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DEVELOPMENT OF THE METHOD OF MILK PRODUCTS ANALYSIS BY HEADSPACE SOLID-PHASE MICROEXTRACTION WITH GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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ABSTRACT

Demand of dairy products, especially yogurts, increases year to year in Kazakhstan; it has a significant contribution to the market, developing the range of yogurts. Due to its wide consumption and acceptance, science interests on the analysis of dairy products. However not all method are able to analyze the composition of yogurts, due to the complex compound and peculiar structure. For this purpose solid-phase micro extraction (SPME) technique was developed and coupled to subsequent gas chromatography–mass spectrometry (GC-MS) analysis. This work describes an analytical procedure for determination of hazardous components in yogurts, using this method that allows identifying the components even in very small concentrations. The proposed method provides a rapid, environmentally friendly and accurate method for identification and quantification hazardous components in yogurt, during the analysis was developed optimization of fiber selection, temperature of extraction, time of extraction and preincubation time. Optimization was provided between the five different fibers for the spme analysis. During the analysis, it was established that polyacrylate fiber is the most selective and allows determining as more as possible components in sample, whereas vials was extracted during the time of 30 min as suitable under 80 °C. Quantification of compounds was performed via external calibration. The newly developed method was subsequently applied to 11 kinds of yogurt, incubating for 30 min as optimal. As expected, the emission of components from yogurt samples depends on the temperature and on fiber selection.

Keywords: yogurt, SPME, GC-MS, optimization, food safety.

INTRODUCTION

Milk and dairy products contribute an important role to human body. They are considered as the source of variety of nutrients, and influence on the exchange of calcium and phosphorus [1-2]. Yoghurt is a product of the lactic acid fermentation of milk produced by homofermentative lactic acid bacteria Streptococcus thermophilus and Lactobacillus delbrueckii subsp. Bulgaricus [3]. Over recent years an increase in the consumption of yoghurt and other acid fermented dairy products has been observed due

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to their nutritional properties and indispensable benefits [4]. Yoghurt is one of the frequently consumed dairy products in Kazakhstan [5]. The milk market reaches in Kazakhstan year by year, leading on the development of the variety and quantity of production of milk products [6]. Food preference of consumers usually depends on the acceptance of flavor especially. The flavor of natural or plain yoghurt is a weak, but in time it has characteristic and own taste, that is affected by such factors as volatile and nonvolatile component, composition, viscosity [7]. The essential indicators of yogurt that influence on the preference of consumer are acidity, aroma perceptions and textural properties of the product [8]. There is a great responsibility and resources are needed to produce high qualitative milk products in large quantities, which lead to the unscrupulous production of manufacturers, adding the harmful things into the contamination of yogurt. Such cheap and available products can be chemicals such as emulsifiers, flavorings, colorants preservatives that are able to replace the natural products [9].

The analysis of processed dairy products, like yogurt, is not only scientific interest, but also a wide consumption and acceptance [10]. Dairy products, especially yogurt, are highly complex compound, which is needed scrupulous analysis, demanding special procedures for sample preparation to achieve an excellent analytical determination [11]. Therefore, it is important to develop new analytical methods that allow to uncover the composition of complex matrices in a simplify way. Chromatographic methods allow identifying the components even in very small concentrations that is why this method is acceptable for analysis of hazardous components in dairy products [12-14]. The solid-phase microextraction followed by gas chromatography-mass spectrometry method (SPME-GC-MS) is widespread method for identify and quantify the volatile compounds in liquid food, such as milk products [15]. In this work, a SPME-GC-MS method was developed, identifying and quantifying contaminants of yogurts, optimizing the fiber selection, temperature of extraction, time of extraction and preincubation time for analysis.

EXPERIMENTAL PART

Materials and samples

Five different fiber coatings were employed in the present work: 85 mμ polyacrylate (PA), 85 mμ carboxen/polydimethylsiloxane (CAR/PDMS), 65 mμ polydimethylsiloxane/divinylbenzene (PDMS/DVB), 50/30 mμ divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 100 mμ polydimethylsiloxane (PDMS). All fibers were purchased from Supelco (Bellafonte, PA, USA). Fibers were conditioned according to manufacturer's recommendations.

For method development, the popular yogurt "ABVGdeika" (Agroproduct LLP, Almaty, Kazakhstan) was chosen as a model sample. For method approbation, 11 different yogurt samples, which were purchased from the stores, were analyzed.

