OPTIMIZATION OF THE AUTOMATED SPME METHOD FOR THE ANALYSIS OF VOLATILE COMPOUNDS IN TRADITIONAL ESTONIAN FOOD (KAMA AND KVASS) USING GC/MS

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INTRODUCTION

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. Until now Estonia has been far behind talking along to food aroma analyses and therefore many traditional Estonian foods have not been investigated of their odor. The aim of this article was to analyze aroma of different Estonian foods, like Kama and Kvass, and optimize an automated solid-phase microextraction method.

Kama (also Talkkuna in Finnish) is a traditional Estonian food that is mostly enjoyed for breakfast, refreshing drink on hot summer days or as a stomach filling drink between main courses. It is a finely milled powder mixture of roasted barley, rye, oat and pea flour mixed with sour milk, yoghurt, butter milk or kefir and mainly sweetened with sugar. Kama present numerous health benefits while consumed: dietary fibers, vitamins and minerals from grains, Ca and beneficial lactic acid bacteria from sour milk and essential amino acids from all ingredients. In this study only Kama flour (without dairy product added) was studied.

Kvass is a refreshing drink of Russian origin, which became very popular after World War II in Estonia and other Baltic states. Kvass tastes similar to beer, but is non-alcoholic (less than

1% ethanol) and sweet. Natural ("real") kvass was originally made by simultaneous acid and alcoholic fermentation of bread or rye flour with addition of sugar and contained living lactic acid bacteria and yeast. It had a very short shelf-life of two days and was served from barrels on the streets usually within one day, but was banned because of health regulations.

A solid-phase microextraction (SPME) is a technique that involves exposing a fused silica fiber coated with a stationary phase to a sample containing vial. The analytes are being absorbed into the stationary phase until an equilibrium has been reached, after which the fiber is removed from the vial and the analytes are thermally desorbed in the injector of a gas chromatograph. So far sample preparation techniques involved static or dynamic headspace extraction, liquid–liquid extraction, supercritical fluid extraction, distillation or solid-phase extraction etc., but the main advantages of the SPME technique are its simplicity, sensitivity, fastness and that the use of solvent is eliminated and only a small amount of sample is needed.

The SPME method has been automated by adapting a CTC CombiPAL autosampler. Factors affecting sample throughput (fiber selection, sample preparation, chromatographic parameters) of the method have been discussed and optimized in this article.

Solid phase micro-extraction with GC/MS can be used for analyzing the whole volatile profile of Kvass and Kama, but does not indicate to the odor-active compounds. Some volatiles are of great importance and may contribute greatly to the Kvass and Kama flavor, while others are important merely in building up the background flavor of the product. Therefore, GC-olfactometry was used to look into the main compounds responsible for Kvass and Kama aromatic notes.

EXPERIMENTAL, RESULTS AND DISCUSSION

SPME requires a previous optimization of the extraction parameters that can affect extraction efficiency, in order to obtain high recoveries of volatiles. First optimization step would be selecting the most efficient fiber. Supelco (Bellefonte, PA) offers many fiber assortment kits, of which the Stableflex would be the best choice for any food aroma analyses. It consists of four different fibers (Car/PDMS, DVB/Car/PDMS, DVB/PDMS and Polyacrylate) mainly used for analyzing food aroma. SPME analyze can be carried out either manually or automatically (in this study CTC CombiPAL was used). The advantages of manual analyze are low cost and simplicity, but disadvantage is low reproducibility. If the goal is qualitative

analysis, then manual SPME would be sufficient, in order to get quantitative data, the CombiPAL autosampler should be used.

Vials for the analysis were first cleaned in a dishwasher, then with acetone (inside and outside) and kept in oven (270 ^{o}C) until sample preparation. After taking out, they were cooled under the hoof to avoid any extraneous peaks from the environment. In addition several various blank experiments (fiber blank, fiber inserted to empty vial) were performed before the samples. The kvass bottles were cooled to +4 C and Kama flour was poured from its original carton package to a glass bottle with a screw cap to minimize, in both cases, the loss of very volatile compounds.

In this study GC oven temperature programming started at -10 ^{o}C (cooled with liquid nitrogen), held for 1 minute and raised 12 ^{o}C/min to 280 ^{o}C, holding time for 1 min. Standard non-polar column HP-5 30 m × 0,25 mm × 1 μm was used. -10 ^{o}C oven starting temperature was used for gaining sharper peaks and thicker column for increasing adsorption efficiency. The oven programming was not changed during the optimization process.

For fiber selection 1,5 g Kama and 2 ml of Kvass was used in 20 ml vials closed with cap and Teflon-faced silicone rubber septa (Supelco, Inc., Bellefonte, PA). Samples were agitated with magnetic stirring (250 rpm, stirrer covered with glass), extraction time of 20 minutes 40 ^{o}C (pre-incubation without the fiber for 5 minutes, 40 ^{o}C). For both, Kvass and Kama, the Car/PDMS (1 cm, 85 um) showed the best efficiency and was used for further optimization.

