DETERMINATION OF FATTY ACIDS IN PEANUTS BY CAPILLARY GAS CHROMATOGRAPHY

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Abstract: A modification of a procedure for the determination of fatty acids: palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), arachidic ($C_{20:0}$), behenic ($C_{22:1}$), and lignoceric ($C_{24:0}$), in peanut oil by capillary gas chromatography is presented. After the extraction of lipids from peanut beans, saponification and isolation of fatty acids was made, fatty acids were converted to methyl esters (FAMES), which were purified and after that separated by capillary gas chromatography. Preparation of FAMES was done with a methanolic solution of sulphuric acid instead of the more common used saponification agent BF_3 which has corrosive properties. The purification of the FAMES was performed by column chromatography using XAD2 resin as an adsorbent. The recovery of the method varied from 89.3 % for palmitic acid (relative standard deviation, RSD, 4.2 %) to 97.3 % for lauric acid (RSD 2.8 %). Using this method 20 different peanut samples from the Strumica region, Macedonia, were analysed on the content of fatty acids.

Key word: Fatty acids, peanut, capillary gas chromatography

1. INTRODUCTION

The newest trend in the food processing industry is notifying the composition of edible oils and other food commodities on the content of each individual fatty acid. Therefore a demand for quantitative methods for fatty acid analysis in food products exists. Although official AOAC methods [1] in some cases propose spectrometric methods, most of the methods reported in the literature involve gas chromatographic separation and quantification of the individual fatty acids after their isolation from lipids [2-5].

In this paper, a modification of a procedure for palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$), arachidic acid ($C_{20:0}$), behenic acid ($C_{22:1}$), and lignoceric acid ($C_{24:0}$), in peanut oil by capillary gas chromatography is presented. After the extraction of lipids from peanut beans, saponification and isolation of fatty acids, preparation, purification and separation of fatty acids methyl esters (FAMES) by capillary gas chromatography was performed.

2. EXPERIMENTAL

2.1. Instrumental

HP model 5890 series II (plus) gas chromatograph equipped with an HP automatic liquid sampler and a flame ionization detector (FID) was used with a fused silica capillary column (30 m x 0.32 mm i.d., 1 μm film thickness) coated with 100% poly(dimethylsiloxane), commercially available as SPB^{TM}-1 (Supelco). The carrier gas (N_2) flow rate was 1.5 mL·min^{-1} and the split ratio was 1:10. The injection port was maintained at 250 ^{0}C and the FID at 280 ^{0}C. Oven temperature was set at 200 ^{0}C (1 min) increasing for 5 ^{0}C·min^{-1}. The final oven

temperature was maintained at 250 °C (20 min). Aliquots of the derivatized extracts were injected into the column. Identification of the individual fatty acid methyl esters was achieved by comparison with reference standards.

2.2. Reagents and standards

All solvents and standards were of analytical grade (Merck). The fatty acid standard oil was obtained from Supelco. Amberlite XAD-2 resin (20-50 mesh) was obtained from Rohm and Haas (USA). Stock solutions of fatty acids, FAMES and of internal standard were prepared in acetone.

2.3. Procedure

2 g of the ground sample was introduced to the Soxhlet extractor and lipids were extracted (8-10 h) with petroleum ether (40-80 °C). When extraction was complete, the solvent was removed under a reduced pressure at 30 °C using a rotary evaporator and the total lipids were calculated. Aliquots of lipid extracts (0.1-0.2 g) were hydrolysed in 2.5 mL methanolic KOH (0.5 mol·L^{-1} solution by refluxing for 35 minutes at 100 °C in tightly sealed Pyrex tubes. After cooling the pH value of the solution was adjusted to 3 with 25% HCl (V/V) and fatty acids were extracted into 10 mL of petroleum ether.

The organic phase was evaporated to dryness under nitrogen. Fatty acids were dissolved in 0.2 mol·L^{-1} H_2SO_4 prepared in anhydrous methanol. Methylation was performed by refluxing (10 min) at 90 °C. After cooling the solution containing FAMES it was filled up to 25 mL with methanol. 5 mL of FAMES extract was applied to the XAD-2 column. The solution was drained under vacuum. The column was washed with 15 mL of distilled water, drained under vacuum and elution was performed with 25 mL of acetone.

3. RESULTS AND DISCUSSION

Most of the analytical methods include the official AOAC method [1-4] for the determination of fatty acids in oils use boron trifluoride (BF_3) in methanol for esterification and preparation of FAMES. BF_3 is a corrosive agent. Therefore the aim of our work was to establish a reliable and applicable analytical method for routine analysis of fatty acids in peanut oil without use of BF_3 for esterification. For that purpose it was necessary to perform saponification of fatty acids, which was done with 0.5 mol·L^{-1} KOH in ethanol. Fatty acids were liberated by adding HCl (25%) to pH 3 and esterified in the presence of methanolic H_2SO_4. Unsaponifiables were removed using XAD-2 column chromatography. Elution of FAMES from the resin was performed by acetone. XAD-2 has already been used as an adsorbent in the analysis of drugs of abuse [6], synthetic food colourings [7] etc., but we have found it to be satisfactory adsorbent of FAMES as well.

