Development of a GC-FID method for the analysis of fatty acids in palm olein to study the formation of C18:1 trans during deep discontinuous frying processes in El Salvador food industry.

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Desarrollo de un método GC-FID para el análisis de ácidos grasos en oleína de palma para estudiar la formación de C18:1 trans durante procesos de fritura discontinua en la industria alimentaria de El Salvador.

Desenvolupament d'un mètode GC-FID per a l'anàlisi d'àcids grassos en oleïna de palma per estudiar la formació de C18:1 trans durant processos de fregit discontinu a la indústria alimentària d'El Salvador.

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SUMMARY

An analytical method for the derivatization of fatty acids to methyl esters using basic catalysis with special emphasis on the identification of the different C18:1 *trans* isomers was developed and validated, showing excellent accuracy, precision and linearity. This method was employed to evaluate the degradation of palm olein during the deep discontinuous frying process of potatoes and bananas over 60 hours at different temperatures, simulating most habitual food processing methods used in El Salvador. It was observed that untreated oils already showed low levels of trans fatty acids (around 0.085%) although these products were marketed as if they did not contain any trans fats. As expected, the concentration of trans fatty acids in the oil increased throughout the deep-frying process, while contents in mono- and polyunsaturated fatty acids decreased. This degradation of the oil varied depending on the temperature and the amount of water of the product being cooked.

Keywords: *Trans* Fatty Acids, FAMEs, PUFAs, Palm olein, method validation.

RESUMEN

Se desarrolló y validó un método analítico para la derivatización de ácidos grasos a ésteres metílicos mediante catálisis básica con especial énfasis en la identificación de los diferentes isómeros trans C18:1, mostrando excelente exactitud, precisión y linealidad. Este método se empleó para evaluar la degradación de la oleína de palma durante el proceso de fritura discontinua de papas y plátanos durante 60 horas a diferentes temperaturas, simulando los métodos de procesamiento de alimentos más habituales utilizados en El Salvador. Se observó que los aceites no tratados ya mostraban niveles bajos de ácidos grasos trans (alrededor del 0,085%), aunque estos productos se comercializaban como si no contuvieran grasas trans. Como era de esperar, la concentración de ácidos grasos trans en el aceite aumentó durante el proceso de fritura, mientras que los contenidos de ácidos grasos mono y poliinsaturados disminuyeron. Esta degradación del aceite variaba dependiendo de la temperatura y la cantidad de agua del producto a cocer.

Palabras clave*:* Ácidos grasos trans, FAME, PUFA, oleína de palma, validación de métodos.

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RESUM

Es va desenvolupar i validar un mètode analític per a la derivatització d'àcids grassos a èsters metílics mitjançant catàlisi bàsica amb especial èmfasi en la identificació dels diferents isòmers trans C18:1, que mostra una precisió, precisió i linealitat excel·lents. Aquest mètode es va utilitzar per avaluar la degradació de l'oleïna de palma durant el procés de fregit discontinu de patates i plàtans durant 60 hores a diferents temperatures, simulant els mètodes de processament d'aliments més habituals utilitzats a El Salvador. Es va observar que els olis no tractats ja presentaven nivells baixos d'àcids grassos trans (al voltant del 0,085%), tot i que aquests productes es comercialitzaven com si no contenguessin greixos trans. Com era d'esperar, la concentració d'àcids grassos trans a l'oli va augmentar durant el procés de fregit, mentre que el contingut en àcids grassos mono i poliinsaturats va disminuir. Aquesta degradació de l'oli variava en funció de la temperatura i de la quantitat d'aigua del producte que es cuinava.

Paraules clau*:* Àcids grassos trans, FAME, PUFA, oleïna de palma, validació del mètode.

INTRODUCTION

Deep Frying is a common procedure widely used in the food industry [1] consisting of immersing food in hot oil or fat at temperatures above the boiling point of water at atmospheric pressure [2]. It involves chemical, physical, and nutritional modifications in food increasing its palatability and organoleptic properties [2][3], through changes such as starch gelatinization, protein denaturation, and non-enzymatic browning [4]. The quality of the deep-frying process depends on factors such as the type and humidity of the food, the quality of the oil used, the temperature of the process, and the residence time of the product in the hot oil [5] [6]. At an industrial scale, an oil-to-food ratio of 6:1 is typically employed [7]. Demand for the oils and fats commonly used for frying has increased in the last 10 years, according to the Central Reserve Bank of El Salvador. In 2022, the import of oils and fats to this country was estimated at around 179.2 million kg of oil, including sunflower, soybean, corn, cotton, palm oil and its fractions (olein and stearate), and mixed oils [8].

In El Salvador, from traditional and domestic cuisine to large-scale production, palm oil is the most common oil used for deep-frying food, which is mostly fried [9], due to its thermal stability, high solid fat content (which does not require hydrogenation), high oxidative stability (long shelf life), wide range of melting points, and its competitive price; however, hydrogenated fats are still used due to their greater oxidation resistance [10] [11] [12]. Palm oil contains C16:0, C18:1Δ9c and C18:2 in its main fatty acid profile [13] [14]. It can be physically modified by fractionation to obtain a liquid part called olein that contains between 70% and 80% of unsaturated fatty acids (UFA) and a solid part known as stearin that contains between 20% and 30% of saturated fatty acids (SFA). Both fractions can be used in different food applications to meet the requirements for *trans* free fats. These fractions are mixed with other oils for better frying performance [14]. The deterioration of frying oils is related to the increase in temperature, accelerating chemical processes like thermal oxidation that can cause isomerization, cyclization, or polymerization of the oil. This degradation is also enhanced by residues from previous frying cycles [15]. The nutritional value is reduced, generating volatile compounds that produce unpleasant odors and flavors [15]. In some Latin American countries, the use of palm oil is common since in its original composition it does not have negative effects on the lipoprotein and cholesterol profile due to the distribution of fatty acids in the triglycerides that form it [16]. However, since the 1980s there have been campaigns claiming that food products containing saturated tropical oils (coconut oil, palm kernel oil and crude palm oil) contributed to the risk of coronary heart disease, which intensified research on palm oil and its fractions[10].

