

P58: ASSESSMENT OF FATTY ACID COMPOSITION OF PEANUT BUTTER FAT EXTRACTED THROUGH DIFFERENT EXTRACTION METHODS

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Abstract – The aim of this work was to evaluate the influence of fat extraction methods on the fat content and the composition of fatty acids (FAs) of a NIST standard reference material, peanut butter (SRM[®]2387). There were used six extraction approaches: Soxhlet automatic with Büchi B-811 unit (Soxhlet standard (SS), Soxhlet warm (SW), hot extraction (HE) and continuous flow (SC)), repeated extraction with solvent (SR EN ISO 17189:2005) (SR) and Soxhlet traditional extraction (ST) by using a Soxhlet extractor. Fatty acid methyl esters (FAMES) determination was realized by using internal standards (IS) and applying relative response factors (RRF), and without IS by applying correction factors (CF). Determination of FAMES was performed on a GC-MS.

Values of fat content and fatty acid composition determined by the 6 variants were compared with the certified values of SRM[®]2387. All four approaches of extraction made with Büchi unit B-811 were effective in fat extraction and analysis of fatty acid composition compared to standard methods.

Keywords: extraction, fat, fatty acids, GC-MS

1. INTRODUCTION

Nowadays, there are different extraction methods for fat determination, depending on the matrix characteristics. The most used methods for fat extraction are Soxhlet method, and the ones described by Folch et al. (1957) and Bligh and Dyer (1959).

For fatty acids determination the most used method is gas chromatography (GC) coupled with mass spectrometry (MS) [1] or flame ionization detector (FID) [2], [3], [4], [5]. For a better identification and quantification of fatty acids, the compounds are converted into corresponding FAMES through derivatisation process. In order to transform FAs into FAMES, a methylation process is

needed and can be realized in base hydrolysis or acid hydrolysis.

The objective of this work was to evaluate the efficiency of six methods for extraction of fat for quantification of the fatty acids profile of peanut butter. Fat extraction of peanut butter was realized through the automatic Soxhlet method with 4 different extraction programs (Soxhlet standard, hot extraction, warm extraction, and continuous flow), the Soxhlet traditional method with an extractor and the SR EN ISO 17189 extraction method, and results were compared. The fatty acids profile of peanut butter fat extracted through these methods was compared.

2. EXPERIMENTAL

2.1. Reference standards

In experiments the following reference standards were used: SRM 2377, with NIST certified mass fraction values (mg/g) for 26 FAMES and F.A.M.E. Mix. C4-C24 with mass percent (%) for 37 FAMES. The extraction methods were compared on a NIST certified reference material, SRM[®]2387, peanut butter, with certified mass fraction for 12 individual fatty acids (C14:0, C16:0, C18:0, C20:0, C22:0, C24:0, C16:1n7, C18:1n9, C18:1n7, C20:1n9, C18:2n6, C18:3n3) and for the sum of saturated fatty acids (SFAs)/ monounsaturated (MUFAs)/ polyunsaturated fatty acids (PUFAs) and reference values for 5 individual fatty acids (C17:0, C17:1n7, C22:1n9, C20:2n6, C22:4n6).

2.2. Extraction of fat from peanut butter

The extraction of peanut butter fat (NIST, SRM[®]2387) was performed with petroleum ether by applying six different extraction approaches: extraction with Büchi B-811 unit (Soxhlet standard, hot extraction, warm extraction, Soxhlet under continuous flow), extraction by applying the method from SR EN ISO 17189, and Soxhlet traditional extraction by using a Soxhlet extractor.

2.3. Preparation of FAME from fat extracted from peanut butter

FAMEs preparation was realised by transesterification of extracted fat from peanut butter with methanolic solutions of sodium hydroxide and boron trifluoride (BF₃) in accordance with SR EN ISO 12966-2:2011 [6].

