VILNIUS UNIVERSITY
CENTER OF PHYSICAL SCIENCE AND TECHNOLOGY

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Headspace gas chromatography for the determination of carboxylic acids and hexanal

SUMMARY OF DOCTORAL DISSERTATION

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FIZINIŲ IR TECHNOLOGIJOS MOKSLŲ CENTRAS

Birutė Bugelytė

Viršerdvės dujų chromatografija karboksirūgščių ir heksanalio nustatymui

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INTRODUCTION

Sample preparation is the most time and labour consuming analytical procedure. Various extraction methods are used when preparing samples for analysis. One of the most popular methods for liquid samples preparation is liquid-liquid extraction. It is widely used because of its simplicity and wide applicability, but requires large amounts of expensive and toxic organic solvents. Preparation of solid samples is even more complicated. For this, Soxhlet extraction is often applied. It requires large amounts of solvents and a long sample preparation time. It is particularly challenging in the case of volatile analytes which are easy to lose when analysing both liquid and solid samples.

Headspace gas chromatographic analysis (HS-GC) is increasingly used to determine volatile analytes from solid and liquid samples. In HS-GC the sample is heated in a closed vessel. Volatile compounds partially pass into the gas phase and when equilibrium is reached a portion of the gas phase is injected into the gas chromatograph. This prevents non-volatile components of the sample from entering a chromatographic system. HS-GC analysis uses small amounts of solvents, requires a shorter analysis time and can use environmentally friendly solvents.

In this work, the advantages of HS-GC are demonstrated in the development of methods for the determination of short-chain carboxylic acids and hexanal.

Carboxylic acids are often found in fats, citrus fruits and fermented foods. Also, they can be added to food products as preservatives. It is Because these substances can affect the taste, texture, aromatic properties of food, it is important to identify them and to consider their amounts.

Edible oils are a very important part of the human diet. They are an important source of energy for the body, are rich in nutrients and vitamins that are soluble only in fat. Some unsaturated fatty acids are not synthesized in the body, so they are considered irreplaceable. It is

necessary that the required amount of vegetable fat enters the body with food. However, unsaturated fatty acids can be oxidized. As a result of their oxidation, the food turns rancid, the nutritional value decreases and there is a risk of poisoning by lipid oxidation products. Because of this, it is important to be able to quickly detect fat oxidation process.

During lipid oxidation, a number of lipid oxidation products are formed. One of secondary oxidation product of linoleic acid is hexanal. Hexanal is considered as an indicator of fat oxidation.

The goal of present work was to investigate the possibilities of headspace gas chromatography for the determination of some short-chain carboxylic acids and hexanal in solid and liquid matrices and to apply the developed methodologies to the real samples analysis.

In order to reach the goal **major tasks** were designed:

1. To select a derivatization reagent and to evaluate the influence of derivatization on the shape of the chromatographic peaks of acetic, propionic, oxalic and succinic acids.

2. To prepare methodologies for headspace gas chromatographic determination of derivatized oxalic, succinic, lactic, malic and citric acids, to compare the determination of carboxylic acids using headspace gas chromatography and traditional gas chromatography.

3. To apply the prepared methods for the determination of carboxylic acids to the analysis of real samples.

4. To select a solvent suitable for the preparation of hexanal calibration solutions; to optimaize the conditions of hexanal HS-GC analysis and to apply the prepared method for the determination of hexanal in edible oils.

5. To investigate the possibility of using deep eutectic solvents (DESs) for the extraction of hexanal from solid matrices.

6. To combine microwave-assisted extraction of hexanal from solid matrices using deep eutectic solvents, and headspace gas chromatographic analysis. To prepare a rapid method for the determination of hexanal in fat rich food.

Main statements for the defence

1. Derivatization of carboxylic acids improves the symmetry of the chromatographic peaks.

2. Headspace gas chromatography can be applied to the determination of short-chain carboxylic acids and hexanal in liquid and solid matrices.

3. Deep eutectic solvents are suitable for microwave-assisted extraction of hexanal from solid matrices.

4. Microwave-assisted extraction of hexanal combined with headspace gas chromatographic analysis simplifies the procedure of complex sample preparation and shortens the analysis time.

