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Original Research Article

Optimization and validation of a simplified methodology for simultaneous extraction of fatty acids and tocopherol homologues in peanuts

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ABSTRACT

A new sample treatment methodology was developed for the simultaneous determination of oleic, linoleic, palmitic and stearic fatty acids (FA), and α-, β-, γ- and δ-tocopherol homologues in peanut samples. Usually, the determination of these compounds is carried out after oil extraction with an organic solvent at boiling temperatures of the solvent. The innovative analytical methodology developed in this work was performed in a 20 mL vial in which the FA and tocopherols were simultaneously extracted, and the derivatization of the FA was, afterwards, conducted. The reduction in analysis time from 1 h 30 min (reference methodology) to 20 min (new methodology) increased the sample throughput. Furthermore, the amount of organic solvent used decreased from 40 mL to 6 mL The new methodology was validated based on the analysis of a certified reference peanut butter sample and the results were compared to those obtained by using the reference methodology and a suitable agreement with the certified values was found. Finally, the new sample treatment approach was used to analyse toasted and fried (with and without tegument) peanuts.

1. Introduction

Peanuts (*Arachis hypogaea*) are considered a highly nutritious food due to their high levels of monounsaturated FA, dietary fiber, proteins, minerals, and antioxidants (Esche et al., 2013). The presence of peanuts in a balanced diet is associated with the prevention of coronary diseases, cancer, and Alzheimer's (Shin et al., 2009). According to FAOSTAT (FAO, 2019), global peanut production has almost doubled from 1994 to 2018, with China and India leading the world production, reaching nearly 46 million tonnes per year.

Health benefits of peanuts are associated with the compounds present in their fats. Peanuts contain around 50 % wt. of fat, with more than 70 % of the FA being unsaturated, mainly oleic acid (42–52 %) and linoleic acid (32–37 %) (Aljuhaimi and Musa Özcan, 2018; Gavrilova et al., 2020; Liu et al., 2011; Yoshida et al., 2005). Nevertheless, tocopherols, although minor compounds of peanuts, are also important due to their antioxidant ability. Tocopherols are mostly present as α- and ϒ-tocopherol homologues. Depending on the peanut varieties, their concentrations fluctuate between 18−57 mg kg^{-1} oil and 36−78 mg kg^{-1} oil, respectively (Carrín and Carelli, 2010; Hu et al., 2019; Stevens-Barrón et al., 2019). In addition, peanuts also contain δ- and β-tocopherol homologues at levels lower than 10 mg kg^{-1} (Costa De Camargo et al., 2016; Hejtmánková et al., 2018). Therefore, monitoring fat composition is important to assess the quality of peanuts (Özcan and Seven, 2003).

Fat composition changes in nuts are usually evaluated after oil isolation using chemical or mechanical procedures (Beltrán Sanahuja et al., 2009). After that, the FA and tocopherol contents of peanuts are determined in the extracted oil (Costa De Camargo et al., 2016; Maguire et al., 2004; Stevens-Barrón et al., 2019), although different oil treatments are used for each of the compound's families.

The chemical extraction is regularly carried out at high temperatures using an organic solvent. This procedure leads to degradation of the components more sensitive to heat and oxygen (Costa De Camargo et al., 2016). For instance, the reported loss in the total tocopherol content was around 35−40 wt. % in peanut oil as a result of the high temperatures (Bramley et al., 2000). On the other hand, although mechanical extraction of the peanut oil is less time-consuming and the use of organic solvents is avoided, the extraction efficiency is quite low and hampers its use in trace analysis (Celenk et al., 2018; Serra et al., 2019).

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Consequently, avoiding the previous fat isolation step, i.e., the direct analysis of samples such as the analytical methodology proposed in this work, would get around these drawbacks. Furthermore, this direct analysis methodology (DAM) would be faster, greener and would reduce experimental errors (Figueiredo et al., 2016). In this sense and related to the FA analysis, Barnes and Holaday (1972) directly determined the FA profile of ground peanuts using hot deionized water, and using BCl_3 as the derivatizing reagent. However, the toxic properties of BCl_3 discourage its use; besides, high temperatures used to evaporate hexane induced losses of methyl esters. In another work, Liu et al. (2013), using microwave radiation, developed an accelerated procedure for the FA extraction and derivatization. The results revealed no significant differences from the data obtained by analyzing the extracted oil.

Regarding the determination of tocopherols, Lee et al. (1998) optimized their direct determination in peanuts and peanut butter. A hexane:ethyl acetate (90:10, v/v) mixture was employed as the extracting solvent, achieving recoveries above 90 % for all tocopherol homologues. Other studies have also analyzed tocopherols from peanuts, walnuts, or pistachios, obtaining successful results after extracting the ground sample in propanol (Hejtmánková et al., 2018).

