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Research Paper / Araştırma Makalesi

Discrimination, Quantitation, and Identification of Edible Vegetable Oil Blends Based on Their Fatty Acid Profiles

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ABSTRACT

Based on the most common vegetable oil blends, binary and ternary analytical mixtures were constructed in mass fractions from 0.50 to 0.97, and their fatty acid profile was determined and represented graphically. The fatty acids with discriminatory power were selected to construct equations to predict commercial oil blend proportions. Three different linear equations resulted from the analysis for i. palm oil-based blends: y = (0.3713 ± 0.0217)x + (11.401 ± 0.68) for $C_{18:2}$ and (0.4357 ± 0.0254)x + (51.281 ± 2.90) for $C_{16:0}$ ii. soybean oil-based blends y = (-0.0789 ± 0.0046)x + (30.686 ± 1.71) for $C_{18:1}$ and (0.0686 ± 0.0040)x - (0.1395 ± 0.0081) for $C_{18:3}$ and iii. sunflower oil-based blends y = (-0.0552 ± 0.0032)x + (12.167 ± 0.6105) for $C_{16:0}$. Finally, the fatty acid profiles of n = 10 commercial samples (i.e., vegetable oil blends) were determined, and the model was applied to them with satisfactory results.

Keywords: Oil quality, Edible vegetable oil blends, Fatty acid profile, GC/FID, Guaranteed label

Yağ Asidi Profillerine Dayalı Yenilebilir Bitkisel Yağ Karışımlarının Ayırt Edilmesi, Nicelenmesi ve Tanımlanması

ÖZ

En yaygın bitkisel yağ karışımlarına dayalı olarak, ikili ve üçlü analitik karışımlar 0.50 ila 0.97 arasında kütle fraksiyonlarında oluşturulmuş ve bunların yağ asidi profilleri belirlenmiş ve grafiksel olarak gösterilmiştir. Ticari yağ karışım oranlarının tahmini için denklemler oluşturmak amacıyla ayırt edici güce sahip yağ asitleri seçilmiştir. Yapılan analizden üç farklı lineer denklem elde edilmiştir: (i) palm yağı bazlı karışımlar, C18:2 için y = (0.3713 ± 0.0217)x + (11.401 ± 0.68) ve C16:0 için (0.4357 ± 0.0254)x + (51.281 ± 2.90), (ii) soya fasulyesi yağı bazlı karışımlar, C18:1 için y = (-0.0789 ± 0.0046)x + (30.686 ± 1.71) ve C18:3 için (0.0686 ± 0.0040)x - (0.1395 ± 0.0081) ve (iii) ayçiçeği yağı bazlı karışımlar, C16:0 için y = (-0.0552 ± 0.0032)x + (12.167 ± 0.6105). Son olarak, ticari numunenin (n = 10, bitkisel yağ karışımları) yağ asidi profilleri belirlenmiş ve model, tatmin edici sonuçlarla bunlara uygulanmıştır.

Anahtar Kelimeler: Yağ kalitesi, Yenilebilir bitkisel yağ karışımları, Yağ asidi profili, GC/FID, Garanti edilen etiket

INTRODUCTION

Cooking oils are food products cataloged as essential commodities [1], accounting for a worldwide demand of 177.5 million tons in 2015 [2]. At least 13 vegetable oils are frequently commercialized, but particular focus is given to palm, soybean, rapeseed, and sunflower oils [2, 3].

Edible oils consist mainly of diacylglycerols, triglycerides, and phospholipids [4]. Since the composition and abundance of fatty acids present in vegetable oils depend on the plant species from which they were obtained [5], the differentiation of pure oil samples and blends can be based on fatty acid profiles.

Fatty acid profiling is a common practice in food analysis, and chromatographic techniques are the most common in analyzing edible oil samples. Several examples can be cited as to the application of gas chromatography in the quality analysis of edible oils [6-8]. Additionally, gas chromatography and discriminatory analysis have already been used to distinguish between argan oil and other edible oils based on the fatty acid profile [9]. Recently, a successful differentiation of edible oils was based on their fatty acid profile and Raman spectra [10]. However, research is mostly focused on the quality assessment of pure vegetable oils [11].