Structure of dissertation

The doctoral dissertation is written in Lithuanian language and consists of eight chapters: abbreviations, introduction, literature review, methodology, results and discussion, conclusions, list of publications and list of references.

1. Literature review

The literature review consists of four parts. The first part provides an overview of the headspace gas chromatographic analysis. The second part reviews deep eutectic solvents. In the third part presents lipid oxidation indicator. The last section provides an overview of carboxylic acids.

2. Methodology

This section consists of seven chapters to describe the experimental part of the dissertation. It describes the reagents, solutions, sample preparation and equipment used during the study. There are also included conditions of gas chromatographic analysis. The seventh chapters describes statistical evaluation of results.

3. Results and discussion

This chapter consists of two sections. The first section describes headspace gas chromatographic determination of carboxylic acids. The second section describes headspace gas chromatographic determination of hexanal.

3.1. Headspace gas chromatographic analysis of carboxylic acids

Short-chain carboxylic acids are found in food, mainly in fermented products as a result of animal metabolism, hydrolysis and microbial activity during manufacture and storage. Usually, short-chain organic acids quantities are not regulated and allowed quantum

satis. However, their determination is important as the acids affect flavour, texture, aromatic properties and bacterial growth, they can serve as authenticity and ageing markers.

3.1.1. Headspace gas chromatographic analysis of acetic, propionic, oxalic and succinic acids

Usually, carboxylic acids are determinated after derivatization. However, there are some works on the direct gas chromatography determination of short-chain carboxylic acids without their derivatization. To verify this possibility, the solutions of the acetic, propionic, oxalic and succunic acids in acetone were directly injected into the gas chromatography system and the peaks symmetry was evaluated (Table 1). This preliminary examination demonstrated that the peak shapes were not acceptable and a derivatization of the acids was indispensable.

Table 1. Peak symmetry at 10 % height.

	Non-derivatized	**Derivatized**
Acetic acid	0.561	1.033
Propionic acid	0.022	0.870
Oxalic acid	–	0.792
Succinic acid	–	0.913

Since the aim was to prepare the simple, solvent extraction-free method for the determination of carboxylic acids in food, the derivatization technique had to be water insensitive.

Derivatization of the carboxylic acids was accomplished by tributyl borate and concentrated HCl as a catalyst. The mixture was heated at 95 °C temperature for 40 min and then derivatization products were extracted with 1 ml of hexane and the hexane phase was injected into the gas chromatography for analysis. As can be seen in Table 1 the symmetry of derivatized acids significantly improved.

Optimization of headspace gas chromatographic conditions. The next step of the work was to examine the possibility to determine the carboxylic acids by headspace gas chromatography.

A derivatization procedure was carried out in the headspace vial at the conditions described above and the gaseous phase was injected for the analysis. Unfortunately, the peaks of oxalic and succinic acids butyl esters were absent in the chromatograms. Probably their volatility at the selected temperature was too low to be detected by headspace gas chromatography. Thus, further investigation was carried out on two acids – acetic and propionic.

Analytical characteristics of the suggested technique at the optimized derivatization/headspace extraction conditions (derivatization/heating temperature – 95 °C; derivatization/ equilibration time – 30 min) were determined. The results are presented in Table 2.

Table 2. Analytical characteristics of acetic and propionic acids.

Characteristics	**Value (acetic acid)**	**Value (propionic acid)**
The limits of detection, mg/l	0.9	3.0
Linear range, g/l	0.003 – 0.5	0.01 – 0.5
R^2	0.9970	0.9950
RSD, % (n = 5, c = 50 mg/l)	2.5	3.4

Table 3. Results of real sample analysis.

	Value (acetic acid, g/kg (n = 3)	**Value (propionic acid, g/kg (n = 3)**
Kefir	1.8 ± 0.1	0.14 ± 0.01
Ketchup	7.8 ± 0.4	–
Tomato paste	2.4 ± 0.1	–
Bread	3.9 ± 0.2	–
Croissant	1.2 ± 0.1	0.83 ± 0.05
Biscuits	1.1 ± 0.1	0.49 ± 0.03

Real sample analysis. The headspace gas chromatographic technique was applied for the determination acetic and propionic acids in food products: bakery products, ketchup, tomato paste and kefir. The results of the analyses are presented in Table 3.