Even though there are studies in which direct extraction from ground peanuts of tocopherols or FA have been successfully approached, to the best of our knowledge, the direct and simultaneous determination in one single step of FA and tocopherol profiles of ground peanuts has not been studied yet.

Based on this, the aim of this study was to develop and validate a novel simultaneous direct analytical methodology (DAM) for the extraction at the same time of both FA and tocopherols in grounded peanuts. To achieve this goal, an optimization of the extraction and derivatization conditions -sample amount, temperature, time, and acid volume- using a response surface methodology (RSM) was carried out. A quantitative comparison of the FA and tocopherol obtained content results to those found by using conventional methods, i.e. after oil extraction, was also performed. When comparing the sample treatment developed in this work to the reference methodology the new method reduced the sample analysis time from 1 h 30 min to 20 min increasing the sample throughput. In addition, the volume of organic solvent used lowered from 40 mL to 6 mL what decreased waste generation without comprising the results.

Table 1
Nutrition facts showed in the packaging.

Code	Treatment	Origin	Total fat (g 100g)	Saturated fat (g 100g)	Salt (g)
A	Toasted, unpeeled, salted	China	43.2	8.2	0.01
B	Toasted, unpeeled, salted	United States	51	11	0.40
C	Toasted, unpeeled, salted	China	49	10	1.1
D	Toasted, peeled, without salt	China	54	10	<0.01
E	Toasted, unpeeled, salted	-	46.2	8.9	0.24
F	Fried	Argentina	52	8.9	2.0
G	Fried, peleed, salted	China	48	8.7	0.4
H	Fried, peleed, salted	China	51	10	1.1
I		China	52	8.9	2.0
J	Toasted, unpeeled, with sugar	Argentina	32	5.4	1.0

2. Materials and methods

2.1. Materials

Peanuts were acquired from a local supermarket in Spain. Table 1 shows their characteristics. Samples were packaged in 250 g units under modified atmosphere (N_2). They were stored at room temperature and controlled light exposure until analysis.

Methanol and isopropanol (high-performance liquid chromatography (HPLC grade) and n-hexane (99 %, gas chromatography (GC) grade) was purchased from Panreac (Barcelona, Spain). Petroleum ether, sodium chloride, sodium methoxide 25 % wt solution in methanol, sulphuric acid, and tocopherol homologues (α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol) standards and tocopherol acetate (internal standard, IS) were acquired from Sigma–Aldrich Inc. (St. Louis, MO, USA). A certified reference mixture of 37 fatty acid methyl esters (FAMEs) in dichloromethane (F.A.M.E. Mix. C4-C24) and nonadecanoic acid methyl ester, used as IS, were purchased from Sigma–Aldrich Inc.

The developed methodology (DAM) was validated using a standard reference material STM® 2387 of peanut butter provided by the National Institute of Standards and Technology (NIST, Gaithersburg, USA).

2.2. Sample preparation

2.2.1. Reference methodology

Salted and fried peanut samples were ground in an electric grinder mill to a fine powder just before oil extraction. Convectional Soxhlet oil extraction procedure was carried out as reported previously (Beltrán Sanahuja et al., 2009; Erol et al., 2011). Briefly, four grams of each sample were transferred to a cellulose thimble and were extracted by using a commercial fat extractor (Selecta, Barcelona, Spain) during 90 min with 40 mL of petroleum ether. According to the instrument's manufacturer, the temperature of the heating module was set at 135 °C. Nevertheless, the actual temperature of the oil extraction was 60 °C because this is the boiling temperature of the solvent. The remains of petroleum ether were removed by using a nitrogen stream. Peanut oils were stored in sealed amber vials at -21 °C until their analysis.

For the determination of FAMEs, the referenced procedure selected was that described in ISO 12966-2 (ISO, 12966-2 (Animal and vegetable fats and oils - Gas chromatography of fatty acid methyl esters - Part 2: Preparation of methyl esters of fatty acids), 2017). The method was applied with appropriate adjustments in reagents amounts. Briefly, 0.3 g of peanut oil, were placed into a 100 mL round-bottom flask and 6 mL of 0.2 M sodium methoxide solution were added. The mixture was shaken and heated in a heating plate until clear visual appearance (10 min, approximately). After cooling to room temperature, 6 mL of n-hexane and 30 mL of saturated sodium solution were added. After shaking, the upper light n-hexane-layer was transferred to a vial. Before the analysis, 0.04 g of n-hexane phase and 0.15 g of IS (1 000 mg kg^{-1} nonadecanoic acid methyl ester) were diluted with 1.0 g of n-hexane.