2.4. FAMEs analysis and operating condition of GC-MS

FAMEs analysis was performed in accordance with ISO 12966-4:2015 [7], with changes from the reference, by using of a gas chromatograph coupled with a mass spectrometer (Trace GC Ultra/TSQ Quantum XLS, Thermo Fisher Scientific, USA). A high polarity capillary column, TR-FAME (60 m x 0.25 mm x 0.25 µm) was used. Analysis of the derivatized extracts samples were performed in the positive electron impact ionization (EI⁺) mode, SIM mode (Selected Ion Monitoring) using 24 segments. The ion source temperature was 250°C, the oven temperature was programmed at 100°C for 0.2 min, increased to 240°C with 2°C/min and hold for 15 min. At this temperature it was used as a mobile phase He of purity 99.9995% (5.0), at a constant flow rate of 1 mL/min. A volume of 0.5 µL of extract was injected at 240°C in split mode with a 1:50 split ratio and a 50 mL/min splitting rate. Injections were performed in duplicate. Instrument control, data acquisition and processing were performed using the Xcalibur Program.

Identification of FAMEs from peanut butter samples was performed by comparison with retention times of FAME components from the reference standards used. The analysis of FAs composition in peanut butter was made based on RRF (g/100 g), the results being corrected by applying of the obtained recovery by using internal standards (TAG-IS, C15:0 and FAME-IS, C23:0) and based on CF (weight %).

2.5. Evaluation of measurement results

2.5.1. Evaluation of results obtained based on RRF

In order to verify that the determined fat content and composition in individual and total SFAs/ MUFAs/ PUFAs of peanut butter by the 6 extraction approaches are within the certified values of the reference material, the results of the measurements were compared with certified values [8]. This consists in comparing the absolute difference between the certified value and the

measured value (Δ_m), with the expanded uncertainty (U_Δ).

Where there is no significant difference between the measurement result and the certified value, Δ_m should be less or equal to U_Δ . Identification of peanut butter peaks was performed by comparison with the retention times of the FAME components in the reference standard SRM 2377 and the mass/charge ratio (m/z) characteristic for each component. The composition in fatty acids in peanut butter was determined as absolute concentration (g/100 g).

2.5.2. Evaluation of results obtained based on CF

The composition in individual and total fatty acids, SFAs, MUFAs and PUFAs from peanut butter extracted by the 6 extraction methods, determined based on correction factors (CF), was evaluated according to the repeatability limit (r) and the absolute difference between 2 results (Δ). Thus, it was intended that the absolute difference between two independent analysis results obtained by the same method on identical test materials in the same laboratory by the same operator using the same equipment within a short timeframe should not exceed the limit of repeatability (r), in no more than 5% of cases [2].

The peanut butter peaks were identified by comparing the retention times with the retention times of FAME components from both reference standards, SRM 2377 and F.A.M.E. Mix. C4-C24 and the mass/charge ratio (m/z), characteristic of each component. The composition in fatty acids of peanut butter was determined as relative concentration (weight percent, %).

3. RESULTS AND DISCUSSION

3.1. Fat content of peanut butter

There were no significant differences between the fat content values determined by the 6 extraction methods and the certified value (CRM) of peanut butter. For each extraction method the condition $\Delta_m \leq U_\Delta$ was fulfilled.

For the standardized SR and ST methods, the differences between the value obtained and the certified value were the lowest (0.4%), and for the 4 automatic extraction methods (SS, HE, SW, SC) the differences between the measured values and those certified were between -0.56 ÷ -1.74%.

Considering that the differences between the determined fat content values and the certified

value were not more than $\pm 2\%$, it can be said that the 6 extraction methods are effective in extracting the fat from the peanut butter.

