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Odour-Active Compounds in Homemade Kvass

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Abstract

Kvass is a refreshing drink of Russian origin existing since the 10th century. Its traditional way of production involves rye bread leftovers which are left to ferment together with water and sugar. Odor-active compounds in homemade kvass were determined by gas chromatography equipped with an olfactometer using the modified frequency (MF) technique. Odor-active compounds with the highest MF value were found to be phenyl ethanol (honey-yeast odor), octanoic acid (rancid-goat odor), 2-acetyl furan (roasted bread odor), β-damascenone (apple odor), isoamyl alcohol (whiskey, fruity, ripened cheese odor), phenylacetaldehyde (honey-rose odor), methional (cooked potato odor), 4-vinyl guaiacol (clove-like odor), 1-often-3-one (mushroom odor), S-carvone (herbal odor), solution (caramel odor), (R)-(+)-γ-octalactone (coconut-almond odor), propenyl propyl disulfide (sulfurous bug-like odor), γ-nonalactone (coconut odor) and acetyloxy-dimethylfuranone (caramel odor). The formation of aromas in kvass fermentation could be explained by the Ehrlich pathway, degradation of amino acids, ester and aldehyde formation, norisoprenoid, organic acid and lactone degradation, yeast cell lysis and Maillard reactions taking place in the bread and/or kvass wort production. The results of this study may be a valuable source of inspiration for the development of new products resembling this traditional fermented beverage.

Keywords: *Homemade Kvass; Traditional Beverage; Odour-Active Compounds; Modified Frequency; Olfactometry*

Abbreviations

FID: Flame Ionization Detector; GC: Gas Chromatography; GC/MS: Gas Chromatography Mass-Spectrometry; GC-O: Gas Chromatography-Olfactometry; HS: Headspace; LRI: linear Retention Index; MF: Modified Frequency Value; OAV: Odor Activity Value; RI: Retention Index; SAFE: Solvent Assisted Flavour Evaporator; SPME: Solid Phase Micro Extraction

Introduction

Kvass is a refreshing drink of Russian origin which already exists since 10th century. Kvass is a sweet, low-alcoholic fermented rye beverage, produced from either bread, malt and/or flour [1-4]. The industrial kvass technology developed in former Soviet Union includes simultaneous acid and alcoholic fermentation of rye bread, malt or flour with addition of sugar. After consumption of sugars by yeast cells, the process is cooled and the yeast is settled down. Then invert sugar or honey was added to give the product sweetness. The final product contains living lactic acid bacteria and yeast cells and had a very short shelf life of 1 - 2 days. Kvass was transferred into barrels, from which it was directly sold on the streets. Later on this was banned, mainly due to health regulations [5,6].

Throughout hundreds of years both marketing and production methods of kvass have changed. Since World War II the beverage became very popular again in Russia as well as the Baltic states. Many investors have entered the market and knowledge of the historical

drink was used as a base for developing new kvass-like products. Since the very short shelf life of the traditional drink, several commercial formulas have come to replace this popular drink. Three different formulas of kvass can be identified, of which the first one is a bottled kvass-like non-fermented soft drink, prepared by mixing sugars, flavorings and carbonated water. The second formula is aimed at home preparation. It includes a sachet with concentrated malt extract (in either syrup or powdered form) and dried yeast. Malt extract and sucrose should be dissolved in water, after which dried yeast is added and left to ferment aerobically for 24 hours at room temperature. The mixture should be cooled down before consumption. The third formula is ready-to-drink natural kvass, which is industrially produced made from kvass wort concentrate, hot water, sugar syrup, baker's yeast and lactic acid bacteria. First kvass wort concentrate is diluted with hot water and mixed with sugar syrup. Then preliminary treated baker's yeast and lactic acid bacteria are introduced, followed by fermentation and blending of kvass wort, filtration, pasteurization and bottling. This soft drink is not containing living microorganisms and sealed bottles shelf-life is three months. After opening it must be consumed in no more than 7 days [1].

Since people look for the real kvass flavor, different manufacturers are trying to make kvass as similar to the authentic product as possible. There are plenty of existing recipes using the addition of different flavoring ingredients such as roots, herbs and spices [4,7,8]. In the past, homemade kvass was often spontaneously fermented, while nowadays baker's yeast (*Saccharomyces cerevisiae*) is used. In this study the odor-active compounds in kvass were analyzed, based on a standardized version of the traditional recipe using commercially available baker's yeast and rye sourdough bread. These ingredients were used to mimic the traditional recipe as close as possible.

Experimental Study

Sample preparation

Kvass samples were produced following the guidelines for a homemade recipe. Black rye bread (Fazer Must Vormileib, Estonia) was used, which consisted of rye flour, water, wheat flour, sugar, rye malt flour, barley malt extract, yeast and salt. It was cut into cubes of 2 - 3 cm, roasted in an oven set at 160°C for 25 minutes. Boiling water was added in ratio 1 : 10 (unroasted bread : water). First, two thirds of the water were added to the toasted bread and left for two hours in a covered vessel to cool down slowly. Bread was removed from the wort by filtration with a sieve of 250 µm pore size, followed by filtration through a sieve of 500 µm pore size. The other one third of boiling water was added to the bread residue and followed by extraction and filtration as previously described. Sugar content was measured with a pocket refractometer (Pal-1, ATAGO, Tokyo, Japan) and adjusted to 10° Brix by addition of sucrose syrup to the two fractions of wort. Sterile fermentation bottles were filled ¾ to allow some headspace for oxygen supply and were closed with a sterilized water lock. Inoculation rate was 0.33 g/L dried commercial baker's yeast from the brand Veski Mati (*Saccharomyces cerevisiae*, Balti Veski AS, Estonia) and incubation conditions were 48 ± 0.5 hours at 30°C. Before analysis, samples were stored at -20°C to prevent flavor loss or changes.