The reference procedure used for determining the tocopherol content followed the ISO 9936 (ISO 9936 (Animal and vegetable fats and oils — Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography), 2016). Briefly, a 25 mg portion of peanut oil was dissolved in 1 mL of n-hexane and filtered thought 0.45 μm nylon-membrane syringe filter. Suitable dilutions were performed and 0.13 g of IS (200 mg kg^{-1} tocopherol acetate) was added.

2.2.2. Direct analysis methodology (DAM)

Peanut samples were milled for 1 min by using an electronic grinder. In order to prevent tocopherol oxidation, 0.05 g ascorbic acid g^{-1} per gram of peanut were added before grinding (Delgado-Zamarreño et al., 2001). Ground peanuts were kept in an amber vial at −21 °C until analysis.

One gram of grounded peanut sample was weighted into a 20 mL vial

and 6.0 mL of n-hexane and BHT (3 mM) were added. A stirring bar (4.5 mm) was introduced into the vial before closing it and placing it in a water bath. The stirring rate was fixed at 900 rpm for 10 min and the temperature was set at 50 °C.

Afterwards, 3 mL of a 0.2 M sodium methoxide solution (Sigma–Aldrich, St. Louis, MO, USA) was added. Derivatization time was fixed at 10 min. Next, 3 mL of a 5 % v/v sulphuric acid solution (Sigma–Aldrich, St. Louis, MO, USA) in methanol were added. The mixture was blended during 1 min. Finally, the supernatant was collected and filtered through 0.45 μm nylon-membrane syringe filter. A small amount of the supernatant (0.04 g) was employed for FAMEs analysis, after its correct dilution, and 0.15 g of 1000 mg kg^{-1} of IS were also added. On the other hand, another amount of the supernatant (0.50 g) was employed for tocopherol analysis, also after its correct dilution, adding 0.13 g of 200 mg kg^{-1} of IS. Fig. 1 summarizes the experimental procedure.

2.3. Instrumental conditions

2.3.1. GC–MS analysis

FAMEs were determined using an Agilent 7890 N GC coupled to a 5977B Mass Spectrometer (MS) (Agilent Technologies, Palo Alto, CA). The column used was a BPX70 column (60 m × 0. 25 mm × 0. 25 μm). Ion source and GC–MS transfer line temperatures were 250 °C and 280 °C, respectively. Helium flow rate was fixed at 1 mL min^{-1} and samples were injected by using a 1:100 split ratio. FAMEs were identified using National Institute of Standards and Technology (NIST) MS library matches. Fig. S1A shows a typical GC chromatogram of a peanut FAMEs profile analysed using DAM. Quantitation was performed via the integration of the total ion current chromatogram. Concentrations were expressed as g FA 100 g^{-1} of peanut.

2.3.2. HPLC analysis

Tocopherol determination was carried out using an Agilent 1260 Infinity Binary System ultra-high-performance liquid chromatography (UHPLC) with a fluorescence detector (Agilent Technologies, Palo Alto, CA). Separation of target analytes was performed using an Inertsil NH_2 NP-HPLC column (5 μm, 250 × 4.6 mm I.D.) thermostat at 40 °C. A mixture of n-hexane:isopropanol (98:2 v/v) was used as mobile phase. The separation was developed in isocratic mode with a flow rate of 1.2 mL min^{-1} and the sample injection volume was 20 μL. Irradiation and emission wavelengths were fixed at 298 nm and 325 nm, respectively. Tocopherols in samples were identified by comparison to the retention times of the standards. Fig. S1B shows a typical HPLC chromatogram of a peanut tocopherols profile analysed using DAM. Concentration values were expressed as mg tocopherol 100 g^{-1} of peanut.

2.4. Box-Behnken Experimental Design (BBD)

To investigate the influence of different variables on the extraction and derivatization of the analytes, a four-variable and three-level BBD was created using the Statgraphics Centurion XVI software (version 7.0.0, Stat-Ease, Inc., Minneapolis, MN, USA). As it is shown in Table 2, sample weight, temperature, time, and acid volume were the four independent variables selected. Time was equally divided for the extraction and derivatization steps. Thus, a set of 27 experiments with 3 centre points was generated. Experiments (see Supplementary Table S1) were carried out in a randomized order.

