EVALUATION OF AROMA VOLATILES IN NATURALLY FERMENTED KVASS AND KVASS EXTRACT

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Abstract
Kvass is a non-alcoholic beverage produced by fermenting kvass mash with yeast; alcohol content in kvass must be less than 1.2% by volume. Kvass extracts have longer shelf-life and they are essentially free of ethanol. The aim of this research was to evaluate and compare aroma compounds in naturally fermented kvass and kvass extracts. Experiments were carried out at the Latvia University of Agriculture, Department of Food Technology from November 2014 to February 2015. Three commercially available kvass samples (Bruveris, Bauskas and Liepkalni) were used to produce kvass extracts applying vacuum evaporation. The investigation of volatile compounds in kvass and kvass extracts was performed using solid phase microextraction and gas chromatography mass spectrometry. Dry matter content in kvass extracts was 32.4 ± 0.3% (ISO 2173:2003). In all kvass and extract samples in total 25 volatile compounds were detected. Ten of them were esters, five alcohols, five acids, four aldehydes and three ketones. Such aroma compounds as ethyl acetate (fruity flavour), hexyl acetate (fruit, herb) and ethyl decanoate (grape) were found only in Bruveris kvass, 2,3-butanedione (buttery) and phenethyl butyrate (floral) were found only in Bauskas alus kvass and three volatile compounds were identified only in Liepkalni kvass – acetic acid (sour), furfuryl alcohol (burnt) and carvone (caraway). Less than a half of the main aroma volatiles in kvass were also identified in kvass extracts and total values of peak areas were significantly lower in kvass extracts compared to kvass ($p = 0.01$).
Key words: kvass, kvass extracts, aroma volatiles, dry matter, vacuum evaporation.

Introduction

Nowadays most of the commercially available beverages sold as kvass are kvass drinks and malt extract drinks. They are made by diluting grain extract concentrates with water and adding colourings, different flavours (Klosse, 2013) and artificial sweeteners. Kvass drinks and malt extract drinks are sometimes produced without the use of yeast, therefore carbon dioxide is added artificially for no fermentation has taken place. Many consumers choose naturally fermented kvass over kvass drinks. Kvass production is similar to the production of beer but alcoholic fermentation is stopped before ethanol level reaches 1.2 % alcohol by volume. Naturally fermented kvass is made from diluted malt extract which is fermented by adding bread yeast *Saccharomyces cerevisiae.* Naturally fermented bread kvass is made from dried rye bread by soaking it in hot water for a few hours. After separating water-bread extract from the soaked bread, it is fermented by adding bread yeast.

The most important sensory characteristics that describe the products are taste and aroma. Food aroma has been investigated in many countries all over the world as the food smell is undoubtedly the most important parameter influencing consumer acceptance. Estonian researchers (Kaseleht and Leitner, 2008) have started analysing volatile compounds in traditional Estonian food (kama and kvass) but the research for aroma compounds in kvass is still ongoing.

Baker's yeast, used for bread fermentation throughout the world, is very important for the bread quality and different commercial baker's yeasts are each highly selected strains of the species *Saccharomyces cerevisiae*. The fermentative activity of baker's yeast is essential not only for the rising action of the dough by production of CO_2, but also in production of wide range of aroma compounds identified in bread (Birch et al., 2013). *Saccharomyces cerevisiae* is also used for kvass fermentation; therefore kvass aroma is highly dependable on the baker's yeast.

Most of the aroma compounds in bread crumb are derived from the metabolism of yeast and the dominating compounds are alcohols, aldehydes as well as 2,3-butanedione (diacetyl), 3-hydroxy-2-butanone (acetoin) and esters. These aldehydes or their corresponding alcohols are formed inside the yeast cell from degradation of the flour amino acids via the Ehrlich pathway (Hazelwood et al., 2008). The esters are produced in the yeast cell by an enzymatic reaction between acetyltransferases, acetyl coenzyme A and various alcohols (Lilly et al., 2000). The di-ketones 2,3-butanedione and 3-hydroxy-2-butanone are formed from acetohydroxy acids leaked from the yeast cell through non-enzymatic chemical reactions outside the yeast cell (Wainwright, 1973). Furthermore, products from oxidation of flour lipids, such as alcohols, aldehydes and ketones, contribute highly to the aroma profile of bread crumb (Birch et al., 2013), whereas the aroma compounds in the crust originates from Maillard reaction occurring at high temperatures and low water activity between reducing sugars and amino acids (Purlis, 2010). Kvass made from bread rusks is fermented twice – once when making bread and the second time when fermenting kvass mash.