Despite that the current legislative Costa Rican framework (RTCA 67.04.40:07) does contain parameters to assess pure oils, it does not contemplate oil mixtures, which are the products mostly found commercially [12]. A blending of oils combines each edible oil's strong points while offering a balance of fatty acids and antioxidants [12]. This approach is also used to enhance oils' oxidative and thermal stability [12].

Herein we describe the use of fatty acid profiling by gas chromatography applied to vegetable oil blends. We demonstrated that certain indicator fatty acids could serve as a guide to verify or identify fraudulent oils mixtures and even help quantify each oil proportion.

MATERIALS and METHODS

Sampling

Samples were randomly procured from local supermarkets by government officials from the Ministry of Economy, Industry, and Commerce. A total of n = 10 commercial oil blend samples were selected and tested.

Sample Preparation and Derivatization

Exactly 0.1 g of vegetable oil were measured and quantitatively transferred to a 15 mL glass vial. Immediately, 2 mL of a boron trifluoride solution (~14 g BF_3/100 mL methanol, B1252, Sigma-Aldrich, St. Louis, Missouri, USA) and 1 mL of toluene were added. The vial was hermetically closed and heated for 45 minutes in a shaking water bath at 100°C and constant gentle agitation (TSSWB15, Thermo Scientific™, Precision™, Waltham, Massachusetts, USA). Once cooled, 5 mL of ultrapure water [type I, 0.055 µS cm^{-1} at 25 °C, 5 µg L^{-1} TOC] was obtained using an A10 Milli-Q Advantage system and an Elix 35 system (EMD Millipore Burlington, MA, USA)] and 1 mL hexane (≥95 mL/100 mL, 650552, Sigma-Aldrich,) were added to the vial and both layers are let to segregate. Then, the upper phase was recovered and pass-through sodium sulfate (798592, Sigma-Aldrich, anhydrous, granular, free-flowing, Redi-Dri™, ACS reagent, ≥99%) which was used as desiccant. Finally, 1 mL of the organic phase was sifted using a syringe filter (hydrophobic PTFE membrane, 0.45 µm, Acrodisc®, PALL®, NY, USA) and transferred to a conical glass 350 µL insert and 2 mL HPLC vial (Agilent Technologies, Santa Clara, CA, USA) for injection.

Oil Blend Preparation

Pure analytical standards were acquired to produce known oil binary and ternary mixtures (i.e., palm and soybean oil, soybean and sunflower oil, sunflower and corn oil, palm, soybean, and sunflower oil; Table 1). Then, the fatty acid profile from each oil and mixture was characterized (for an example see Figure 1A). All standards were purchased from SUPLECO (47122, 47123, 47112-U, and 46962 for soybean, sunflower, corn, and palm oil, respectively (Bellefonte, Pennsylvania, USA). All mixtures were prepared and measured individually five times. Additionally, fatty acid methyl ester (FAME) standard mixes (SUPELCO, 18919 and CRM 47885, > 99%, C_4-C_{24}) were dissolved at 10 mg mL^{-1} in dichloromethane and containing 0.01 g/100 mL 2,6-di-*tert*-butyl-4-methylphenol. These mixtures were separated quantitatively, used to calibrate the chromatographic equipment, and set each FAME retention time. A reagent blank and a standard mix were run in parallel before each determination as quality control.

Chromatographic Equipment Used during Fatty Acid Determination

All fatty acid profiles were performed using OMA℠ AOAC and AOCS methods 996.06 and Ce 1e-91 using a GC/FID system model GC-2014 equipped with an AOC-20i automatic liquid sample injection system (Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan) and J&W DB-23, 20 m × 0.18 mm × 0.2 µm (50% polybiscyanopropylsiloxane, Agilent Technologies Santa Clara, Ca, USA).

Chromatographic Conditions for Fatty Acid Determination

A gradient was used to separate the FAMEs, the temperature program was established as follows: Initial temperature 80°C, hold for 0.5 min, ramp 3°C min^{-1} to 85°C, ramp 60°C min^{-1} to 175°C, ramp 10°C min^{-1} to 185°C, hold 5 min, and finally ramp 2.5°C min^{-1} to 210°C, hold 2 min. The flame ionization detector temperature was set to 260°C, operating with hydrogen, helium (as a makeup gas) and air flow set at 75, 65, and 60 kPa (Ultra-high purity gases, Praxair, Uruca, San José, Costa Rica). The linear velocity of hydrogen carrier gas was kept constant at 41 cm s^{-1} (for a resulting total and column flow of 225.8 and 1.11 mL min^{-1}, respectively). A 2 µL 200:1 split injection was performed were the port was set at 250°C and 193 kPa.