The selected responses were the concentrations of four major FA

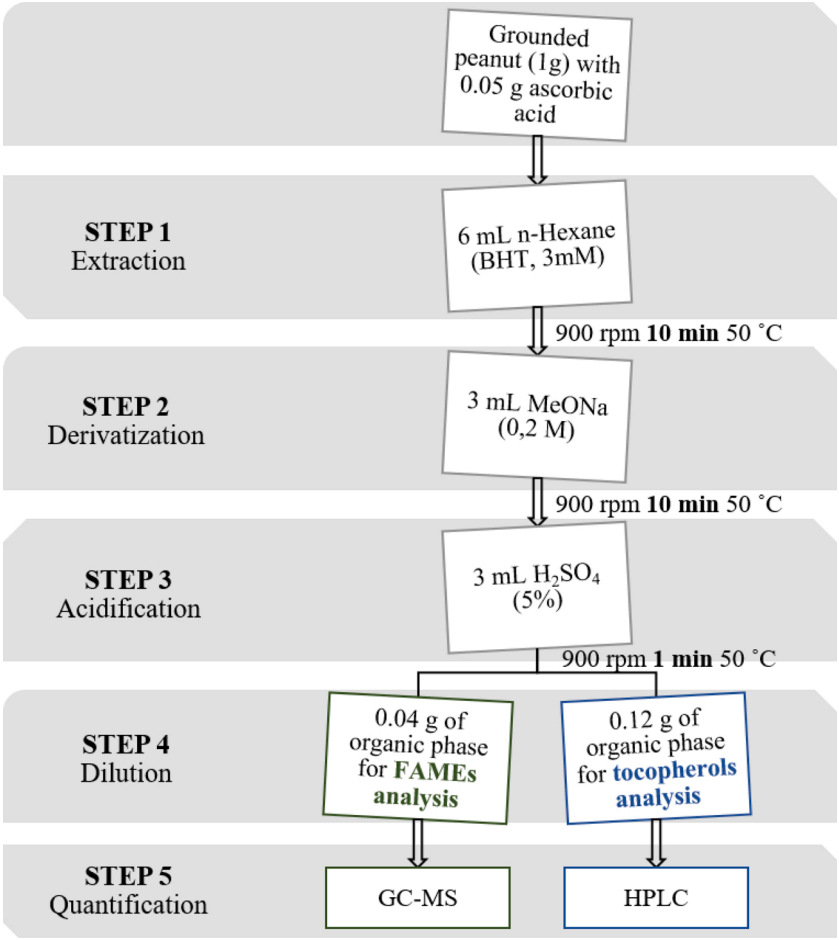

Fig. 1. Sample preparation steps for DAM.

Table 2
Independent variables tested at three different levels by using a BBD.

	Unit	Symbol	Levels		
			−1	0	+1
Sample weight	g	A	0.20	0.60	1.00
Temperature	ºC	B	25.0	50.0	75.0
Time	min	C	5.0	12.5	20.0
Acid volume	mL	D	1.0	2.5	4.0

(palmitic, stearic, oleic, and linoleic) and of the four tocopherol homologues (α-, β-, γ-, and δ-tocopherol) (Özcan, 2010; Salamatullah Mohammad et al., 2021). The evaluation of the results of experimental design was performed by selecting the conditions which maximized the analytes concentrations

The significance of the effects was checked by analysis of variance (ANOVA). Multiple regression analysis was performed to illustrate the effect of factors on the response. In a second-degree polynomial equation (Eq. 1), where Y is the concentration β_0 is the model constant; β_i represents the linear coefficients; β_{ii} the quadratic coefficients; β_{ij} the interaction coefficients and X_i the different factors, the different β coefficients were determined (Sahu et al., 2018).

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_{ii}^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} \beta_{ij} X_i X_j \quad (1)$$

2.5. Analytical figures of merit of the developed method

Sample analysis was performed in quintuplicate (n = 5). Accuracy and precision values were evaluated analysing a peanut butter certified reference material. Accuracy was determined by comparison of the results obtained in DAM with the certificated values by means of a two-sided Student's *t*-test using the SPSS commercial software (Version 15.0, Chicago, IL). Precision was estimated performing the analysis on the same day and on non-consecutive days to obtain intra-day and inter-day precision, respectively (Bánguena-Polo et al., 2008).

To quantify LOD and LOQ dilutions of the target analytes solutions were prepared. The LOD and LOQ were calculated as Eqs. 2 and 3:

$$\mathrm{LOD} = 3.3\frac{h}{H}\cdot\left[\mathrm{analyte}\right] \quad (2)$$

$$\mathrm{LOD} = 10\frac{h}{H}\cdot\left[\mathrm{analyte}\right] \quad (3)$$

Where H is the height of the chromatographic peak corresponding to a dilution of the analyte near the LOD and h is the peak-to-peak background noise in the chromatogram. The analyte concentration in the standard solution was set to provide the lowest detectable signal.

Finally, the DAM methodology was applied to the analysis of 10 different peanuts samples. Results were compared to those obtained with the conventional methodology using an analysis of variance (ANOVA).

3. Results and discussion

3.1. DAM optimization

The design matrix for the independent variables together with all the response values studied by both predicted and experimental trials are shown in Table S1. The RSD between the experimental and predicted values was, on average, 7 % for fatty acids and 3 % for tocopherol. Additionally, an ANOVA was applied to compare both data and the results are shown in Table S2.