Trans fatty acids (*TFA*) present in the diet have different origins, including industrial hydrogenation or deodorization of unsaturated vegetable oils, particularly when subjected to high temperatures exceeding 200°C. The reuse of these oils in frying processes contribute to its thermal decomposition through hydrolysis, oxidation, and polymerization, resulting in the formation of isomerization products (*TFA* generators), and cyclization [7] [17][18]. These transformations alter the chemical composition of the oils, producing toxic compounds, that pose potential risks to consumer safety and can have significant negative consequences for the human organism [19].

Although the metabolic effect of *trans* isomers is somewhat controversial, with different views based on biochemical, nutritional and epidemiological studies, they are generally considered as unhealthy [20]. The main concern about their health effects arose due to the structural similarity of these isomers with SFA [21] having a more adverse effect than saturated fats, since they increase the concentration of low-density lipoproteins cholesterol (LDL) and reduce that of high-density lipoproteins cholesterol (HDL); thus, increasing the total cholesterol [22].

Consequently, the Pan American Health Organization has launched the REPLACE Action Plan that outlines six strategic areas of action (Review, Promote, Legislate, Analyze, Raise Awareness and Demand) to eliminate *TFA* from industrial production between 2020 and 2025 [23]. The World Health Organization and the European Food and Nutrition Action Plan 2015-2020 suggested that *TFA* should be less than 1% of the recommended daily intake of total fats, including those from natural sources [24].

The requirements of FDA 21CRF100.9 "Food Labeling" (2018), based on a daily intake of 2000 calories, established a limit of 78 g/day of total fat, 20 g/day of saturated fats, and 300 mg/day of cholesterol, but did not establish any value for *trans* fats [25]. According

to the Nutrition Institute of Central America and Panama [26], the countries of America have agreed on a new plan to reduce cardiovascular diseases, which involves the elimination of *TFA* from industrial food production by 2025.

In El Salvador, nutritional labeling is based on the RTCA 67.01.60.23 standards, where total *TFA* are declared per 100 g, 100 mL or serving, although a recommended daily value has not been established [27] . RTCA 67.04.40:07 outlines the general specifications that prepackaged and processed oils and fats must be suitable for human consumption [28]. In this guideline an analysis methodology of TFA is proposed; however, in the determination of the identity and composition of FAME by gas chromatography, *trans* isomers are not usually determined. Moreover, there is no technical regulation overseeing the quality and compositional changes in oils reused in frying processes.

For these reasons, the present study aims to implement a method for the validation of an analytical methodology to identify and quantify the formation and variation of the fatty acid content in palm olein oil during the frying process by gas chromatography, with a special focus on the formation of *trans* fatty acids like C18:1 methyl oleate during both industrial and artisanal frying processes conducted in El Salvador. To do so, reference materials were internally developed in concordance with EURACHEM standards [29], which mandate that these materials be stable, homogeneous, and not require extensive characterization.

MATERIALS AND METHODS

Seeds of yellow and white corn (*Zea mays*), sunflower (*Helianthus annuus*), soybean (*Glycine max*), mustard (*Sinapis alba*) and flaxseed (*Linum usitatissimum*) were purchased in the local market of Antiguo Cuscatlán in San Salvador. Palm oil and its olein and stearate fractions were purchased at Price Smart (Orisol, El Salvador). Analytical reagent grade of sodium hydroxide and petroleum ether were acquired from Merk (Darmstadt, Germany), HPLC grade methanol and glacial acetic acid from J.T. Baker (Phillipsburg, NJ, USA), and isooctane from VWR (Radnor, PV, USA). Hydrogen gas grade UHP, air grade zero and helium grade UAP were purchased at INFRA (San Salvador, El Salvador).

The chromatograph used was a Shimadzu GC-2014 Systems with Shimadzu AOCS/20i Automatic Injector, series: C11504300615 SA, and an FID detector (Kyoto, Japan). Chromatographic separation was carried out using a capillary column TG-POLAR 100% biscyanopropylmethylsiloxane (103 m x 0.25 mm x 0.2 µm Series: N1159321) from Thermo Scientific (Waltham, MA, USA). For the quantification of the samples, software Class VP TM 7.4 SP1, EZ Start from Shimadzu was used (Kyoto, Japan).

The semi-industrial fryer used to carry out the experiments was from Waring Pro (Stamford, CN, USA). The digital thermometer employed for monitoring the temperatures was from Fluke (Everett, WA, USA).

Extraction method

One hundred g of seeds were placed in a mortar and homogenized with a porcelain pestle until a fine powder was obtained. The powder was placed in porcelain crucibles and dried in a convection oven at 60°C for 12 h until constant weight. The virgin oil was extracted with petroleum ether in a Soxhlet at reflux temperature for 8 h. Identification of the fatty acids was carried out based on Kovats Retention Index [30].

