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Static headspace-gas chromatographic analysis of volatile yogurt components

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Static headspace-gas chromatographic analysis is suggested for acetaldehyde, ethanol, acetone, and acetoin determination in yogurt. Headspace extraction conditions were optimized: thermostatting at 90 °C temperature for 20 min, pressurisation for 2 min, injection for 0.09 min. Prior to the headspace extraction, yogurt was diluted with water. For the quantification of the analytes a standard addition method was applied.

Keywords: headspace-gas chromatography, volatile compounds, yogurt

INTRODUCTION

Yogurt and related dairy products are produced from milk by fermentation and are widely consumed as a healthy and nutritious food [1]. The main fermentation product of milk sugar lactose is lactic acid, but small amounts of volatile by-products are also produced during the fermentation process. The content of the fermentation products depends on the nature of cultures of lactic acid bacteria used for the manufacture of yogurt. Volatile fermentation products impart the specific aroma and flavour that are an important part of yogurt's identity [2]. More than 100 volatile compounds have been identified in yogurt, however, only some of them such as acetaldehyde, ethanol, acetone and acetoin have relatively high concentrations and are the most important components responsible for the characteristic aroma of plain yogurt [2–5]. During storage the concentrations of volatile constituents in yogurt change [6, 7]. This leads to the formation of off-flavours. Thus, the analysis of key flavour compounds enables manufactures to produce desirable dairy products and can serve for the determination of yogurt shelf-life. However, despite the extreme importance of volatile yogurt components as an indicator of quality and product conformity, the chemical analysis of them is complicated by high levels of lipids, proteins, and carbohydrates [2]. As volatile constituents in yogurt are present in small or even trace concentrations, their isolation from the complex matrix and enrichment are often necessary for their analysis.

The analytical techniques that have been applied for isolating volatile compounds include simultaneous distillation–extraction, solvent extraction, dynamic purge and trap and headspace (HS) methods [8–14]. Headspace sampling followed by gas chromatographic analysis is the fastest and cleanest method for analysing volatile organic compounds [2, 13]. Headspace-gas chromatography (HS-GC) allows simplifying the determination of volatile yogurt compounds by their isolation in the headspace of a sample vial and subsequent automatic delivery of an aliquot of the vapour to a GC system for separation of the volatile components [15].

The aim of this study was to adjust the HS-GC method for quantification of the key aroma compounds (acetaldehyde, ethanol, acetone and acetoin) in different species of yogurt.

EXPERIMENTAL

Reagents and solutions

Acetaldehyde (≥99%), acetone (≥99.9%), ethanol (≥96%) and acetoin (≥95%) were obtained from Sigma-Aldrich (Germany). Stock solution of volatile flavour compounds (acetaldehyde, acetone, ethanol and acetoin) (1 mg ml^{-1} each) was prepared in distilled water. Working solutions of volatile flavour compounds were prepared by dilution of the stock solution with distilled water. All solutions were stored in the dark at 4 °C.

Yogurts "Turkisk", "Aistė Natural with Honey", "Dobilas Natural" and "Dobilas with Lemon and Ginger" were purchased from the local supermarket.

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Sample preparation

To the weighed yogurt sample an equivalent quantity of distilled water was added. The mixture was mixed with a spatula and 12 g of the mixture were transferred into a 22 ml HS vial. The vial was sealed hermetically with a polytetrafluoroethylene-coated rubber septum and an aluminium cap and transferred to HS equipment for the analysis.

Instrumentation

The chromatographic analysis was performed on a Perkin-Elmer Clarus 580 series gas chromatograph (PerkinElmer, USA) equipped with a flame ionisation detector. The GC system was equipped with the Elite-Wax capillary column (30 m × 0.32 mm id, 0.25 μm film thickness) (PerkinElmer, USA). Headspace extraction and sample introduction were performed on a PerkinElmer Headspace Sampler Turbomatrix 16 (PerkinElmer, USA) equipped with a balanced pressure system.

Headspace-gas chromatographic conditions

A headspace vial with the sample was positioned in the HS autosampler and equilibrated for 20 min at 90 °C. Needle temperature was 90 °C. The settings of the headspace sampler were 2 min for pressurization and 0.09 min for injection. Helium was employed as a carrier gas with a constant flow of 1 ml min^{-1}. The injector temperature was held at 110 °C. Injection was performed in the split mode (split 10:1). The detector temperature was held at 250 °C. The oven temperature was programmed as follows: 40 °C for 1 min and from 40 to 100 at 10 °C min^{-1}.