The *F* value indicates model was significant for all analytes; There is only a 0.01 % chance of occurring due to the noise. Terms of the model with *F* values smaller than 0.500 indicate they were significantly different. Consequently, non-significant terms were deleted.

According to the obtained experimental results, empirical coefficients of second-order polynomial model (Eq.1) were established for each analyte (Table 3). The highest contributions to FAMEs equations were related to the sample amount. Based on previous studies that used BBD for optimization in the oil extraction process (Ketenoglu, 2020), (Kemerli-Kalbaran and Ozdemir, 2019), a maximum sample amount of 1 g was chosen to the extraction of both FAMEs and tocopherols. In the quoted works, an optimum solvent/sample ratio was found to be around 10 ml g^{-1} of the sample. Hence, a fixed value of this parameter, i.e, 6 mL, was selected in this study. This value was in agreement with Badwaik et al. (2012) who concluded that the optimal sample to solvent ratio for peanut oil extraction was 6:1. This value, while guaranteeing a high solvent-sample ratio in the worst-case scenario, enabled the rest of the procedure to be developed in a 20 mL vial.

The interaction of sample amount and temperature (AB) was not negligible for FAMEs determination. Fig. 2 shows that higher values of the sample amount required higher temperature. An increase in temperature affected positively all FAMEs responses until a temperature around 50 °C. On the other hand, at higher temperature values, the responses declined. An increase in temperature enhanced the extraction of the lipids and the derivatization efficiency, thereby increasing the responses. However, higher temperature values (from 50 °C to 75 °C) may accelerate the oxidation of lipids or/and methyl esters becoming thermally unstable alkyl esters (Liu et al., 2013). Similar results were also reported by Liu et al. (2013) in nut oils showing that FAMEs content was negatively affected when applying high extraction temperatures (Liu et al., 2013).

Temperature was not a significant factor in tocopherol responses. However, the temperature-time interaction affected negatively to the extraction of alpha-tocopherol. In this sense, Kemerli-Kalbaran and Ozdemir (2019) reported that although the extraction efficiency of antioxidants increased by increasing the temperature and time, its interaction had a negative impact on antioxidant activity in pine nuts. This may be caused by oxidation of antioxidant compounds such as tocopherols when high temperatures were applied for prolonged times. Even so, optimal temperature, i.e, 50 °C, was high enough to extract the homologues and low enough to preserve tocopherols. (Fig. 3)

Regarding the acid volume, it has been reported that it directly influences the FA derivatization step in soybean seed (Braga et al., 2019). To the best of our knowledge, the influence of the acid addition in the tocopherol extraction has not been described yet. However, preliminary studies (data not shown) pointed out that acid was required for making complete α-tocopherol extraction viable, so this factor was also included in the design.

Integrating the results of the optimization of FAME and tocopherols extraction, the optimal conditions for general sample treatment were fixed. Sample amount and acid volume, which were more significant in tocopherol than in FAME analysis, were established at 1.0 g and 4.0 mL, respectively. Higher values for both factors were not tested because they were limited by vial capacity (20 mL), which was employed to scale down the procedure.

Since approximately the same optimal extraction temperature was obtained for both analyte groups, its value was established at 50 °C, and finally the optimal time for the simultaneous extraction and derivatization was set at 20 min.

At the optimal extraction conditions, different ground peanut samples were analysed by using DAM and the results were compared to those provided by the reference methodology (Table 4). Chromatograms of FAMEs and tocopherols are shown as supplementary material (Fig. S1). It is important to highlight that significant differences between chromatograms obtained by DAM and by conventional methodology were not detected.

Although the optimization of the DAM was not carried out for C20:1, C22:0 and C24:0 FAMEs, their results were included because the peanut

Table 3
Empirical coefficients of second-order polynomial model.

	ANALITES							
	C16:0	C18:0	C18:1	C18:2	α-TOC	β-TOC	γ-TOC	δ-TOC
Intercept (β_0)	294.61	135.04	14167.55	412.04	73.71	1.64	45.98	2.06
Linear								
β_I(A)	141.41	3.67	−8494.47	579.61	42.11	−0.37	19.87	0.86
β_{II}(B)	26.46	14.59	705.15	46.77	0.11	−0.01	0.19	0.02
β_{III}(C)	12.02	7.48	238.82	19.43	0.57	0.01		−0.03
β_{IV}(D)	28.88	22.25	900.91	40.67	1.71	0.25		−0.17
Quadratic								
β_{VI}(AA)	−149.48	−99.79		−415.51	−14.07			
β_{VII}(BB)	−0.22	−0.13	−6.39	−0.39				
β_{VIII}(CC)	−0.53	−0.33	−11.25	−0.91				
β_{IX}(DD)						−0.05		
Interaction								
β_X(AB)		2.45	127.58			0.02		
β_{XI}(AC)								
β_{XII}(AD)								
β_{XIII}(BC)					−0.01			
β_{IXX}(BD)								
β_{XX}(CD)								0.01