Acetic acid was determined in all the examined samples. Propionic acid was present in kefir, croissant and biscuits. The croissant was the only product for which the concentration of calcium propionate was indicated by producers (< 0.1 %). This is consistent with the results obtained.

3.1.2. Headspace gas chromatographic determination of lactic, malic, citric, oxalic and succinic acids

In order to expand the limits of the method, it was decided to change the derivatization reagent. For derivatization, we have chosen N,O-Bis-(trimethylsilyl)trifluoroacetamide (BSTFA), as the reaction can be expected to be complete at room temperature.

Direct gas chromatographic determination of carboxylic acids. BSTFA reagent is moisture sensitive, therefore three solvents – acetone, diethyl ether (DEE) and dimethylformamide (DMF) – were tested as a derivatization medium. For this, to 1 ml of succinic acid solutions (1 g/l) 50 µl of BSTFA was added and after 20 min direct GC analysis was carried out. The analysis revealed that derivatization in acetone was not complete. In the chromatogram obtained after the derivatization a relatively small peak of a succinic acid derivatization product emerges at 12.25 min. However, a huge asymmetric peak of underivatized succinic acid at 10.70 min is still present as in the chromatogram obtained before derivatization (Fig. 1A). When the solvent acetone was replaced with DEE or DMF, the derivatization was complete (chromatograms of succinic acid in DEE before and after derivatization are presented in Fig. 1B).

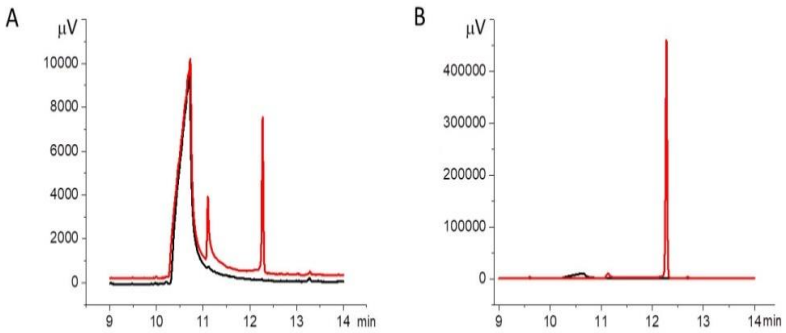

Figure 1. Chromatograms of succinic acid (1 g/l) in acetone (A) and in DEE (B) before (black) and after (red) derivatization.

We decided in favour of less volatile solvent DMF (boiling point 152 °C).

5 – 50 μl of BSTFA was added to 1 ml of 1 g/l solutions of oxalic, lactic, succinic, malic and citric acids in DMF, and derivatization was carried out for 20 min. The results of the gas chromatography analysis demonstrated that for all the acids 30 μl of BSTFA was sufficient.

Further, the effect of derivatization time on the derivatization yield was studied. The results showed that gas chromatographic analysis of the carboxylic acids can be performed immediately after the derivatization reagent is added.

In order to correct the loss of analyte during sample handling, an internal standard should be added to the analyte solutions. For lactic, malic, citric and oxalic acids, n-tridecane was selected as an internal standard. Only in the case of succinic acid, the peak of n-tridecane overlapped with the peak of the analyte, so n-tetradecane was chosen.

Analytical characteristics of the suggested method at the optimized derivatization conditions were determined. The results are presented in Table 4.

Table 4. Analytical characteristics of carboxylic acids obtained by direct gas chromatography.