RESULTS AND DISCUSSION

Headspace extraction conditions

In order to achieve the best performance when using headspace-gas chromatography, careful attention should be paid to headspace extraction conditions. Prior to the GC analysis, the vial with the sample should be thermostatted until the equilibrium of the analytes is reached between the yogurt and the headspace. The equilibrium distribution of an analyte between the sample phase and the gas phase is defined by the partition coefficient K that depends on the temperature. Samples should be prepared to maximize the concentration of the volatile components in the headspace (i. e. to minimize the partition coefficients). K can be lowered by increasing the temperature at which the vial is equilibrated. The influence of temperature is an analyte-specific function and must be evaluated separately in each case [15].

Thus, the first HS parameter optimized was the sample's thermostatting temperature. In order to examine an influence of the temperature on the sensitivity, 10 g of "Turkisk" yogurt were taken for the analysis. This yogurt was selected because of its heavy consistency and large fat content (10%). Fat content is the decisive parameter in the partition and release of aroma volatiles [16]. Thus we selected the yogurt with the biggest fat quantity available, and expected that the HS-GC conditions optimized on this yogurt would fit also for the analysis of yogurts with less problematic matrices.

The maximum allowable temperature depends on the solvent's nature as the solvent's vapour mainly determines the pressure in the headspace. If the pressure is too high, some instrumental problems may result, the vial may cause leakage or even break [15]. As the main solvent in yogurt is water, in order to not exceed solvent's vapour pressure the maximum equilibration temperature used was 90 °C and the temperature range examined was 50–90 °C. The results presented in Fig. 1 demonstrate that for all the analytes the peak areas increased with the temperature and reached the maximum value at 90 °C. Based on the results, 90 °C equilibration temperature was selected for the HS analysis.

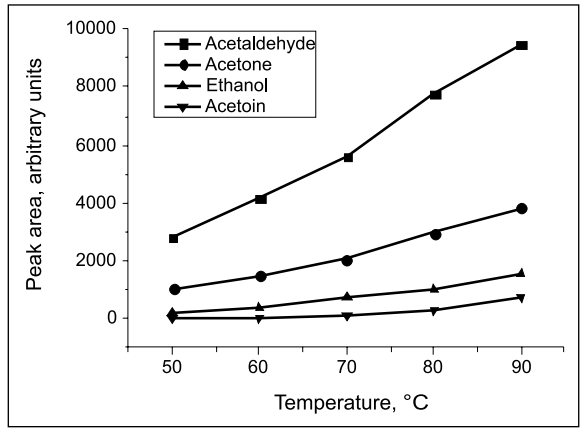

Fig. 1. Influence of temperature on headspace extraction efficiency. Extraction time 10 min, pressurization time 1 min, injection time 0.04 min

Further, the time necessary to reach equilibrium of the analytes between the yogurt and the headspace was determined. The equilibration time up to 50 min has been investigated. As is seen in Fig. 2, the peak areas of the analytes

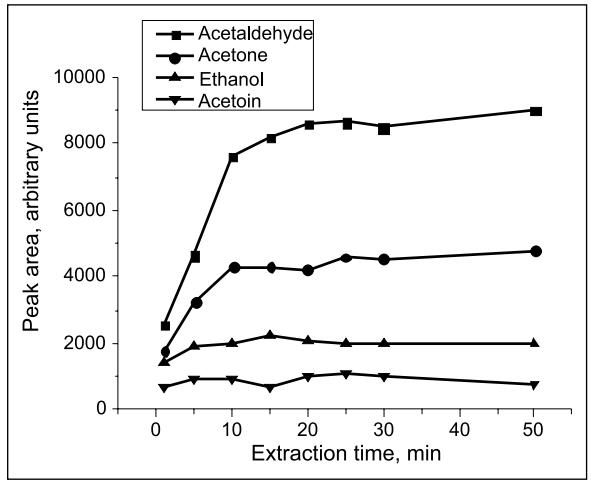

Fig. 2. Influence of extraction time on headspace extraction efficiency. Extraction temperature 90 °C, pressurization time 1 min, injection time 0.04 min

levelled off after 10–20 min. The optimum equilibration time of 20 min was therefore chosen to achieve the maximum sensitivity without extending the time of analysis.