Malt is the main ingredient in other naturally fermented kvass and the roasting process clearly influences its aroma and colour. According to Hoff at al. (2012), compounds from the Maillard reaction are responsible for the colour change during roasting and the volatile composition. Pyrazine compounds, including 2,6-dimethylpyrazine, 2-methylpyrazine and 2-ethyl-3-methylpyrazine, and furans such as furfural, 5-methylfurfural, and 2-furanmethanol are Maillard reaction products formed during the roasting process (Riu-Aumatell et al., 2014), these compounds are mainly found in alcohol-free beers. Methionol was also found and is formed during the Maillard reaction or alcoholic fermentation (Pinho et al., 2006). According to Perpète and Collin (2000), ethanol could induce the retention of some volatile compounds, indicating that in low-alcohol beers the perception of some volatile compounds could be more pronounced.

Kvass extracts have longer shelf-life, they are substantially free of ethanol and can be used by consumers that abstain from alcohol for various reasons; kvass extracts can be diluted with still or carbonated water to produce drinks at desired water to kvass extract proportion (the taste could be mild or stronger).

The aim of this research was to evaluate and compare aroma compounds in naturally fermented kvass and kvass extracts.

Materials and Methods

Experimental design

Experiments were carried out at the Department of Food Technology, Latvia University of Agriculture from November 2014 to February 2015. The object of the research was vacuum evaporated kvass extracts. To produce kvass extracts three commercially available kvass samples were used: 'Liepzeme' Ltd. Liepkalni naturally fermented non-pasteurised, non-filtered bread kvass, 'Bauskas alus' Ltd. naturally fermented kvass and 'Bruveris' Ltd. naturally fermented kvass (Table 1). Liepzeme Ltd. kvass was initially experimentally developed at the Department of Food Technology and then adapted to the industrial production process.

Different ingredients and heat treatment or the lack of it can influence aroma composition in kvass and kvass extracts. All investigated kvass samples are made by naturally fermented kvass mash. The main difference among the samples is that only Liepzeme Ltd. produces kvass from rye bread rusks baked at the factory, therefore using the traditional method for kvass production. The aroma compounds in Liepkalni kvass and kvass extract could be more similar to aroma compounds in bread crust and crumb as natural rye bread is used in kvass production. Bruveris and Bauskas alus use water and malt and/or malt extract to obtain kvass mash. These two producers also filter and pasteurize their products contrary to Liepzeme Ltd. When it is pasteurized the microflora in kvass is eliminated and it can be stored for longer periods of time compared to non-pasteurized kvass.

Kvass extract production

Carbon dioxide content was reduced in all kvass samples using Magnetic stirrer MSH 300 for 15 min to reduce foaming during vacuum evaporation. The initial dry matter content was determined in kvass samples with digital refractometer DR301-95 (ISO 2173:2003), as well as in kvass extracts. Kvass extracts were produced using rotary vacuum evaporator Heidolph Laborata 4000 Efficient in two stages; kvass samples were 200 ml. The parameters of extraction method were 50 °C temperature of all extraction process, 30 rpm for the first 30 min, from 50 to 60 rpm for over an hour until fixed dry matter content. Dry matter content was continuously measured during the second evaporating process every 10 minutes.