3.2. Fatty acid composition obtained based on RRF

Fig. 1 shows a chromatogram of a sample of peanut butter. Based on reference standard SRM 2377, 12 peaks of fatty acid methyl esters were identified, of which 6 SFAs: myristic acid (C14:0),

palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0); 4 MUFAs: palmitoleic acid (C16:1n7), oleic acid (C18:1n9), cis-7-vaccenic acid (C18:1n7) and cis-11-eicosenoic acid (C20:1n9) and 2 PUFAs: cis-9,12-octadecadienoic/linoleic (C18:2n6) and cis-9,12,15-octadecatrienoic/linolenic (C18:3n3).

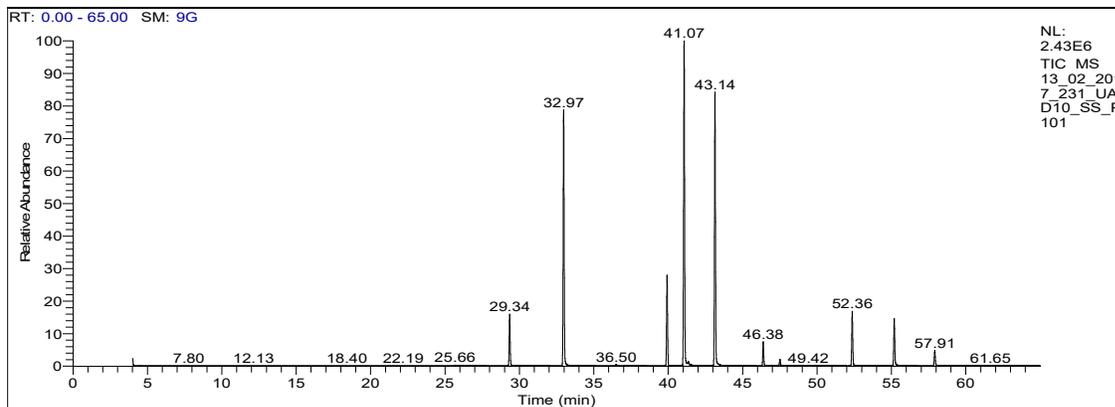


Figure 1. Chromatogram of fatty acids of peanut butter (C14:0 (RT=25.66), C15:0 (RT=29.34), C16:0 (RT=32.97), C16:1n7 (RT=34.41), C17:0 (RT=36.50), C17:1n7 (RT=37.79), C18:0 (RT=39.92), C18:1n9 (RT=41.07), C18:1n7 (RT=41.36), C18:2n6 (RT=43.14), C18:3n3 (RT=45.64), C20:0 (RT=46.38), C20:1n9 (RT=47.50), C20:2n6 (RT=49.53), C20:4n6 (RT=51.65), C22:0 (RT=52.36), C22:1n9 (RT=53.49), C24:0 (RT=57.91))

Between the measured values of the individual SFA content there were no significant differences from the certified value, the condition $\Delta_m \leq U_\Delta$ being fulfilled for the 6 SFAs from the peanut butter composition. Regarding the measured values for total SFAs content, all fat extraction methods were effective, with no significant difference from the certified value ($\Delta_m \leq U_\Delta$): CRM (10.4 ± 0.2 g/100g), SS (9.6 ± 0.9 g/100 g), HE ($10.6 \pm 0.7/100$ g), SW (10.8 ± 0.3 g/100 g), SC (9.2 ± 1.0 g/100 g), ST (10.9 ± 0.5 g/100 g), SR (10.6 ± 0.6 g/100 g).

In the case of MUFA content, there were no significant differences between the measured individual content and the certified value, the condition $\Delta_m \leq U_\Delta$ being fulfilled for cis-7-vaccenic acid (C18:1n7) and cis-11-eicosenoic acid (C20:1n9) for all 6 extraction methods (SS, HE, SW, SC, SR, ST). In the case of palmitoleic acid (C16:1n7), the condition $\Delta_m \leq U_\Delta$, was not fulfilled for all 6 extraction approaches, there being significant differences between the measured values and the certified value. The determined values were lower with $-31.82\% \div -38.64\%$ compared to the certified value.

