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Characterisation of selected volatile organic compounds in Rwandan indigenous beer 'Urwagwa' by dynamic headspace gas chromatography-mass spectrometry

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The volatile organic compound profile of alcoholic beverages consists of a wide range of compounds, comprising of acids, alcohols, esters, aldehydes, and other trace level aroma compounds. These compounds play an important role as they provide relative information on the product quality and enhance product acceptability. In this study, two brands of commercially available traditional banana beer samples were collected from a local market in Kigali, Rwanda; and were analyzed for volatile organic compounds. Dynamic headspace, using gas chromatography-mass spectrometry (DHS/GC-MS), was used to identify volatile compounds at three different temperatures. Fifty volatile compounds, inclusive of 14 acids, 14 alcohols, and 22 esters were identified and quantified in the two brands of indigenous banana beer samples. Only 12 volatile components (three alcohols, three acids and six esters) were common in all banana beer samples. Among all the identified compounds, ethanol and ethyl acetate were the dominant compounds in all beer samples. Ethanol levels were found to be 8.7 and 18.1% (v/v) for brand A and B, respectively using gas chromatography (GC). The two major toxic compounds, methyl alcohol and ethyl carbamate/urethane, were detected in lower concentration levels compared to other identified compounds, thus suggesting negligible risk.

Key words: Indigenous banana beer, volatile compounds, dynamic headspace, gas chromatography-mass spectrometry (GC-MS), 'Urgwagwa'.

INTRODUCTION

The production of indigenous traditional fermented foods and beverages from local agricultural products, such as millet, sorghum, corn (that is, maize), wheat or plantains, are important especially considering the socio-economic status and heritage preservation in developing countries (Gadaga et al., 1999; Lasekan and Lasekan, 2000; WHO, 2004). Indigenous fermented foods and beverages form a great part of the diet in many African countries as they serve as both a food item and a beverage (Jespersen, 2003; Kayodé et al., 2007).

In Rwanda, home-brewed banana beer is used to signify hospitality and to affirm bonds of social cohesion; brewing beer for sale has increased the economic status for many households. In modern times, indigenous beers

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have entered the commercial market, both in rural areas and townships, where many people earn their living through selling these traditional beer products (Lyumugabe et al., 2010; Muyanja et al., 2003; Shackleton, 2003). Indigenous traditional beer, like any other fermented beverage, contains a variety of volatile compounds formed during the fermentation process (Lasekan and Lasekan, 2000), and/or from raw material employed in brewing process. Moreover, some of the volatile compounds are produced as a result of the prevailing microbes, using and producing various compounds, which may serve as carbon source for other microbes.

Volatile organic compounds are considered essential in the brewing industry as they affect the quality of the beer and enhance consumer acceptance (Guillaume et al., 2009; Lui et al., 2005). Volatile organic compounds are normal constituents of many fermented foods and beverages and are classified into esters, alcohols, aldehydes, acids, terpenes, ketones, sulphur compounds, amines, phenol etc. (Cortacero-Ramírez et al., 2003; Lui et al., 2005).

Examination of volatile compounds in beer is of great importance in modern brewing technology as it helps in the proper selection of raw materials, yeast strains and fermentation condition, brewing procedure, quality control of the beer and product development (Cajka et al., 2010; Castiňeira et al., 2002; Silva et al., 2008).

A great variety of organic acids present in indigenous beer possess significant roles, as they affect the organoleptic properties of the product (for example, taste, aroma and colour), stability, nutrition, acceptability and quality (Aka et al., 2008; Castiňeira et al., 2002; Santalad et al., 2007). Moreover, the level of organic acids in beverages such as beer, juice, wine and others, contributes much to tartness, other flavour attributes and longer product shelf-life (Aka et al., 2008; Guillaume et al., 2009). The volatile organic profile of indigenous Rwandan banana beer has not been extensively covered over the past years, although there are several studies on other traditional beers in Africa (Aka et al., 2008; Kayodé et al., 2007; Santalad et al., 2007).