Identification of odour-active compounds

Volatile compounds were determined by Gas Chromatography-Mass Spectrometry (GC/MS). Triplicate samples of 2 ml were put into 20 ml headspace (HS) vials that contained a 1-cm glass covered magnetic stir bar. For sample preparation and injection, a CTC CombiPAL auto-sampler (CTC Analytics, Zwingen, Switzerland) with Solid Phase Micro Extraction (SPME) option was used. The incubation time was 5 min at 60°C, after which a 2-cm SPME fiber (50/30-µm DVB/Car/PDMS Stableflex, Supelco, Bellefonte, PA) was injected into the vial for 20 minutes at 60°C. Samples were mixed in a single magnetic mixer (SMM, Chromtech) at 250 rpm. Volatiles were desorbed in a GC/MS-TOF (Waters Inc.) in EI+ mode on a DB-5 column with a 1 µm thickness, 30 m length and 250 µm diameter as well as in a GC/qMS 5975 (Agilent Technologies, USA) on a ZB-WAX plus column (30 m x 0.25 mm x 0.25 µm) from Phenomenex (USA). The polar ZB-WAX column was used to confirm the identification of compounds found by the non-polar column DB-5. Helium was used as carrier gas (purity 5.0, AGA Eesti, Estonia). The column temperature was raised from 35°C to 280°C with a rate of 10°C/min and holding time 1 minute at 280°C (total runtime 25.5 minutes) for GC/MS-TOF and raising also from 35°C, but to 245°C with a rate of 10°C/min (total runtime 21.5 min-

utes) for GC/qMS. The column effluent was used in splitless mode for identification purposes, while for quantitation purposes samples were analyzed in split mode 1:5 to decrease the amount of oversaturated peaks. Volatile compounds were identified by comparing their spectra and retention indices to those present in the NIST library. If GC/MS-TOF was used, then the identification was carried out using Chromalynx software (Waters Inc.).

To determine which volatile compounds were odour-active, samples were analyzed by GC-Olfactometry. 0.5 ml of sample was put into 20 ml HS vials that contained a 1-cm glass covered magnetic stir bar. For sample preparation and injection, the same conditions were used as GC/MS-TOF analysis. Volatiles were desorbed in an Agilent 7890 gas chromatograph equipped with a flame ionization detector (FID) and a sniffing port ODP-3 (Gerstel, Germany). The column effluent was split 1:1 between the FID and the sniffing port using deactivated fused silica capillaries (1-m length, 0.15-mm i.d.). The sniffing port was supplied with humidified air at 30 ml/min. The transfer line temperature was 280°C. A capillary column DB-5 MS (30m length, 250 µm i.d. and 1 µm film thickness; J&W Scientific, Folsom, CA) was used in the GC. Helium gas (purity 5.0, AGA Eesti, Estonia) was used as a carrier at a constant flow of 2 ml/min. Splitless mode was used in a split/splitless injector at 250°C. The initial oven temperature was 35°C followed by a rate of 45°C/min to 85°C, then by 9°C/min to 200°C and then by 45°C/min to 280°C and held for 1 minute (total run time 16.7 min). Intensity measurement was performed with the Gerstel ODP recorder program. If an assessor recognized an odour, he/she activates a microphone by pushing the specific remote control button for intensities 1 - 5 and describes the odour by quality (intensity scale: 1 = very weak, not identifiable; 2 = weak, but identifiable; 3 = moderate, easily recognizable, but not strong; 4 = strong; 5 = extremely strong). The sniffing panel was composed of 4 experienced and 1 inexperienced assessors (1 male, 4 female, aged 22-30). All samples were sniffed in duplicate. Results were expressed in modified frequency (MF), calculated with the following formula as proposed by Dravnieks [9]:

Odour-active compounds were identified based on their mass spectrum, calculated Van den Dool and Kratz Retention Indices (LRIs), and odour description by the GC-O panel. This data was compared with data from online databases and literature sources [10-14]. When determining the most important odour-active compounds based on either OAVs or MFs, often a certain value is chosen (e.g. OAV > 1 [15] or MF > 40% [16]). Since these values often change from author to author, in this research MF above 50% was chosen as a distinction between 'relevant' and non-relevant odor-active compounds. In this study, odour recombination and omission testing were not carried out.

Semi-quantification of odour-active compounds

For quantitation purposes, relative quantitation by HS-SPME was performed in triplicate. Peaks were integrated and compared using TargetLynx software (Waters Inc.) and Microsoft Excel 2010 (Microsoft Corp.). 5 µl of 1 mg/ml internal standard (IS) 1,2,3-trichloropropane (Sigma-Aldrich, USA) dissolved in methanol was used in each vial. Prior to quantification a calibration curve of IS was measured in the same sample matrix.

