0 | 0.6 | 0.9 | 0.8 | 1.0 | 0.6 | 0.9 |
| 12 | 5.7 | 3.3 | 2.3 | 4.0 | 4.8 | 1.4 | 2.9 | 3.6 | 2.8 | 2.0 | 0.9 | 1.5 | 1.8 | 1.3 |
| 0.6 | 0.5 | 0.5 | 0.4 | 0.6 | 0.4 | 0.5 | 0.4 | 0.5 | 0.4 | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 |
| 1.4 | 4.4 | 2.1 | 1.7 | 0.8 | 2.6 | 0.8 | 1.3 | 0.8 | 3.2 | 1.7 | 1.3 | 0.8 | 2.7 | 0.9 |
| 0.6 | 0.5 | 0.5 | 0.4 | 0.6 | 0.4 | 0.5 | 0.4 | 0.5 | 0.4 | 0.4 | 0.4 | 0.4 | 0.5 | 0.4 |
| ND | 0.4 | 0.3 | 0.3 | ND | 0.3 | 0.3 | 0.2 | ND | 0.2 | 0.2 | 0.1 | ND | 0.2 | 0.2 |
| ND | 3.8 | 1.6 | 1.3 | ND | 2.4 | 0.8 | 1.0 | ND | 1.0 | 0.9 | 0.5 | ND | 0.7 | 0.7 |
| 0.6 | ND | ND | ND | 0.5 | ND | ND | ND | 0.4 | ND | ND | ND | 0.4 | ND | ND |
| Furan derivative | | | | | | | | | | | | | | |
| ND | 0.3 | 0.1 | 0.1 | ND | 0.2 | 0.1 | 0.1 | ND | 0.1 | 0.1 | 0.1 | ND | 0.1 | 0.1 |
| Terpenes | | | | | | | | | | | | | | |
| – | ND | ND | ND | 0.2 | ND | ND | ND | 0.1 | ND | ND | ND | 0.2 | ND | ND |
| 0.6 | ND | ND | ND | 0.4 | ND | ND | ND | 0.3 | ND | ND | ND | 0.5 | ND | ND |
| 1.0 | ND | ND | ND | 0.8 | ND | ND | ND | 0.3 | ND | ND | ND | 0.6 | ND | ND |
| 0.7 | ND | ND | ND | 2.7 | ND | ND | ND | 0.4 | ND | ND | ND | 0.4 | ND | ND |

2-furanmethanol, however its impact on beer aging is discussed in Section 3.4.6 under furan derivatives. On the other hand, maltol (sweet caramel) and 3-buten-2-ol were observed to increase until Day 4 reaching 4.9 and 8.6-fold respectively.

For phenolic alcohols detected in the beer samples, maltol and *p*-cresol were present in all beer samples while creosol was only detected in

the *Chibuka Banana* beer. The phenolic compounds detected in the South African traditional beer, the Rwandese and the Zimbabwean traditional beers are all different (Lyumugabe et al., 2013; Bvochora et al., 2005). Generally phenolic flavours give a spicy clove or medicinal taste in beer. Only creosol and *p*-cresol increased during beer maturation with the *Chibuku Banana* beer recording up to 24-fold of both phenol derivatives

at Day 7 compared to Day 1. The two phenols give beer that undesirable spicy medicinal taste which might contribute to sensory defect as the beer matures. In addition, the sweet terpene alcohols (terpenoids) were present notably levomenthol, isopulegol, geraniol, *trans*-farnesol and eugenol. These are discussed under terpenes in Section 3.4.5.