	Retention times, min	Relative standart deviations, % (c = 10 mg/l, n = 5)	R^2	Limits of detection, mg/l
Lactic acid	8.48	2.2	0.9990	0.27
Oxalic acid	9.61	4.9	0.9960	0.55
Succinic acid	12.25	4.5	0.9970	0.59
Malic acid	14.65	4.2	0.9980	0.51
Citric acid	18.58	3.1	0.9990	0.33

Headspace gas chromatographic determination of carboxylic acids. The next step of the work was to examine the possibility to determine the analytes of interest by headspace gas chromatography. The derivatization conditions were the same as in the case of direct gas chromatography determination. The samples were heated at 140 °C temperature for 20 min. Unfortunately, the concentrations of the derivates in the headspace were very low, thus the limits of detection were high and not of practical use. Citric acid was not detected at all.

In order to obtain lower LOD, the solvent with a higher boiling point should be chosen. On the other hand, headspace gas chromatography is suitable for identifying the acids in dry samples.

Real sample analysis. The possibility to apply headspace gas chromatography for identifying of carboxylic acids is illustrated by the determination of succinic acid in amber. We have analysed the amber collected at the seaside of Palanga, excavated in the Kaliningrad region and originating from Japan. In the chromatograms of Palanga and Kaliningrad amber, a peak of succinic acid is clearly visible, while it is absent in the chromatogram of Japanese amber.

Headspace gas chromatography was also applied to detect citric acid in boiled smoked chicken sausages. Unfortunately, for citric acid quantification, the sensitivity of the headspace gas chromatographic method was not sufficient. Thus the direct gas

chromatography method was applied. It was determined that the sausages contained 0.12 mg/g of citric acid. However, differently from the headspace chromatogram, many peaks were observed in the chromatogram of the extract.

3.2. Headspace gas chromatographic determination of hexanal

During storage and especially during heating, unsaturated fatty acids of edible oils undergo oxidation. Oxidation has a great impact on the quality of food products because of off-flavours, of a decrease in the nutritional properties. Thus there is a great interest on a fast and simple detection of lipid oxidation.

Hexanal seems to be a suitable marker as it is the main secondary oxidation product of linoleic acid which is one of the principle fatty acids of many edible oils.

This chapter presents an investigation of the application of headspace gas chromatography for the determination of hexanal in liquid (edible oils) and solid matrices.

3.2.1. Determination of hexanal in edible oils

Four commonly used edible oils (olive oil, sunflower oil, rapeseed oil and linseed oil) were selected for the investigation. When developing a method for the determination of hexanal in edible oils, it is necessary to select the solvent, to optimize the amount of solvent, to select the heating temperature and time, to select the time of introduction of the gas phase into the gas chromatograph.

Solvent selection. the amount of hexanal in the gas phase increases with temperature. This was demonstrated by heating a solution of hexanal in DMF (1 g/l) at 90 – 140 °C for 10 min. On the other hand, it was also determined that when sunflower oil is heated above 110 °C temperature, the hexanal content increased because of the degradation of linoleic acid. The maximum equilibration

temperature that does not generate hexanal formation was determined to be 110 °C.

order to prepare standard hexanal solutions with a stable hexanal concentration that does not change for a long time, coconut oil was tested as a solvent. It was heated at 180 °C for 30 min and headspace gas chromatographic analysis was performed. The absence of a hexanal peak (at 5.74 min) in the chromatogram (Fig. 2) suggests that coconut oil can be used as a solvent for calibration solutions of hexanal.

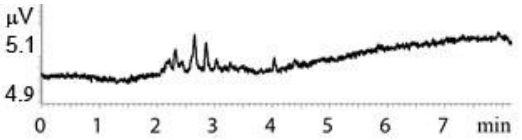

Figure 2. Headspace gas chromatography chromatogram of coconut oil heated at 180 °C for 30 min.

Sample amount and equilibration time. 1 – 10 g of coconut oil containing 1 g/kg of hexanal was equilibrated at 110 °C temperature for 30 min and headspace gas chromatographic analysis was performed. The results demonstrated that with the increase of the sample weight, the hexanal peak area initially increased but started to decrease when the sample size exceeded 5 g indicating that for big samples longer equilibration time is desired.

For the optimisation of equilibration time, 2 – 5 g of sample were investigated (Fig. 3). According to the results, for further work 3 g of sample was equilibrated for 22 min.

