Characterisation of the key aroma compounds in alcohol-free beer base by gas chromatography-olfactometry

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Abstract

The pleasant fruity flavour of lager beers is one of the most appreciated features of these beverages, whereas alcohol-free beers (AFB) also exhibit a flavour reminiscent of wort. Even though several studies have been carried out to characterise the key odorants in different alcoholic beers, there are no similar works for AFB. Hence, the aim of this research is to identify the compounds contributing to the characteristic aroma of AFB. In this work, the volatile fraction of an AFB-base (without added flavourings) was isolated using solvent assisted flavour evaporation (SAFE) and analysed by GC-MS and GC-Olfactometry. Twenty-three odour regions showed odour activity in GC-O experiments, amongst which the most potent were methional, phenylacetaldehyde, 2-methoxyphenol, β-damascenone, 2-phenylacetic acid, 2-phenylethanol, and 5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone. The presence of these compounds plays a crucial role in AFB aroma.

Introduction

AFB consumption has increased over the last few years, mainly in response to strict drink driving legislation, medical recommendation or religious grounds, but also due to a growth in health awareness. According to current UK legislation, the description "alcohol-free" may be applied to products containing "an alcoholic strength by volume of not more than 0.05 per cent".

These beers usually exhibit a flavour reminiscent of wort. Recent literature shows that Strecker aldehydes, particularly 2-methylbutanal, 3-methylbutanal and methional, are responsible for the negative attributes associated with AFB flavour [1], and these compounds are also present in barley malt [2]. These aldehydes have exceptionally low odour thresholds (1.25 μg/L, 0.6 μg/L and 0.25 μg/L for 2-methylbutanal, 3-methylbutanal and methional, respectively [3]) and impart potent worty, malty aromas even at very low concentrations.

Although worty aroma of AFB has been related to Strecker aldehydes [1], there is no information in literature about the possible contribution of other odour-active compounds to the overall aroma of AFB. Sensomic methodology has been employed to identify the key odorants in different beers, such as pale lager [4] and wheat beers [5]. In the latter example, the authors found more than 30 odorants contributing to the characteristic aroma of wheat beer, Strecker aldehydes being amongst them. The aim of this study was to identify a more complete set of odour-active volatile compounds present in AFB by means of Sensomic methodology.

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Experimental

Materials

An alcohol-free beer-base (AFB-base), without any external flavour added, was brewed, bottled and pasteurised in Heineken's pilot brewery (Zoeterwoude, The Netherlands) in January 2016 following a standard cold-contact fermentation procedure (brewing conditions not specified). Diethyl ether and saturated alkane standards were purchased from Sigma (Dorset, UK).

Isolation of the volatile fraction

For the isolation of volatiles from the AFB-base, the procedure described by Langos et al. was employed with slight modifications [5]. Briefly, 1 kg of sample was extracted with redistilled diethyl ether (250 mL × 4). The organic phase was dried over anhydrous Na_2SO_4 and filtered before concentration using a Vigreux distillation column (60 cm, 1 cm i.d.) at 40 °C until a final volume of approximately 100 mL was reached. To separate the non-volatile materials from the extract, this was submitted to a high-vacuum distillation process known as solvent assisted flavour evaporation (SAFE) technique (evaporation at 25 °C and 10^{-5} Pa). The distillate was fractioned into an acidic and a basic/neutral fraction using $NaHCO_3$ 0.5 M solution (60 mL × 3). After washing with 30 mL of a saturated NaCl solution three times, the organic layer was kept for further treatment (basic organic extract). In parallel, the basic aqueous phase was acidified to pH 2.25±0.10 by adding HCl solution (10 M or 1 M) and extracted using redistilled diethyl ether (60 mL × 3) and the extracts combined (acidic organic extract). Both basic and acidic organic extracts were concentrated using a Kuderna-Danish concentrator at 45 °C (final volume ~400 µL for each extract). The concentrated aroma extracts were kept at -80 °C until use.

Gas chromatography analyses of concentrated aroma extracts

In order to identify odour-active compounds in the concentrated aroma extracts, these were analysed by GC-Olfactometry (GC-O) using a 5890 Series II gas chromatograph (Hewlett Packard, Waldbronn, Germany) provided with an FID detector held at 250 °C. A sample (2 µL) was injected and two capillaries with different polarities were employed: Rxi®-5 Sil MS capillary (30 m, 0.25 mm i.d., 1.0 µm df) non-polar column and a Stabilwax®-DA (30 m, 0.25 mm i.d., 0.25 µm df) polar column, both from Restek (Bellefonte, Pennsylvania, USA). The temperature gradients were set as follows: 40 °C for 2 min, then a rise of 5 °C/min up to 200°C and 15 °C/min from 200 °C to 300 °C, and then held for 19 min for the non-polar column; 40 °C for 2 min, then rise of 4 °C/min up to 200 °C, then from 200 °C up to 250 °C at 15 °C/min, and then held for 15 min for the polar column. Helium was used as a carrier gas (2 mL/min). The sample was split 1:1 at the end of the column, followed by two untreated silica-fused capillaries of the same dimensions (1 m, 0.32 mm i.d.). An ODO II sniffing port (SGE, Ringwood, Victoria, Australia), where the flow was diluted with a moist make up gas, was utilised. Every sample was analysed by at least 3 assessors in duplicate. The assessors scored the intensity of the aromas perceived on a scale from 1 ("very weak") to 10 ("very strong"). These results were reported as the modified frequency, defined as $MF(\%)=[F(\%)\cdot I(\%)]^{1/2}$, where F(%) is the detection frequency and I(%) is the average intensity expressed as the percentage of the maximum intensity [6].