After the fiber selection sample preparation should be optimized. The amount of sample, salt and water addition was investigated. In case of Kvass, the work is still ongoing, but with Kama, first only the flour was added in amounts of 0,5; 1 and 1,5 grams into the 20 ml vial. When solid samples are analyzed by SPME, water or other surface-active compounds should be added to improve the transport of compounds from the sample to the gaseous phase. For this reason, 5 ml of HPLC water was added into the vial together with either 0,5; 1 or 1,5 g of Kama flour. Then 2 g of NaCl was added for salting-out effect to 0,5; 1; and 1,5 g of Kama flour (all included 5 ml of HPLC water).

Surprisingly, it was seen from the chromatograms that water and salt addition actually decreased the peaks' heights and a few compounds had disappeared. And since the reproducibility with water was not as good as without water, 1,5 g of pure Kama flour was chosen for the continuation of the optimization process. pH effect was not investigated due to the choice of pure flour use.

In addition chromatographic conditions should be investigated. As mentioned earlier, the oven temperature programming has proven to be adequate and was not optimized. Although

the analytes are fully desorbed in some cases even with 0,5 minute, the desorption time was set to 10 minutes to avoid carry-over effect and was not changed. In case of split/splitless injector, splitless mode is always used because of desorption phenomena (all compounds do not desorb at the same time). In order to get better looking peaks, decreased initial oven temperature is used and if the fiber is saturated, less sample amount should be used.

Incubation temperature should not exceed 80 ^{o}C, over which the forming of artifacts may occur. Volatile compounds show an increased response upon heating to 40-45 ^{o}C, above these temperatures, the response goes down due to migration of analytes out of the fiber. For analyzing very volatile compounds, the extraction time and temperature should be decreased. In aroma experiments 35 ^{o}C could be used as it is closest to the human body temperature and could indicate best for the aroma compounds people feel while consuming. In this study, for determining the effects of heating temperature, the sample bottles were maintained at 40, 50, 60 and 65 °C.

From the results it was clear, that increasing the temperature, the chromatographic response increased as well. It was seen, that aldehydes, pyrazines and higher molecular compounds increased with higher temperatures while smaller molecules, like isobutyraldehyde, 2-propanone and 2,3-butanedione would not depend on the temperature. The amount of analyte extracted depends also on the extraction time and sample agitation. In some cases, increasing temperature and time would not be always beneficial due to the high boiling compounds overloading the fiber and replacing the low boiling compounds as the active sites of the fiber are being filled up. Concerning to Kama, capacity problems did not occur and, therefore, temperature 65 ^{o}C was chosen. Different exposure times (5, 10, 20, 30 and 40 min) were then investigated.

From the results it was seen that generally lower boiling compounds, like isobutyraldehyde, 2-propanone, 2,3-butanedione etc. were not affected by extraction time. For pyrazines and other higher boiling compounds, it was seen that 40 minutes extraction gave better chromatographic response. Since 40 minutes made the analyzing procedure too long and there was not too much difference in the chromatographic response with 30 minutes, then 30 minutes was chosen as optimum.

As mentioned earlier, solid phase microextraction with GC/MS can be used for analyzing the whole volatile profile of kvass and kama, but in order to find the odor-active compounds, GC-Olfactometry should be used. For GC-O analysis, the fiber was injected in a HP GC model 5890 equipped with a split/splitless injector. At the end of the capillary, the effluent is split into the FID (flame ionization detector) and a sniffing port ODP (Gerstel, Germany). The

sniffing port is held at 300 ^{o}C to prevent any condensation of volatile compounds. Humidified air was added in the sniffing cone to reduce fatigue and drying of the assessor's nasal passage. The column and operating conditions were the same as those used for GC-MS, except for the temperature programming, which was 35 C 8 C/min to 280 C.