3.4.4. Aldehydes and fatty acids

Aldehydes are also responsible for the off flavours during beer aging (Rodrigues et al., 2011). Their presence in beer is attributed to various processes including Strecker degradation reactions (alkyl aldehydes), Maillard reactions (furyl aldehydes) and other enzymatic reactions (linear aldehydes) (Andrés-Iglesias et al., 2016). Fig. 3 shows that most of the aldehydes were present in all beer samples except nonanal, 4-nonenal and undecanal which could not be detected in the *Chibuku Banana* beer samples. However, the most abundant aldehydes in all beer samples on Day 1 were furfural, 2-heptenal and 2-butenal, 2-methyl. Notably, most aldehydes had reduced by more than 50% on Day 2 and were almost undetectable by Day 7. A similar observation has been reported on American beers during a 35-day aging period (Wei et al., 2001). This is in contrast to what has been observed on commercial Belgian lagers where aldehydes increased with aging time (Vanderhaegen et al., 2007). However, a notable observation similar to western beers was that of 2-methyl-2-butenal which somehow increased only in *Ijuba* beer samples reaching 6.7-fold at Day 4 but reducing down to 1.5-fold by Day 7. Hydroxymethylfurfural also increased in all beer samples reaching up to 77-fold in *Chibuku Original* at Day 4 followed by a gradual decrease to concentrations ranging between 2 and 7-fold for all beer samples. Hydroxymethylfurfural is a furan derivative and its behaviour is explained in Section 3.4.6. The decrease in aldehyde content is due to oxidation processes yielding mainly fatty acids. In this regard, fatty acids detected in this study were expected to increase drastically but only a slight increase was observed throughout the shelf life of the beers with a maximum 1.9-fold levels on Day 4 for *n*-decanoic acid in the *Chibuku Original* beer. A similar rising trend for decanoic acid and octanoic acid has been observed during shelf life of some lager beers (Rendall et al., 2015). All the four fatty acids detected in the beer samples exert an undesirable waxy soapy taste (Horák et al., 2008) but are themselves precursors in the formation of fruity esters.

3.4.5. Ketones

Of the 12 ketones identified in the beer samples, it was observed that the furan derivatives, cyclopentyl ketones and diones all increased during beer shelf life peaking at Day 4 eventually decreasing to levels below the quantities initially observed at Day 1. The only exception was furyl hydroxymethyl ketone and 1-(2-furanyl)-ethanone in *Ijuba* beer samples where they were still 1.8 and 2.7-fold their initial amounts. The behaviour of ketones observed in the current study is rather unique to African opaque beer since a study on Portuguese beers observed that their ketones reduced over a six months period (Rendall et al., 2015) while in Belgian lagers, a gradual increase in the targeted ketones was observed over a one year aging (Vanderhaegen et al., 2007). The cyclopentyl ketones and diones induce a caramellic flavour. The potential impact of furyl derivatives is discussed in Section 3.4.7.

3.4.6. Terpenes

Terpenes are responsible for the citrusy taste in food. There were various terpenes consisting of three monoterpenes (α -myrcene – clovy aroma, D-limonene – orange flavour and 3-carene – lemon flavour), a sesquiterpene (caryophyllene – spicy pepper), 5 terpenoids (also listed under alcohols) and three terpenyl esters (citronellyl heptanoate – lemon flavour, gerynyl acetate and geranyl isobutyrate – both sweet rosy) identified in the *Chibuku Banana* beer only (Table 2 and Fig. 3). The three esters are derived from terpenoids, citronellol and geraniol. Citronellol usually exists as a yeast catalysed biotransformation product of geraniol. Geraniol was one of the terpenoids identified in the *Chibuku Banana* beer.

The terpenes with high peak responses on Day 1 were D-limonene and eugenol both contributing 35 and 23% of the total terpene peak responses, respectively. Of the five terpenoids identified in *Chibuku Banana* beer, *trans*-farnesol (sweet floral flavour) was also detected in the other three beers. Only D-limonene, *trans*-farnesol and 3-carene have also been detected in the traditional sorghum beer elsewhere in which some traditional herbs were added (Lyumugabe et al., 2013). Most of the terpene content generally reduced during beer shelf life losing between 40 and 80% in agreement with other studies on western beers (Rodriguez Madrera et al., 2013; Rendall et al., 2015) except for *trans*-farnesol and levomenthol (peppermint flavour). The increase in *trans*-farnesol was also observed in the *Chibuku Original* and *Ijuba* beer samples where it reached 8 and 13-fold respectively at Day 4 before reducing down to 4.1 and 5.6-fold respectively at Day 7.