Table 1. Preparation of the standard oil blends for fatty acid determination

Mixture proportions and mass fraction in mg						
50/50	60/40	70/30	80/20	90/10	94/6	97/3
Soybean and sunflower						
250/250	300/200	350/150	400/100	450/50	470/30	485/15
Sunflower and corn oil						
75/75	90/60	105/45	120/30	135/15	470/30	485/15
Palm and soybean oil						
75/75	90/60	105/45	120/30	135/15		
Ternary mixture						
Palm, soybean, and sunflower						
45/45/10	60/30/10	70/20/10	80/15/5	85/10/5		

Statistical Analysis

Fano factors (i.e., index of dispersion for windowed data expressed as variance-to-mean ratios, VMR) were obtained for the most promising fatty acids to serve as markers that might help discriminate between oils. Pearson Product Moment α = 0.05 was selected to prove variable association, where positive correlation and *p* values below 0.05 suggest an increasing trend. Linear regression models were constructed from the fatty acids selected as markers to prepare a model that permits quantification. Absolute values of determination close to one were an assessment of the goodness of fit. All tests were performed using Sigma Plot 14.0 (Systat Software Inc, San José, California, USA).

RESULTS and DISCUSSION

Overview of the Fatty Acid Profile of Oil Blends

Standard Oil Blends

Fatty acid profile of palm based oil blends has a higher content of saturated fatty acids with the 50:50 mixture of palm and soybean oil exhibiting a ratio of 1:1:1 poly, mono, and saturated fatty acids with $C_{16:0}$, $C_{18:1}$, and $C_{18:2}$ as the most abundant acids (Figure 2A, Table 2). In terms of the least representative fatty acids within the profile, of palm oil blends are the sole source of $C_{12:0}$ (input of ca. 2 g/100 g). Palm oil based blends are characterized for myristic and α-linoleic acids at ca. 2 and 5 g/100 g, respectively (Figure 2A and Table 2).

In contrast, for soybean and sunflower based blends the majority of the profile relies on unsaturated fatty acids (ca. 88 g/100 g of the total profile consists in MUFA and PUFA with the prevalence of $C_{18:1}$ and $C_{18:2}$) (Table 2). Dietary $C_{16:0}$ input of palm oil or palm oil-based blends (from ca. 36 to 51 g/100 g, Table 2) is the reason as for the demonstrated increase in low density lipoprotein [13] and reduction in antioxidant capability despite of palm oil being the only oil tested here which inputs beneficial fatty acids such as EPA and DHA (ca. 0.20 g/100 g, respectively, Table 2) [14].

The blends prepared herein behave as expected as the main input. For example, as soybean oil is included to palm oil, the resulting blends reflect an increase fatty acids such as $C_{18:2}$ and $C_{18:3}$. These trend has been also observed in other experiments involving oil blends where palm oil fraction is substituted; even by fish oil [15]. Finally, an interesting result is that all oil mixtures have more or less similar levels of $C_{18:0}$ and $C_{18:1}$ (ca. 2-4 and 28-35 g/100 g, respectively, Table 2).

Commercial Oil Blends

Despite some differences in processing techniques (see below) both palm olein and palm oil based blends does not reflect any significant ($p < 0.05$) differences in both general and individual fatty acid profiling (Table 3). In general terms the profile of commercial palm oil/olein blends (Figure 1B, Table 3) is similar to the one obtained for the 50-60 palm oil inclusion standard mixture (Table 2). This includes minor fatty acids such $C_{14:0}$, $C_{18:0}$, α- and γ-linolenic (with levels < 4 g/100 g, Table 2 and Table 3).