Results and Discussion

First of all, fermentation conditions and recipes were adjusted to obtain a reproducible production procedure resulting in constant quality homemade kvass with desired organoleptic properties. Fermentation times were varied between 12 and 48 hours, temperature 20°C up to 30°C. Together with inoculation rate the final fermentation conditions were adjusted to be representative of the authentic production process while preserving reproducibility. Sensory analysis was used as a guideline to decide on which raw materials should be used. The criteria for designing the recipe were to aim for kvass with a rich malty flavor, slightly viscous mouthfeel and a dark brown color. Since ordinary rye sourdough bread gave very light- colored and mild kvass, black rye bread (darkened with rye malt flour and barley malt extract) was chosen to improve color, taste and aroma intensity. The ratio of bread against water was adjusted to the optimal level giving a tasty product that is not too bitter or too watery. Two commercially available baker's yeasts of the brands Veski Mati (*Saccharomyces*

cerevisiae, Balti Veski AS, Estonia) and Nordic Yeast (*Saccharomyces cerevisiae*, Nordic Yeast Eesti OÜ, Estonia) were compared in kvass fermentation. The Veski Mati strain was chosen because it resulted in a faster fermentation with a richer overall aroma profile (Data not shown).

Kvasswort without yeast inoculation was used to determine the initial odour compounds present in kvass wort. The odor -active compounds (Modified Frequency > 50%) in unfermented kvass wort and in homemade kvass are presented in table 1. The raw material - from which the kvass is produced - plays a crucial role in the overall flavor profile, because most of the relevant odor -active compounds present in unfermented kvass wort, could also be found in the fermented kvass. However, they do differ in relative concentrations based on their MF values.

Nr	Compound name	RI DB-5	RI ZB WAX	Odour description	Qualification	Modified frequency (%) Kvass (kvass-wort)	Quantity (µg/kg) Home-made kvass	Quantity (µg/kg) Kvass-wort
1	Phenylethanol	1130 [1103-1127]	1900 [1892-1914]	**Honey-like**, **sweet**, **yeast-like**, **floral,** spicy, **herbal**, rose, pollen, **bready with a rose-honey nuance**	MS-TOF DB-5, qMS ZB WAX plus, RI, O, standard	81 (55)	183 ± 111	13 ± 0
2	Octanoic acid	1172 [1160-1192]	2069 [2062-2079]	**Fatty acid**, cheese, fresh, **moss, waxy, rancid**, oily, vegetable	MS-TOF DB-5, qMS ZB WAX plus, RI, O, standard	77 (33)	29 ± 41	6 ± 1
3	2-Acetylfuran	885 [893-918]	1498 [1500]	Sweet, almonds, **nutty, brown and toasted with a milky, lactonic undertone**, **balsamic**-cinnamon note, cereal	MS-TOF DB-5, qMS ZB WAX plus, RI, O	77 (56)	56 ± 16	404 ± 369
4	**β**-damascenone	1397 [1379-1397]	1802 [1798-1811]	**Grape, fruity, sweet**, waxy, **apple**	qMS ZB WAX plus, RI, O, standard	71 (59)	N.D	N.D
5	3-methyl-1-butanol	738 [726-750]	1196 [1194-1225]	**Pungent**, **balsamic**, **alcohol**, fusel, ethereal, **fruity**, banana, malty, ripe onion, burnt, **cheese**, bitter, harsh, cognac, **whiskey**, molasses, **diacetyl-like**	MS-TOF DB-5, qMS ZB WAX plus, RI, O, standard	69 (-)	16358 ± 1241	240 ± 25
6	Phenylacetaldehyde	1061 [1038-1053]		**Honey-like, sweet, rose, green**, **grassy, floral**, powdery, fermented, **chocolate with a slight earthy nuance**	MS-TOF DB-5, RI, O, standard	69 (69)	326 ± 10	46 ± 24
7	Methional	918 [894-918]		**Cooked potato**, musty, tomato, earthy, **vegetable**, creamy	RI, O, standard	65 (64)	N.D	N.D
8	4-vinylgiauacol	1328 [1309-1324]		**Spicy, clove**, smoky, **phenolic**, pepper, wood	RI, O	63 (57)	N.D	N.D
9	1-octen-3-one	982 [958-985]		Garlic, **mushroom**, spicy, rubbery, carrots, herbaceous, dirty, dust, earthy, green, oily, **vegetative, fungal, savoury**	RI, O, standard	62 (81)	N.D	N.D
10	S-carvone	1302 [1217-1244]	1729 [1721-1742]	**Fresh, herbal**, **caraway,** minty, liquorice	MS-TOF DB-5, qMS ZB WAX plus, RI, O, standard	58 (49)	80 ± 113	87 ± 124

