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Static Headspace GC Method for Aldehydes Determination in Different Food Matrices

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Abstract**—**Aldehydes as secondary lipid oxidation products are highly specific to the oxidative degradation of particular polyunsaturated fatty acids present in foods. Gas chromatographic analysis of those volatile compounds has been widely used for monitoring of the deterioration of food products. Developed static headspace gas chromatography method using flame ionization detector (SHS GC FID) was applied to monitor the aldehydes present in processed foods such as bakery, meat and confectionary products.

Five selected aldehydes were determined in samples without any sample preparation, except grinding for bakery and meat products. SHS–GC analysis allows the separation of propanal, pentanal, hexanal, heptanal and octanal, within 15min. Aldehydes were quantified in fresh and stored samples, and the obtained range of aldehydes in crackers was 1.62±0.05 – 9.95±0.05mg/kg, in sausages 6.62±0.46 – 39.16±0.39mg/kg; and in cocoa spread cream 0.48±0.01 – 1.13±0.02mg/kg. Referring to the obtained results, the following can be concluded, proposed method is suitable for different types of samples, content of aldehydes varies depending on the type of a sample, and differs in fresh and stored samples of the same type.

Keywords**—**Lipid oxidation, aldehydes, crackers, sausage, cocoa cream spread.

I. Introduction

DURING processing, storage and handling of lipid containing food, lipid oxidation causes its quality deterioration, off-flavors formation and thus contributes to the development of its specific taste and aroma. Series of lipid oxidation reactions lead to the formation a large number of decomposition products. Primary products of lipid oxidation are lipid hydroperoxides which are unstable and rapidly decompose yielding a wide range of secondary lipid oxidation products.

A small proportion of the formed secondary lipid oxidation products are volatile compounds. Some of them, such as aldehydes, are highly specific to the oxidative degradation of particular polyunsaturated fatty acids [1]. Aldehydes are important lipid-derived volatiles as they have low odor threshold values and can produce a wide range of flavors and odors. Since their perception thresholds are very low, they are important to the aroma even at trace amounts [2].

Assessing the oxidative damage of lipids in plants, food and in biological systems could be achieved applying numerous methods thoroughly reviewed in the literature [1], [3]-[6]. These methods include determination of the formation of primary or secondary lipid oxidation products. When the targeted compounds are volatiles, gas chromatographic analysis of those compounds has been widely used as a method of choice for monitoring the food deterioration. Since food products contain ingredients with different fatty acids profile yielding in series of volatile compounds, there is a necessity to monitor not only the main present aldehyde – hexanal, but also other aldehydes typical for rancidity development.

The main objective of this work was to apply the optimized static headspace gas chromatographic method to different processed food products of plant and animal origin and to determine the aldehydes content in both, fresh and stored food samples.

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II. Material and methods

A. Materials

Three different types of processed foods such as bakery, meat and confectionary products were used in these experiments for determining the lipid oxidation of products during storage.

Bakery product, crackers was prepared of commercially available refined wheat flour, and contained 30% of fat [7], [8]. Crackers were stored at ambient temperature for 12 months.

Meat product, traditional dry fermented sausage, was made of lean pork meat and fat in the ratio of 80:20 and spices: red hot paprika powder, salt, garlic, caraway and sugar [9], [10]. After two and seven months of storage, sausages were subjected to analyses.

Confectionary product, cocoa spread cream, was prepared using 50% of powdered sugar, 24% of vegetable fat, 6% of refined sunflower oil, 7% of cocoa powder, 12% of milk powder, 0.5% of lecithin, and vanilla and hazelnut flavors. Refined sunflower oil was partially or completely replaced with soybean oil and the following samples of cocoa spread cream were obtained in order to get the following samples:

CONTROL SAMPLE – sample with 100% of sunflower oil,

CS50 – 50% of sunflower oil replacement with soybean oil,

CS70 – 70% of sunflower oil replacement with soybean oil,

CS100 – 100% of sunflower oil replacement with soybean oil.

Oxidative stability of cocoa spread samples was monitored during six months of storage in the dark at ambient temperature.