3.4.7. Furan and furan derivations

Furan and its derivatives are responsible for the golden brown colour and the caramellic breadly flavour in foodstuff. During aging of alcohols, they are produced through Maillard reaction mechanisms involving sugars and amino acids in the absence of enzymes. Some of the most common furan aldehydes, furfural and 5-hydroxymethylfurfural were detected in the beer samples. While furfural (almond flavour) reduced by about 70% in all beers, 5-hydroxymethylfurfural was observed to increase significantly during beer maturation with levels reaching 77-fold in *Chibuku Original* beer at Day 4 before reducing gradually down to between 2 and 7-fold for all beers at Day 7. An increase in 5-hydroxymethylfurfural during beer aging has also been observed in lager beer (Rodrigues et al., 2011). The only furan alcohol detected in the beer samples was 2-furanmethanol. It occurs as a result of biotransformation of furfural and therefore it increased to between 1.2 and 5.6-fold at Day 3 finally dropping to 30% at Day 7. While reduction in furfural is in contrast with what happens in most western beers, an increase in 5-hydroxymethylfurfural and 2-furanmethanol during beer aging has also been observed in lager beers (Rodriguez Madrera et al., 2013; Rendall et al., 2015; Rodrigues et al., 2011).

Furanone slightly increased during the first days reaching between 1.2 and 4.1-fold at Day 4 and eventually reducing to less than 50% of the original amounts. However, its derivative dihydro-5-pentyl-2(3H)-furanone remained almost constant throughout the beer shelf life. Furyl hydroxymethyl ketone also increased reaching between 3.3 and 12-fold on Day 4 and eventually reducing to less than twice the initial amounts at Day 7. A similar trend was observed for 1-(2-furanyl)-ethanone, maximizing 4-fold in *Ijuba* beer samples at Day 4. Another furan derivative (2-pentyl furan) notably present in *Chibuku Original*, *Ijuba* and *Leopard* beers reduced drastically and was almost non-detectable within the first 4 days. Of the furan derivatives detected in this study, only 2-pentyl furan was reported in the Rwandese sorghum beer (Lyumugabe et al., 2013).

4. Conclusions

A variation in beer volatile compounds during deterioration of opaque beer traditional to South Africa has been traced over a 7-day period using an SBSE-TD-GC/HRT technique. The study identified 84 volatile compounds and traced them throughout the beer shelf life as a way of understanding how they may be contributing to deterioration of the opaque beer. The study has observed that while a general decrease in the amount of volatile compounds occurs over the aging period, the amount of some compounds (notably esters) that contribute to important beer flavours maximizes at Day 4 before dropping to levels below their initial concentrations. Day 4 was therefore identified as an important day during opaque beer shelf life. With considerations that the volatile compounds were not quantified in this study and therefore could not be linked to their flavour threshold values, more research is needed for a better understanding of South Africa's traditional opaque beer. The current study has opened future research perspectives around opaque beer traditional to African rural communities.

Credit author statement

Somandla Ncube: Conceptualization, Methodology, Data mining, Results analysis and discussion, Manuscript writing. **Simiso Dube:** Mentorship, Reviewing and editing manuscript. **Mathew Muzi Nindi:** Conceptualization, Mentorship, Results analysis and discussion, Reviewing and editing manuscript

