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

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Introduction

Kama is a traditional Estonian food, mostly enjoyed for breakfast. It is a finely milled flour mixture (roasted barley, rye, oat, pea) mixed with sour milk or yoghurt. Kama presents numerous health benefits while consumed: dietary fibres, vitamins, minerals, beneficial lactic acid bacteria, essential amino acids etc. In this study only Kama flour (without dairy product added) was studied.

Kvass is a refreshing non-alcoholic and sweet drink. Natural kvass is made by simultaneous acid and alcoholic fermentation of rye bread or malt with addition of sugar. It contains living lactic acid bacteria and yeasts.

An automated solid-phase microextraction (SPME) method was developed to analyze volatile compounds in Kama and Kvass using gas-chromatography with mass detection. Factors affecting sample throughput (fiber selection, sample preparation etc) of the method were optimised in this study. Odour-active compounds were investigated with GC-olfactometry.

Experimental

Four StableFlex fibers (85µm Carboxen/PDMS, 50/30µm DVB/Carboxen/PDMS, 65µm DVB/PDMS, 85µm Polyacrylate) commonly used for foodstuff were tested.

The amount of Kama (0,5g; 1g; 1,5g); water (5ml) and salt (2g), addition to Kama, was investigated.

For determining the effects of heating temperature, sample bottles were maintained at 40, 50, 60 and 65 ℃.

Different fiber exposure times (5, 10, 20, 30 and 40 min) were also investigated.

The odour-active substances were analysed with an HP 5890 equipped with FID and Gerstel ODP2 Olfactometry.

Retention Indices according to Farkas et al. (1994) were calculated and compared with Flavornet database.

Odour thresholds (in water) were found in a handbook (Gemert, 2003).

Parameters of Analysis

GC-MS: HP 5890 coupled with HP MSD

Column: J&W HP-5, 30m×0,25mm×1µm

Carrier gas: He, 0,8 ml/min

Inlet: Split/Splitless, 0,75mm liner, 280 ℃ (splitless mode)

Oven: -10 ℃, for 1 min, 12 ℃/min to 280 ℃, for 1 min

Sample prep: 20 ml vial,1 cm magnet stirrer (glass cover) cleaned with acetone, kept 270 ℃.
Vials sealed with PTFE cap.
Preincubation without fiber 5 min.

SPME autosampler: CTC CombiPAL

GC-O: 35 ℃, for 1 min, 8 ℃/min to 280 ℃ for 1 min.
Effluent was split 1:1 between FID and ODP,
ODP transfer line was set to 300℃ with humidification.

MS Library: Wiley275

Results and Discussion

For both, Kvass and Kama, the Car/PDMS fiber showed the best efficiency. Surprisingly water and salt addition to Kama flour decreased the peaks' heights and a few compounds had disappeared, and also decreased the reproducibility, therefore 1,5 g of pure flour was chosen for the optimization process. It was found, that increasing the extraction temperature and time increased the chromatographic response and 65 ℃ 30 min was chosen for sample preparation. In this study 19 compounds were found to form Kama aroma. Mostly pyrazines forming in Maillard reaction during roasting process.

Tab.1: **Compounds correlative to the chromatogram on Fig.1.** (calculated/*www.flavornet.org**)

Nr	R_t	Compound name	Odour impression	RI^{a}/RI(DB5*)	Threshold (mg/L)
1	7.30	2,3-butanedione	Butter	-/593*	0,0003-2,3
2	7.83	Acetic acid	Sour	-/600*	22-1000
3	9.40	2,3-pentanedione	Butter, creamy	-/700*	0,02-0,03
4	11.34	Hexanal	Grass	807/801*	0,0025-0,75
5		Unknown	Algae	899/?*	
6	13.29	2,6-dimethylpyrazine	Roasted potato	905,5/-*	0,4-54
7	13.29	2,5-dimethylpyrazine	Roast, ether-like	908,2/[905]*	0,08-1,8
8	13.45	2,3-dimethylpyrazine	Roast, cheese	920,5/892*	0,4-2,5
9	-	1-octen-3-one	Mushroom	976,4/976*	0,000005-0,01
10	14.73	2-ethyl-5-methylpyrazine	Roasted, cocoa	996,1/[993]*	0,016-0,1
11	14.74	Trimethylpyrazine	Roast, ether-like	1000,0/1000*	0,023-9
12	14.67	2-ethyl-6-methylpyrazine	Hazelnut	1003/1003	0,04
13	15.07	Acetylpyrazine	Roast	1021,8/1023*	0,06-0,062
14		Unknown	Rubber	1032/?*	
15		Unknown	Roast, grain	1052/?*	
16	15.85	2-ethyl-3,5-dimethylpyrazine	Roast, rancid	1084/1083*	0,00016-15
17		Unknown	Roast, ether-like	1091/?*	
18	16.89	2,3-diethyl-5-methylpyrazine	Grass, roast	1157/1158*	0,00009-0,001
19		Unknown	Roast	1214,5/?*	