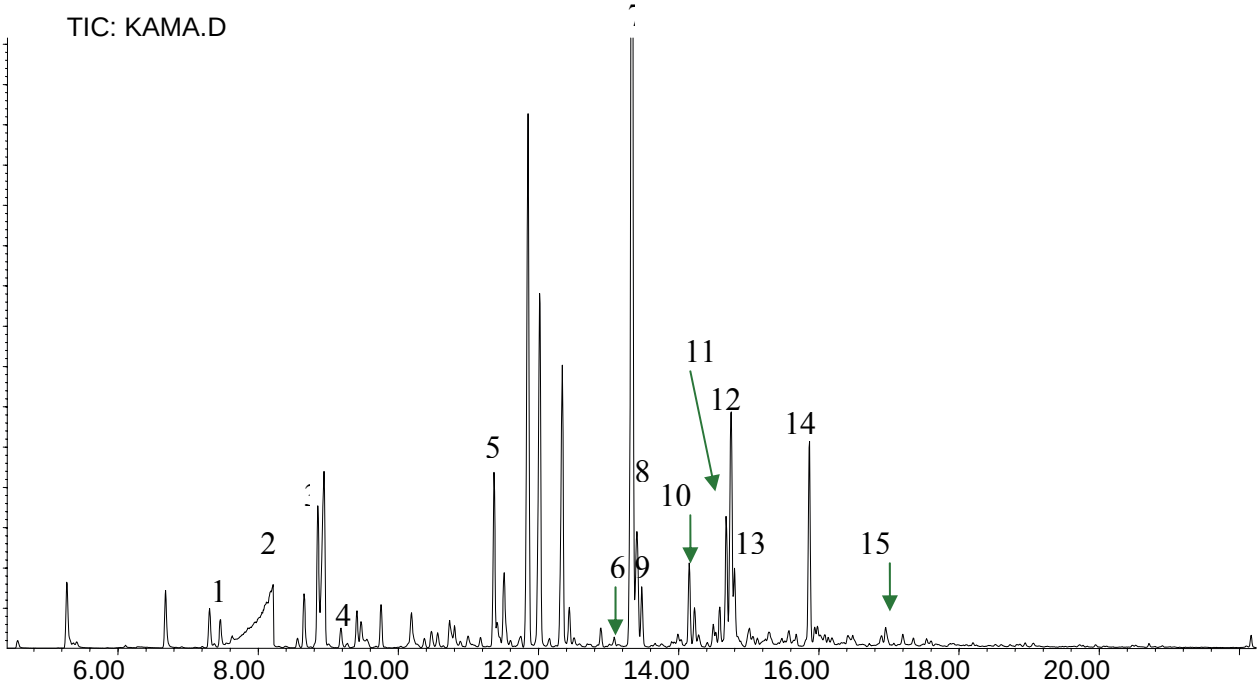

Figure 1. Odor-active key compounds responsible for Kama aroma (GC/MS chromatogram, aroma detected with GC-O)

For determination of odor-active compounds [Figure 1], an assessor is sitting in front of the sniffing port and smelling the effluent of the column. An "olfactometer button" was depressed when an aroma was detected. The initiation and termination of aroma detection was recorded by an ODP recorder. In this study 15 compounds were found to form Kama aroma. Mostly pyrazines forming from Maillard reaction.

Table 1. Compounds correlative to the chromatogram.

Nr.	Ret.time	Compound name	Kovats RI$^{\infty}$	Odor$^{\infty}$	Threshold*
1	7.30	2,3-butanedione	593	Butter	0,000007-0,02
2	7.83	Acetic acid	600	Sour	0,25-500
3	8.88	2-methyl butanal	641	Cocoa, almond	0,001-0,1
4	9.17	3-penten-1-ol	686	Butter, creamy	0,66-4,3
5	11.34	Hexanal	801	Grass	0,02-0,33
6	12.88	1-heptanone	895	Algae	0,45-3,3
7	13.29	2,5-dimethylpyrazine	[905]	Roasted potato	0,17-1,82

8	13.39	Ethylpyrazine	[907]	Roast	0,25-2
9	13.45	2,3-dimethylpyrazine	892	Roasted meat	0,88
10	14.15	5-methylfurfural	978	Champignon	0,005-6
11	14.67	2-ethyl-3-methylpyrazine	1003	Roasted, cocoa	-
12	14.73	Trimethyl-pyrazine	1000	Must, Roasted, potato	-
13	14.78	2-ethyl-3-methylpyrazine	1047	Roast	0,035-0,15
14	15.85	2-ethyl-3,5-dimethylpyrazine	1083	Coffee	0,000007-0,000011
15	16.95	3,5-diethyl-2-methylpyrazine	1160	Baked	-

Preliminary work (also in this study) about the odor-active compounds can be done using databases available in the internet. www.flavonet.org[∞] is one of the biggest, where the description of the odor of the identified compound can be found. In addition, compound's odor threshold (lowest concentration of compound that is detectable by the human sense of smell) can be found from internet or books (Odour threshold values in air, water and other media, L.J.van Gemert, 2003*).

CONCLUSION

In conclusion, SPME technique has been successfully applied to analyze the volatile compounds from Kama flour and Kvass. The sample preparation process is simple, does not involve the use of organic solvents, is less time consuming, and can be used as a routine method. The SPME fiber, sample amount, water and salt addition, extraction time and temperature were optimized to obtain the maximum sensitivity for the greater number of compounds. GC-Olfactometry was adapted to investigate the odor-active compounds.

Owing to the large number of volatile compounds extracted and good reproducibility the developed methodology can be used to compare volatile profile from different types of kvass, or to compare Kama flour and Kama drink (with the dairy product added) or to study the aroma evolution during aging or for correlation with sensory analysis results. A better understanding of aroma would be also valuable for the production technology, quality control and product development.