Alcohols form a significant group of volatile compounds in beer and ethanol is amongst alcohols mostly found in beer, at higher levels compared to the others. Their presence contributes to the beer flavour, giving rise to a warming character and plays an important role in the flavour perception of consumers (Campillo et al., 2009; Pinho et al., 2006). To quantify present organic compounds, various rapid and reliable techniques are needed for the quantification of volatile organic com-pounds in beer, wine, and other food products.

Different methods that are widely used for the determination of volatile compounds include: gas chromategraphy (GC) (Cortés et al., 2005; Dragone et al., 2009; Wang et al., 2004), capillary zone electrophoresis (CZE) (Cortacero-Ramírez et al., 2003; Santalad et al., 2007), nuclear magnetic resonance (NMR) (Rodrigues et al., 2010), and high-performance liquid chromatography (HPLC) (Loret et al., 2005) amongst others. However, prior to the actual analysis, various extraction methods are employed to extract volatile components from the beer matrix, such as liquid-liquid extraction (LLE) (Castro et al., 2004) and solid-phase micro extraction (SPME) (Campilloetal.,2009;Horáketal.,2010;Isidorovetal., 2003).

In recent years, dynamic headspace, in conjunction with gas chromatography, coupled to mass spectrometry (DHS-GC/MS), have been employed in analysis of volatile compounds produced by various bacterial strains, through their Lipopolysaccharides (LPS) structures (Venter et al., 2006). This method has been widely employed in analysis of volatile compounds in beers and other food products. Literature shows that this dynamic headspace gas chromatography-mass spectrometry (DHS-GC-MS) has been recorded as an effective and rapid technique in analyzing volatile compounds in cheese, honey and microbiologically contaminated canned tomatoes (Bianchi et al., 2009; Radovic et al., 2001; Thierry et al., 1999). This method, has increased advantages, such as requiring fewer working hours, being highly sensitive and solvent-free (Thierry et al., 1999; Venter et al., 2006).

Other reports further described GC-MS as a simple, effective, sensitive and accurate method of quantifying volatile compounds in beverages and other samples, as it combines the two effective techniques (Nigel et al., 1995; Soria et al. 2008). It was therefore the primary objective of this study to apply DHS-GC-MS in the extraction of volatile components at different appropriate temperatures, to detect a wide range of volatile compounds that are likely to be present in commercially produced indigenous banana beer. The secondary objective was to determine the quantity of ethanol in beer.

MATERIALS AND METHODS

Analysis of volatile components using DHS-GC-MS

Sample preparation and dynamic headspace extraction

Two different brands of commercially produced banana beer were purchased from a retail market in Kigali, Rwanda and samples were transported to the laboratory at low temperatures. Fifty milliliter (50 ml) of the samples were held at different temperatures of 40, 60 and 80°C, respectively at intervals of 15 min. These elevated temperatures drive volatile compounds from the beer sample matrix into gas phase above the sample (headspace), and 1 ml of each headspace was immediately sampled, using a syringe and injected onto GC-MS for analysis. All the analyses were done at least in triplicate for statistical purposes.

Gas chromatography/mass spectrometry

Separation and identification of volatile components in commercially produced banana beer samples were carried out, using a Finnigan Focus GC (Abel- Bonded 60-325/350) gas chromatography, equipped with a capillary column AB-1MS (30 m × 0.25 mm × 0.25 µm), with helium used as gas carrier (constant flow- 3.0 ml min^{-1}) and operated in a split-less mode of injection at 20°C. The GC oven program was held at 50°C for 30 min, ramped at 15°C min^{-1} to 250°C, and held for 10 min at the final temperature. The column was connected to a Finnigan Focus DSQ mass spectrometer for mass detection of fragments with an m/z smaller than 1000. Mass analysis was performed at 70 eV with an ion source temperature of 200°C. Integration of the peaks was performed on the TIC using Xcalibur software (Finnigan) with MS spectral libraries. All analysis was performed in triplicate at different set temperatures.