The concentrated aroma extracts were also analysed by GC-MS using equivalent capillaries and chromatographic conditions as used for the GC-O analyses. The instrument employed for these analyses was a gas chromatograph model 7890A coupled to a 5975C inert XL EI/CI MSD triple axis mass spectroscopy detector and a 7683B Series autosampler (Agilent Technologies, Santa Clara, CA, USA). The carrier gas was helium at a flow rate of 1mL/min. Mass spectra were recorded in the electron-impact mode at an ionisation voltage of 70 eV and source temperature of 200 °C.

Results and discussion

The sensomic approach was applied for the identification of key odorants in alcohol-free beer. Recently, this methodology has been applied to identify key flavour compounds in a wide variety of foodstuff and beverages, such as hazelnuts [7] and rapeseed oil [8]. For this reason, concentrated aroma extracts (basic and acidic fractions) were prepared from AFB-base using the methodology described previously [5].

Table 1: Odour regions and attributed compounds found by GC-Olfactometry (n=3 in duplicate) in acidic and/or basic fractions of a SAFE extract of an alcohol-free beer-base

LRI					
Rxi-5	*StabW*	*Odour quality*[a]	*Odorant*[b]	*Fn*[c]	*%MF*[d]
579	1000	cream, butter	butanedione	b	80
648	950	malty, cocoa	3-methylbutanal	a	65
664	1429	vinegar	acetic acid	a	76
680	950	cocoa	2-methylbutanal	b	60
725	1225	banana, alcoholic	3-methyl-1-butanol	b,a	44
845	1609	cheese	butanoic acid	a	76
886	1646	cheese, rancid	3-methylbutanoic acid	a	83
917	1470	boiled potato	methional	b,a	91
992	1354	cooked rice	2-acetyl-1-pyrroline[e]	b	31
1059	1649	rose, honey	phenylacetaldehyde	b,a	95
1103	1872	smoky	2-methoxyphenol	b,a	92
1109	2188	smoky, spicy	3-hydroxy-4,5-dimethyl-2(5*H*)-furanone	a	56
1125	2074	candy floss	5-ethyl-4-hydroxy-2-methyl-3(2*H*)-furanone	a	48
1127	1930	rose, honey	2-phenylethanol	b,a	86
1130	2223	cloves, woody	2-methoxy-4-vinylphenol	b	67
1154	2223	curry, spicy	5-ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	b,a	89
1180	1983	spicy, smoky	2-methoxy-4-methylphenol	b,a	55
1206	2380	leather	4-vinylphenol	b,a	68
1293	2540	honey, floral	2-phenylacetic acid	a	87
1382	2022	honey, rubber	2'-methoxyacetophenone	b	73
1389	1835	apple, apricot	β-damascenone	b	87
1400	-	hospital, phenolic	unknown.	a	56
1472	2556	vanilla	vanillin	a	73

[a]Odour perceived at the sniffing port of the GC-O.
[b]Compounds were identified by comparison of their mass spectrum and LRI on two columns with those of authentic standards, and confirmed by detection in the extract by GC-MS
[c]Fraction where the compound was found: basic/neutral (b) or acidic (a).
[d]$MF(\%)=[F(\%)\cdot I(\%)]^{1/2}$, where F(%) is the detection frequency and I(%) is the average intensity expressed as the percentage of the maximum intensity
[e]Tentative identification based on odour description and LRI.

These extracts from the AFB-base were sniffed by GC-O on columns of different polarity and mass spectra were obtained from GC-MS analyses. Twenty-three odour regions were found in total in both basic and acidic fractions from the AFB-base. Table 1 shows the most active odour regions found in the SAFE extracts. Amongst them, the highest MF values corresponded to 2-methoxyphenol, β-damascenone, 5-ethyl-3-hydroxy-4-methyl-2(*5H*)-furanone, 2-phenylacetic acid, 2-phenylethanol and the Strecker aldehydes methional and phenylacetaldehyde. The presence of these compounds might explain the honey-like, worty aroma of alcohol-free beers brewed by cold contact fermentation. Moreover, two Strecker aldehydes were found to be important: 2-methylbutanal and 3-methylbutanal. These two, along with methional, have been previously reported as contributors to malty and worty aromas in alcohol-free beers [1, 2].

Similar work has been carried out in other beers, such as wheat beer [5] and pale lager beer [4], where higher alcohols and esters were found to be main contributors to the overall aroma. Examples of these are ethyl hexanoate, ethyl butanoate, 3-methylbutyl acetate, and 3-methyl-1-butanol. In our case, no fruity esters were detected by GC-O. This was associated with the mild conditions for cold contact fermentation process, where yeast was not active enough to synthesise esters throughout the Ehrlich pathway [9]. Butanedione, also found in this study, has been reported as an off-flavour in lager beers [10].

Note, however, that in this study we used an alcohol-free beer "base" which was prepared without the addition of external flavours which provide the desirable fruity note which is not generated during cold contact fermentation. The addition of external flavours to commercial alcohol-free beers is common practice of brewers to improve the flavour of AFB.

We conclude that the information generated from this study will help in the identification of the less desirable worty notes in alcohol-free beers. Further quantitative and sensory analysis will elucidate the actual role of the key odorants in the overall aroma of these beverages.

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