Parameters of analysis

GC-MS analysis was performed using Multi-Purpose Sampler (Gerstel, Germany) installed on 7890N/5975C GC/MS system (Agilent, USA) equipped with a

split/splitless injector. The sample was injected into the inlet heated to 250 °C in splitless mode. Separation was conducted on DB-WAXetr column (60 m × 0.25 mm × 0.50 μm, Agilent, USA) at a constant flow rate of helium of 1.0 mL/min. The oven temperature was programmed from 40 °C to 100 °C at a rate of 15 °C/min, then to 240 °C (held for 20 min) at a heating rate of 10 °C/min. Temperatures of MS source, quadrupole and were set to 230 and 150 °C, respectively. Total run time was 38 min.

2 g of each yogurt in a 20 mL headspace vial were taken for analysis. Effect of extraction time, extraction temperature, incubation time was studied. Extraction time and incubation time were selected by 15, 30, 45 min occasionally. Extraction temperature was 23 °C, 40 °C, and 80 °C exceedingly.

RESULTS AND DISCUSSION

Selection of the Fiber Coating

The absorption of the fiber coating is the key parameter that governs the selectivity and recovery of SPME. Thus, the selection of a suitable fiber coating is an important step in SPME optimization. In this study, five different fiber coatings: PDMS, CAR/PDMS, PA, PDMS/DVB, and DVB/CAR/PDMS were evaluated. The extraction efficiency of different fibers was expressed as the number of obtained compounds. The higher extraction efficiency was achieved with PA. However, CAR/PDMS had higher extraction capacities for benzoic and sorbic acids (Figure 1).

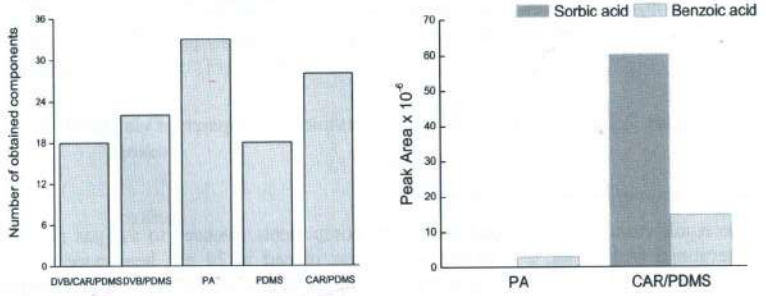

Figure 1. Number of total components and the peak area of selected compounds identified by different fiber coatings

As result from the fiber screening it could be concluded that the extraction of yogurt ingredients from the headspace can be carried out with all fibers. Desorption from the polar PA material was more complete. By contrast, extraction with CAR/PDMS fiber revealed that certain analytes like benzoic and sorbic acids has more efficient

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desorption, and it was selected as the optimal for them. However PA was more suitable for further optimization accordingly its extraction efficiency.

Optimization of extraction temperature

To figure out the appropriate extraction time 2 g of the yogurt was placed in 20 mL headspace vials and incubated for 30 min at 23, 40 and 80 °C. After incubation, the PA fiber was exposed to the headspace for 15, 30 and 45 min. Figure 2 illustrates the time-, and temperature-dependency of total peak areas of all components in yogurt. The optimum of the extraction efficiency already occurs at high temperature of 80 °C. As shown on the left of Fig. 2 no increase was observed until 30 min, and further peak area decreased. For this reason, an extraction time of 15 min has been chosen for all subsequent HS-SPME GC-MS analyses performed at 80 °C.

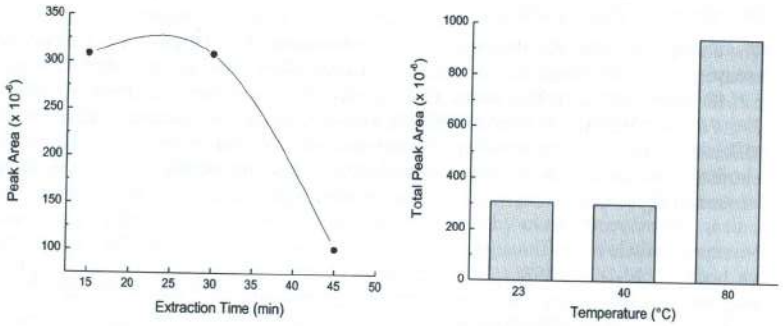

Figure 2. Effect of extraction time and extraction temperature via HS-SPME

Optimization of preincubation time

The equilibration time for total and target components released to the gas phase was determined as follows: 2 g of the yogurt was placed in 20 mL headspace vials and incubated for 15, 30 and 45 min at the selected optimal temperature 80 °C. Experiments were carried out to optimize the preincubation temperature. But it was decided to incubate the sample at the selected extraction temperature, that is, at 80 °C.

The extraction time was set up to 15 min. Whereas the equilibrium was already reached after 15 min, then peak areas started to decrease after this period (Fig. 3). This effect might be due to desorption processes already started during equilibration. As shown in Figure 3, at time of 30 min and 45 min the amount of determined compounds were almost equal.