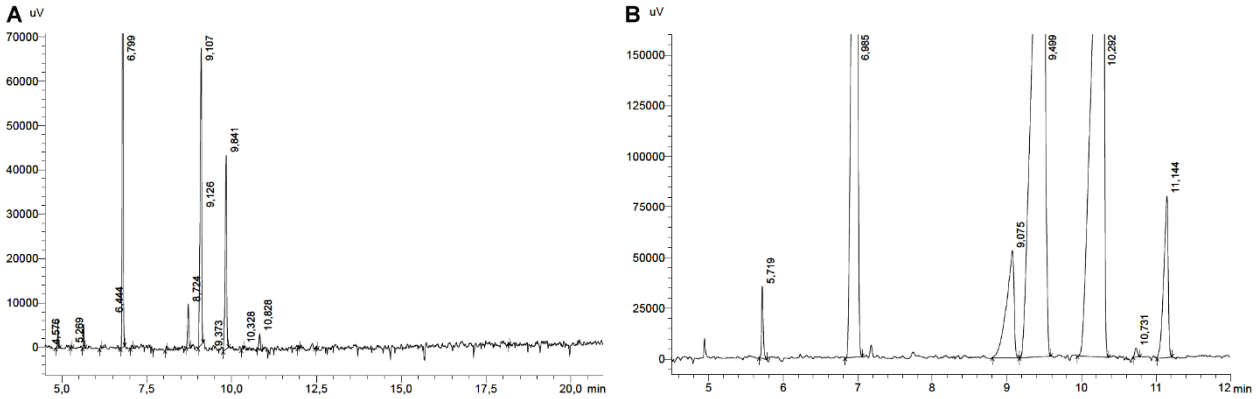

Figure 1. Experimental chromatograms for A. Palm:soybean oil standard blend at 70:30 ratio. B. a selected section of obtained for sample E, for which n = 7 signals are evident.

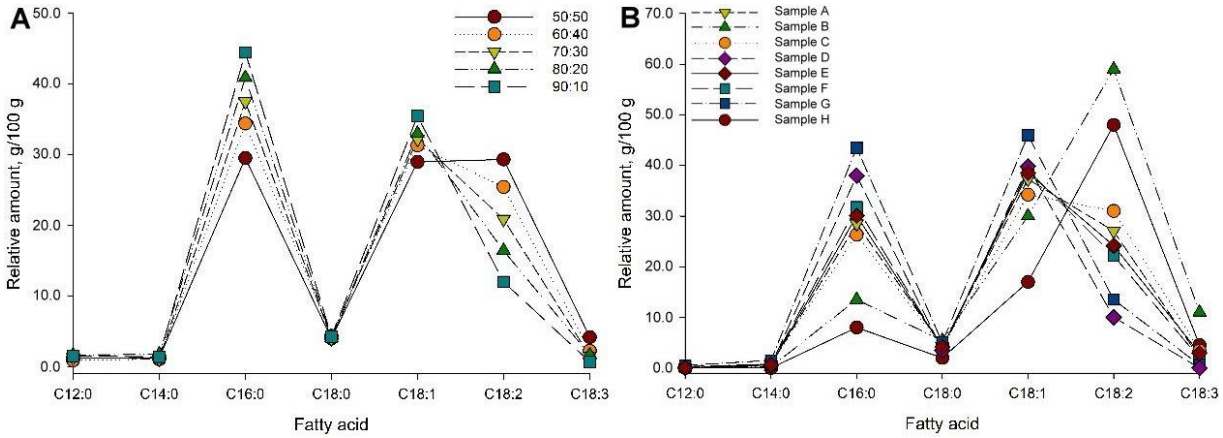

Figure 2. Fatty acid profile for A. Palm and soybean oil blends using pure standards B. Commercial oil samples labeled as palm or palm olein mixtures with soybean oil. Key: Sample A, Palm olein 1; Sample B, Palm olein 2; Sample C, Palm olein: Soybean oil 1, Sample D, Palm olein: Soybean oil 2; Sample E, Palm:Soybean oil; Sample F, Soybean oil: Palm olein; Sample G, Soybean oil 1; Sample H, Soybean oil 2.