11	3-hydroxy-4,5-dimethyl-5H-furan-2-one (sotolon)	1109 [1082-1131]		**Sweet, caramel,** maple, brown sugar, **cotton candy, coffee**	RI, O	54 (-)	N.D.	N.D.
12	(R)-(+)-γ-octalactone	1228 [1215-1268]		**Fatty,** spicy, **green, coconut, almond**	RI, O	53 (64)	N.D.	N.D.
13	Propenyl propyl disulfide	1104 [1116]		**Sulphurous,** bug, cucumber, **hearty,** gouache, unpleasant, fresh, fruity, estery, coffee	RI, O	52 (-)	N.D.	N.D.
14	**γ-**nonalactone	1376 [1360-1372]		**Sweet**, creamy, **coconut**, fatty with oily buttery nuances	RI, O	52 (77)	N.D.	N.D.
15	Acetyloxy-dimethylfuranone	1362 [1339]		**Sweet, caramel,** tropical, fruity, **brown sugar, toffee, molasses, baked bread**	RI, O	52 (-)	N.D.	N.D.
16	Eugenol	1356 [1348-1358]		**Sweet**, spicy, **clove like, woody**, with **phenolic** savoury ham and bacon notes and **cinnamon and allspice nuances**	RI, O, standard	48 (11)	N.D.	N.D.
17	Dimethyl trisulfide	999 [950-961]	1360 [1354-1408]	**Sulphurous, alliaceous, cooked**, **savoury**, **meaty,** eggy, fresh, green, **rotten food**, fishy, cauliflower, **cabbage**	MS-TOF DB-5, qMS ZB WAX plus, RI, O	46 (42)	378 ± 62	N.D.
18	Methyl decanoate	1346 [1324-1326]		Oily, wine-like, **fruity, floral**	MS-TOF DB-5, RI, O	46 (-)	124 ± 67	N.D.
19	Furaneol™	1058 [1043-1090]		**Cotton candy, sweet, caramelized sugar, strawberry, green**	RI, O, standard	43 (62)	N.D.	N.D.
20	Benzoic acid	1161 [1170-1190]		Buggy, **acidic**, pungent, solvent, grain, rubber, **urine**, **unpleasant**, artificial	RI, O, standard	42 (9)	N.D.	N.D.
21	Nonanal	1093 [1084-1110]	1389 [1375-1412]	**Gravy, green**, **tallow-like**, fruity, gas, chlorine, floral, **waxy**, sweet, melon, **soapy**, fatty, lavender, citrus fruit	MS-TOF DB-5, qMS ZB WAX plus, RI, O, standard	41 (-)	28 ± 27	40 ± 40
22	Unknown	1686 [-]		**Bad dishwashing cloth, mop, unpleasant**	-	40 (-)	N.D.	N.D.

23	Unknown	1194 [-]		**Hearty, roasted, bacon, nutty, umami, malty, yeasty, sweaty**		38 (52)	N.D.	N.D.
24	Benzaldehyde	997 [947-996]	1507 [1498-1513]	Burnt sugar, almond, woody, **roasted, sulphurous**	MS-TOF DB-5, qMS ZB WAX plus, RI, O, standard	36 (-)	62 ± 45	25 ± 31
25	Ethanol	<500 [<500-668]	937 [900-955]	Strong, **alcoholic**, **ethereal**, medical	MS-TOF DB-5, qMS ZB WAX plus, RI, O, standard	35 (-)	Oversaturated	Trace
26	Phenylethyl acetate	1254 [1250-1260]	1805 [1811]	**Floral,** jasmine, linalool, **flower, citrus, candy, sweet**	qMS ZB WAX plus, RI, O, standard	34 (40)	N.D.	N.D.
27	Unknown	1561 [-]		**Coconut, very disturbing, pungent, unpleasant, grass, green, soapy**	-	31 (-)	N.D.	N.D.
28	**β-ionone**	1476 [1477-1503]		**Woody, sweet, fruity**, berry-like with a green berry background	RI, O, standard	30 (20)	N.D.	N.D.
29	Unknown	1100 [-]		**Sweet, hearty, bacon-like, roasted, unpleasant, pungent**	-	30 (-)	N.D.	N.D.
30	Dimethyl sulfide	504 [500-505]		**Sulphurous, dimethyl sulfide**, creamy, tomato, **fishy**, scallop, berry fruity and **vegetative nuances**	RI, O, standard	29 (49)	N.D.	13 ± 6
31	Unknown	1432 [-]		**Metallic, artificial, dried, soil-like, green, unpleasant, chemical**	-	29 (-)	N.D.	N.D.
32	Ethyl (2E,4Z)-deca-2,4-dienoate	1489 [1465-1479]		Pear**, green**, waxy, vegetative, **tropical, fruity**	MS-TOF DB-5, RI, O	27 (-)	14 ± 20	N.D.
33	Ethyl acetate	607 [568-616]	896 [904]	Caramel, **sweet**, solvent-like, ethereal, fruity, acid, **buttery**, pungent, orange, grape, **rum-like**	MS-TOF DB-5, qMS ZB WAX plus, RI, O, standard	26 (42)	663 ± 30	N.D.
34	Butyric acid	826 [821-834]		**Sharp**, **dairy-like**, **cheesy, buttery** with a fruity nuance, **rancid, sweaty**	MS-TOF DB-5, RI, O, standard	26 (40)	N.D.	4 ± 3
35	Unknown	987 [-]		**Metallic, dried, wood, grass**	-	24 (18)	N.D.	N.D.
36	Toluene	760 [746-797]		**Sweet**, Pungent, **Caramel**, Ethereal, Synthetic, **Fruity**, Rubbery, Solvent-like	MS-TOF DB-5, RI, O, standard	23 (9)	6 ± 3	Trace
37	Unknown	1247 [-]		**Green, fruity, citrus, sweet, soapy, leave-like**	-	21 (-)	N.D.	N.D.