Gas phase injection time. The more gas phase isjected to GC, the bigger peak of hexanal should be observed. On the other hand, with the increase of injected gas phase volume, peaks can broaden and tail. We used an equipment supplied by pressure balanced sampling that allows direct control of the time width of the vapour plug entering the GC column. Injection time widths from 0.01 to 0.2 min have been examined. Based on the results (Fig. 4), in order to

establish the optimal conditions for the maximum recovery without a loss of efficiency, 0.07 min injection time was chosen.

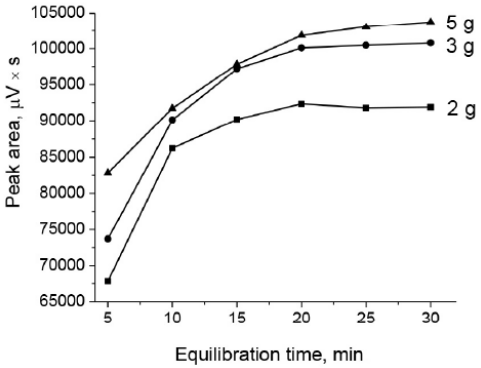

Figure 3. Influence of coconut sample (2, 3 and 5 g) equilibration time on the hexanal peak area. Hexanal concentration 0.1 g/kg, equilibrated at 110 °C temperature.

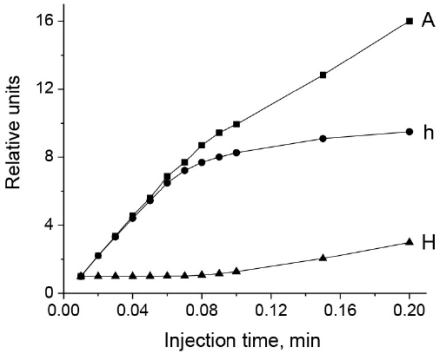

Figure 4. Relative hexanal peak area (A), height (h), and theoretical plate height (H) dependence on the injection time. The parameters at 0.01 min are considered equal to 1. Hexanal concentration 0.1 g/kg, equilibrated at 110 °C temperature for 22 min.

Method analytical characteristics. Analytical characteristics of the suggested method at the optimized conditions were determined. The results are presented in Table 5.

Table 5. Analytical characteristics of the method for headspace gas chromatographic determination of hexanal.

Characteristics	Value (coconut oil)
The limits of detection, μg/kg	30
Linear range, g/kg	0.0001 - 2
R^2	0.9977
RSD, % (n = 5, c = 10 mg/kg)	1.1

Real sample analysis. The developed headspace gas chromatographic method was applied for hexanal determination in four edible oils before and after their heating. The results are presented in the Table 6.

Table 6. Hexanal content in untreated and heated edible oils, mg/kg.

Oil	Untreated	180 °C, 10 min	200 °C, 10 min	200 °C, 30 min
Sunflower	3.3	20.6	31.2	36.1
Olive	3.1	7.3	26.1	26.9
Linseed	2.6	10.5	15.8	16.2
Rapeseed	2.5	7.7	10.7	11.8

The biggest quantity of hexanal was observed in sunflower oil and it significantly increased after heating. Rapeseed oil is the most resistant to the oxidation at elevated temperatures. For linseed oil hexanal is not the most relevant oxidation marker as hexanal is not the main volatile oxidation product.

3.2.2. Determination of hexanal in fat rich food

Tetradecane was suggested as a solvent for hexanal release from the sample. Solvent amount has an influence on the analytes concentration in the headspace. An increase in solvent volume can improve the extraction of hexanal from a solid matrix. On the other hand, diffusion of the analyte through a thick layer of a solvent can require longer equilibration time. For solvent amount optimization,

0.5 – 5 ml of n-tetradecane containing 0.1 g/l of hexanal was equilibrated at 110 °C temperature for 30 min. The results demonstrated that for 1.5 – 5 ml solution volume hexanal peak areas were quite stable and a slight decrease in the peak area was observed only up to the 1.5 ml liquid phase volume. Thus 2 ml n-tetradecane amount has been chosen.

When 2 ml of n-tetradecane was used, the hexanal peak area has maximized at about 10 min and after remained stable. The optimum equilibration time was set to 12 min.