Derivatization method

The fatty acid to its methyl ester derivatization method is based on the European Pharmacopoeia 6.2 01/2008:20429 corrected 6.2 [31]. In this case, 0.25 g of oil sample was passed into a glass vial with a septum cap. 2 mL of 0.5M sodium hydroxide/methanol were added and dissolved for 30 s, then placed in a water bath at 60°C for 10 min., allowed to cool for 10 min. While stirring, 1 mL of glacial acetic acid and 5 mL of distilled water were added. 4 mL of isooctane were added, stirring for 30 seconds until the two phases separated. The organic phase was separated and placed in a 25 mL volumetric flask, repeating the same procedure 4 times. Isooctane extracts were combined and stirred for 10 min.

Chromatographic analysis

Isothermal method:

An aliquot of 1 mL of a derivatized oil is diluted up to 5 mL with isooctane. 1 µL of this sample is injected in split mode and chromatographed at 185°C for 60 min. This method was used for the identification of the FAMEs.

Temperature gradient method:

An aliquot of 1 mL of a derivatized oil is diluted up to 5 mL with isooctane. 1 µL of this sample is injected in split mode, and chromatographed at 165°C for 35 min, increasing 5.0°C/min to 230°C held for 33 min, for a total analysis run of 80 min. Injector and detector temperatures were set at 260°C, and high-purity helium was used as carrier gas, and high-purity hydrogen and air to feed the FID. This method was used for the frying process study.

Quantification method

Area, standard deviation (SD), coefficient of variation (CV), and uncertainty were evaluated for each peak. To ensure the correct integration of the chromatographic peaks, normalization calculations were carried out with the retention times of the analytes, using the theory of Kolthoff and Hortwitz [30] to verify that the obtained data in the three repetitions of each sample met the acceptance parameters.

RESULTS AND DISCUSSION.

Deep frying of bananas and potatoes simulation.

Three experimental methodologies of deep and discontinuous frying of ripe bananas (*Musa paradisiaca*) and fresh potatoes (*Solanum tuberosum*) were carried out to determine possible variations in the frying pro-

Table 1. *Samples that were obtained during the frying process simulation.*

Experimental simulation	Samples withdrawn	Total
Fried Bananas 1	Virgen Olein, 6h, 12h, 18h, 24h, 30h, 36h, 42h,48h, 54h and 60 hours	11
Fried Bananas 2	Virgen Olein, 6h, 12h, 18h, 24h, 30h, 36h, 42h,48h, 54h and 60 hours	11
Fried Bananas 3	Virgen Olein, 6h, 12h, 18h, 24h, 30h, 36h, 42h,48h, 54h and 60 hours	11
Fried Potatoes 1	Virgen Olein and 60 hours	2
Fried Potatoes 2	Virgen Olein and 60 hours	2
Fried Potatoes 3	Virgen Olein and 60 hours	2
White Oil (without any food)	Virgen Olein and 60 hours	2

Table 2. *Actual weights and theoretical concentration calculations of the five concentration levels of the Reference Materials derivatized for method validation*

Levels of concentration		[C18:1t] theorical	Assay (n)	injection order	Virgin Olein sample (g)	Olein 60 h reused sample (g)	Area [C18:1 t] Experim. %	Relative response factor (RRF)	Recovery (%)	% Recovery must be 80-110%*
Bk Rx	WHITE REAGENT	0.0000	1	↓	0.0000	0.0000	0.0000	0.00		
		0.0000	2		0.0000	0.0000	0.0000			
		0.0000	3		0.0000	0.0000	0.0000			
					1 white isooctane SELECTIVITY					
LOW LEVEL	LEVEL 0 K=1 100% m/m Virgin Olein (OV)	0.0769	1	↓	0.2502	0.0000	0.0805	1.00	100.02	Accepted
			2		0.2500	0.0000	0.0846			
			3		0.2501	0.0000	0.0737			
					1 Bk Rx, Bk isooctane, 1 BkRx					
			4		0.2502	0.0000	0.0725			
			5		0.2500	0.0000	0.0726			
			6		0.2501	0.0000	0.0774			
					REPEATABILITY					
MEDIUM LEVEL	LEVEL 1 K=2 75% m/m OV and 25% m/m O. 60 h of reuse	0.1568			1 Bk Rx, Bk isooctane, 1 BkRx			0.93	99.34	Accepted
			1		0.1875	0.0625	0.1528			
			2		0.1875	0.0625	0.1581			
			3		0.1875	0.0625	0.1563			
					1 Bk Rx, Bk isooctane, 1 BkRx					
	LEVEL 2 K=3 50% m/m OV and 50% m/m O. 60 h of reuse	0.2367	1	LINEARITY RECOVERY	0.1251	0.1251	0.2368	1.01	100.63	Accepted
			2		0.1251	0.1251	0.2345			
			3		0.1252	0.1251	0.2432			
					1 Bk Rx, Bk isooctane, 1 BkRx					
	LEVEL 3 K=4 25% m/m OV - 75% m/m O of 60 h reuse	0.3166	1	↓	0.0625	0.1876	0.3221	1.00	100.32	Accepted
			2		0.0625	0.1875	0.3084			
			3		0.0625	0.1875	0.3224			
					1 Bk Rx, Bk isooctane, 1 BkRx					
HIGH LEVEL	LEVEL 4 K=5 100% m/m Olein with 60 hours of reuse.	0.3965	1		0.0000	0.2500	0.3916	1.00	100.00	Accepted
			2		0.0000	0.2500	0.3942			
			3		0.0000	0.2501	0.3944			
					1 Bk Rx, Bk isooctane, 1 BkRx					
			4		0.0000	0.2502	0.3978			
			5		0.0000	0.2501	0.4054			
			6	↓	0.0000	0.2500	0.3956			
					ACCURACY					

cess. In addition, an olein blank was analyzed without being used for frying. 6 L of palm olein were placed in the semi-industrial fryer, heating up to 185°C, and temperature was constantly monitored with a digital thermometer. When the selected temperature was reached, the food was fried for 7 min. The cooked food was removed, and the excess of oil was drained into the frying vat. After 53 min, a new batch of raw food was introduced, repeating the cycle until completing 12 hours in one day. This process was repeated for five consecutive days without replacing oil.