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Adebiyi, J.A., Obadina, A.O., Adebo, O.A., Kayitesi, E., 2018. Fermented and malted millet products in Africa: expedition from traditional/ethnic foods to industrial value-added products. *Crit. Rev. Food Sci. Nutr.* 58, 463–474. <https://doi.org/10.1080/10408398.2016.1188056>.
- Adekoya, I., Obadina, A., Adaku, C.C., De Boevre, M., Okoth, S., De Saeger, S., et al., 2018. Mycobiota and co-occurrence of mycotoxins in South African maize-based opaque beer. *Int. J. Food Microbiol.* 270, 22–30. <https://doi.org/10.1016/j.jfoodmicro.2018.02.001>.
- Alvim, R.P.R., de Cássia Oliveira Gomes, F., Garcia, C.F., de Lourdes Almeida Vieira, M., de Resende Machado, A.M., 2017. Identification of volatile organic compounds extracted by headspace solid-phase microextraction in specialty beers produced in Brazil. *J. Inst. Brew.* 123, 219–225. <https://doi.org/10.1002/jib.416>.
- Andrés-Iglesias, C., Montero, O., Sancho, D., Blanco, C.A., 2015. New trends in beer flavour compound analysis. *J. Sci. Food Agric.* 95, 1571–1576. <https://doi.org/10.1002/jsfa.6905>.
- Andrés-Iglesias, C., Nešpor, J., Karabín, M., Montero, O., Blanco, C.A., Dostálek, P., 2016. Comparison of carbonyl profiles from Czech and Spanish lagers: traditional and modern technology. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 66, 390–397. <https://doi.org/10.1016/j.lwt.2015.10.066>.
- Azzi-Achkouty, S., Estephan, N., Ouaini, N., Rutledge, D.N., 2017. Headspace solid-phase microextraction for wine volatile analysis. *Crit. Rev. Food Sci. Nutr.* 57, 2009. <https://doi.org/10.1080/10408398.2014.957379>, 20.
- Baltussen, E., Sandra, P., David, F., Cramers, C., 1999. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. *J. Microcolumn Sep.* 11, 737–747. [https://doi.org/10.1002/\(SICI\)1520-667X\(1999\)11:10<737::AID-MCS7>3.0.CO;2-4](https://doi.org/10.1002/(SICI)1520-667X(1999)11:10<737::AID-MCS7>3.0.CO;2-4).
- Biernacka, P., Wardencki, W., 2012. Volatile composition of raw spirits of different botanical origin. *J. Inst. Brew.* 118, 393–400. <https://doi.org/10.1002/jib.55>.
- Bvochora, J.M., Danner, H., Miyafuji, H., Braun, R., Zvauya, R., 2005. Variation of sorghum phenolic compounds during the preparation of opaque beer. *Process Biochem.* 40, 1207–1213. <https://doi.org/10.1016/j.procbio.2004.04.005>.
- David, F., Ochiai, N., Sandra, P., 2019. Two decades of stir bar sorptive extraction: a retrospective and future outlook. *TrAC Trends Anal. Chem. (Reference Ed.)* 112, 102–111. <https://doi.org/10.1016/j.trac.2018.12.006>.
- Elpa, D., Durán-Guerrero, E., Castro, R., Natera, R., Barroso, C.G., 2014. Development of a new stir bar sorptive extraction method for the determination of medium-level volatile thiols in wine. *J. Separ. Sci.* 37, 1867–1872. <https://doi.org/10.1002/jssc.201400308>.
- Gonzalez Viejo, C., Fuentes, S., Torrico, D.D., Godbole, A., Dunshea, F.R., 2019. Chemical characterization of aromas in beer and their effect on consumers liking. *Food Chem.* 293, 479–485. <https://doi.org/10.1016/j.foodchem.2019.04.114>.
- Horák, T., Kellner, V., Čulík, J., Jurková, M., Čejka, P., 2007. Determination of some beer flavours by stir bar sorptive extraction and solvent back extraction. *J. Inst. Brew.* 113, 154–158. <https://doi.org/10.1002/j.2050-0416.2007.tb00272.x>.