The gas phase injection time was chosen based on the results describes in Section 3.2.1 and was 0.07 min.

In order to achieve a better repeatability of the results benzaldehyde was selected as an internal standard.

Analytical characteristics of the suggested method at the optimized conditions were determined. The results are presented in Table 7.

Table 7. Analytical characteristics of the method for headspace gas chromatographic determination of hexanal.

Charakteristics	**Value (n-tetradecane)**
The limits of detection, µg/l	15
Linear range, g/l	0.00005 – 2
R^2	0.9994
RSD, % (n = 5, c = 1 g/l)	1.2

Real sample analysis. The developed headspace gas chromatographic method was applied for hexanal determination in potato chips and fried potatoes. 1 g of a grinded sample was placed into a 20 ml headspace vial and 2 ml of a 0.1 g/l benzaldehyde solution in n-tetradecane was added. The vial was hermetically capped, ultrasonicated for 10 min and subjected for headspace gas chromatographic analysis. The chromatograms of potato chips and fried potatoes headspace is presented in Fig. 5. and Fig. 6.

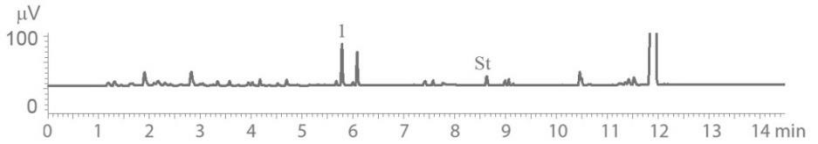

Figure 5. Chromatogram of fried potatoes extract. 1 – hexanal, St – benzaldehyde.

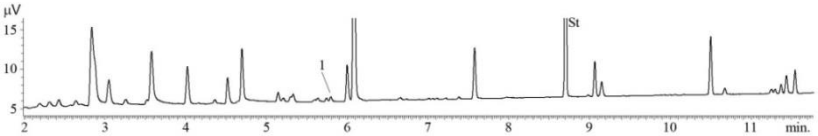

Figure 6. Chromatogram of chips extract. 1 – hexanal, St – benzaldehyde.

The results demonstrated that the concentration of hexanal in the examined potato chips was 1.2 mg/kg and in the fried potatoes 280 mg/kg. Such a big quantity of hexanal suggests that the edible oil for potatoes frying was heated at elevated temperatures for a long time.

3.2.3. Determination of hexanal using microwave-assisted extraction with deep eutectic solvents

The next step of the work was to improve the determination of hexanal in fat rich food by replacing n-tetradecane with another, more environmentally friendly and less volatile solvent. These requirements are met by deep eutectic solvents.

Moreover, more efficient extraction method – microwave-assisted extraction – was applied and combined with headspace gas chromatographic analysis.

Preparation of deep eutectic solvents. Eight DESs – choline chloride-ethylene glycol (ChCl-Eg), choline chloride-glycerol (ChCl-Gly), choline chloride-glucose-H_2O (ChCl-Glu-H_2O), choline chloride-oxalic acid (ChCl-Ox. a.), choline chloride-urea (ChCl-Urea), tetrabutylammonium hydrosulfate-octanol (TBAHS-Octanol), tetrabutylammonium hydrosulfate-octanoic acid (TBAHS-Oct. a.) and menthol-octanoic acid (Menthol-Oct. a.) – were prepared using microwave heating. Microwave-assisted preparation in comparison

with conventional heating reduced the preparation time from several hours to only 30 s. All the DESs were composed of two components except ChCl-Glu-H_2O which contained 30 % of water.

Headspace chromatograms of the synthesized DESs were obtained. For this, 0.5 g of DES were placed into the headspace vial and heated for 20 min. At 80 °C all the eutectic solvents tested were stable with no peaks in their headspace chromatograms. Above 100 °C heating temperature for all the DESs some peaks are visible in their chromatograms. The optimum temperature was chosen 80 °C.