Table 3 also demonstrates that general profile of the commercial soybean and sunflower oil and sunflower and corn oil blends are quite similar with polyunsaturated fatty acid proportion varying from ca. 50-60 g/100 g. Interestingly, soybean and sunflower oil blends show concentrations (ca. 0.5 g/100 g) of γ-linolenic acid (Table 3). Meanwhile, commercial blends based on sunflower and corn oil are devoid of this fatty acid. This indicates that soybean oils are responsible for the input of γ-linolenic acid in the oil blends. The impact of soybean oil in the input of γ-linolenic acid has been also tested *in vivo* [16]. A similar scenario is observed with erucic acid in blends containing palm oil [see binary and ternary standard blends containing palm oil (inputs of 0.35 and 0.64 g/100 g, respectively), Table 2] [17]. On another hand, when compared to the sunflower and corn oil blends, the main difference observed for the profile of the ternary mixture of sunflower, corn, and canola oil lies within a couple minor fatty acids (i.e., the presence palmitoleic and tricosanoic acids; 0.15 and 1.21 g/100 g, respectively, Table 3).

Selected Fatty Acid Content in Pure Oils and With Highest Differentiation Potential

Palm oil contains approximately 50 g/100 g saturated fatty acids [i.e., palmitic acid ($C_{16:0}$) and stearic acid ($C_{18:0}$) 44 and 5 g/100 g, respectively]. Unsaturated fatty acids are oleic acid ($C_{18:1}$) and linolenic acid (ca. 40 and 10 g/100 g respectively) [18]. Commercially, some oils contain palm olein instead of palm oil in their formulations. In this regard, palm olein is the liquid fraction obtained during the fractionation of palm oil, which involves crystallization under controlled temperature and removal of crystals by filtration. Palm olein contains higher amounts of oleic (i.e., $C_{18:1}$, 39–45 g/100 g) and linoleic acids ($C_{18:2}$, 10–13 g/100 g) compared to palm oil [19].

Sunflower oil contains mostly unsaturated fatty acids (ca. 85 g/100 g) and consisting of oleic ($C_{18:1}$) and linoleic acids ($C_{18:2}$), ranging from 14–43 and 44–75 g/100 g, respectively [20].

Table 2. Quantitative fatty acid profiles obtained for each oil blend obtained from analytical standards[a]