- Horák, T., Čulík, J., Jurková, M., Čejka, P., Kellner, V., 2008. Determination of free medium-chain fatty acids in beer by stir bar sorptive extraction. *J. Chromatogr. A* 1196–1197, 96–99. <https://doi.org/10.1016/j.chroma.2008.05.014>.
- Johnson, A.J., Hopfer, H., Heymann, H., Ebeler, S.E., 2017. Aroma perception and chemistry of bitters in whiskey matrices: modeling the old-fashioned. *Chemosens. Percept.* 10, 135–148. <https://doi.org/10.1007/s12078-017-9229-3>.
- Kobayashi, M., Shimizu, H., Shioya, S., 2008. Beer volatile compounds and their application to low-malt beer fermentation. *J. Biosci. Bioeng.* 106, 317–323. <https://doi.org/10.1263/jbb.106.317>.
- Lyumugabe, F., Gros, J., Nzungize, J., Bajjana, E., Thonart, P., 2012. Characteristics of African traditional beers brewed with sorghum malt: review. *Biotechnol. Agron. Soc. Environ.* 16, 509–530.
- Lyumugabe, F., Bajjana Songa, E., Wathelet, J.P., Thonart, P., 2013. Volatile compounds of the traditional sorghum beers “ikigage” brewed with Vernonia amygdalina “umubirizi”. *Cerevisia* 37, 89–96. <https://doi.org/10.1016/j.cerevis.2012.12.001>.
- Marsili, R.T., Laskonis, C.R., 2019. Evaluation of sequential-SBSE and TF-SPME extraction techniques prior to GC-TOFMS for the analysis of flavor volatiles in beer. *J. Am. Soc. Brew. Chem.* 77, 113–118. <https://doi.org/10.1080/03610470.2019.1590070>.
- Mascia, I., Fadda, C., Karabín, M., Dostálek, P., Del Caro, A., 2016. Aging of craft durum wheat beer fermented with sourdough yeasts. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 65, 487–494. <https://doi.org/10.1016/j.lwt.2015.08.026>.
- Matumba, L., Monjerezi, M., Khonga, E.B., Lakudzala, D.D., 2011. Aflatoxins in sorghum, sorghum malt and traditional opaque beer in southern Malawi. *Food Contr.* 22, 266–268. <https://doi.org/10.1016/j.foodcont.2010.07.008>.
- Matumba, L., Van Poucke, C., Biswick, T., Monjerezi, M., Mwatseteza, J., De Saeger, S., 2014. A limited survey of mycotoxins in traditional maize based opaque beers in Malawi. *Food Contr.* 36, 253–256. <https://doi.org/10.1016/j.foodcont.2013.08.032>.
- Morales, M.L., Fierro-Risco, J., Callejón, R.M., Paneque, P., 2017. Monitoring volatile compounds production throughout fermentation by Saccharomyces and non-Saccharomyces strains using headspace sorptive extraction. *J. Food Sci. Technol.* 54, 538–557. <https://doi.org/10.1007/s13197-017-2499-6>.
- Moyano, L., Serratos, M.P., Marquez, A., Zea, L., 2019. Optimization and validation of a DHS-TD-GC-MS method to wineomics studies. *Talanta* 192, 301–307. <https://doi.org/10.1016/j.talanta.2018.09.032>.
- Nešpor, J., Karabín, M., Hanko, V., Dostálek, P., 2018. Application of response surface design to optimise the chromatographic analysis of volatile compounds in beer. *J. Inst. Brew.* 124, 244–253. <https://doi.org/10.1002/jib.493>.
- Pérez-Jiménez, M., Pozo-Bayón, M.Á., 2019. Development of an in-mouth headspace sorptive extraction method (HSSE) for oral aroma monitoring and application to wines of different chemical composition. *Food Res. Int.* 121, 97–107. <https://doi.org/10.1016/j.foodres.2019.03.030>.
- Rendall, R., Reis, M.S., Pereira, A.C., Pestana, C., Pereira, V., Marques, J.C., 2015. Chemometric analysis of the volatile fraction evolution of Portuguese beer under shelf storage conditions. *Chemometr. Intell. Lab. Syst.* 142, 131–142. <https://doi.org/10.1016/j.chemolab.2015.01.015>.