HS instrumentation used in this work is equipped with the balanced pressure system: after equilibration the vial is pressurized by a carrier gas to the pressure equal to the carrier gas inlet pressure of the column. After a few minutes (pressurization time) the carrier gas flow is temporarily interrupted and the pressurised gas in the vial expands onto the column, resulting in the flow of the mixed headspace-gas from the vial to the column for the determined time (injection time). Pressurization time of several minutes is needed to assure a homogeneous distribution of carrier gas in the vial. We examined 1–4 min pressurization time and determined that 2 min are sufficient.

For balanced pressure systems, the sample volume transferred into the column depends of the injection time. Longer injection time results in higher sensitivity as a larger quantity of the sample is introduced. On the other hand, a long injection time causes band broadening at the start of the chromatogram. Thus a compromise for resolution and sensitivity has to be found. In this work 0.01–0.1 min injection times have been examined. The results showed (Fig. 3) that the peak areas of the analytes increased up to 0.09 min injection time. At 0.1 min injection time, the peak areas decreased probably because of a bad resolution of the peaks that resulted in bad peak integration. Thus for further work 0.09 min injection time was used.

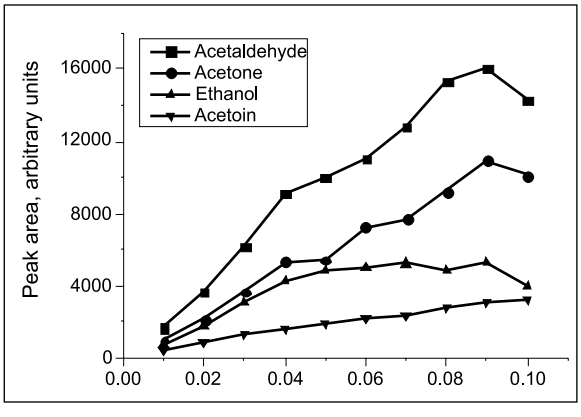

Fig. 3. Influence of injection time on headspace extraction efficiency. Extraction temperature 90 °C, extraction time 20 min, pressurization time 2 min

Validation of the method

For aqueous solutions containing the analytes of interest, quality parameters such as linearity, limits of detection and repeatabilities were determined under the optimized HS conditions. The calibration curves were drawn with 8 calibration points with three replicate injections. Limits of detection (LOD) were defined as three times of base-line noise. The repeatabilities were determined by five-repetition analysis for 10 and 100 mg l^{-1} of the analytes. Linear ranges, correlation coefficients, limits of detection and relative standard deviations are presented in Table 1.

Table 1. **Analytical characteristics for aqueous solutions**

Analyte	Linear range, mg l^{-1}	R^2	LOD, µg l^{-1}	RSD, %	
				10 mg l^{-1}	100 mg l^{-1}
Acetaldehyde	0.003–200	0.997	1.8	2.5	3.4
Acetone	0.005–200	0.995	3.0	3.4	4.2
Ethanol	0.011–200	0.999	6.6	4.1	4.4
Acetoin	0.064–200	0.998	38.4	8.8	4.9

Yogurt analysis

The results obtained indicate that the proposed static HS-GC method could be successfully used for the determination of acetaldehyde, acetone, ethanol and acetoin in water. However, yogurt is a much more complicated matrix and the transition of the volatiles to the headspace could be aggravated by its heavy consistency and large fat content. Thus further experiments were carried out in order to check the repeatability of the results obtained for yogurt.

To 10 g of yogurt 400 µl of an aqueous standard solution containing 100 mg l^{-1} of each analyte were added, the vial was closed and left for 5 min in an ultrasonic bath to mix. After that, an HS-GC analysis under the above-presented conditions was accomplished. Unfortunately, the repeatability of the results obtained was unsatisfactory, relative standard deviations of five replicate analyses for all the analytes exceeded 25%. We made an assumption that two reasons can be responsible for a poor repeatability. The first one is that a transition of the analytes to the headspace could be impeded by a thick and dense yogurt matrix. The second probable reason is inhomogeneity of a mixed yogurt–standard solution.