Transition of hexanal from DESs to the headspace. Transition efficiency of hexanal from the DESs to the headspace was studied. For this, 5 μL of a 100 mg/mL solution of hexanal in n-tetradecane and 0.5 g of a DES were placed to the headspace vial and the vial was heated at 80 °C for 20 min. The results are presented in Fig. 7.

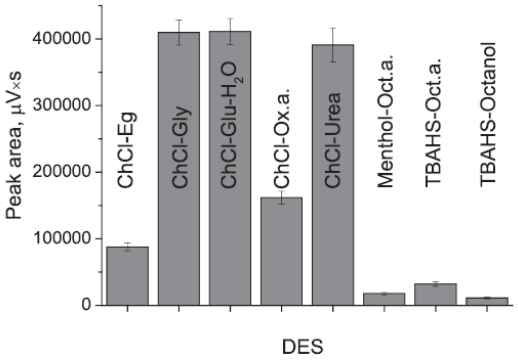

Figure 7. Hexanal peak area obtained by headspace gas chromatography using different extraction solvents. DES content 0.5 g, hexanal concentration 1 mg/g. Headspace vial (20 mL) heated at 80 ^{o}C for 20 min.

ChCl-Gly, ChCl-Glu-H_2O, and ChCl-Urea demonstrated the best release of hexanal to the headspace. These DESs were selected for the further work.

Microwave-assisted extraction of hexanal. Microwave-assisted extraction of hexanal from potato chips using ChCl-Gly,

ChCl-Glu-H_2O and ChCl-Urea was examined. Unfortunately, the areas of hexanal peaks obtained after microwave-assisted extraction from potato chips were 20 – 50 times smaller than those of direct headspace gas chromatorafy analysis of the solutions. This fact can be explained by a partial redissolution of hexanal in the fat present in potato chips after the mixture was cooled.

In order to increase the extraction efficiency, one more solvent, hydrophobic coconut oil, was applied. To prevent coconut oil from mixing with the DES, 100 μl of coconut oil was placed into a porous polypropylene capillary. A coconut oil-filled polypropylene capillary was fixed in an microwave-assisted extraction vial over a mixture of the sample and DES. Upon exposure to microwaves, the polar DES heats up, the volatile hexanal is extracted from potato chips to the DES, then to the gas phase, and subsequently dissolves in the coconut oil. After extraction, the microwave-assisted extraction vial is cooled. The capillary with the cooled extract is transferred to the headspace gas chromatographic vessel and the headspace gas chromatographic analysis is performed.

Microwave-assisted extraction and subsequent headspace gas chromatographic analysis of a mixture containing 5 μL of a 100 mg/mL solution of hexanal and 1 g of DES were performed. The results demonstrated that the highest hexanal yield was obtained using ChCl-Glu-H_2O.

Optimization of headspace gas chromatographic conditions. The influence of the headspace vial heating temperature (80 – 180 °C) on the hexanal peak area was examined. As expected, with the increase in heating temperature the area of the hexanal peak increased. However, when the temperature reached 160 °C, the polypropylene capillary melted. The headspace chromatograms obtained at 160 °C and higher heating temperatures, contain many extraneous peaks, probably polypropylene decomposition products (Figure 8). Therefore, 140 °C temperature was chosen.

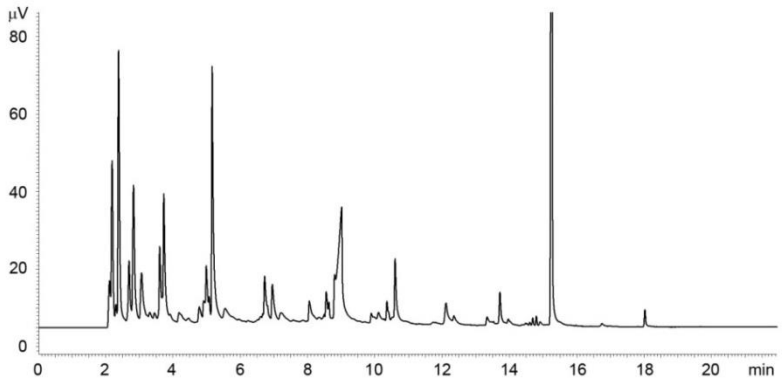

Figure 8. Headspace gas chromatogram of a polypropylene capillary heated to 160 ^{o}C.