Ethanol analysis using GC with FID detection

Samples and standard preparation

Ethanol (99.98% purity, Merck, RSA) standard solutions in the concentration of 0, 5, 10 and 20% were prepared by diluting ethanol with distilled water and methanol (99.9% purity, Merck, RSA) and were used as an internal standard in this study. Beer samples were first filtered using Acrodisc® PF Syringe Filter (0.8/0.2 µm Supor Membrane, PALL Life Science). Ethanol standard solutions and beer samples were dispensed into individual GC capped vials and placed in an automated sample injector (Thermo, A1 3000, Italy) where 0.5 µl of each solution was injected into GC for analysis.

GC conditions for ethanol analysis

The analysis of ethanol was conducted in a Finnigan Focus GC (Abel- Bonded 60-325/350) gas chromatograph, equipped with a flame ionization detector and an auto-injector (Thermo, A1 3000, Italy). Chromatography was performed with AB-1MS capillary column (30 m × 0.25 mm × 0.25 µm) with gradient of 100% dimethyl polysiloxane. The GC conditions were: 40°C for 1 min ramped at 50°C min^{-1} to 180 °C held 1 min at final temperature and then remaining at maximum temperature 260°C for 10 min. The flow rates of Helium (H_2) and air were set at 4 and 20 ml min^{-1}, respectively. The temperature of the FID detector and injection port was set at 230 and 65°C, respectively. Helium was used as gas carrier (constant split flow- 16 ml.min^{-1}) split-less mode of injection was selected and the injection volume was 0.5 µl. For GC quantification of ethanol, the peak area and retention time of each compound was used.

RESULTS AND DISCUSSION

Volatile organic acids characterization of alcoholic beers were analysed using GC-MS which is known to provide molecular information with high sensitivity and selectivity (Riu-Aumatell et al., 2004). This study employed the DHS-GC/MS technique for analysis of volatile organic compound in commercially-produced traditional banana beer. The method used (DHS-GC/MS), made it possible to identify fifty volatile compounds in the two brands of commercially produced traditional banana beer samples, identified selected compounds are listed in Tables 1 and 2 for beer brand A and B, respectively. Only twelve volatile compounds were commonly identified in both brands of banana beer samples (three alcohols, three acids and six esters). The most abundant classes (group) of compounds identified were from esters followed by acids, and alcohols as shown in Tables 1 and 2. Tables 1 and 2 lists all identified selected volatile compounds from both beer samples at different temperatures using DHS-GC-MS.

Alcohols constitute an important group of volatile compounds in beer as they contribute to the beer's strong and pungent smell as well as taste. However, only ethanol, 1-propanol and 2-hexanol were found in all banana beer samples investigated, in significantly varying amounts at different temperatures. The concentration of ethanol was noted to be high in all analysed banana beer samples, compared to other identified alcohols (Tables 1 and 2). The ethanol contents determined for beer brand A and B were 8.7 and 18.1% (v/v), respectively.

The high content of ethanol in banana beer samples could be explained by the high quantity of fermentable sugars present in banana juice (Kyamuhangire et al., 2002). These results are in agreement with those previously stated by Mugula et al. (2003) and Namugumya and Muyanja, (2009) who reported relative higher concentration of ethanol in 'togwa' (8.7% v/v) and 'kwete' (13.30% v/v) both Tanzanian and Ugandan traditional beverages. Several other authors have reported higher levels of ethanol in most beers in double-fold magnitude, compared to other alcohol (Cortacero-Ramírez et al., 2003; Wang et al., 2004).

Ethanol content is very important for the mouth-feel and flavour of alcoholic beverages. It greatly influences beer flavour, giving rise to a warming character and playing a role in the flavour perception of other beer components (Pinho et al., 2006). The higher-alcohol groups such as amyl alcohol, isoamyl alcohol, isobutyl also serve as the important precursors of flavor-active esters (Lui et al., 2005; Pinho et al., 2006; Silva et al., 2008) although they were not identified in the current study.