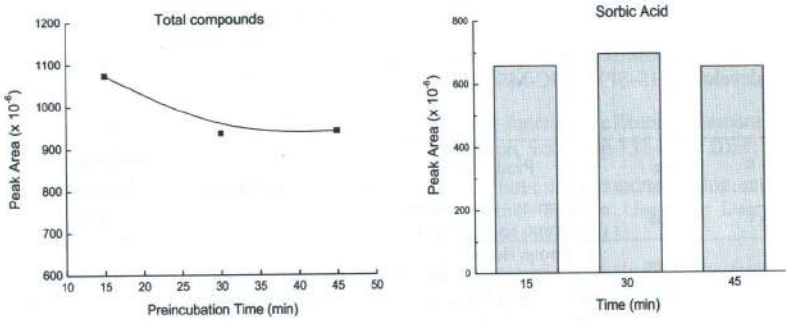

Figure 3. Effect of preincubation time on extraction efficience of total and certain components in yogurt

As the consequence from this testing, the equilibration time used for further experiments at 80 °C was 30 min.

Approbation of method and investigation of real yogurt samples

The established HS-SPME GC-MS method (qualification and quantification amount) were applied to 11 samples of yogurt. Among volatile ingredients, sorbic and benzoic acids were predominant (Figure 4).

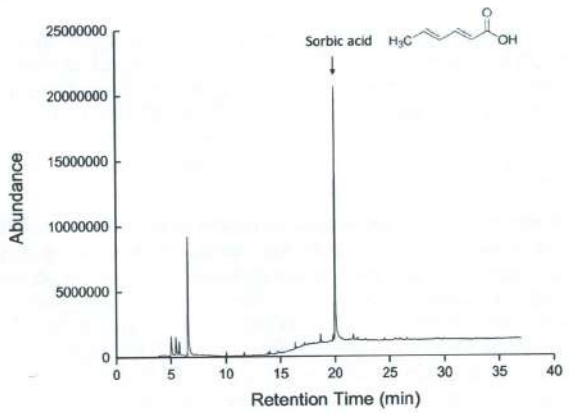

Figure 4. The chromatogram of the sample No. 9

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The content of priority preservatives, sorbic and benzoic acids, is shown in Table 1.

Table 1 – The results of determining the priority preservatives in yogurt samples by developed HS-SPME GC-MS method

#	Name	Producer	Fat, %	Content, mg/kg	
				Sorbic acid	Benzoic acid
1	Fruttis	"Campina" Ltd, Russia, Stupino t., Sitenko st.	5.0	0.1	0.2
2	Danone	"Danone Berkut" LLP, Kazakhstan, Almaty	1.5	-	0.2
3	Uslada	"Ehrmann" LLP, Russia, Moscow d., Ramen st.	1.2	-	0.1
4	Alpenland	"Ehrmann" LLP, Russia, Moscow d., Ramen st.	5.0	-	0.1
5	Ermigurt	"Ehrmann" LLP, Russia, Moscow d., Ramen st.	3.2	-	0.1
6	Chudo	Vim-Bil-Dan LLP, Rossia, Moscow, Dmitrivskoe st.	3.2	-	0.1
7	Alpengurt	"Ehrmann" LLP, Russia, Moscow d., Ramen st.	5.0	-	0.1
8	Alpengurt	"Ehrmann" LLP, Russia, Moscow d., Ramen st.	7.0	-	0.1
9	Abvgdeika	"Agroproduct" LLP, Almaty, Kazakhstan	8.0	3.0	0.1
10	Dolce	"FoodMaster" Ltd, Kazakhstan, Almaty	3.2	0.4	0.1
11	Fruttis	"Campina" Ltd, Russia, Stupino t., Sitenko st.	8.0	0.1	0.1

Benzoic acid have been detected in all yogurt samples, and the highest amount of sorbic acid was established in sample 9, which was chosen like model sample for method optimization. Since these products do not belong to the class of baby food, the established content of found preservatives does not exceed the permissible standards.

CONCLUSION

In this research was established and verified a particular HS-SPME GC-MS approach for detection and quantification of total and certain components in yogurts. The parameters of analysis were optimized. It was investigated that the most absorption of components from the sample achieves PA fiber, however for some components CAR/PDMS is selective, so the fiber selection depends on the analyte that is going to be studied. The optimum of the extraction efficiency occurs at high temperature of 80 °C, for these conditions of analyzes 15 min for extraction is relevant. Also, at all times of preincubation, means 15, 30, 45 minutes were not noticed a big difference in the rate of determined components, however for the more precise analysis it is more expedient to

use only 30 min for preincubation. Adhering to the conditions for analysis provided above, we get alternative results, which would be optimal for the further research.

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