- Richter, T.M., Eyres, G.T., Silcock, P., Bremer, P.J., 2017. Comparison of four extraction methods for analysis of volatile hop-derived aroma compounds in beer. *J. Separ. Sci.* 40, 4366–4376. <https://doi.org/10.1002/jssc.201700676>.
- Ríos-Reina, R., Segura-Borrego, M.P., García-González, D.L., Morales, M.L., Callejón, R.M., 2019. A comparative study of the volatile profile of wine vinegars with protected designation of origin by headspace stir bar sorptive extraction. *Food Res. Int.* 123, 298–310. <https://doi.org/10.1016/j.foodres.2019.04.071>.
- Rodrigues, J.A., Barros, A.S., Carvalho, B., Brandão, T., Gil, A.M., Ferreira, A.C.S., 2011. Evaluation of beer deterioration by gas chromatography-mass spectrometry/multivariate analysis: a rapid tool for assessing beer composition. *J. Chromatogr. A* 1218, 990–996. <https://doi.org/10.1016/j.chroma.2010.12.088>.
- Rodríguez Madrera, R., García Hevia, A., Suárez Valles, B., 2013. Comparative study of two aging systems for cider brandy making. Changes in chemical composition. *LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.)* 54, 513–520. <https://doi.org/10.1016/j.lwt.2013.05.037>.
- Rossi, S., Sileoni, V., Perretti, G., Marconi, O., 2014. Characterization of the volatile profiles of beer using headspace solid-phase microextraction and gas chromatography-mass spectrometry. *J. Sci. Food Agric.* 94, 919–928. <https://doi.org/10.1002/jsfa.6336>.
- Ruvalcaba, J.E., Durán-Guerrero, E., Barroso, C.G., Castro, R., 2019. Development of a stir bar sorptive extraction method to study different beer styles volatile profiles. *Food Res. Int.* 126, 108680. <https://doi.org/10.1016/j.foodres.2019.108680>.
- Tang, K., Hu, J., Fan, W., Xu, Y., Li, J.M., 2019. Chemometric analysis of Chinese red wines using stir bar sorptive extraction combined with GC-MS analysis. *Eur. Food Res. Technol.* <https://doi.org/10.1007/s00217-019-03380-3>.
- Ubeda, C., Callejón, R.M., Troncoso, A.M., Peña-Neira, A., Morales, M.L., 2016. Volatile profile characterisation of Chilean sparkling wines produced by traditional and Charmat methods via sequential stir bar sorptive extraction. *Food Chem.* 207, 261–271. <https://doi.org/10.1016/j.foodchem.2016.03.117>.
- Vanderhaegen, B., Delvaux, F., Daenen, L., Verachtert, H., Delvaux, F.R., 2007. Aging characteristics of different beer types. *Food Chem.* 103, 404–412. <https://doi.org/10.1016/j.foodchem.2006.07.062>.
- Vera, L., Aceña, L., Guasch, J., Boqué, R., Mestres, M., Busto, O., 2011. Characterization and classification of the aroma of beer samples by means of an MS e-nose and chemometric tools. *Anal. Bioanal. Chem.* 399, 2073–2081. <https://doi.org/10.1007/s00216-010-4343-y>.
- Wei, A., Mura, K., Shibamoto, T., 2001. Antioxidative activity of volatile chemicals extracted from beer. *J. Agric. Food Chem.* 49, 4097–4101. <https://doi.org/10.1021/jf010325e>.
- Zhang, M., Pan, Q., Yan, G., Duan, C., 2011. Using headspace solid phase micro-extraction for analysis of aromatic compounds during alcoholic fermentation of red wine. *Food Chem.* 125, 743–749. <https://doi.org/10.1016/j.foodchem.2010.09.008>.
- Zhao, Y.P., Zheng, X.P., Song, P., Sun, Z.L., Tian, T.T., 2013. Characterization of volatiles in the six most well-known distilled spirits. *J. Am. Soc. Brew. Chem.* 71, 161–169. <https://doi.org/10.1094/ASBCJ-2013-0625-01>.