Detection of volatile (aroma) compounds in kvass and kvass extracts

Volatile compounds were determined in kvass and kvass extract samples using solid phase

Table 1

Comparison of kvass used in the research

Kvass	Producer	Ingredients	Shelf-life
Classic naturally fermented kvass	Bruveris Ltd.	Water, malt, sugar, rye malt extract, acidifier: lactic acid, yeast	6 months
Naturally fermented kvass	Bauskas alus Ltd.	Water, sugar syrup, barley and rye malt extract, carbon dioxide, acidifiers: lactic acid and citric acid, yeast	4 months
Liepkalni bread kvass (non-pasteurised and non-filtered)	Liepzeme Ltd.	Water, rye bread rusks 10% (rye flour, wheat flour, sugar, rye malt, salt, yeast, barley malt extract caraway), sugar, barley malt, wheat malt, acidifier: citric acid, yeast	2 weeks

micro extraction (SPME) in combination with gas chromatography/mass spectrometry (GC/MS) according to the methods of Sabovics et al., 2010; 2013. The SPME fibre was coated with a thin bipolar polymer film – Carboxen/Polydimethylsiloxane (CAR/PDMS). The film thickness was 85 μm with polarity (Supelco, Inc., USA).

A sample of 5.00±0.05 g was weighed into 20 ml glass vial which was covered with a rubber gasket and sealed with a cork. The vial with the sample was heated for 30 min at 40 °C to excrete volatile compounds above the liquid phase. After 30 min CAR/PDMS fibre was inserted into the vial through the rubber gasket and held above the sample for 30 min at 40 °C temperature. During this time volatile compounds were absorbed onto the fibre.

Volatile compounds from the fibre were thermally desorbed in GC/MS injector. Separation of volatiles was carried out in the Elite-Wax (PerkinElmer, Inc., USA) capillary column (60 m × 0.25 mm i.d., polyethylene glycol coating thickness 0.25 μm).

GC–MS analysis was performed with the following parameters: the initial temperature was 40 ºC, held for 7 min, then ramped from 40 °C to 160 °C at a rate of 6 °C min^{-1} and from 160 ºC to 210 °C at a rate of 10 °C min^{-1} then held for 15 min at 210 °C. The total run time was 47 min for a sample. Mass spectrometer in Electron impact Ionization mode was set to 70 eV as the electron energies, while the ion source temperature was set to 250 °C and the inlet line temperature was set to 250 °C. Injections were performed in split mode (2:1) and helium (He) was used as the carrier gas at a constant flow of 1 ml min^{-1}; acquisition parameters in full scan mode – m/z 40-300. Compounds were identified by comparison of their mass spectra with mass spectral library Nist98 and the amount of compounds was measured as peak area units (PAU).

Data analysis

The obtained data processing was performed with the Microsoft Excel 13 for Windows; mean values and standard deviations were calculated. ANOVA and Tukey's test were used for data cross-comparison. For the interpretation of the results it is assumed that α=0.05 with 95% confidence. The Principal component analysis (PCA) was done using Multibase2015 statistics program.

Results and Discussion

Dry matter in kvass and kvass extracts

The initial dry matter content was different in all kvass samples: 8.6% (Liepkalni), 7.9% (Bauskas alus) and 12.1% (Bruveris), it could be explained with

Table 2

Volatile compounds (PAUx10^7) in Bruveris kvass and kvass extract

Volatile compounds	Odour (Gas chromatography ..., 2004; Odor Descriptors, 2015)	Kvass	Kvass extract
Ethylacetate	fruity	0.40±0.00	-
3-methylbutanal	fruity, almond-like, toasted, malty, green, herbaceous	-	0.08±0.00
4-penten-2-ol	fruity	**6.81±0.38**	**3.89±0.07**
isoamyl acetate	banana	**1.37±0.06**	-
ethyl hexanoate	apple peel, fruit	0.54±0.08	-
3-methyl-1-butanol	whiskey, malt, burnt	0.63±0.04	-
hexyl acetate	fruit, herb	0.20±0.00	-
3-hydroxy-2-butanone	butter, cream	-	0.07±0.00
5-methyl-1-hexanol	-	0.09±0.01	-
ethyl octanoate	fruit, fat	0.79±0.04	-
acetic acid	sour	-	0.28±0.04
furfural	bread, almond, sweet	0.27±0.01	-
ethyl decanoate	grape	0.07±0.01	-
furfuryl alcohol	burnt	-	0.12±0.01
2-phenylethyl acetate	rose, honey, tobacco	0.69±0.07	0.02±0.01
hexanoic acid	fatty type	0.12±0.01	0.10±0.00
phenylethylalcohol	rose	0.25±0.01	0.29±0.00
octanoic acid	sweat, cheese	**1.55±0.10**	0.23±0.02
decanoic acid	rancid, fat	0.36±0.08	0.06±0.01
	The sum of peak area	**14.15±0.89**	**5.14±0.17**