38	Unknown	815 [-]		**Whiskey, sour, sweaty, diacetyl-like**	-	19 (-)	N.D.	N.D.
39	Unknown	1753 [-]		**Spicy, rotundone-like, pepper, pungent, acidic, Asian spice mix (curry-like)**	-	19 (-)	N.D.	N.D.
40	n-Decanoic acid	1387 [1374-1404]	2278 [2270-2282]	**Unpleasant, rancid**, sour, fatty, citrus, **soapy**	MS-TOF DB-5, qMS ZB WAX plus, RI, O	19 (-)	54 ± 8	N.D.
41	Unknown	905 [-]		**Artificial, unpleasant, solvent-like, perfume**	-	18 (38)	N.D.	N.D.
42	Unknown	1119 [-]		**Beer, sour**	-	18 (-)	N.D.	N.D.
43	Isoamyl acetate	878 [869-881]	1115 [1117]	**Sweet**, banana, **fruity with a ripe estery nuance**, fresh, **pear odour**	MS-TOF DB-5, qMS ZB WAX plus, RI, O, standard	17 (-)	369 ± 40	N.D.
44	2,6-Dimethylpyrazine	934 [906-922]		Cocoa, **roasted nuts, roast,** beef coffee	RI, O	17 (-)	N.D.	N.D.
45	Unknown	1266 [-]		**Caraway, sweet, green, grass, coriander, herbs**	-	17 (-)	N.D.	N.D.
46	Cinnamyl alcohol	1291 [1259-1312]		**Cinnamon spice, floral,** green and **fermented with powdery balsamic nuances**	RI, O	13 (-)	N.D.	N.D.
47	Isoamyl hexanoate	1262 [1208-1254]	1464 [1452]	**Fruity**, sweet, pineapple with a slightly **pungent sour** cheesy note	MS-TOF DB-5, qMS ZB WAX plus, RI, O	13 (-)	15612 ± 5355	N.D.
48	Unknown	962 [-]		**Roasted, coffee, caramel, pyrazine**	-	12 (-)	N.D.	N.D.
49	Unknown	1250 [-]		**Urine, artificial, rubber-like, plastic-like**	-	12 (-)	N.D.	N.D.
50	Isobutyl benzoate	1371 [-]		**Fruity**, chemical, **sweet**, **cherry fruity**, with a balsamic nuance	MS-TOF DB-5, RI, O	12 (-)	21 ± 16	39 ± 0
51	Unknown	851 [-]		**Candy, sweets-like**	-	11 (11)	N.D.	N.D.
52	o-cresol	1031 [1037]		Musty, **phenolic, burnt**	RI, O	11 (-)	N.D.	N.D.
53	**γ**-octalactone	1271 [1255-1268]		**Coconut**, sweet, creamy, milky, soapy, coumarin, fruity, peach, apricot	RI, O	11 (22)	N.D.	N.D.
54	Ethyl butanoate	804 [759-804]		**Sweet, fruity, tutti frutti**, lifting and diffusive, banana, strawberry, **bubblegum**, **pineapple**, acetone, caramel	MS-TOF DB-5, RI, O, standard	10 (31)	28 ± 7	N.D.

55	**γ-**hexalactone	1053 [1047-1068]		Coumarin, sweet, iactonic, **tobacco**, green, **coconut**	RI, O	10 (46)	N.D.	N.D.
56	2,3-butanedione	664 [574-621]		**Buttery**, sweet, cream, caramel, pungent, **spirit**, fruit, pineapple	RI, O, standard	8 (63)	N.D.	N.D.
57	S-(2,2-dimethylpropyl) 2,2-dimethylpropane thiosulfinate	731 [-]		**Roasted, alliaceous**	MS-TOF DB-5, RI, O	8 (-)	N.D.	N.D.
58	Unknown	1081 [-]		**Urine**	-	8 (-)	N.D.	N.D.
59	Ethyl heptanoate	1087 [1083-1099]	1333 [1328]	Fruity, pineapple, **sweet**, estery, banana, berry, **cognac** and slightly green with a seedy nuance	MS-TOF DB-5, qMS ZB WAX plus, RI, O	8 (36)	Trace	N.D.
60	Dodecanoic acid	1534 [1537-1592]		Dry, metallic, weak, fatty, **waxy, coconut oil**	MS-TOF DB-5, RI, O	6 (36)	19 ± 26	N.D.
61	2-methylbutyric acid	836 [837-876]		**Overripe fruit**, sweaty, cashew, **sweet**, acidic, **dirty**, cheesy with a fermented nuance, **pungent**, Roquefort cheese	MS-TOF DB-5, RI, O	5 (-)	N.D.	N.D.
62	2-Ethyl hexanol	1012 [1024-1040]	1476 [1446-1505]	Mild, **oily**, floral, rosy, **citrus**, fresh, sweet	MS-TOF DB-5, qMS ZB WAX plus, RI, O	5 (13)	3517 ± 583	N.D.
63	Ethyl dodecanoate	1597 [1554-1598]	1660 [1634]	**Mango-like, sweet**, waxy, soapy, rummy with a creamy-floral nuance	MS-TOF DB-5, qMS ZB WAX plus, RI, O	3 (-)	78 ± 111	N.D.
64	Carbon dioxide	<500 [-]	[-]	-	MS-TOF DB-5, qMS ZB WAX plus	-	1772 ± 243	N.D.
65	Acetaldehyde	<500 [-]	711 [694-712]	-	MS-TOF DB-5, qMS ZB WAX plus	-	137 ± 21	N.D.
66	3-methyl butanal	657 [650-669]	926 [934]	-	MS-TOF DB-5, qMS ZB WAX plus, RI	-	N.D.	35 ± 4
67	2-methyl butanal	667 [627-668]		-	MS-TOF DB-5, RI	-	N.D.	40 ± 2
68	Acetic acid	676 [646-663]	1462 [1434-1474]	-	MS-TOF DB-5, qMS ZB WAX plus, RI	-	N.D.	Trace
69	Ethyl propanoate	709 [704-714]		-	MS-TOF DB-5, RI	-	24 ± 17	N.D.
70	2-Methyl-1-butanol	739 [728-743]	1210 [1189-1217]	-	MS-TOF DB-5, qMS ZB WAX plus, RI	-	9081 ± 498	168 ± 5