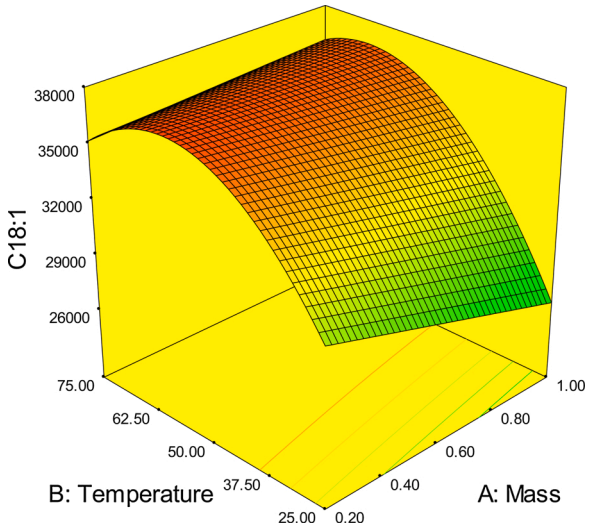

Fig. 2. Effects of sample amount and temperature on the response of C18:1 using DAM.

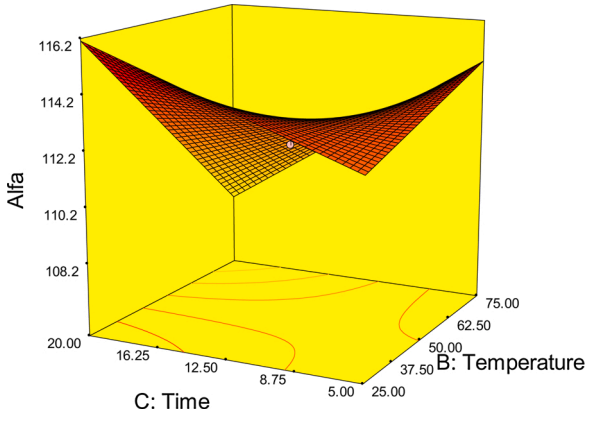

Fig. 3. Effects of time and temperature on the response of alpha-tocopherol using DAM.

Table 4
Fatty acids and tocopherol composition in peanuts analysed using direct analysis methodology (DAM) and the reference methodology.

Determination	DAM		Reference methodology		SS
	Content	RSD (%)	Content	RSD (%)	
Fatty acids (g 100 g^{-1})					
C16:0	1.2 ± 0.1	3 %	1.1 ± 0.1	4 %	NS
C18:0	0.42 ± 0.03	2 %	0.35 ± 0.03	3 %	NS
C18:1	38 ± 2	3 %	36 ± 4	6 %	NS
C18:2	2.2 ± 0.2	5 %	2.1 ± 0.3	5 %	NS
C20:1	0.71 ± 0.05	3 %	0.61 ± 0.06	3 %	NS
C22:0	0.62 ± 0.05	3 %	0.48 ± 0.06	6 %	NS
C24:0	0.24 ± 0.02	4 %	0.17 ± 0.02	6 %	NS
∑ SFA	2.48 ± 0.12	5 %	2.1 ± 0.12	6 %	NS
∑ MUFA	39 ± 2	5 %	37 ± 4	11 %	NS
∑ PUFA	2.2 ± 0.2	9 %	2.1 ± 0.3	14 %	NS
∑ TUFA	41 ± 2	5 %	39 ± 4	10 %	NS
Tocopherols (mg 100 g^{-1})					
α-tocopherol	139 ± 14	4 %	117 ± 13	4 %	***
β-tocopherol	2.5 ± 0.4	6 %	2.4 ± 0.5	9 %	NS
γ-tocopherol	88 ± 7	3 %	89 ± 10	5 %	NS
δ-tocopherol	3.8 ± 0.4	4 %	4.2 ± 0.8	8 %	NS
Total tocopherols	233 ± 16	3 %	212 ± 16	3 %	***

Results expressed as mean ± confidence interval of four replicates (α = 0.05). n = 3.
NS, no significance difference.
*** No significant difference at α = 0.01.

oil also contained remarkable concentrations of these compounds (Özcan and Seven, 2003). Significant differences were not found between the evaluated methodologies (α = 0.05), except for α-tocopherol, for which the concentration obtained by the DAM was higher. It could be explained as applied temperatures in the oil extraction process (around 60 °C) and longer extraction periods needed in the reference methodology could oxidize partly of α-tocopherol, the least stable isomer (Setiadi et al., 2003). Moreover, RSDs values were lower using DAM in comparison with the reference methodology, which reflected the

simplification of the DAM procedure. In addition, a reduction in the sample treatment time was achieved, i.e., from 1 h and 30 min in the reference methodology to 21 min in the developed DAM.