This can be explained by the fact that the values in which this compound is found are detectable but can not be quantified. The measured values for this compound are between the detection limit (LOD, 0.021 g/100 g) and the quantification limit (LOQ, 0.064 g/100g).

Also, for oleic acid (C18:1n9), in the case of ST extraction method, the condition $\Delta_m \leq U_\Delta$ was not fulfilled, the determined value being higher than the certified value, with a difference of + 9.67%.

In terms of MUFA content measured, only 5 fat extraction methods were effective, with no significant difference from the certified value ($\Delta_m \leq U_\Delta$): CRM (24.4 ± 0.9 g/100 g), SS (24.3 ± 2.2 g/100 g), HE (25.8 ± 0.9 g/100 g), SW (26.1 ± 1.1 g/100 g), SC (23.0 ± 2.2 g/100 g), SR (26.5 ± 1.3 g/100 g). For the Soxhlet traditional extraction method (ST), the determined value was higher than the certified value, with a percentage difference of + 9.01% due to the non-fulfillment of the condition for palmitoleic acid and oleic acid.

Table 1. Values of repeatability limit, *r*, (weight %) for fatty acids and fat extracted from peanut butter through different extraction methods

FA codification	SS			HE			SW			SC			SR			ST		
	Average % (n = 5)	<i>r</i> %	Δ^a %	Average % (n = 5)	<i>r</i> %	Δ^a %	Average % (n = 5)	<i>r</i> %	Δ^a %	Average % (n = 5)	<i>r</i> %	Δ^a %	Average % (n = 5)	<i>r</i> %	Δ^a %	Average % (n = 5)	<i>r</i> %	Δ^a %
C14:0	0.05	0.01	0.01	0.04	0.01	0.01	0.04	0.01	0.00	0.04	0.01	0.01	0.05	0.01	0.01	0.05	0.00	0.00
C16:0	10.42	0.39	0.36	10.12	0.49	0.46	9.99	0.33	0.30	10.20	0.53	0.49	10.29	0.22	0.19	9.98	0.19	0.18
C16:1n7	0.06	0.02	0.02	0.06	0.01	0.00	0.06	0.01	0.00	0.06	0.00	0.00	0.06	0.01	0.01	0.06	0.01	0.01
C17:0	0.10	0.00	0.00	0.10	0.00	0.00	0.10	0.00	0.00	0.10	0.01	0.01	0.09	0.06	0.05	0.10	0.01	0.01
C17:1n7	0.04	0.02	0.02	0.05	0.01	0.01	0.05	0.00	0.00	0.05	0.01	0.01	0.04	0.03	0.03	0.05	0.01	0.01
C18:0	4.15	0.20	0.19	4.23	0.28	0.22	4.30	0.12	0.11	4.06	0.57	0.49	4.25	0.09	0.09	4.31	0.12	0.10
C18:1n9	48.44	0.87	0.81	48.04	1.18	1.04	47.92	0.76	0.64	48.40	0.46	0.42	48.86	0.54	0.53	47.92	0.39	0.38
C18:1n7	0.48	0.06	0.04	0.52	0.06	0.05	0.50	0.04	0.04	0.50	0.08	0.08	0.52	0.05	0.04	0.51	0.06	0.06
C18:2n6	29.00	0.04	0.03	28.80	0.36	0.33	28.82	0.24	0.19	28.80	0.52	0.47	28.20	0.29	0.26	28.65	0.24	0.20
C18:3n3	0.06	0.02	0.02	0.06	0.01	0.01	0.05	0.00	0.00	0.05	0.01	0.01	0.06	0.01	0.01	0.05	0.00	0.00
C20:0	1.27	0.12	0.10	1.35	0.19	0.18	1.38	0.12	0.10	1.30	0.09	0.08	1.30	0.08	0.08	1.40	0.07	0.06
C20:1n9	1.10	0.11	0.10	1.18	0.12	0.10	1.21	0.13	0.12	1.15	0.09	0.07	1.14	0.06	0.06	1.21	0.07	0.06
C20:2n6	0.02	0.01	0.01	0.02	0.00	0.00	0.02	0.00	0.00	0.02	0.00	0.00	0.02	0.01	0.01	0.02	0.01	0.01
C20:4n6	< LOQ																	
C22:0	3.41	0.51	0.43	3.76	0.55	0.52	3.85	0.38	0.28	3.63	0.29	0.23	3.57	0.28	0.24	3.69	0.20	0.20
C22:1n9	0.12	0.07	0.05	0.13	0.04	0.04	0.13	0.04	0.03	0.14	0.04	0.03	0.17	0.02	0.02	0.12	0.01	0.01
C24:0	1.27	0.27	0.22	1.55	0.28	0.27	1.58	0.23	0.20	1.49	0.12	0.10	1.38	0.15	0.13	1.61	0.08	0.07
SFA (%)	20.7	0.77	0.72	21.2	0.82	0.73	21.2	0.58	0.52	20.8	0.60	0.60	20.9	0.42	0.36	21.4	0.28	0.28
MUFA (%)	50.2	0.77	0.72	50.0	1.11	0.99	49.9	0.64	0.54	50.3	0.48	0.41	50.8	0.54	0.53	49.9	0.37	0.35
PUFA (%)	29.1	0.05	0.04	28.9	0.35	0.32	28.9	0.24	0.19	28.9	0.52	0.47	28.3	0.29	0.26	28.7	0.24	0.21

