SUPPLEMENTARY MATERIAL

Fatty acids determination in Bronte pistachios by gas chromatographic method

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A gas chromatographic with Flame Ionization Detector (GC-MS FID) method for the identification and quantification of fatty acids based on the extraction of lipids and derivatization of free acids to form methyl esters was developed and validated. The proposed method was evaluated to a number of standard FAs, and Bronte's pistachio's samples were used for that purpose and to demonstrate the applicability of the proposed method. In this regard, repeatability, mean and standard deviation of the analytical procedure were calculated. The results obtained have demonstrated Oleic acid as the main component of Bronte's pistachios (72.2 %) followed by linoleic acid (13.4 %) and showed some differences in composition with respect Tunisian , Turkish and Iranian pistachios.

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Experimental

A gas chromatographic method for the identification and quantification of FAs, based on the extraction of lipids and derivatization of the FAs into corresponding methyl esters (FAMES) using acid catalysed (Christie 1982) was developed and validated.

Sampling plan

Ten different cultivations from Bronte (Sicily) were examined for fatty acids determination. About 500 g of pistachios were collected from each cultivation. Every pool of samples were divided in two parts for repeatability tests and quantification of FAs.

Reagents and standards

All chemicals, solvents and reagents employed were analytical grade. Standards that were used for qualitative identification of fatty acids, stearic (SA) C18:0, palmitic (PA) C16:0, oleic (OA) C18:1 and linoleic (LA) C18:2 c9, c12, (Sigma Aldrich, Amsterdam) were purchased. Fatty acids standards at 10000 mg/L were prepared by transferring 100 mg of the pure standard to a volumetric flask and diluting to 10 ml with n-hexane. A standard solution mixture at 1000 mg/L was prepared by diluting 0.1 mL of each FAs standard solution to 1 mL with n-hexane. Standard fatty acid mixture was used for the identification of the peaks. The fatty acids in the experimental samples were identified by comparing their retention time and peak positions by the formula:

$$\text{Equation 1} \qquad \qquad \qquad TR = TR_{st} \pm 0,5$$

Where TR is the determined retention time (min), TR_{st} is the retention time for each FAs standard. The relative percentages of the fatty acids were also determined. Quantitation of individual FA is thus, based on the comparison of their peak areas (A_i), and the peak area of suitable Standard. The relative percentages of fatty acids (C) were determined by the formula:

$$\text{Equation 2} \qquad \qquad \qquad C = \frac{A}{\sum A} 100$$

Preparation of samples and fats extraction

Approximately 3 g of homogenized sample was weighted and mixed with about 4 g of diatomaceous earth. The mixture was transferred into the extraction cells and extracted using Accelerated Solvent Extraction ASE 200 Dionex. Lipids were extracted with Petrol ether/Acetone (7:3). After extraction, the mixture was dried with Na_2SO_4 and filtered through fluted filter paper. The solvent was removed by evaporation using rotary evaporator.

Preparation of FAMES

It is difficult to analyze directly FAs because of their high polarity and low volatility (Christie 1982). In order to overcome these difficulties, volatile derivatives were prepared (Antolin et al. 2008). Esterification increases the volatility of fatty acids, improves the peak configuration, the separation, and the sensitivity of detectors. For the preparation of FAMES, 200 mg portion of fat was weighed into a test tube with screw cap. After adding 2 ml portion of 2% hydrogen chloride in methanol, the sample tube was closed and heated at 120°C for 1 hour. After allowing the tube to cool down, a 2 ml portion of water and 1 ml of n-hexane were added. The contents of the tube were shaken and allowed to undergo phase separation. The supernatant of the solution was transferred into a 2-ml vial.

GC analysis

The FAMES solution was injected on an Gas Chromatograph equipped with a capillary column Famewax (30 m length, 0.25 mm internal diameter, 0.25 μm film thickness) and a Flame Ionization Detector (FID) Trace GC Ultra (Thermo Fisher Scientific, Inc. (NYSE:TMO)). The initial oven temperature was 130° C, programmed by 6°C/min until 225°C. Helium was used as the carrier gas at a flow rate of 1.2 ml min⁻¹ with a split ratio of 20:1. The injector temperature was set at 220°C. Identification of FAMES was achieved by comparing their retention times with those of known standards and the amount of fatty acids was expressed as percent of total.

Validation

The method was subject to validation according to guidelines for validation of chromatographic methods (Taverniers et al. 2004; Antolin et al. 2008). In this regard, repeatability, mean and standard deviation of the analytical procedure were all calculated. Separate FA standards were used to calculate the mean retention times (RT) in the FID detector. The precision of the quantitative method was checked through the repeatability experiment. Repeatability of the proposed method

was assessed on the basis of the standard deviation (σ) values of the content of FAMES in the 1000 ppm mixture.

Equation 3

$$\sigma = \sqrt{\frac{1}{N} \sum_{i=1}^N (x_i - \mu)^2}$$

Estimation of repeatability and further statistical indicators were based on ten series of experiments. The area of each peak was then measured and corrected. The area of an analyte was divided by the sum of areas for all peaks. This value represents the percentage of an analyte in the sample.

Equation 4

$$P_{A_i} = \frac{A_i}{\sum A_i} 100$$

According to the method, the concentrations of FAs in ten samples of Bronte Pistachios were determined. The FAs in the experimental samples were identified by comparing their retention time and peak positions. Relative percentage of the fatty acids were also determined by comparing their peak areas.

Fatty acid	RT(min)	Area
Palmitic	10.88	37074810
Stearic	13.72	16755894
Oleic	13.98	13095438
Linoleic	14.61	29594305

Table S1. Retention Times (RT) and area of the examined fatty acids.

N. Exp	Palmitic	Stearic	Oleic	Linoleic
1	26.8	22.7	25.8	25.1
2	26.2	22.3	25.5	25.2
3	25.6	22.3	25.5	25.2
4	26.9	22.7	25.8	25.2
5	26.5	22.5	25.6	25.3
6	26.3	22.3	25.6	25.2
7	26.7	21.9	25.3	25.2
8	26.9	22.3	25.5	25.2
9	26.2	22.7	25.7	25.3
10	25.8	22.3	25.4	25.1
Mean±SD	26.4±0.45	22.4±0.25	25.6±0.16	25.2±0.07
CV%	1.7	1.1	0.6	0.3

Table S2. Results from repeatability test after ten trials expressed in fatty acids composition percentage.

References

- Antolín EM, Delange DM, Canavaciolo VG. 2008. Evaluation of five methods for derivatization and GC determination of a mixture of very long chain fatty acids (C24:0–C36:0). J Pharmaceut Biomed. 46:194–199
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