On the other hand, different acids were identified in the two brands of banana beer samples and only acetic acid, 5-hexanoic acid, and benzoic acid (although not volatile), were detected in all beer samples, but in significantly varying amounts at different temperatures. In all beer samples, acetic acid was detected in the same lower amounts (Tables 1 and 2) at different temperatures.

Propanoic acid was only detected in brand A (Table 1) at 40°C with high total signal. Beer is considered to be acidic due to the presence of organic acids predominantly present in beer. This is in agreement with low values of pH detected in both brands A (4.23) and B (3.35) in this study. Organic acids in beer are found in low concentration and considered essential as they affect pH stability and taste of beer such as sourness, tartness, acidity (Guillaume et al., 2009; Montanari et al., 1999).

In beer, organic acids play significant roles, contributing to flavour, colour, and aroma properties, but also the levels of organic acids in foods and beverages are good indicators of fermentation processes (Rodrigues et al., 2010;

Table 1. Selected volatile compounds identified in brand A.

Selected compound	Total signals achieved at different temperatures (°C)			Sensory characteristic	Reference
	40	60	80		
Alcohols					
Methyl Alcohol	[1]ND	ND	1.5 x10^5	Alcoholic, solvent	Cortacero-Ramírez et al. (2003)
Ethanol	2.1x10^8	1.8 x10^7	4.2 x10^4	Alcoholic, strong, fruity	Cortacero-Ramírez et al. (2003) and Annan et al. (2002)
1-propanol	1.8 x10^3	3.4 x10^5	3.7 x10^6	Alcoholic, ripe fruit	Liu et al. (2005)
Propane-1,3-diol	3.6 x10^6	ND	ND	[2]NA	[3]N/A
Cyclohexanol	ND	ND	1321	Camphor like odor	Mahn (1991)
2-Hexanol	ND	6.0 x10^3	ND	Herbaceous, pungent	Mahn (1991)
1,2,3-Butanetriol	9.2 x10^3	ND	ND	NA	N/A
3-Hepten-1-ol	ND	ND	2.7 x10^5	Herbaceous, melon-like	Mahn (1991)
2-undecen-4-ol	1.1 x10^4	ND	ND	NA	N/A
Acids					
Acetic acid	ND	7.3 x10^2	ND	Vinegar, sour	Liu et al. (2005)
Propanoic acid	1.1 x10^4	ND	ND	Acidic, pungent odor	Diéguez et al. (2002)
Methyphosphonic acid	1.5 x10^3	ND	ND	NA	N/A
Benzoic acid	4.6 x10^3	ND	ND	NA	N/A
5-Hexanoic acid	ND	2.6 x10^4	ND	NA	N/A
5-Undecenedioic acid	ND	ND	8.7 x10^2	NA	N/A
1, 3,7-Trimethyluric acid	7.8 x10^3	ND	ND	NA	N/A
Benzeneacetic acid	ND	ND	5.5 x10^3	NA	N/A
Esters					
Ethyl isocyanoacetate	ND	1.3 x10^4	ND	NA	N/A
Ammonium acetate	5.0 x10^3	ND	2.1 x10^4	NA	N/A
Formic acid, ethyl ester	3.1 x10^6	3.2 x10^4	ND	Lemon, rum,strawberry	Wikipedia (2011)
Ethyl acetate	1.7 x10^8	2.1 x10^6	1.5 x10^6	Sweetish, fruity,solvent	Kobayashi et al. (2008)
Propanoic acid, ethyl ester	6.8 x10^4	ND	ND	Sweet, fruity, gum	Annan et al. (2002)
Propanoic acid, 2-methyl-, ethyl ester	9.7 x10^4	4.3 x10^4	ND	Fruity	Dragone et al. (2009)
Butanoic acid, ethyl ester	ND	3.1 x10^4	6.6 x10^4	Papaya, butter, sweetish, apple, fruity	Dragone et al. (2009)
Propanoic acid, 2-hydroxy-, ethyl ester	8.0 x10^4	ND	ND	Strawberry, raspberry	Dragone et al. (2009)
1-Butanol, 3-methyl-, acetate	2.2 x10^5	1.5 x10^5	9.0 x10^5	Fruity, banana aroma	Verstrepen et al. (2003)
Hexanoic acid,ethyl ester	3.9 x10^4	ND	9.9 x10^4	Apple, fruity,sweetish, estery	Dragone et al. (2009)
Octanoic acid, ethyl ester	ND	ND	2.3 x10^5	Ripe fruits, pear, sweety	Liu et al. (2005)
Decanoic acid, ethyl ester	ND	ND	6.9 x10^4	Sweety, fruity, dry	Liu et al. (2005)