By comparing the measured values of the individual PUFA content, there were no significant differences the certified values and the obtained values, the condition $\Delta_m \leq U_\Delta$ being fulfilled for the two polyunsaturated fatty acids from the peanut butter composition. From the point of view of the measured values in terms of total PUFA content, all fat extraction methods were effective, with no significant difference from the certified value, the condition $\Delta_m \leq U_\Delta$ being fulfilled: CRM (13.2 ± 0.4 g/100 g), SS (13.2 ± 1.2 g/100 g), HE (14.0 ± 0.6 g/100 g), SW (14.4 ± 0.5 g/100 g), SC (12.5 ± 1.2 g/100 g), ST (14.4 ± 0.5 g/100 g), SR (14.0 ± 0.7 g/100 g).

The results obtained regarding the composition of fatty acids of peanut butter, based on RRF, were calculated by applying the recovery. Recovery evaluation was performed by addition of internal standard, triglyceride of pentadecanoic acid (TAG-IS, C15:0) to the fat sample and addition of tricosanoic acid methyl ester (FAME-IS, C23:0) to the final extract, before injection into GC. The data obtained show that the FAME preparation method is effective for triglyceride esterification, obtaining an average recovery of 90.74% ($81.56\% \div 98.79\%$).

3.3 Fatty acids composition obtained based on CF

Correction factors established based on reference standards (SRM 2377, F.A.M.E. Mix C4-C24) were used to determine the composition in fatty acids, in weight p% (relative concentration), of the fat extracted through the 6 extraction approaches (table 1). Based on these reference standards, 17 peaks of fatty acids methyl esters were identified, of which 12 FAMEs common to the certified values (C14:0, C16:0, C16:1n7, C18:0, C18:1n9, C18:1n7, C18:2n6, C18:3n3, C20:0, C20:1n9, C22:0, C24:0), and 5 FAMEs common to CRM reference values (C17:0, C17:1n7, C20:2n6, C20:4n6, C22:1n9).

For the 6 fat extraction methods from peanut butter, the absolute difference between two independent tests (Δ) is less than or equal to the repeatability limit (*r*) ($\Delta \leq r$), both as the sum of SFAs/ MUFAs/ PUFAs, as well as individual fatty acids (Table 1).

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