71	Dimethyl disulfide	751 [747-761]		-	MS-TOF DB-5, RI	-	Trace	N.D.
72	3-Furaldehyde	836 [815-831.7]		-	MS-TOF DB-5	-	Trace	N.D.
73	Furfural	837 [817-848]	1456 [1448-1503]	-	MS-TOF DB-5, qMS ZB WAX plus	-	N.D.	321 ± 453
74	Styrene	900 [888-915]		-	MS-TOF DB-5	-	Trace	N.D.
75	2-Butyl-4-methyl-1,3-dioxolane	953 [-]		-	MS-TOF DB-5	-	N.D.	21 ± 18
76	2-n-Butyl furan	956 [892.1-894]		-	MS-TOF DB-5	-	N.D.	6 ± 0
77	Hexanoic acid	963 [959-1020]	1844 [1803-1866]	-	MS-TOF DB-5, qMS ZB WAX plus	-	1250 ± 0	N.D.
78	2-Pentyl furan	994 [974-998.5]		-	MS-TOF DB-5	-	N.D.	3 ± 2
79	Ethyl hexanoate	996 [996-1014]		-	MS-TOF DB-5	-	Trace	N.D.
80	Octanal	1006 [999-1023]		-	MS-TOF DB-5	-	N.D.	16 ± 6
81	(Z)-2-Octene	1030 [806-812]		-	MS-TOF DB-5	-	N.D.	95 ± 121
82	D-limonene	1041 [1031-1039]	1177 [1175-1190]	-	MS-TOF DB-5, qMS ZB WAX plus	-	N.D.	208 ± 139
83	2-Acetylpyrrole	1068 [1038-1087]	1980 [1949-2006]	-	MS-TOF DB-5, qMS ZB WAX plus	-	56 ± 6	N.D.
84	Propanal	1093 [506]		-	MS-TOF DB-5	-	153 ± 31	N.D.
85	1-Nonanol	1172 [1168-1173]		-	MS-TOF DB-5	-	N.D.	93 ± 6
86	Ethyl octanoate	1194 [1173-1209]	1440 [1428]	-	MS-TOF DB-5, qMS ZB WAX plus	-	4366 ± 709	12 ± 17

87	(1-ethylpropyl)-benzene	1201 [-]		-	MS-TOF DB-5	-	N.D.	6 ± 5
88	2-methyl-5-(1-methylethenyl)-cyclohexanone	1225 [1196-1205]		-	MS-TOF DB-5	-	N.D.	6 ± 6
89	Nonanoic acid	1256 [1288-1297]		-	MS-TOF DB-5	-	173 ± 56	9 ± 6
90	Ethyl nonanoate	1293 [1294-1320]	1528 [1520-1530]	-	MS-TOF DB-5, qMS ZB WAX plus	-	1704 ± 284	N.D.
91	2-Acetyl-4-methylphenol	1326 [-]		-	MS-TOF DB-5	-	N.D.	4 ± 4
92	Ethyl 9-decenoate	1384 [1386-1389]	1689 [1685-1712]	-	MS-TOF DB-5, qMS ZB WAX plus	-	2696 ± 1572	N.D.
93	Ethyl decanoate	1392 [1381-1391]	1634 [1595-1650]	-	MS-TOF DB-5, qMS ZB WAX plus	-	784 ± 417	227 ± 27
94	Isopentyl octanoate	1447 [1450]	1659 [1651-1674]	-	MS-TOF DB-5, qMS ZB WAX plus	-	15779 ± 6620	N.D.
95	Propargyl alcohol	1466 [-]		-	MS-TOF DB-5	-	773 ± 329	N.D.
96	2-heptyl-1,3-dioxolane	1532 [-]		-	MS-TOF DB-5	-	N.D.	10 ± 9
97	Hexadecane	1599 [-]		-	MS-TOF DB-5	-	N.D.	1 ± 0
98	Isoamyl decanoate	1645 [1653]		-	MS-TOF DB-5	-	4532 ± 1637	N.D.
99	Phenethyl butyrate	1658 [1440-1444]		-	MS-TOF DB-5	-	568 ± 137	N.D.

100	Benzoic acid, 2-ethyl-hexyl ester	1728 [-]		-	MS-TOF DB-5	-	N.D.	Trace
101	2-Phenylethyl octano-ate	1864 [1854]		-	MS-TOF DB-5	-	525 ± 153	N.D.
102	Methyl hexadecanoate	1924 [1915-1928]		-	MS-TOF DB-5	-	1040 ± 256	N.D.