Table 1. Contd.

Selected compound	Total signals achieved at different temperatures (°C)			Sensory characteristic	Reference
	40	60	80		
4-Ethylbenzoic acid, heptadecyl ester	ND	ND	2.0 x10^3	NA	N/A
4-Ethylbenzoic acid, octadecyl ester	ND	ND	2.0 x10^3	NA	N/A

[1]ND: Not detected. [2]NA: No odor description available. [3]N/A: No literature. Alternative nomenculeture: formic acid, ethyl ester (ethyl formate), propanoic acid, ethyl ester (ethyl propionate), propanoic acid, 2-methyl-, ethyl ester (ethyl isobutyrate), butanoic acid, ethyl ester (ethyl butyrate), propanoic acid, 2-hydroxy-, ethyl ester (ethyl lactate), 1-butanol, 3-methyl-, acetate (isoamyl acetate), Hexanoic acid, ethyl ester (ethyl hexanoate), octanoic acid, ethyl ester (ethyl octanoate/ethyl caprylate), decanoic acid, ethyl ester (ethyl decanoate/ethyl caprate), 4-ethylbenzoic acid, heptadecyl ester (heptadecyl4-ethylbenzoate), 4-ethylbenzoic acid, octadecyl ester (octadecyl4-ethylbenzoate).

Santalad et al., 2007). However, according to Bvochora and Zvauya (2001) some organic acids, such as acetic acid, propanoic acid, formic acid and butyric acid are undesirable in beer as they impart off-flavour.

Generally, acids constitute an important group of volatile compounds that can contribute with vinegary, cheesy, and fatty odors to the beer's sensory properties as well as bitterness, stringency and rancidity (Pinho et al., 2006) . The short-chain volatile organic acids (C_2-C_6) are, principally the compounds responsible for the rancid odour in beer and spirits under improper storage condition (Diéguez et al., 2002).

In addition to alcohols and acids, seventeen different esters were detected in the two brands of banana beer samples. Only formic acid, ethyl acetate (ethyl formate/ethyl methanoate), ethyl acetate, propanoic acid 2-methyl-ethyl ester (Ethyl isobutyrate), butanoic acid, ethyl ester (ethyl butyrate), 1-Butanol 3-methyl-ethyl ester (Isoamyl acetate) and hexanoic acid ethyl ester (ethyl hexanoate) were identified in all samples, in different amounts. Among all the esters identified, ethyl acetate was the dominating volatile compound in all samples, followed by 1-Butanol 3-methyl-ethyl ester (Isoamyl acetate) and butanoic acid, ethyl ester (ethyl butyrate) (Tables 1 and 2).

Volatile esters are known to be present in small quantities in fermented beverages such as beer and wine where they are important in influencing the flavour. The most important flavour-active esters in beer are ethyl acetate (solvent-like aroma), isoamyl acetate (fruity, banana aroma), ethyl caproate and ethyl caprylate (sour apple) and phenyl ethyl acetate (flowery, roses, honey) (Kobayashi et al., 2008; Verstrepen et al., 2003).

Beer flavour becomes fruity and undesirable when the concentrations of esters are too high. Most of these ester compounds are produced by yeast during fermentation. Therefore, the control and formation of esters is very important to maintain consistent quality of the beer and other beverages (Riverol and Cooney, 2007).