To verify the first reason, repeatabilities for 2 and 10 g of yogurt were examined without an addition of a standard solution. In the both cases the repeatabilities were similar and did not exceed 10% (Table 2).

Table 2. **Yogurt peak area repeatabilities (n = 5)**

Analyte	RSD, % (n = 5)	
	2 g of yogurt	10 g of yogurt
Acetaldehyde	6.3	4.3
Acetone	5.6	5.6
Ethanol	5.3	5.5
Acetoin	8.0	9.8

In order to check the second probable reason of bad repeatability, a standard solution of the analytes was added to 10 g of yogurt, to 2 g of yogurt and to homogenized mixtures containing: 2 g of yogurt and 1 g of water, 2 g of yogurt and 2 g of water, and 2 g of yogurt and 4 g of water. As can be seen from the results presented in Table 3, better repeatability of the results was obtained from the yogurt–water mixtures. On the other hand, an addition of water results in the decrease

of extraction efficiency (Fig. 4) as the analytes are soluble in water. For further work, in order to get satisfactory extraction efficiency and at the same time a good repeatability of the results, the yogurt–water ratio 1:1 was maintained.

Table 3. **Spiked yogurt peak area repeatabilities (n = 5)**

Analyte	RSD, %				
	10 g of yogurt	2 g of yogurt	2 g of yogurt and 1 g of water	2 g of yogurt and 2 g of water	2 g of yogurt and 4 g of water
Acetaldehyde	21.2	4.4	2.5	2.2	5.6
Acetone	25.2	4.5	4.0	2.6	2.9
Ethanol	23.9	8.7	4.8	5.0	7.0
Acetoin	24.2	7.5	4.3	3.6	4.2

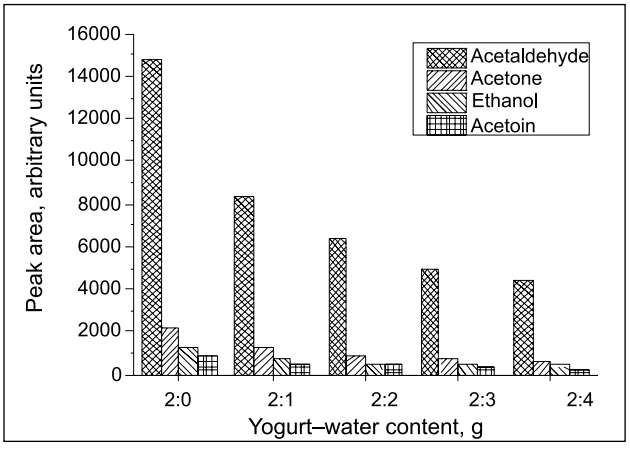

Fig. 4. Influence of the yogurt and water content on peak areas of the analytes. Extraction temperature 90 °C, extraction time 20 min, pressurization time 2 min, injection time 0.09 min

The influence of the yogurt–water (1:1) content on the extraction efficiency was determined. As the results presented in Fig. 5 demonstrate, the sample content had a negligible effect on acetone, ethanol and acetoin peak areas, only peak areas of acetaldehyde slightly increased with the sample content. Based on the results, 12 g of the yogurt–water (1:1) mixture was used for the analysis.

Application

The prepared HS-GC method was applied for the analysis of four different yogurts: "Turkisk", "Aistė Natural with Honey", "Dobilas Natural" and "Dobilas with Lemon and Ginger". The quantification was made using the standard addition method. Yogurt was diluted with distilled water at the ratio 1:1. Three portions of the mixture (12 g each) were analysed: the first portion was not spiked, the second portion was spiked with 100 µl of the standard solution containing 100 mg l^{-1} of each analyte, the third portion was spiked with 200 µl of the standard solution.

The results of the analysis are presented in Table 4. They are in correlation with those presented in the literature: 2–80 mg kg^{-1} for acetaldehyde, 1.8–11 mg kg^{-1} for acetone, 0.2–45 mg kg^{-1} for ethanol and 2.2–28.2 mg kg^{-1} for acetoin [12]. The only exception is ethanol concentration in "Dobilas with Lemon and Ginger". This yogurt is flavoured and likely the peak of added flavour interferes with that of ethanol. Thus for the analysis of this yogurt type, gas chromatographic conditions should be modified in order to achieve better separation of volatile components.