At the beginning of each simulation and every 6 hours of frying, oil samples were taken, identified, and stored in 25 mL amber bottles for subsequent analysis. At the end of the twelve daily cycles, the oil was filtered with a fine mesh cotton blanket to remove solids and stored in a glass bottle. It was left to rest for 12 hours until the remaining food residues settled, and then it was filtered again. In each experiment, the oil was reused until a total of 60 hours of heating was reached. The samples obtained from the simulations are shown in Table 1.

Preparation of olein samples used as reference material (RM)

Virgin olein with the minimum content of C18:1*trans* and samples of olein used for frying potatoes over 60 hours having the maximum content of said fats were used as reference materials. By combining both fresh and used oils, three levels of intermediate concentrations of TFAs were obtained. A series of previous chromatographic analyzes were carried out to correctly identify the minimum and maximum concentration of the FAME of C18:1*trans*.

Theoretical calculations of the concentrations were carried out based on internal normalization thanks to the FID similar response factors for different fatty acids [32]. At level 0 the theoretical content of C18:1*trans* was 0.077g and for level 4 it was 0.396 g. The preparation of the RM was carried out by placing the glass tubes containing the individual oil samples in a water bath at 60°C for 15 minutes until a homogeneous colored liquid was obtained, and immediately vortexed for 3 minutes then let it rest for 24 hours. The vials were covered with aluminum foil to protect them from light and stored at 20°C and a relative humidity of 40%.

The RM of each calibration level was weighed in five different tubes, each replica was derivatized individually as described previously and injected into a GC with an FID detector.

To verify the purity of the reagents, a Reactive Blank (RB) and Isooctane Blank (IB) were carried out. The theoretical calculations of the different concentration levels of C18:1*trans* and those weighed in the laboratory, as well as the number of replications carried out, are shown in Table 2.

Validation of the method

Eight analytical parameters were validated for the methodology used in the identification and quantification of FAME's, specifically C18:1*trans*. The official document of the Salvadorian Accreditation Body (OSA) G9.6 "Validation of Analytical Methods" physicochemical, version 2, was approved on May 23rd, 2017, [33].

The main goal of the validation process is to demonstrate that the analytical method used meets the appropriate analytical performance, with a high degree

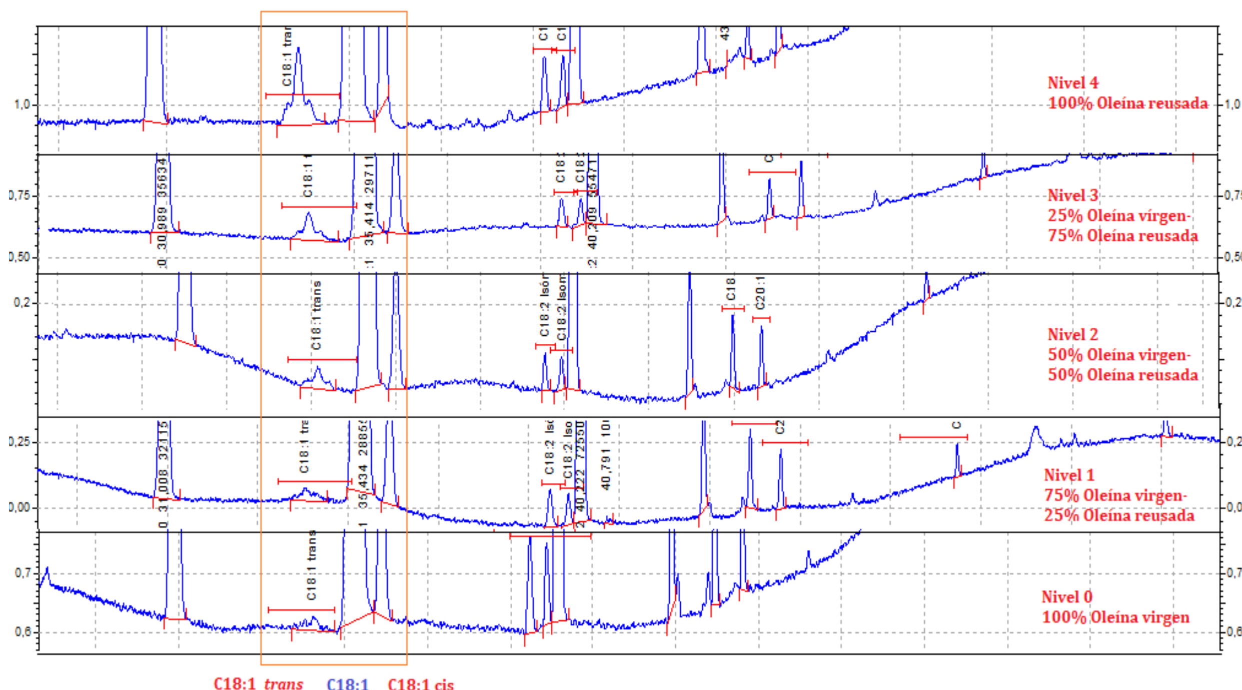

Figure 1. *Chromatograms of the 5 different concentration levels of C18:1trans*

of certainty in obtaining precise and accurate results [34]. For this, the injection of the RM was carried out in order of increasing concentration of C18:1*trans*, to minimize possible carry-over effects. After every 3 consecutive injected samples, an isooctane blank and a reactive blank (also referred to as virgin olein) samples were analyzed- to confirm that there were no interferences such as FAME residues that could remain in the column, as shown in Table 2.