At 140 °C temperature an influence of heating time on hexanal peak area was examined and 10 min heating time was chosen.

Optimization of microwave-assisted extraction conditions. The solvent quantity must be sufficient to guarantee that the entire sample is immersed in the solvent. On the other hand, too much of the extracting solvent may result in lower recoveries because of nonuniform distribution and exposure to microwaves. In many applications a solvent/sample ratio 10:1 was found to be optimal. Thus in our work 0.2 g of potato chips was extracted using 2 g of ChCl-Glu-H_2O.

The use of stirring in MAE accelerates the extraction by enhancing desorption of the analyte bound to the sample matrix and the mass transfer process in DES phase. Stirring also induces convection in the headspace. However, at high stirring speeds, potato chips were splashed on the walls of the vessel and their contact with DES was poor. Thus moderate stirring rate of 200 rpm was selected.

Extraction time from 5 to 30 min was examined. The highest extraction efficiency was obtained using 10 min extraction time.

Method analytical characteristics. Analytical characteristics of the method at the optimized conditions were determined. The results are presented in Table 8.

Table 8. Analytical characteristics of the method for headspace gas chromatographic determination of hexanal.

Charakteristics	Value (ChCl-Glu-H_2O)
The limits of detection, μg/kg	3.1
Linear range, mg/g	0.01 - 12.5
R^2	0.9915
RSD, % (n = 5)	11.7 (c = 0,05 mg/g)
	8.7 (c = 0,1 mg/g)
	5.6 (c = 1 mg/g)

Real sample analysis. The developed headspace gas chromatographic method was applied for hexanal determination in fat-rich food (potato chips, fried potatoes and fried black bread). The results demonstrated that the concentration of hexanal in potato chips was below the detection limit. The concentration of hexanal in fried potatoes purchased in a local restaurant was 5.5 μg/g. The biggest hexanal concentration (7.1 μg/g) was determined in fried black bread. It suggests that the edible oil for potatoes and bread frying was used several times.

CONCLUSIONS

1. It was found that the derivatization of acetic, propionic, oxalic and succinic acids with tributylborate in aqueous medium is complete and symmetric chromatographic peaks are obtained. A headspace gas chromatographic method for the determination of derivatized acetic and propionic acids has been prepared and applied for food analysis. The derivatization products of oxalic and succinic acids are not volatile enough to be determined in aqueous media by headspace gas chromatography.

2. The derivatization conditions of oxalic, succinic, lactic, malic and citric acids in non-aqueous solutions with the reagent BSTFA were investigated. The solvent chosen is DMF, in which the derivatization takes place completely. The methods of gas chromatographic analysis of solutions and of headspace gas chromatographic analysis of the derivatized acids have been prepared. Citric acid was not detected by headspace gas chromatography, when analyzing solutions. The method was applied to detect succinic acid in amber and to detect and identify citric acid in sausages.

3. Coconut oil is a suitable solvent for the preparation of hexanal calibration solutions for the determination of hexanal in edibles oils by headspace gas chromatography because: 1) the distribution of hexanal between coconut oil and the gas phase is close to that between rapeseed oil and the gas phase; 2) hexanal is not produced when coconut oil is heated at 180 °C.

4. The prepared coconut oil headspace gas chromatographic method for the determination hexanal was applied to the determination of hexanal in olive, rapeseed, sunflower and

linseed oils. The highest amount of hexanal was found in heated sunflower oil.

5. The extraction efficiency of hexanal from fat-rich food using microwave-assisted extraction with eutectic solvents was studied. Of the 8 solvents tested, hexanal was found to be best extracted ChCl-Glu-H_2O.

6. Two separated solvents – ChCl-Glu-H_2O and coconut oil – were used and microwave-assisted extraction of hexanal was combined with headspace gas chromatographic analysis was combined. A rapid method for the determination of hexanal in solid matrices was prepared.The method was applied for hexanal determination in potato chips, fried potatoes and fried black bread.

LIST OF PUBLICATIONS

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