Binary mixtures							
Palm and soybean oil							
	Mass fraction						
Fatty acid[b]	50-50	60-40	70-30	80-20	90-10		
	Concentration, g/100 g						
Lauric $C_{12:0}$	1.21	0.90	1.29	1.64	1.49		
Tridecanoic $C_{13:0}$	-	-	0.12	-	-		
Myristic $C_{14:0}$	1.23	1.06	1.11	1.74	1.40		
Pentadecanoic $C_{15:0}$	-	-	0.15	-	-		
Palmitic $C_{16:0}$	29.48	34.36	37.46	40.83	44.40		
Palmitoleic $C_{16:1}$	-	-	0.32	-	0.30		
Margaroleic $C_{17:1}$	-	-	0.30		-		
Stearic $C_{18:0}$	4.01	4.34	3.97	4.04	4.24		
Oleic $C_{18:1}$ (ω-9)	28.93	31.27	32.14	32.94	35.41		
Linoleic $C_{18:2}$ (ω-6)	29.28	25.39	20.87	16.41	11.98		
γ-linolenic $C_{18:3}$ (ω-6)		0.16	0.28	-	0.09		
α-linolenic $C_{18:3}$ (ALA, ω-3)	4.18	2.23	1.55	1.68	0.68		
8,11,14-Eicosatrienoic $C_{20:3}$ (ω-9)	0.64	-	-	-	-		
Arachidic $C_{20:0}$	-	-	0.18	-	-		
Behenic $C_{22:0}$	0.30	-	-	0.56	-		
cis-11-eicosenoic $C_{20:1}$ (ω-9)	-	-	0.15	-	-		
Erucic $C_{22:1}$ (ω-9)	0.35	-	-	0.17	-		
cis-5,8,11,14,17-eicosapentaenoic EPA $C_{20:5}$ (ω-3)	-	0.28	-	-	-		
cis-13,16-docosadienoic $C_{22:2}$ (ω-6)	0.20	-	-	-	-		
Lignoceric $C_{24:0}$	-	-	0.13	-	-		
Docosahexenoic DHA $C_{22:6}$ (ω-3)	0.18	-	-	-	-		
General profile, g/100 g[b]							
ΣSFA	36.23	40.66	44.41	48.81	51.53		
ΣMUFA	29.28	31.27	32.91	33.11	35.71		
ΣPUFA	34.48	28.06	22.70	18.09	12.75		
ΣPUFA/ΣSFA	0.95	0.69	0.51	0.37	0.25		
ω-6/ω-3	6.75	10.20	13.67	9.79	17.82		
Soybean and sunflower oil							
	Mass fraction						
Fatty acid	50-50	60-40	70-30	80-20	90-10	94-6	97-3
	Concentration, g/100 g						
Palmitic $C_{16:0}$	8.84	9.25	9.67	10.23	10.62	10.79	10.86
Stearic $C_{18:0}$	3.22	3.60	3.61	3.78	3.65	3.37	3.67
Oleic $C_{18:1}$ (ω-9)	26.59	25.94	25.28	24.69	23.46	23.11	22.90
Linoleic $C_{18:2}$ (ω-6)	58.19	57.18	56.63	56.02	55.30	55.89	54.86
α-Linolenic $C_{18:3}$ (ALA, ω-3)	3.17	4.03	4.81	5.28	6.12	6.83	6.41
γ-Linolenic $C_{18:3}$ (ω-6)	-	-	-	-	0.21	-	0.37
Arachidic $C_{20:0}$	-	-	-	-	0.27	-	0.43
cis-11-eicosenoic $C_{20:1}$ (ω-9)	-	-	-	-	0.16	-	0.50
cis-11,14-eicosadienoic $C_{20:2}$ (ω-6)	-	-	-	-	0.10	-	-
Lignoceric $C_{24:0}$	-	-	-	-	0.11	-	-
ΣSFA	12.06	12.85	13.28	14.01	14.65	14.16	14.96
ΣMUFA	26.59	25.94	25.28	24.69	23.62	23.11	23.40
ΣPUFA	61.36	61.21	61.44	61.30	61.73	62.72	61.64
ΣPUFA/ΣSFA	5.09	4.76	4.63	4.38	4.21	4.43	4.12
ω-6/ω-3	84.10	57.22	57.28	57.19	54.60	58.26	58.60

[a]Values expressed as means. Both, standard deviation among replicates or uncertainty is not presented as invariably lies below 5.73% (using a conservative approach). [b]Σ represents the summation of SFA: Saturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, and PUFA: Polyunsaturated Fatty Acids.

Like sunflower oil, corn oil is also mostly unsaturated with linoleic ($C_{18:2}$), oleic ($C_{18:1}$), and palmitic ($C_{16:0}$) acids with 54, 27, and 11 g/100 g [21].

Finally, soybean oil is mostly composed of stearic acid ($C_{18:0}$), palmitic acid ($C_{16:0}$), linoleic acid ($C_{18:2}$), linolenic acid ($C_{18:3}$), oleic acid ($C_{18:1}$). These fatty acids in soybean oil average 4, 10, 13, 18, and 55 g/100g, respectively [22]. We suggest the reader toward the work of Dorni and coworkers [23], which show the complete profile of several pure oils as reference.

Table 2. Quantitative fatty acid profiles obtained for each oil blend obtained from analytical standards[a] (Continuing)