Figure 1 shows the chromatograms obtained from the RM from level 0 to level 4, where the increasing area of the C18:1*trans* peak is observed at a retention time of 34.2 minutes, followed by C18:1*cis* at a retention time of 36.1 minutes. Percentages of experimental areas that were used to calculate the validation parameters (Table 2) were determined from the chromatograms. Average relative areas for C18:1*trans* from levels 0 to 4 were 0,0769%, 0,1557%, 0,2382%, 0,3176% and 0,3965% respectively.

Selectivity

As seen in Table 2, the validation began by injecting the Reactive Blank (RB) and Isooctane Blank (IB) in triplicate. After three injections of olein samples, the injection process of three RBs and one IB was repeated. Eighteen injections of RB and 6 of IB were performed. The acceptance criterion was that peak interference should not appear in the chromatograms in the two types of blanks, specifically at the retention time where C18:1*trans* elutes. With the chromatograms of the RB and IB, it was verified that no peaks were detected in the retention time where the C18:1*trans* elutes. No signals associated with the residual presence were detected where the FAMEs elute and, according to the OSA (2017), the analytical procedure used to determine C18:1*trans* is suitable for differentiating and quantifying it from the other esters, therefore it is considered a selective method [33].

Linearity

Linearity was calculated by analyzing C18:1*trans* at five different concentration levels, from the lowest to the highest level detected in oil used to fry bananas and potatoes. With the experimental results for each sample, the average area percentage of each FAMEs was calculated, as well as standard deviation (SD), coefficients of variation (CV) and residuals per level, and the relative response factor (RF) between the theoretical calculated concentration (based on historical data obtained from analyses carried out on the same types of oils during frying processes) and the concentrations obtained in the validation. Minitab-19 statistical analysis software was used to evaluate linearity.

The linearity acceptance criterion was set at a determination coefficient $R^2 \geq 0.99$, with residual values for each calibration level within 95-105%. Experimental R^2 was 0.9991, and residuals for level 0 (virgin olein) were 100.00%, level 1 were 99.98%, levels 2, 3, and 4 were 100.00%, and level 5 were 99.99%. Since the value of R^2 is greater than 0.99 and residual values are within 99.8 and 100%, it is accepted that there is linearity between the theoretical C18:1*trans* and experimental C18:1*trans*. Therefore, the level of relationship between the concentration levels and the responses detected by the equipment proves to be higher than those required in the acceptance criterion. Moreover, the analysis of variance returned a P-Value of 0.000, demonstrating that the experimental C18:1*trans* values presented a linear behavior as the C18:1*trans* values increased.

Accuracy - recovery percentage

The accuracy of the method was determined as the percentage of recovery. As shown in Table 2, for this purpose, 5 concentration levels (K=5) between 0.073% and 0.405% of C18:1*trans* were analyzed. For the lowest and highest levels, the number of replicates analyzed were N=6 and for the intermediate levels N=3. The procedure was carried out on the same day, with the same equipment and by the same analyst. The recovery percentage for each level was calculated by dividing the value obtained experimentally by the value calculated theoretically, multiplied by 100. The recovery acceptance criteria were determined at a range between 80% and 110% [33].

Table 2 shows the percentages of the theoretically calculated levels, and the data of the arithmetic means obtained from the same experimental levels. The values obtained as a percentage of recovery for each level and the replicates analyzed meet the acceptance criteria according to the AEFI since they are within the range of 99. 34-100.63% [35].

Precision and Repeatability

Based on OSA (2017), this analytical parameter is the closeness of agreement between the results of independent tests under stipulated conditions, which can also be as Repeatability [33]. This was calculated with the areas obtained from the chromatographic results of the five levels analyzed (Table 2), then, the Relative Standard Deviations (RSD) of the percentages of C18:1*trans* areas of each level were calculated.

Repeatability was estimated for levels 0 and 4 with N=6 different trials, and intermediate levels with N=3. For level 0 the RSD was 7.37%, considered acceptable according to Kolthoff's theory where the RSD can be up to 10%. RSD for levels 1 to 4 were of 1.74%,1.91%, 2.52% and 1.21% respectively, which meets the recommendations by the AOAC, stating RSD should be less than 3%.

Limit of Detection (LOD) and Limit of Quantification (LOD)

In accordance with the OSA, a series of comparisons were made by overlapping the chromatograms of the Reactive Blanks (RB) with those of level 0. The detection limit can be calculated as the concentration that generates a signal equal to three times the noise and the limit quantification as the concentration that generates a signal equal to 10 times the noise. To calculate the LOD, the signal of the RB was digitized in the region equivalent to the width of the entire C18:1*trans* peak, from 33.4 to 34.4 min, and the average standard deviation of the chromatographic signal that corresponds to the noise was calculated.