Table 1: *Odour-active compounds in kvass and kvasswort sorted on Modified Frequency (MF%) in homemade kvass. Kovats and van der Dool Retention Indices (RI) were determined with GC-O or with GC/MS-TOF for non-odourous compounds. In the table they are followed by their RI range found in literature between brackets. Odour descriptions are those found in literature, bold odour descriptions were mentioned by the GC-O panel in the current study. MF% of kvass is presented followed by the MF% of kvasswort between brackets. Quantities were determined by semi-quantification based on internal standard response on a GC/MS-TOF DB-5 column, expressed in µg/kg. Literature sources used: [11-14].*

GC/MS peak identification of both homemade kvass and kvass wort resulted in 65 identifiable peaks of which 24 were odour-active. These include common metabolites and intermediates of alcoholic fermentation but also aldehydes, ketones, phenols, pyrazines, furans, pyrans, pyrroles, pyridines and sulfur compounds. Relatively large number of unknown odour-active compounds (20%) is due to the fact, that they were detected only by GC-O, but not GC/MS. The main products of alcoholic fermentation are carbon dioxide (RI < 500) and ethanol (RI < 500), which were both detected by GC/MS. Carbon dioxide is not odour-active, but it does play an important role in the overall sensorial perception of homemade kvass.

Part of the odour-active compounds formed upon fermentation of kvass can be explained by the Ehrlich pathway. Fuel aldehydes, alcohols and acids can be derived from amino acid catabolism. The amino acids taking part in the Ehrlich pathway (leucine, isoleucine, valine, phenylalanine and methionine) are taken up by yeast cells. After transamination, the resulting α-keto acids cannot be redirected into central carbon metabolism. Therefore, they are excreted followed by conversion to Fuel aldehydes, alcohols and acids [18]. Examples of compounds which were detected in homemade kvass and which were probably formed by the Ehrlich pathway are the aldehydes 3-methylbutanal (RI 656.7), 2-methylbutanal (RI 666.7), phenylacetaldehyde (RI 1061.7) and methional (RI 918.0) and the alcohols isoamyl alcohol (RI 738.7), amyl alcohol (739.0) and phenylethanol (RI 1130.4) (Table 1). The presence of several Ehrlich metabolites in kvass wort can be explained by previous fermentation taking place in the production process of rye sourdough bread. Although some of the Ehrlich metabolites, such as isoamyl alcohol and phenyl ethanol, are more odour-active in homemade kvass compared to kvass wort, as can be concluded from their MF values (Table 1).

Sulfur -containing compounds such as propenyl propyl disulfide (RI 1103.6), dimethyl sulfide (RI 503.6), dimethyl disulfide (RI 751.4) and dimethyl trisulfide (RI 999.5) are probably degradation products of the sulfur containing amino acid methionine. In beer production the presence of such compounds is often associated with bacterial contamination of wort, however - since traditional kvass wort is made from sourdough bread - its presence can be explained by the presence of lactic acid bacteria in sourdough cultures [19-21]. Another explanation is that sulfur compounds can be formed upon Maillard reaction [22].

Ethyl and acetate esters are common metabolites of fermentations by baker's or brewer's yeast. An example of an acetate ester found in kvass is ethyl acetate (RI 606.6), the ester formed from ethanol and acetic acid. It was previously reported to be present in kvass by Behrmann., *et al*. [23] and Oganesy-

ants., *et al* [24]. Ethyl esters are formed by reaction of ethanol and fatty acids [21]. They typically give pleasant sweet fruit-like odours, however, not all of the detected ethyl esters by GC-MS, were found to be odour-active in GC-O analysis.

1-octen-3-one (RI 982.3, MF 81% in kvass-wort, 62% in homemade kvass) is an oxidation product of 1-octen-3-ol, an alcohol formed by *S. cerevisiae* and many other yeasts and bacteria [21]. This ketone has a very low odour threshold (0.005 ppb) and has a mushroom smell accompanied with metallic, earthy and vegetative nuances [11,13,14]. Less odour-active ketones and aldehydes were also found in homemade kvass, for example nonanal (RI 1093.2, MF 0% in kvass-wort, 41% in homemade kvass) and benzaldehyde (RI 997.1, MF 0% in kvass-wort, 36% in homemade kvass). Probably these were also formed upon oxidation reactions of either fatty acid derivatives or fusel alcohols.

Octanoic acid (RI 1172.4) is one of the short chain fatty acids that is present in yeast cell walls. Due to autolysis of yeast cells, octanoic acid ends up in the product [25,26]. In beer production this is usually seen as undesired because high levels of short chain fatty acids are responsible for off-flavours. In sparkling wines octanoic acid is an important contributor to its odour, and is formed during its secondary fermentation in the bottle, where yeast autolysis takes place upon maturation [25,26]. Ethyl acetate (RI 606.6), isoamyl acetate (RI 878.2) and several esters can also be formed from fatty acid degradation by either direct action of lipases and/or through β-oxidation followed by alcohol acetyltransferase and lipase action [27].