Table 4. **Results of yogurt analysis, mg kg^{-1} (n = 3)**

Yogurt	Acetaldehyde	Acetone	Ethanol	Acetoin
Turkisk	33.5 ± 2.8	4.9 ± 0.2	13.6 ± 1.4	2.9 ± 0.4
Aistė Natural with Honey	30.3 ± 3.9	4.2 ± 0.3	16.6 ± 1.3	3.2 ± 0.5
Dobilas Natural	35.1 ± 4.1	7.7 ± 0.6	35.4 ± 5.7	5.7 ± 0.9
Dobilas with Lemon and Ginger	46.9 ± 2.5	12.8 ± 1.3	188 ± 15	3.1 ± 0.5

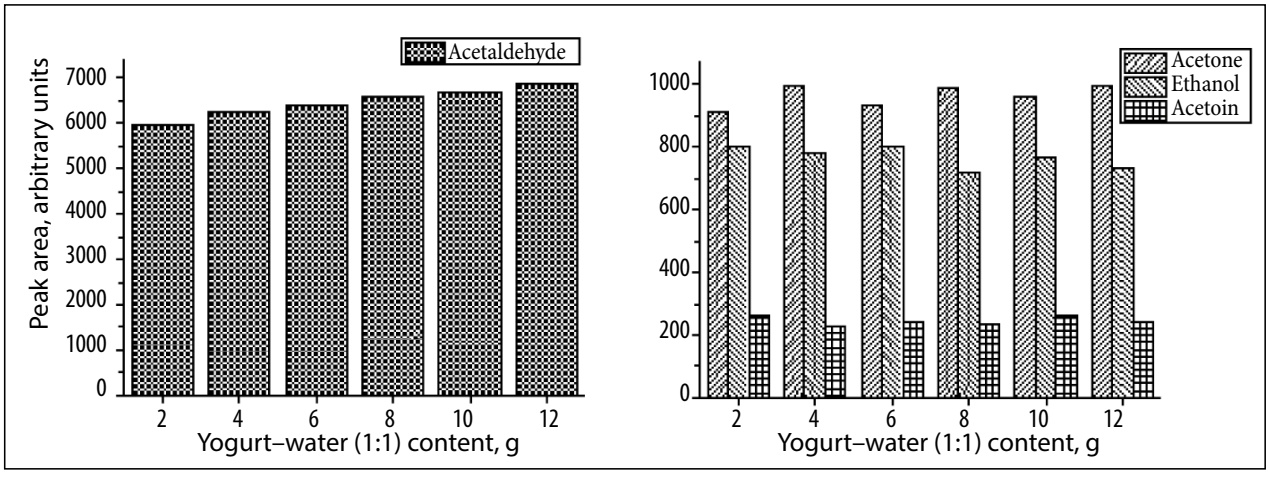

Fig. 5. Influence of the yogurt–water mixture (1:1) content on peak areas of the analytes. Extraction temperature 90 °C, extraction time 20 min, pressurization time 2 min, injection time 0.09 min

CONCLUSIONS

In this work, headspace-gas chromatographic conditions for the analysis of yogurt were optimized and it was demonstrated that static headspace-gas chromatography can be successfully applied for the determination of volatile yogurt components. For the quantification of the analytes a standard addition method should be applied. Before the analysis dense yogurts should be diluted with water in order to facilitate the transition of the analytes to the headspace and to reach a homogeneous distribution of the spiked standard in the yogurt.

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LAKIŲ JOGURTO KOMPONENTŲ STATINĖ DUJŲ CHROMATOGRAFINĖ VIRŠERDVĖS ANALIZĖ

S a n t r a u k a

Acetaldehidui, acetonui, etanoliui ir acetoinui jogurte nustatyti pasiūlyta statinė dujų chromatografinė viršerdvės analizė. Optimizuotos viršerdvės ekstrakcijos sąlygos: termostatavimas – 20 min. 90 °C temperatūroje, suslėgimo trukmė – 2 min., mėginio įleidimo trukmė – 0,09 min. Prieš viršerdvės ekstrakciją jogurtas buvo skiedžiamas vandeniu. Analičių kiekis įvertintas naudojant priedų metodą.