The LOD value obtained was 0.0061%. The LOQ value obtained was 0.0203. The lowest value of the experimental virgin olein was 0.0725%, which means that the values obtained in all the measurements carried out in the frying processes are considered above detection and quantification limits and therefore valid.

Identification of FAMEs including C18:1*trans* methyl esters in palm olein.

Repeatability in the retention times of the FAMEs peaks was observed in the chromatograms obtained from the samples of palm oil, palm olein and palm separate analyzed. In all of them, the six major peaks belonged to the characteristic fatty acids found in palm oil: C12:0, C14:0, C16:0, C18:0, C18:1 and C18:2, while no signal characteristic of C18:1*TFA* methyl esters was observed [13] [36]. The virgin oils extracted from six seed samples, also showed the characteristic fatty acids observed in their respective oleins. With the areas obtained in the chromatographic analysis of each sample, averages area percentages were calculated by internal normalization. The methyl esters of C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3 and the C22:1 characteristics of mustard seed were identified. Predicted retention times of the rest of FAMEs were calculated by extrapolation, observing that the results obtained were very close to the retention times of the fatty acids that had yet to be identified in the olein and that by bibliographic comparison their identity could be predicted, which were: C14:1, C20:0, C20:1, C22:0, and C24:0. The characteristic peaks of virgin palm olein are shown in the chromatogram of Figure 2.

Chromatographic analysis of fried foods.

Fried bananas

The analysis of the virgin olein was carried out without heating the oil to confirm that the peaks of the chromatograms corresponded to the main components of the olein. Based on their retention times, 20 FAMEs could be identified. Averaged results obtained from 3 frying processes over the 60 hours of reuse are shown in Figure 3. FAMEs were grouped by class; the saturated fatty acids in red (C8:0, C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C20: 0, C22:0 and C24:0); monounsaturated FAMEs in blue (C14:1, C16:1, C18:1, C18:1 cis, C20:1); polyunsaturated FAMEs in green (C:18:2 isomer 1, C18:2 isomer 2, C18:3) and C18:1*trans* identified in purple, It was observed that as the olein reuse time increases during the frying processes, the saturated FAMEs also increased from 43.8 to 50.3%, monounsaturated FAMEs decreased from 50.0% to 42.7%, C18:1*trans* FAME increased from 0.08 to 0.41%, while the polyunsaturated FAMEs decreased from 12.2% to 6.6%. The increase in the FAME of C18:1*trans* during the frying process at 185°C (Figure 4) is remarkable. Furthermore, although the nutritional information on the commercial olein bottle indicated it was TFA-free, a small amount of

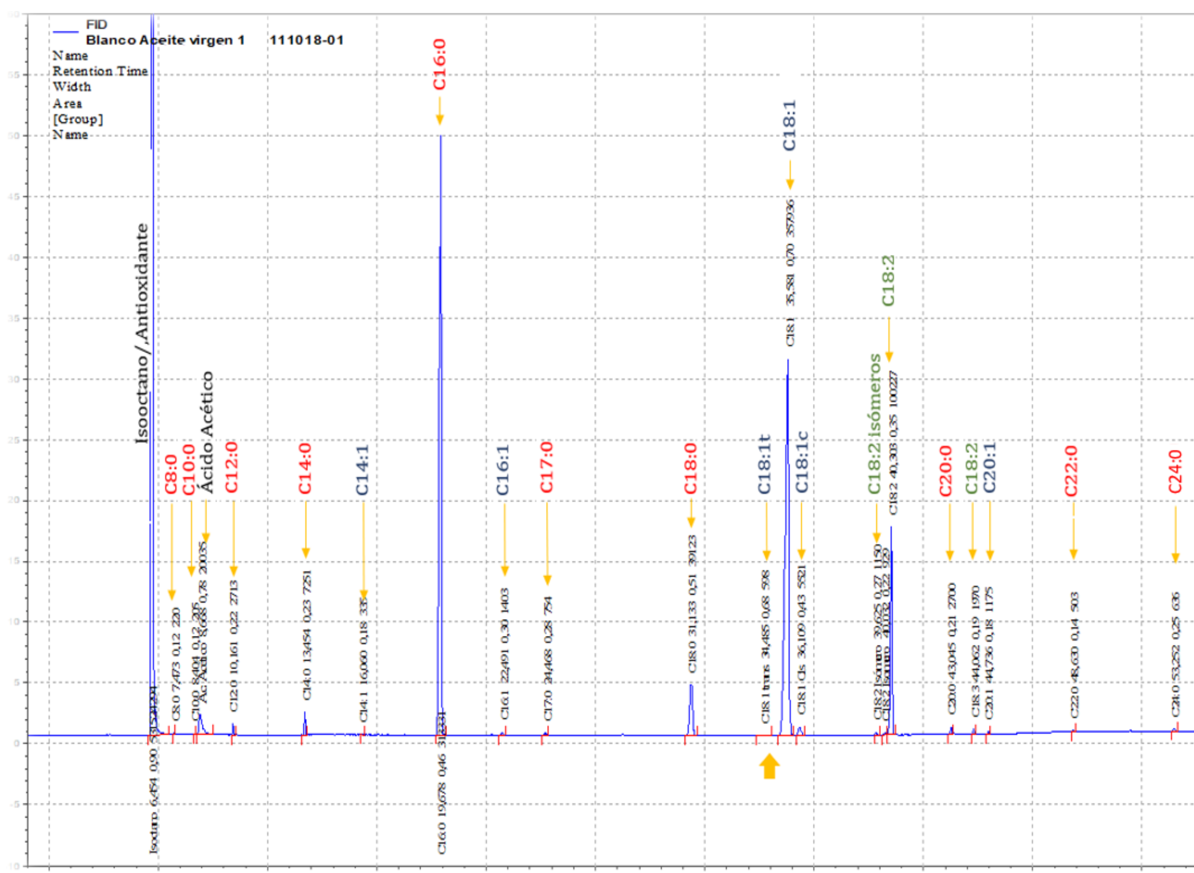

Figure 2. *Chromatograms with the characteristic peaks of virgin palm olein : methyl esters of saturated fatty acids (red), unsaturated fatty acids (blue), and polyunsaturated fatty acids (green).*