3.2. Method validation

The method validation parameters were determined after the DAM optimization (Table 5). The precision and the accuracy of the DAM was evaluated with five replicate analysis of a reference material. The $RSD_{intra\text{-}day}$ values were around 1 % for FAMEs and 1–5 % for tocopherols. The obtained $RSD_{inter\text{-}day}$ values were higher (3 % for FAMEs and 5–11 % for tocopherols), however, results were acceptable (Bánguena-Polo et al., 2008).

Concentration values of FAMEs and tocopherol content in the reference material (peanut butter) obtained by using the DAM and the certified values are shown in Fig. 4. C16:0 concentration was not specified in reference material. The reproducibility and recovery values obtained after the application of DAM were between 95 and 105 % which are considered acceptable values (CIPAC, 2021).

Furthermore, the DAM greenness (Płotka-Wasylka, 2018) has been improved in comparison with the reference methodology since DAM avoids the use of petroleum ether as an organic solvent and the amount of energy employed in the process has been reduced as the oil extraction step has been avoided.

3.3. Application of DAM to different peanut samples

The quantification of FAMEs and tocopherols by using DAM was applied to the analysis of several peanut samples. Ten samples, which had been differently processed, were selected for this purpose (Table 1). Samples were harvested in three of the world's primary peanut exporting countries: Argentina, China and United States (Di, 2014).

Results obtained by using DAM are depicted in Table 6. The RSDs values for tested samples were 4 % for FAMEs and 7 % for tocopherols.

When comparing the analyzed peanut samples, the results agreed with those published in the literature (Aljuhaimi and Özcan, 2018; Juhaimi et al., 2018). Although it is important to note that the concentration of tocopherol was quite variable due to the different geographical origins as well as the sample processing method (Juhaimi et al., 2018). It was reported that the content of α-tocopherol decreased by 34 % upon the dry-blanching process (Costa De Camargo et al., 2016). Thus, the samples with tegument had a higher concentration of alpha tocopherol, except for the sample E.

As regards the FAME content of the samples, the results obtained agreed with those obtained previously for peanuts (Özcan, 2010; Salamatullah Mohammad et al., 2021) with Oleic, Linoleic, Palmitic and Stearic being the main fatty acids in decreasing order. Differences in concentration values has been attributed to harvesting conditions (M. Özcan and Seven, 2003).

Table 5
Validation parameters for the direct analysis methodology (DAM) optimized.

	Validation parameters			
	LOD (mg Kg^{-1})	LOQ (mg Kg^{-1})	Intra-day repeatability * (%)	Inter-day repeatability *(%)
C16:0	2	6	1	2
C18:0	2	5	1	3
C18:1	2	5	1	2
C18:2	2	7	1	2

	LOD ($\mu g \cdot Kg^{-1}$)	LOQ ($\mu g \cdot Kg^{-1}$)	Intra-day repeatability * (%)	Inter-day repeatability * (%)
α-tocopherol	17	53	5	11
β-tocopherol	10	31	2	5
γ-tocopherol	15	45	1	6
δ-tocopherol	10	30	1	7

* Intra-day and inter-day repeatability (peak area relative standard deviation (RSD) %).

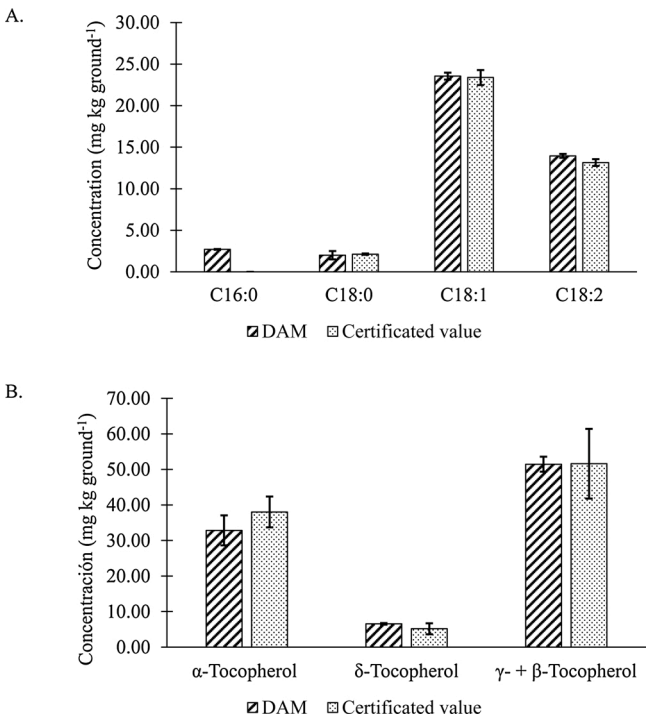

Fig. 4. Concentration values for A. FAMEs and B. tocopherols by DAM compared with certified values.