Sunflower and corn oil							
	Mass fraction						
Fatty acid	50-50	60-40	70-30	80-20	90-10	94-6	97-3
	Concentration, g/100 g						
Palmitic $C_{16:0}$	9.41	8.81	8.22	7.98	8.65	6.64	6.72
Stearic $C_{18:0}$	2.78	2.78	2.93	3.18	3.86	3.84	3.55
Oleic $C_{18:1}$ (ω-9)	30.09	31.19	31.57	31.66	32.90	31.27	31.13
Linoleic $C_{18:2}$ (ω-6)	56.70	57.22	57.28	57.19	54.60	58.26	58.60
α-Linolenic $C_{18:3}$ (ALA, ω-3)	0.67	-	-	-	-	-	-
Arachidic $C_{20:0}$	0.34	-	-	-	-	-	-
ΣSFA	12.53	11.59	11.15	11.16	12.51	10.48	10.27
ΣMUFA	30.09	31.19	31.57	31.66	32.9	31.27	31.13
ΣPUFA	57.37	57.22	57.28	57.19	54.6	58.26	58.6
ΣPUFA/ΣSFA	4.58	4.94	5.14	5.12	4.36	5.56	5.71
ω-6/ω-3	18.34	14.18	11.76	10.62	9.09	8.18	8.61
Ternary mixtures							
	Mass fraction						
Fatty acid	45-45-10	60-30-10	70-20-10	80-10-10	85-10-5		
	Concentration, g/100 g						
Lauric $C_{12:0}$	0.73	0.87	0.91	1.11	1.27		
Myristic $C_{14:0}$		0.74	1.24	1.09	1.38		
Palmitic $C_{16:0}$	28.55	31.12	36.06	40.08	42.51		
Stearic $C_{18:0}$	3.86	4.03	4.44	6.27	4.20		
Oleic $C_{18:1}$ (ω-9)	30.98	32.59	33.05	34.27	34.89		
Linoleic $C_{18:2}$ (ω-6)	33.60	28.05	22.08	17.18	15.40		
α-Linolenic $C_{18:3}$ (ALA, ω-3)	1.98	1.41	2.22	0.51			
8-11,14- Eicosatrienoic $C_{20:3}$ (ω-9)	0.30	-	-	-	-		
cis-5,8,11,14,-Eicosatetraenoic $C_{20:4}$ (ω-6)	-	0.54	-	-	-		
Erucic $C_{22:1}$ (ω-9)	-	0.64	-	-	-		
Docosahexenoic DHA $C_{22:6}$ (ω-3)	-	-	-	-	0.34		
ΣSFA	33.13	36.77	42.66	48.54	49.37		
ΣMUFA	30.98	33.23	33.05	34.27	34.89		
ΣPUFA	35.88	30.00	24.29	17.18	15.74		
ΣPUFA/ΣSFA	1.08	0.82	0.57	0.35	0.32		
ω-6/ω-3	15.14	14.44	15.33	33.70	45.51		

[a]Values expressed as means. Both, standard deviation among replicates or uncertainty is not presented as invariably lies below 5.73% (using a conservative approach). [b]Σ represents the summation of SFA: Saturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, and PUFA: Polyunsaturated Fatty Acids.

Selected Quality Parameters of Commercial Oil Blends

Moisture was not detected in any samples (i.e., below limit of quantitation, 0.1 g/100 g). Two samples exceeded the maximum of 0.1 g/100 g free fatty acids expressed as palmitic acid with a median and maximum values for the *n* = 10 samples of 0.067 and 0.266 g/100 g, respectively. The use of free fatty acids as a shelf-life indicator has a structural reason as triglycerides are an amalgamation of glycerol and free fatty acids [24]. An increase in free fatty acids will indicate a higher oil hydrolysis rate [25]. All samples were below the five meq O_2 kg^{-1} threshold for peroxide index with values ranging from 1.33 to 2.33 meq O_2 kg^{-1}. These values are well below those reported for oil parameters elsewhere [26, 27]. The quality of the blends justifies applying a model to quantitate the oil proportion.

Selection of Marker Fatty Acids per Oil Blend

With the data generated from the standard fatty acid profiles, the information was plotted to analyze fatty acids whose concentration changes could be fitting to use as indicators [to achieve this, different oil proportions were selected and analytes exhibiting significant ($p<0.05$) differences were preferred] and thus propose a predictive model for the commercial samples analyzed. In this regard, in palm/soybean oil blends the fatty acids that exhibited discrimination capabilities were $C_{18:2}$ and, to a lesser extent, $C_{16:0}$ (Figure 2A, 2B). Interestingly most blend proportions can be estimated using similar makers (i.e., discrimination limited to $C_{16:0}$, $C_{18:1,}$ and $C_{18:2}$; Figures 3A, 3B, 3C) even when a ternary mixture is prepared (Figure 4). In fact, under our conditions, the standard binary blend of palm and soybean oil (Figure 3A) has striking resemblance to the palm, soybean, and sunflower oils ternary blend (Figure 4) and its fatty acid input does not seem to disturb considerably the profile.