Table 3

Volatile compounds (PAUx10^7) in Bauskas kvass and kvass extract

Volatile compounds	Odour (Gas chromatography ..., 2004; Odor Descriptors, 2015)	Kvass	Kvass extract
3-methylbutanal	fruity, almond-like, toasted, malty, green, herbaceous	-	0.07±0.00
4-penten-2-ol	fruity	**2.03±0.00**	**1.32±0.13**
2,3-butanedione	buttery type	0.64±0.05	-
isoamyl acetate	banana	0.33±0.05	-
3-methyl-1-butanol	whiskey, malt, burnt	**1.27±0.02**	0.14±0.03
furfural	bread, almond, sweet	0.12±0.01	-
benzaldehyde	almond, burnt sugar	-	0.25±0.01
furfuryl alcohol	burnt	-	0.07±0.00
2-phenylethyl acetate	rose, honey, tobacco	0.09±0.00	-
phenylethylalcohol	floral type	0.37±0.03	**0.93±0.06**
octanoic acid	sweat, cheese	0.24±0.03	0.14±0.00
phenethyl butyrate	floral type	0.15±0.01	-
decanoic acid	rancid, fat	0.31±0.01	0.26±0.00
palmitic acid	waxy type	-	0.09±0.01
	The sum of peak area	**5.54±0.21**	**3.28±0.24**

a higher water to malt ratio in Bruveris Ltd. kvass. After vacuum evaporation, the dry matter content in all kvass extract samples was 32.4 ± 0.3%.

Aroma volatiles in kvass and kvass extracts

Headspace-solid phase micro extraction was used to characterize the volatile compounds present in three types of kvass and kvass extracts. Twenty five volatile compounds were isolated and characterized by GC–MS analysis. The identified volatile compounds belong to esters, alcohols, acids, aldehydes and ketones.

There were 19 volatile compounds identified in Bruveris kvass and the total sum of peak areas of Bruveris kvass was 14.15×10^7 PAU. The amount of compounds in kvass extract was about 3 times lower (5.14×10^7), a total of 10 volatiles were found in the kvass extract. The highest value of peak area (6.81×10^7) among all detected volatile compounds was detected for 4-penten-2-ol (alcohol) in Bruveris kvass, which gives fruity aroma, but in Bruveris kvass extract it was about 50% lower (Table 2). Fatty acid – octanoic acid (1.55×10^7) and ester – isoamyl acetate (1.37×10^7) had the second and third highest peak area values, thus giving odours of sweat and cheese, and banana, respectively.

A more various volatile compounds profile and higher total sum of peak area Bruveris kvass compared to other two kvass samples could possibly be explained by significantly higher dry matter content ($p = 0.011$) in Bruveris kvass, however, the dry matter can also consist of compounds that do not affect the aroma or compounds from which aroma volatiles are not synthesized, and non-volatile flavour-active compounds (lipids, sugars, etc.) which can affect the final flavour perception (Lozano, 2011).

The total sum of peak area in Bauskas kvass extract (3.28×10^7) is about 1.6 times lower than in kvass (5.54×10^7). In total there were 10 volatiles identified in Bauskas kvass and 9 volatiles in Bauskas kvass extract. Analysing the volatile compounds in kvass of Bauskas alus Ltd. alcohol 4-penten-2-ol has the highest peak area value (2.03×10^7) as well, but it was lower comparing to Bruveris kvass (Table 3). Significant amount of 3-methyl-1-butanol (1.27×10^7) can be found in Bauskas alus kvass and smaller amounts in kvass extract, giving to both samples whiskey and malt burnt odour. But in kvass extract phenylethylalcohol has the second highest peak area value (0.93×10^7) after 4-penten-2-ol, forming floral type odour in Bauskas kvass extract.