Comparing the obtained results related to FAMEs content with nutrition information provided in the labels (Table 1), the sum of the two main saturated fatty acids (i.e. C16:0 and C18:0) content was lower than the values declared. Samples E and F presented higher oleic acid content and lower linoleic acid due to they are high oleic peanuts. This relation between those FAMEs content reaches an improvement in shelf-life of peanuts (Davis et al., 2016).

4. Conclusions

In this study, a novel methodology for the direct fatty acid and tocopherol extraction and FA derivatization (DAM) was developed. To this end a multivariate optimization of the experimental conditions was carried out. After setting a Box-Behnken experimental design the optimal conditions for the extraction of the analytes and FA derivatization were found by fitting the analyte concentrations to a response surface. The selected conditions were successfully employed to the analysis of a certified reference peanut. Butter and different peanut samples.

The proposed method reduced the total preparation time of extraction and derivatization to 21.0 min (10.00 min of extraction, 10.00 min of derivatization and 1.0 min of acidification), that is less than one fourth of the time needed when the reference methodology was employed. In addition, a significant reduction of the amount of organic solvent employed was achieved what implies that the method developed can be considered more environmental-friendly.

The proposed method met the general requirements for the characterisation of FAMEs in different peanuts samples (toasted or fried) with different FAMEs and tocopherols content. Results by DAM indicated lower rates of oxidation and decomposition of the unsaturated FAMEs and lower losses of the components compared with the conventional method, which employs heated petroleum ether during the lipid extraction procedure.

In conclusion, DAM is a new and efficient methodology and a

Table 6
Fatty acids (g 100 g^{-1} peanut) and tocopherols (mg 100 g^{-1} peanut) concentration in different samples of processed peanuts by using the DAM and the reference methodologies. n = 3.

	FATTY ACIDS (g 100 g^{-1} peanut)				TOCOPHEROL (mg 100 g^{-1} peanut)			
	C16:0	C18:0	C18:1	C18:2	ALPHA	BETA	GAMMA	DELTA
A	4 ± 0.3	1.14 ± 0.07	25.5 ± 1.3	14.6 ± 0.9	6 ± 2	0.4 ± 0.3	3.6 ± 1.2	0.31 ± 0.44
B	2.40 ± 0.10	0.63 ± 0.03	27.8 ± 1.3	8.2 ± 0.4	7.8 ± 0.6	0.366 ± 0.012	3.9 ± 0.2	0.223 ± 0.005
C	3.04 ± 0.18	0.78 ± 0.05	16.1 ± 0.9	17.3 ± 0.9	7.5 ± 0.8	0.135 ± 0.015	3.5 ± 0.6	0.39 ± 0.06
D	3.9 ± 0.2	0.91 ± 0.08	22.2 ± 0.6	19.1 ± 0.4	3.12 ± 0.14	0.192 ± 0.012	1.45 ± 0.06	0.1 ± 0.02
E	1.7 ± 0.3	0.6 ± 0.2	38 ± 5	1.8 ± 0.4	10 ± 3	0.5 ± 0.3	6 ± 2	0.4 ± 0.3
F	1.7 ± 0.2	0.5 ± 0.07	44 ± 3	3.6 ± 0.4	4.8 ± 0.3	0.31 ± 0.03	2.7 ± 0.13	0.2 ± 0.009
G	3.7 ± 0.2	0.91 ± 0.07	23 ± 2	17.0 ± 1.1	3.2 ± 0.3	0.183 ± 0.010	1.23 ± 0.04	0.09 ± 0.006
H	3.0 ± 0.3	1.02 ± 0.10	22 ± 2	16 ± 2	4.4 ± 0.2	0.154 ± 0.012	4.59 ± 0.13	0.47 ± 0.06
I	2.96 ± 0.17	0.82 ± 0.04	16.5 ± 0.8	20.6 ± 1.1	1.9 ± 0.13	0.079 ± 0.008	1.2 ± 0.2	0.16 ± 0.02
J	1.4 ± 0.2	0.31 ± 0.04	8.8 ± 1.4	7.3 ± 1.2	2 ± 0.2	0.073 ± 0.012	1.39 ± 0.11	0.056 ± 0.008

powerful tool for the rapid and simultaneous characterisation of the most important analytes in nuts oxidation: FAMEs and tocopherols.

Author statement

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Declaration of Competing Interest

All authors have participated in (a) conception and design, or analysis and interpretation of the data; (b) drafting the article or revising it critically for important intellectual content; and (c) approval of the final version.

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Appendix A. Supplementary data

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