B. Preparation of Standard Solutions

Propanal, pentanal, 1-heptanal, and 1-octanal were purchased from Dr. Ehrenstorfer (Augsburg, Germany), 1-hexanal from Sigma-Aldrich (Steinheim, Germany), and HPLC grade ethanol from J.T. Baker (Deventer, Holland). Water used for preparing the solutions was purified using Elix UV followed by Simplicity Water Purification System, Millipore (Molsheim, France). Stock standard solutions were prepared by dissolution of volatile compounds in ethanol, and stored at -23ºC. By diluting the stock solutions with water, series of working standard solutions and their mixtures were prepared daily. Concentration range of aldehydes in the mixture used for the external calibration was as follows: 0.050-50.0µg/mL for propanal; 0.02-8.00µg/mL for pentanal; 0.04-4.00µg/mL for hexanal; and 0.01-8.00µg/mL for heptanal and octanal. The mixture (2mL) was analyzed from the 10mL scrue-capped headspace vials.

C. Sample Preparation

Crackers were ground in a laboratory blender (Waring, Commercial, Torrington, USA) to obtain coarse powder which passed through an 800µm sieve. The traditional dry sausage was homogenized. Cocoa spread cream needed no sample preparation. All samples were accurately weighed (2.00g) into 10mL screw-capped headspace vials.

D. Instrumentation

Static headspace gas chromatographic (SHS–GC) analyses were performed on Agilent 7890A GC System (Agilent Technologies, USA) equipped with a capillary split/splitless inlet, total electronic pneumatic control of gas flow, headspace autosampler and flame ionization detector, FID. Chromatographic data were collected and analysed using Agilent ChemStation Software (Rev. B.03.02). Static headspace (SHS) sampling was performed with the headspace sampler, CombiPAL System (CTC Analytics, Zwingen, Switzerland) equipped with a 2.5mL HS headspace syringe for CombiPAL for the injection of 2.0mL from the 10mL headspace vials. Settings were as described in Mandić et al. [11].

E. GC Analysis Procedure

A 30m x 0.250mm i.d. DB-WAX column with 0.50µm film thickness (J&W Scientific, Agilent Technologies, USA) was utilized for the gas chromatographic separation of the aldehydes. Helium at a constant flow rate of 0.5mL/min was used as a carrier gas. The injector and FID temperatures were 200 and 240ºC, respectively. Split ratio was set at 1:10. Nitrogen was used as a makeup gas for FID. Temperature program setting is given in Table I. According to these parameters, a total run time was 16.333min.

TABLE I
GC OVEN SETTINGS

Time	Temperature (°C)	Rate (°C/min)
0	50	0
5	100	10
10	200	30

F. Statistical Analyses

All analyses were performed in three replications. The mean values with the standard deviations (SD) are reported. Statistical data analysis software system STATISTICA (StatSoft, Inc. (2008.) data analysis software system, version 8.0. www.statsoft.com) was used for data analysis. P values < 0.05 were regarded as significant.

III. RESULTS AND DISCUSSION

Hexanal as the most frequently used marker which indicates the level of lipid oxidation in food can be determined by static headspace gas chromatography, SHS GC, or solid phase microextraction gas chromatography, SPME GC, using FID or MS. Besides, hexanal, propanal, pentanal, octanal, nonanal, and decanal content was reported in the literature [12]-[15].

SHSGC FID method used in this study enables quantification of propanal, pentanal, hexanal, heptanal, and octanal in all of the samples and was accomplished from the external calibration curves of these individual aldehydes. The validation parameters of the method have been previously reported [11].

Lipid oxidation in crackers was monitored at the first day when they were baked, after 6 and 12 months of storage at ambient temperature (22±2ºC). Aldehydes content in crackers are summarized in Table II.

TABLE II
ALDEHYDES CONTENT IN CRACKERS DURING THE STORAGE

Aldehydes content (mg/kg)			
Storage time (months)	0	6	12
Propanal	0.760±0.094	5.425±0.307	3.957±0.070
Pentanal	0.278±0.003	0.397±0.016	1.711±0.101
Hexanal	0.055±0.001	0.315±0.015	1.717±0.071
Heptanal	nd	nd	0.542±0.006
Octanal	0.531±0.140	0.166±0.024	2.025±0.021
Sum	1.624	6.303	9.953

nd – not detected

Content of all determined aldehydes, expressed as a sum of aldehydes, was 6 times higher after 12 months of storage in comparison to fresh crackers, indicating the progress of lipid oxidation during time. Propanal content was higher in comparison to all other aldehydes. Heptanal was not detected in cracker samples during first 6 months of storage. Content of hexanal, which is used as a marker of lipid oxidation, was 30 times higher at the end of the experiment than at the beginning. Purcaro et al. [16] concluded that hexanal can be a realistic index of oxidation process after determining the hexanal content of 2.5 and 3.0mg/kg in crispy bread and biscuits after 6 and 12 month of storage, respectively. Comparing those and the obtained data it could be concluded that the values of hexanal content in bakery products are of the same order of magnitude.