17 volatile compounds were identified in Liepkalni kvass and 13 in Liepkalni kvass extract. Alcohol 4-penten-2-ol had the highest peak area value (3.64×10^7) in Liepkalni kvass (Table 4) and it was lower comparing to Bruveris kvass (Table 2) and higher comparing to Bauskas kvass (Table 3). Peak area of carvone is the second highest (2.03×10^7) in Liepkalni kvass, but it was not detected in Liepkalni kvass extract. Rye bread which is used in Liepkalni kvass production contains caraway; this explains carvone in Liepkalni kvass aroma profile as carvone and limonene form the main portion of essential oils in caraway fruits (Sedláková et al., 2003).

Volatile compounds found in all three kvass sample forms base aroma profile which includes fruity (4-penten-2-ol), banana (isoamyl acetate), whiskey,

Table 4

Volatile compounds (PAUx10^7) in Liepkalni kvass and kvass extract

Volatile compounds	Odour (Gas chromatography ..., 2004; Odor Descriptors, 2015)	Kvass	Kvass extract
4-penten-2-ol	fruity	**3.64±0.11**	**1.40±0.02**
isoamyl acetate	banana	0.22±0.04	-
ethyl hexanoate	apple peel, fruit	0.13±0.01	-
3-methyl-1-butanol	whiskey, malt, burnt	0.63±0.02	0.39±0.00
3-hydroxy-2-butanone	butter, cream	-	0.01±0.00
nonanal	aldehydic type	-	0.03±0.01
ethyl octanoate	fruit, fat	0.39±0.05	0.02±0.00
acetic acid	sour	0.37±0.06	0.48±0.03
furfural	bread, almond, sweet	-	0.10±0.01
furfuryl alcohol	burnt	0.20±0.03	0.10±0.01
carvone	caraway	**2.03±0.06**	-
2-phenylethyl acetate	rose, honey, tobacco	0.10±0.02	-
hexanoic acid	fatty type	0.09±0.01	0.07±0.00
phenylethylalcohol	floral type	0.16±0.01	0.69±0.02
octanoic acid	sweat, cheese	0.72±0.04	0.46±0.08
decanoic acid	rancid, fat	0.25±0.00	0.19±0.00
palmitic acid	waxy type	-	0.11±0.03
	The sum of peak area	**8.94±0.46**	**4.05±0.20**

malt, burnt (3-methyl-1-butanol), rose, honey, tobacco (2-phenylethyl acetate), floral (phenylethylalcohol), sweat, cheese (octanoic acid) rancid and fat (decanoic acid) aroma.

Alcohols can be formed in the metabolism of yeast when long-chain and complex alcohols are produced, but aldehydes and ketones can be formed from alcohols (de Smidt et al., 2008). Acetic acid is formed in yeast fermentation process from yeast and gives acidic and vinegar flavour. Secondary metabolism of yeast can form 3-methyl-1-butanol, which gives malty flavour to product. According to Schieberle (1996), the amount of flavour compounds can be affected by yeast amount and activity, fermentation time and fermentation temperature.

The identified isoalcohols, 3-methylbutanal and phenylacetaldehyde are typically fermentation compounds likely formed via the Erhlich pathway in