It has been recognized for a very long time that traditional beer can make an important contribution to the diet, especially in developing countries (Jespersen, 2003; Kayodé et al., 2007). Beer analysis is important for the evaluation of organoleptic characteristics, quality, nutritional aspects and safety. The quick and easy method for quantification and determination of volatile organic compounds becomes increasingly important for controlling the quality of beer and other beverages (Pinho et al., 2006).

Dynamic headspace, in conjunction with gas chromatography-mass spectrometry, was successively performed for the quantification of volatile compounds from commercial banana beer samples at different temperatures. Dynamic headspace, a solvent-free extraction method, proved to be suitable for analysis of volatile compounds in traditional banana beer samples by gas chromatography-mass spectrometry. These techniques allow identification of a wider range of volatile components, including esters, acid and alcohols. Those with low boiling points were almost completely extracted at lower temperature. The two major toxic volatile compounds, methyl alcohol and ethyl carbamate/urethane, were detected in traditional banana beer samples. The results indicated that they were detected far below other identified compounds, sugges-ting very small concentrations of these compounds and thus possibly of negligible risk.

Table 2. Volatile organic compounds identified in beer brand B.

Selected compound	Total signals achieved at different temperatures (°C)			Sensory characteristics	Reference
	40	60	80		
Alcohols					
Ethanol	1.8×10^8	[1]ND	7.3×10^8	Alcoholic, fruity	Annan et al. (2002)
1-propanol	3.4×10^5	7.9×10^2	ND	Alcoholic, ripe fruit	Cortacero-Ramírez et al. (2003)
2-Hexanol	1.2×10^4	ND	ND	Herbaceous, pungent	Mahn, 1991
1,2-Benzenediol	1.1×10^4	ND	ND	[2]NA	[3]N/A
Benzene-1,3-diol	ND	ND	1.2×10^5	NA	N/A
Acids					
Acetic acid	7.3×10^2	ND	ND	Vinegar, sour	Liu et al. (2005)
5-Hexanoic acid	8.5×10^3	ND	ND	NA	N/A
Benzoic acid	ND	ND	1.1×10^4	NA	N/A
Ethanedioc acid	1.4×10^4	ND	ND	Odorless	Mahn (1991)
5-ndecenedioc acid	8.7×10^2	ND	ND	NA	N/A
Octadecanoic acid	6.5×10^3	ND	ND	NA	N/A
Esters					
Formic acid, ethyl ester	3.2×10^4	ND	ND	Lemon, rum, strawberry	Wikipedia (2011)
Ethyl acetate	1.7×10^8	2.1×10^6	1.5×10^8	Solvent, sweetish, fruity	Kobayashi et al. (2008)
Carbamic acid, acetyl-ethyl ester	ND	ND	7.9×10^3	NA	N/A
Propanoic acid, 2-methyl-, ethyl ester	4.3×10^4	6.3×10^4	ND	Fruity	Dragone et al. (2009)
Ethyl isocyanoacetate	1.3×10^4	ND	ND	NA	N/A
Butanoic acid, ethyl ester	3.1×10^4	4.0×10^4	2.6×10^5	Papaya, butter, sweetish, apple, fruity	Dragone et al. (2009)
1-Butanol, 3-methy-, acetate	1.5×10^5	ND	ND	Fruity, banana aroma	Verstrepen et al. (2003)
Hexanoic acid, ethyl ester	ND	ND	7.4×10^4	Apple, fruity,sweetish, estery	Dragone et al. (2009)

[1]ND: Not detected, [2]NA: No odor description available, [3]N/A: No literature. Alternative nomenculature: Carbamic acid, ethyl ester (ethyl carbamate/urethane), propanoic acid, 2-methyl-, ethyl ester (ethyl isobutyrate), hexanoic acid, ethyl ester (ethyl hexanoate), formic acid, ethyl ester (ethyl formate/ ethyl methanoate), hexanoic acid, ethyl ester (ethyl hexanoate).

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