C18:1*trans* was detected and quantified above the detection limits, showing a concentration of 0.085%.

Statistical analysis of the chromatographic peak of C18:1*trans* in fried bananas

With the previous results, a 2-way analysis of variance (2-Way ANOVA) was carried out using Minitab 19, to determine if there were significant differences in the % C18:1*trans* before and after the frying process. The test determined that there is a correlation between the amount of C18:1*trans* and the frying time (the p-value for the time factor was found to be less than 0.001), as evidenced in Figures 4 and 5. The p-value for the frying replicate factor was also low (0.153), confirming that the mean for the % C18:1*trans* does not change between each frying replicate (Figure 5).

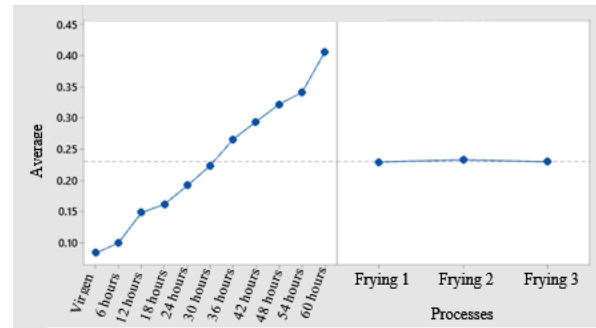

Figure 5. *On the left % C18:1trans versus frying time. To the right average % of trans C18:1 for each frying replicate*

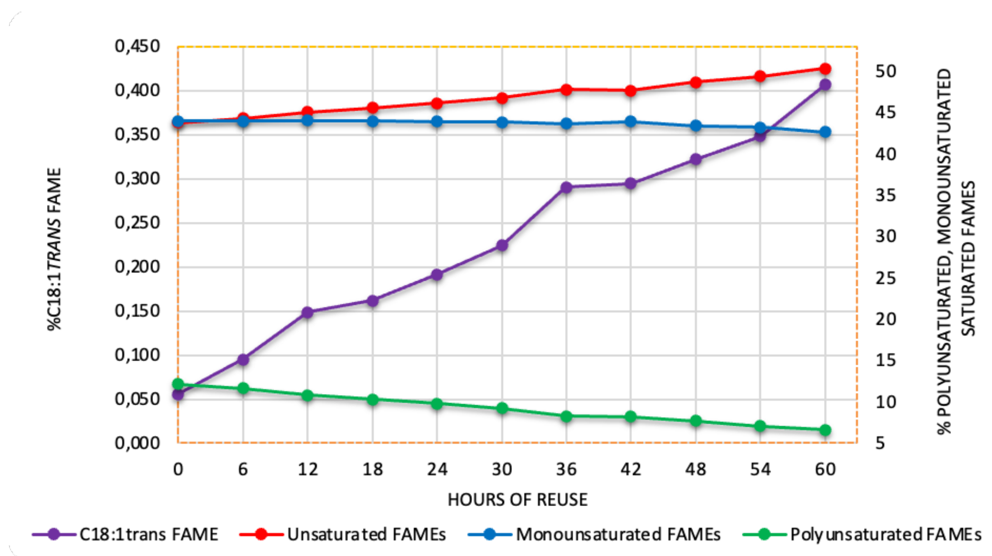

Figure 3. *Average variation of FAMEs in palm olein reused for 60 hours to obtain fried bananas*

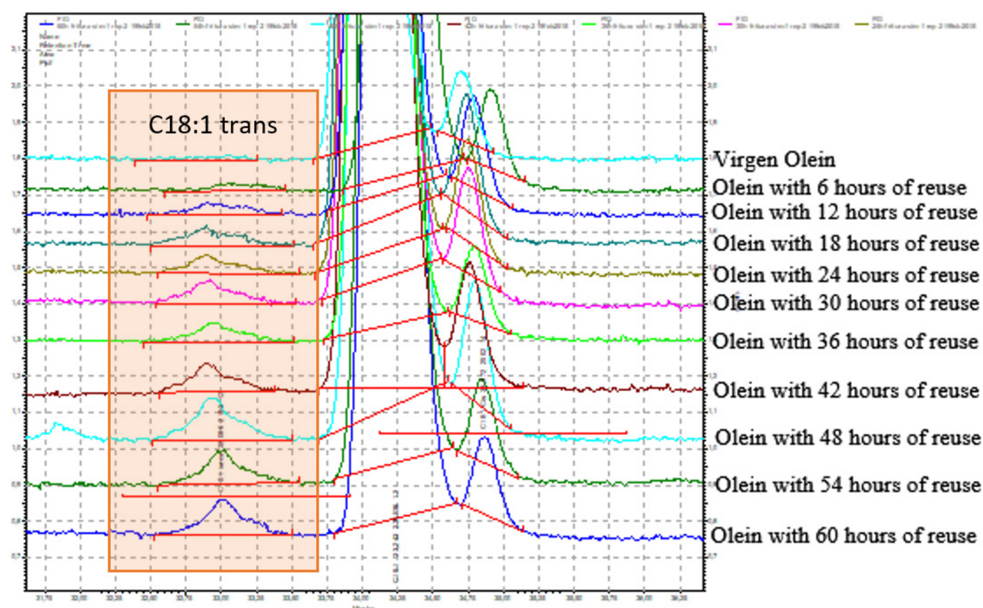

Figure 4. *FAME of C18:1trans in olein with 60 hours of reuse in fried bananas 1*