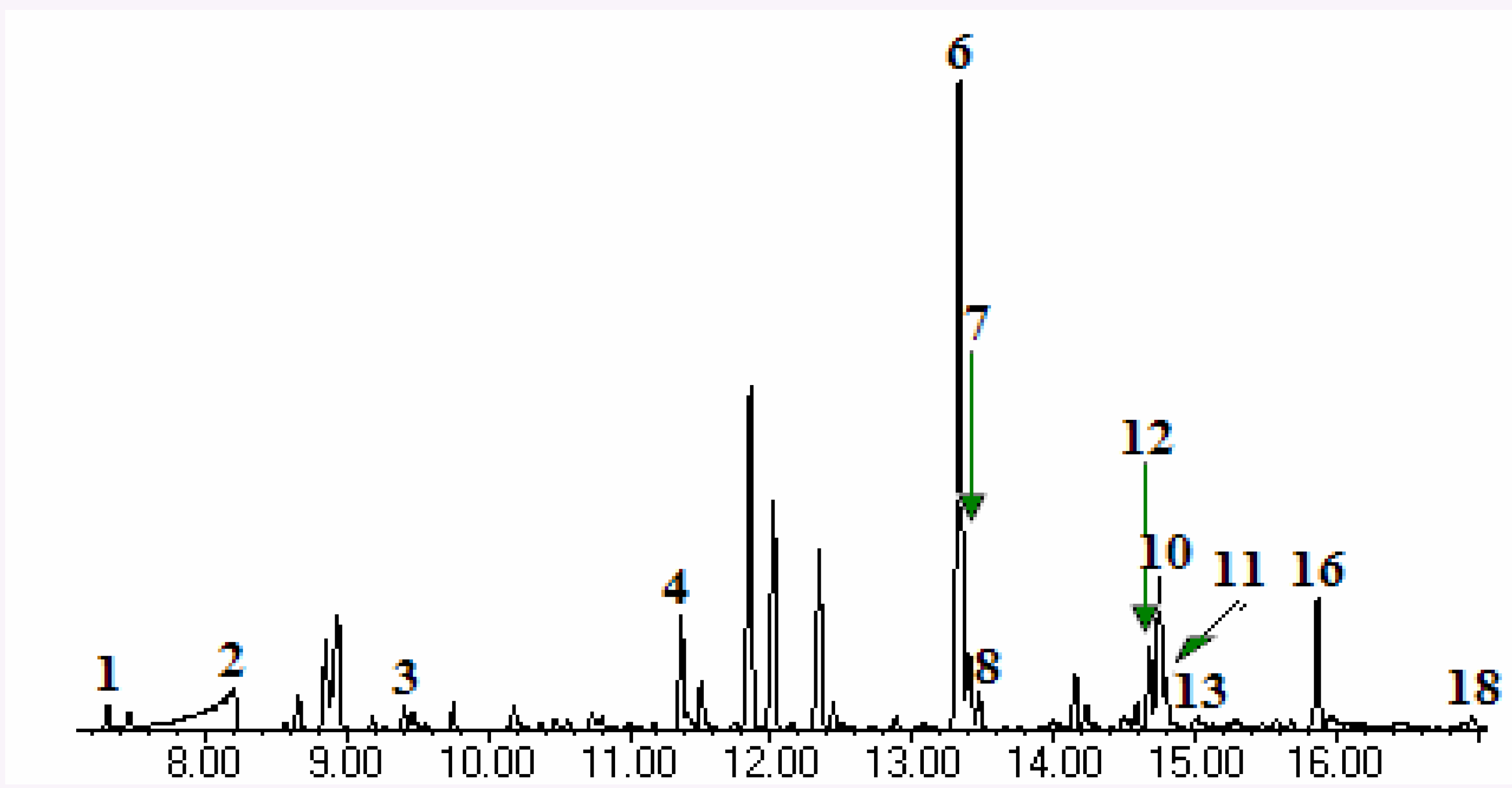

Fig.1: **Odor-active key compounds responsible for Kama aroma (GC/MS chromatogram, aroma detected with GC-O)**

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.

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Literature:

P. Farkas, J.L. Le Quere, H. Maarse and M. Kovac, in: H. Maarse and D.G. van der Heij (Eds.), Trends in Flavour Research, Elsevier, Amsterdam, 1994, p.145.

L.J.van Gemert. Odour threshold values in air, water and other media. Oliemans Punter & Partners, The Neatherlands, 2003.