Odor-active furans such as 2-acetyl furan (RI 885.1, MF 56% in kvass wort, 77% in homemade kvass), sotolon (RI 1108.9, MF 0% in kvass wort, 54% in homemade kvass), acetyloxy-dimethylfuranone (RI 1361.8, MF 0% in kvass wort, 52% in homemade kvass) are formed upon thermal decomposition of carbohydrates. During roasting of bread and subsequent addition of boiling water, sugars from the bread are able to form these furans. However, furans are also present in food products that are not extensively heat treated [28]. In research on Maillard model-systems by Lancker and co-workers [29], furans could even be formed by heat treatment of glucose alone, although the presence of alanine and serine provided additional furan formation pathways. Maillard reactions as such might continue during fermentation, even at milder temperatures (30°C incubation temperature). This could explain why 2-acetyl furan, sotolon and acetyloxy-dimethylfuranone are more important odor compounds in kvass compared to kvass wort.

Norisoprenoids such as β-damascenone (RI 1397.2, MF 59% in kvass wort, 71% in homemade kvass) are degradation products of carotenoids (e.g. neoxanthin, β-carotene). Carotenoids can be degraded by either acid or enzymatic hydrolysis [30]. They degrade directly or via glycosylated intermediates. β-damascenone levels are rising during beer maturation [31]. At lower pH, acid hydrolysis of precursor molecules explains the increase in β-damascenone during aging. Since these precursors are known to be linked to sugars in beer and wine, chemical hydrolysis of sugars probably also contributes to the increase in β-damascenone in kvass [30-32]. S-Carvone (RI 1302.6) is a terpenoid with a fresh herbal, caraway-like odour (MF 49% in kvasswort, 58% in homemade kvass). Dihydrocarveol was also detected by GC-O in both kvass wort and homemade kvass, but only by one of the 5 assessors. It was identified by GC-MS but only in trace amounts. Due to its low response factor, it is hard to determine its precise quantity before or after fermentation [33].

4-Vinylguaiacol (RI 1328.8, MF 57% in kvass wort, 63% in homemade kvass) is an odor compound with a phenolic, clove-like odour, reported to be also one of the key odour-active compounds in wheat beer [15]. 4-Vinylguaiacol is formed from thermal or enzymatic degradation of ferulic acid, a hydroxycinnamic acid present in the outer layers of grains. Ferulic acid and its dehydrodimers are known to be present in rye [34]. Ferulate decarboxylase is an enzyme reported in several strains of *S. cerevisiae* and other yeasts and bacteria which are capable to form 4-vinylguaiacol [35]. Styrene (RI 900.6) can be formed by enzymatic or thermal decarboxylation of cinnamic acid. The same enzyme is involved in ferulic acid decarboxylation resulting in the formation of 4-vinylguaiacol [36].

γ-nonalactone (RI 1376.3, MF 77% in kvass wort, 52% in homemade kvass) and (R)-(+)-γ-octalactone (RI 1227.8, MF 64% in kvass wort, 53% in homemade kvass) are lactones which can be produced by *S. cerevisiae* in (rye) bread, beer and other fermented foods [37]. Since their intensity is higher in kvass wort compared to the fermented product, it might be that these lactones are degraded during kvass fermentation. Their initial presence in kvass wort can be explained by the fact that they are common in bread and/or bread crust [37].

Quantification of volatiles in homemade kvass and kvass wort

Regarding the quantification of odour-active compounds, several problems are arising: part of the compounds have either a too low response factor on GC-MS and/or a very low odor threshold. This made it impossible to quantify these compounds based on their GC-MS response in this study. For those odour-active compounds which could be detected, standard deviations among replicates (n = 3) are often large (Table 1). It is recommended to conduct further research in order to accurately determine the quantities of potentially relevant odour-active compounds in homemade kvass. This could be done by odor extraction (e.g. SAFE - Solvent Assisted Flavour Evaporation) followed by liquid injections into a GC apparatus. With more precise quantification, a recombinant odour mixture could be compared to the original sample to check the correctness of the list of potentially relevant odour-active compounds as found in this study.

Conclusion

The relevant odor-active compounds in homemade kvass were identified as phenyl ethanol (honey-yeast odor), octanoic acid (rancid-goat odor), 2-acetyl furan (roasted bread odor), β-damascenone (apple odor), isoamyl alcohol (whiskey, fruity, ripened cheese odor), phenylacetaldehyde (honey-rose odor), methional (cooked potato odor), 4-vinyl guaiacol (clove-like odor), 1-often-3-one (mushroom odor), S-carvone (herbal odor), sotolon (caramel odor), (R)-(+)-gamma-octalactone (coconut-almond odor), propenyl propyl disulfide (sulfurous bug-like odor), γ-nonalactone (coconut odor) and acetyloxy-dimethyl furanone (caramel odor). Part of these key odor compounds was also found in kvass wort but was present in different concentrations which explains why they were found to be more or less odor-active based on modified frequencies (MF%). The relevant odor-active compounds were formed by yeast fermentation according to the Ehrlich pathway, carotenoid and lactone degradation, degradation of amino acids, yeast cell lysis, and Maillard reactions taking place in bread or kvass wort production. Not all key odors could be identified and quantified, since their low odor thresholds and response factors. It is recommended to conduct further research in order to accurately determine the quantities of potentially relevant odor-active compounds in homemade kvass. More precise quantification results could be used to construct a recombinant odor mixture to compare it to the original sample to check the correctness of the list of relevant odor-active compounds as determined in this study.

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