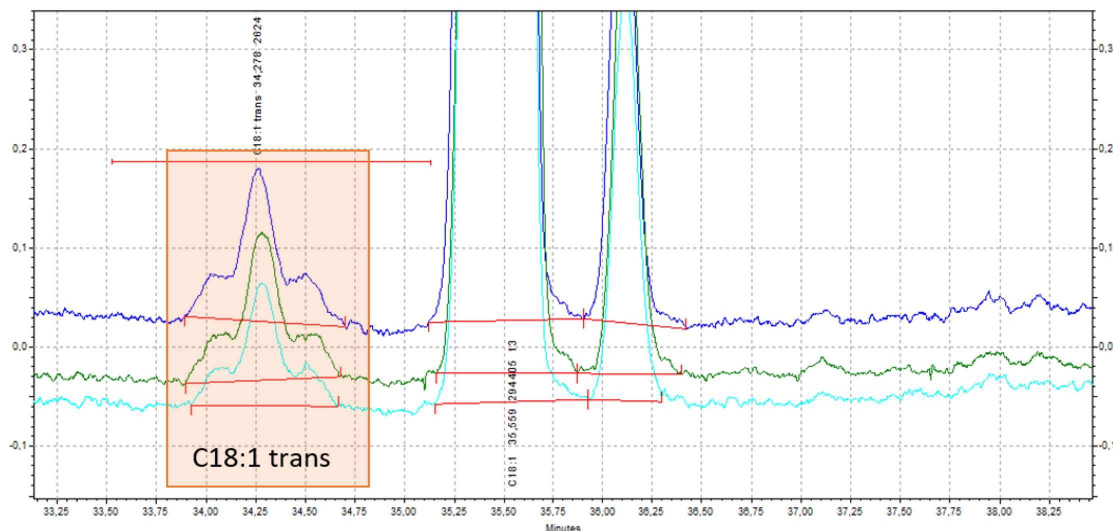

Figure 6. *Chromatograms of the three olein replicates after 60 hours of reuse to fry fresh potatoes.*

Fresh potato fries

To verify the chemical changes in olein, three simulations of frying fresh potatoes cut into sticks were carried out under the same experimental conditions as the bananas. One liter of oil sample was extracted for each simulation. In this case, only virgin samples, and samples with 60 hours of reuse were analyzed, using the same derivatization method, chromatographic conditions, and quantification method. The oil samples were analyzed in triplicate and injected individually. Figure 6 shows the overlay of the three used oil replicates.

Just like bananas, it was observed how the peak of C18:1*trans* increased from the virgin olein from 0.09% without heating to the olein after 60 hours of reuse to 0.39%.

Palm olein heating without food.

To determine the influence of food on the formation and increase of C18:1*trans* during frying processes, palm olein was subjected to the same experimental conditions without food. Virgin oil samples before and after 60 hours of reuse were analyzed in triplicate.

To verify that the virgin palm olein used in all frying products contained an initial amount of C18:1*trans*, a one-sample t-hypothesis test was carried out. P values of 0.000 were obtained with MiniTab in the three simulations of virgin oleins, for C18:1*trans,* confirming that the virgin palm contained C18:1 *trans* even before usage. Table 3 shows the values obtained in the 7 frying processes carried out for the C18:1*trans* determined in the virgin oils and with 60 hours of reuse.

It could be observed that the virgin olein contains a small amount of C18:1*trans* that increases over time when the palm olein is heated to 185°C, even without food.

CONCLUSIONS

A method for the analysis of fatty acids in oils was developed using basic derivatization to FAMEs prior to GC-FID analysis. The validated method showed great linearity (R^2 = 0.9991), accuracy (a range of recoveries between 99.34% and 100.32%), precision (0.004% RSD)

Table 3. *Averages of C18:1 trans in virgin olein and with 60 hours of reuse*

FRIED	C18:1*trans*		
	Virgin	60 hours of reuse	Average
Fried bananas 1	0.085	0.404	0.406
Fried bananas 2	0.082	0.408	
Fried bananas 3	0.084	0.407	
Fried potatoes 1	0.096	0.407	0.388
Fried potatoes 2	0.097	0.383	
Fried potatoes 3	0.087	0.375	
Virgin Olein	0.065	0.361	0.361

and low detection limits (0.0061%) and quantification limits (0.02%).

Thanks to this low detection limits, it could be evidenced that commercial olein from El Salvador contained small amounts of trans-fatty acids, although its label claimed it was trans-free.

This method was used to study the impact of using virgin olein to deep fry bananas and potatoes during a long time (60 h) without replacing the oil. It was observed that during the frying process, mono- and polyunsaturated fatty acid concentrations decreased, while amounts of saturated and trans-fatty acids increased.

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