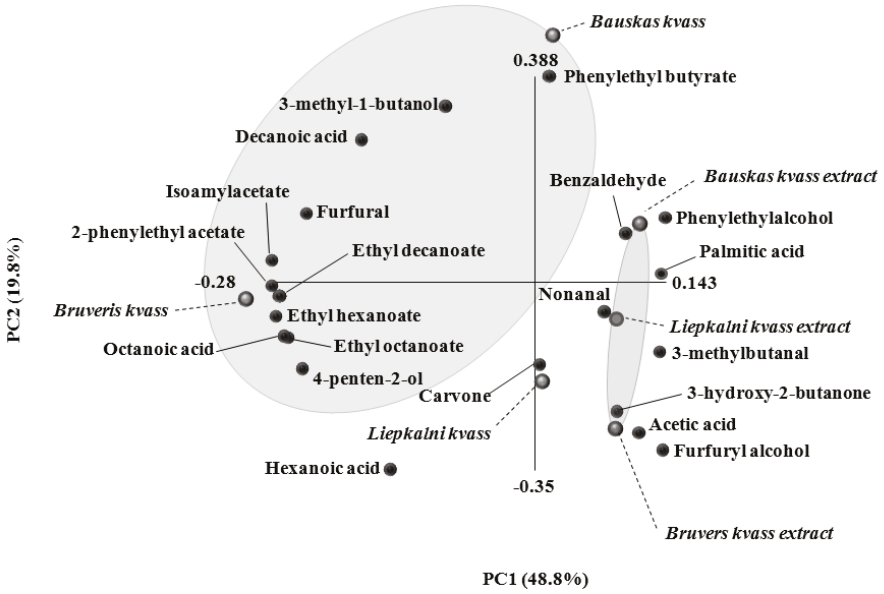

Figure 1. Distribution of volatile compounds in the kvass and kvass extract samples.

the yeast cell (Hazelwood et al., 2008). The identified 2,3-butanedione and 3-hydroxy-2-butanone were most likely formed by oxidative decarboxylation and decarboxylation, respectively of acetohydroxy acids outside the yeast cell. The yeast cell has been found to be responsible for the synthesis and excretion of these acetohydroxy acids (Wainwright, 1973).

The principal component analysis of volatile compounds in kvass and kvass extract samples is shown in Figure 1.

The samples of kvass and kvass extract in the score plot show how the samples relate to each other. Samples close to each other have similar volatile compounds profile, whereas samples far from each other have dissimilar volatile compounds profile. The volatile compounds loading plot shows which volatile compounds are influential from the model and how the volatile compounds are correlated to each other. Forty nine percent of contribution (component 1) means that 51% of original information is lost and component 1 represents about half of the original data. The contribution of the second component is 21% and accumulated contribution of component 1 and component 2 goes up 69%, which means that the scatter plot between PC1 and PC2 covers 69% of original data (Figure 1).

In principal component analysis it can be seen that the volatile compounds profile and value of peak areas are dissimilar in each sample of kvass, where the highest amount of volatile compounds is in Bruveris kvass. In kvass extract samples aroma volatiles profile changes and the value of peak areas decreases comparing to kvass which can be explained by evaporation of water and volatiles during extraction process; evaporation can concentrate new volatile compounds which cannot be detected in kvass samples because of their small amount, and it can also promote the synthesis of new aroma volatiles. Evaporation process has a significant influence ($p=0.01$) on the volatile compounds profile and value of peak areas.

Such aroma compounds as ethylacetate (fruity flavour), hexyl acetate (fruit, herb) and ethyl decanoate (grape) were found only in Bruveris kvass, 2,3-butanedione (buttery) and phenethyl butyrate (floral) were found only in Bauskas alus kvass and three volatile compounds were identified only in Liepkalni kvass – acetic acid (sour), furfuryl alcohol (burnt) and carvone (caraway). 2-phenylethyl acetate can be found in all kvass samples, but from extracts it was found only in Bruveris kvass extract. Benzaldehyde which gives almond and burnt sugar flavour was identified only in Bauskas kvass extract. Liepkalni kvass extract had 3 volatile compounds which were not identified in other extracts – nonanal (aldehydic odour), ethyl octanoate (fruit, fat) and furfural (bread, almond and sweet). Furfural and its derivatives are Maillard reaction products formed during the roasting process of geminated grains and bread baking.

Conclusions

1. Twenty five volatile compounds were identified in all analysed kvass samples and extracts: eight esters, five alcohols, five acids, four aldehydes and three ketones.
2. The highest peak area value of alcohol 4-penten-2-ol, which gives fruity odour was detected in Bruveris kvass, but the lowest in Bauskas kvass; it means that the 4-penten-2-ol is the main volatile compound of aroma.
3. Evaporation process has a significant influence ($p=0.01$) on the volatile compounds profile and value of peak areas: less than a half of the main aroma volatiles in kvass were also identified in kvass extracts and the total values of peak areas were significantly lower in kvass extracts compared to kvass.
4. Liepkalni kvass extract had three volatile compounds which were not identified in other extracts – nonanal, ethyl octanoate and furfural which add aldehydic, fruit, fat, bread, almond and sweet odour to the kvass extract.

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