Aldehydes content in traditional dry sausage produced in a traditional way under controlled conditions [17], after drying (0), 2 and 7 months of storage are shown in Table III.

TABLE III
ALDEHYDES CONTENT IN TRADITIONAL DRY SAUSAGE DURING THE STORAGE

Aldehydes content (mg/kg)			
Storage time (months)	0	2	7
Propanal	1.859±0.526	0.944±0.010	32.59±1.573
Pentanal	1.158±0.377	2.747±0.122	4.034±0.246
Hexanal	0.115±0.077	0.051±0.002	1.665±0.072
Heptanal	0.244±0.068	1.646±0.092	0.520±0.025
Octanal	1.258±0.461	0.222±0.027	0.355±0.013
Sum	**3.241**	**5.609**	**39.16**

Content of all determined aldehydes, expressed as a sum of aldehydes, was 12 times higher after 7 months of storage in comparison to the sausage after drying, indicating the progress of lipid oxidation during time. Content decrease of some aldehydes during the time occurs due to their further oxidative changes.

Propanal is produced during storage as a typical degradation product derived from linolenic acid (C18:3) [18]. This aldehyde is the most abundant one in a dry sausage (month 0) and obtained results are in accordance with the results of Josquin et al. [19]. Hexanal as a secondary lipid oxidation product of linoleic acid (C18:2) significantly affects the flavor of pork [18]. Hexanal was present in all sausage samples, but was not the most abundant aldehyde. This result is not in accordance with the results of some other authors [2], [20], [21]. Possibly, the low content of hexanal influences the specific aroma of traditional dry sausage [10].

Cocoa cream spread is rich in fats, since the presented spread formulation contains fats originating from vegetable fat, refined oil, cocoa powder and milk powder. Aldehydes content in 4 different cocoa cream spreads over storage period of 6 months in dark at ambient temperature are shown at Fig. 1. One of the aims of this experiment was to investigate the influence of the type of oil in the spread formulation on the oxidation stability of the formulated spread.

During 6 months period of time, all of the spread samples containing sunflower oil in their formulation regardless its percentage showed no significant difference in aldehydes content. Cocoa cream spread containing only soybean oil in the formulation showed significantly lower content of aldehydes in the first two months of storage comparing to the samples containing 70%, 50% or 0% of soybean oil.

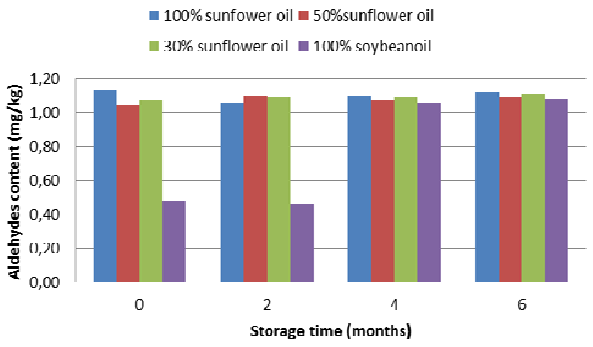

Fig. 1 Aldehydes Content in Different Cocoa Cream Spreads During the Storage

Content of aldehydes in the spreads prepared with soybean oil ranged between 0.46±0.02 and 0.48±0.01 during first two months of experiment, and then reached the values of all other samples which were in the range 1.06±0.01 – 1.11±0.04 mg/kg. Possible explanation for the obtained differences could be the composition of fatty acids in sunflower and soybean oil. Their fatty acids profile differs particularly in the content of linoleic acid (18:2). Sunflower oil is more abundant, and it contains 55-75% of this unsaturated fatty acid, while the soybean oil contains 50-57% of linoleic acid [22].

IV. CONCLUSIONS

Referring to the obtained results, applied SHS GC method is suitable for determination of aldehydes in different types of samples. Content of aldehydes varies depending on the type of a sample, and differs in fresh and stored samples of the same type.

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