A recovery test of the method was made with standard mixed oil with the final concentration of fatty acids which was: palmitic acid 10 %; stearic acid 5 %; oleic acid 60 %; linoleic acid 20.0 %; arachidic acid 1.0 %; behenic acid 3 % and lignoceric acid 1.0 %. Ten milligrams of lauric acid ($C_{12:0}$) was added to the oil mixture as an internal standard. Analytical recoveries and relative standard deviation (RSD) obtained after saponification and methylation of fatty acids, purification of FAMES and their gas chromatographic separation are satisfactory (relative standard deviation, RSD, ranges from 3-4.5 % and the recovery from 89.2 % for Palmitic acid to 97 % for lignoceric acid). Using this method the investigated fatty acids were determined in some peanut samples using a standard addition method. The limit of detection ranges from 0.10 % for lignoceric acid to 0.25 % for linoleic acid).

Applying proposed procedure fatty acids were determined in 20 samples of peanuts from the region of Strumica, Macedonia. The obtained results show (Table I) that oleic acid $C_{18:1}$ accounted the 58.9 % of total fatty acids. The major saturated fatty acid was palmitic acid $C_{16:0}$.

Table I. Fatty acid composition of mature peanut beans

Fatty acid	Mean content of total fatty acids (%)	RSD (%)
Palmitic acid ($C_{16:0}$)	7.8	4.0
Stearic acid ($C_{18:0}$)	3.2	4.3
Oleic acid ($C_{18:1}$)	58.9	5.0
Linoleic acid ($C_{18:2}$)	23.2	3.2
Arachidic acid ($C_{20:0}$)	1.4	3.4
Behenic acid ($C_{22:0}$)	2.3	3.0
Lignoceric acid ($C_{24:0}$)	1.0	3.2
Unknown	2.2	3.0

The use a nonpolar methylsilicone column and the column temperature gradient raising for 5 $^0C\,min^{-1}$ from 200 ^0C to 250 ^0C during chromatographic separation provides good separation of investigated fatty acids within a relatively short time of 20 minutes (Fig. 1).

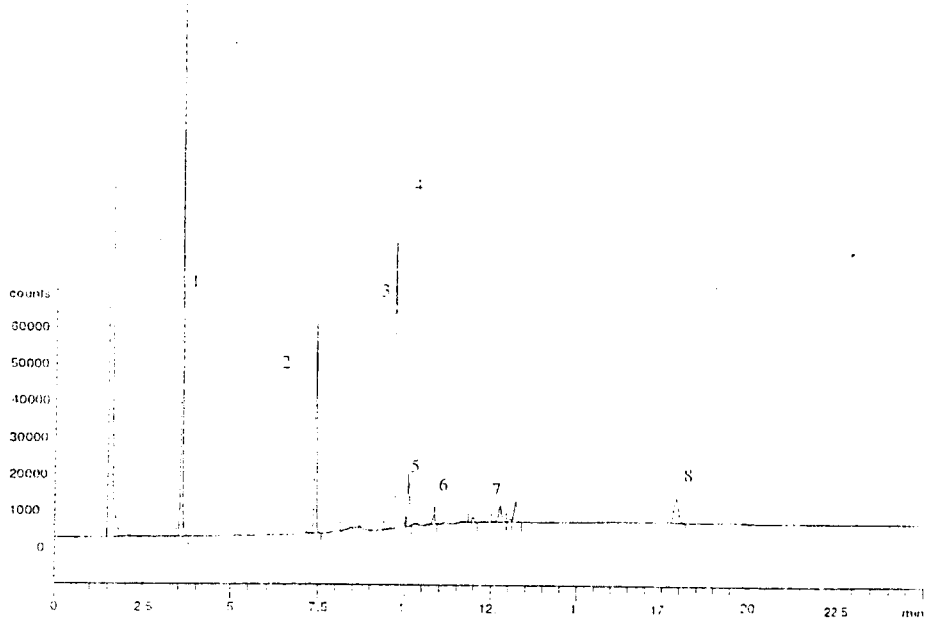

Figure 1. Chromatogram of fatty acids in peanut oil on SPB^{TM}-1
1. lauric acid, internal standard ($C_{12:0}$); 2. palmitic acid ($C_{16:0}$); 3. linoleic acid ($C_{18:2}$); 4. oleic acid ($C_{18:1}$); 5. stearic acid ($C_{18:0}$); 6. arachidic acid ($C_{20:0}$); 7. behenic acid ($C_{22:1}$); 8. lignoceric acid ($C_{24:0}$)

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Апстракт. Презентирана е модификација на метода за определување на масните киселини: палмитинска ($C_{16:0}$), стеаринска ($C_{18:0}$), олеинска ($C_{18:1}$), линолеинска ($C_{18:2}$), арахидинска ($C_{20:0}$), бехенична ($C_{22:1}$) и лигноцеринска ($C_{24:0}$), во масло од кикирики со капиларна гасна хроматографија. По екстракција на липидите од зрната на кикирики, извршена е сапонификација и изолација на масните киселини, нивно преведување во метил естри (FAMES), пречистување на метил естрите и нивно издвојување со капиларна гасна хроматографија. Метилирањето на масните киселини е вршено со метанолен раствор на сулфурна киселина, со што е избегната употребата на најчесто користениот реагенс за дериватизација BF_3, кој има корозивни особини. Пречистувањето на метил естрите е вршено со столбна хроматографија, при што како атсорбенс е користена смолата XAD2. Аналитичкиот принос на методата се движи од 89,3 % за палмитинска киселина (релативна стандардна девијација, RSD, 4,2 %) до 97,3 % за лауринска киселина (RSD 2,8 %). Со примена на оваа метода извршено е определување на масните киселини кај 20 примероци на кикирики од струмичкиот регион.