Table 3. Quantitative fatty acid profiles obtained for each oil blend obtained from the commercial samples tested

Sample C (Palm olein and soybean oil 1)				
Fatty acid		Retention time, min	Mean ± SE_x, g/100 g[a]	
Myristic $C_{14:0}$		5.604	0.85±0.21	
Palmitic $C_{16:0}$		6.744	26.32±2.07	
Stearic $C_{18:0}$		8.663	3.93±0.46	
Oleic $C_{18:1}$ (ω-9)		9.038	34.23±1.91	
Linoleic $C_{18:2}$ (ω-6)		9.776	31.03±3.83	
γ-linolenic $C_{18:3}$ (ω-6)		10.361	0.65±0.16	
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.748	3.41±0.67	
General profile, g/100 g[b]				
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
31.10	34.10	35.10	1.13	9.29
Sample D (Palm olein and soybean oil 2)				
Lauric $C_{12:0}$		4.700	0.27±0.01	
Myristic $C_{14:0}$		5.584	0.75±0.02	
Palmitic $C_{16:0}$		6.734	31.77±0.32	
Palmitoleic $C_{16:1}$		7.002	0.16±0.01	
Stearic $C_{18:0}$		8.634	4.01±0.23	
Oleic $C_{18:1}$ (ω-9)		9.022	39.13±0.23	
Linoleic $C_{18:2}$ (ω-6)		9.731	22.18±0.77	
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.693	1.93±0.23	
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
36.80	39.28	24.11	0.65	11.51
Sample E (Palm and soybean oil)				
Myristic $C_{14:0}$		5.584	0.64 ± 0.03	
Palmitic $C_{16:0}$		6.734	30.07 ± 1.98	
Stearic $C_{18:0}$		8.634	4.14 ± 0.17	
Oleic $C_{18:1}$ (ω-9)		9.022	38.52 ± 1.42	
Linoleic $C_{18:2}$ (ω-6)		9.731	24.14 ± 2.41	
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.693	2.92 ± 0.71	
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
34.85	38.52	27.05	0.78	8.27
Sample F (Soybean and palm olein)				
Myristic $C_{14:0}$		5.591	0.68±0.11	
Palmitic $C_{16:0}$		6.738	28.48±3.19	
Stearic $C_{18:0}$		8.639	4.24±0.23	
Oleic $C_{18:1}$ (ω-9)		9.028	37.28±2.85	
Linoleic $C_{18:2}$ (ω-6)		9.734	27.04±5.46	
γ- linolenic $C_{18:3}$ (ω-6)		10.339	0.30±0.09	
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.696	2.07±0.73	
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
33.40	37.28	29.42	0.88	13.19
Sample I (Soybean and sunflower oil 1)				
Palmitic $C_{16:0}$		6.732	10.14±0.22	
Stearic $C_{18:0}$		8.668	4.47±0.18	
Oleic $C_{18:1}$ (ω-9)		9.035	23.14±0.52	
Linoleic $C_{18:2}$ (ω-6)		9.822	55.80±0.65	
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.744	6.45±0.20	
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
14.61	23.14	62.25	4.26	8.65
Sample J (Soybean and sunflower oil 2)				
Palmitic $C_{16:0}$		6.727	10.69±0.21	
Stearic $C_{18:0}$		8.671	5.15±0.43	
Oleic $C_{18:1}$ (ω-9)		9.041	23.17±1.32	
Linoleic $C_{18:2}$ (ω-6)		9.832	54.34±1.48	
γ- linolenic $C_{18:3}$ (ω-6)		10.339	1.16±0.40	
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.736	5.87±0.39	
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
15.85	23.17	61.37	3.87	9.45

Table 3. Quantitative fatty acid profiles obtained for each oil blend obtained from the commercial samples tested (Continuing)

Sample K (Soybean and sunflower oil 3)				
Palmitic $C_{16:0}$		6.759		10.68 ± 0.42
Stearic $C_{18:0}$		8.719		4.92 ± 0.13
Oleic $C_{18:1}$ (ω-9)		9.091		24.01 ± 0.08
Linoleic $C_{18:2}$ (ω-6)		9.891		54.20 ± 0.64
γ-linolenic $C_{18:3}$ (ω-6)		10.305		0.58 ± 0.02
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.797		6.00 ± 0.25
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
15.60	24.01	60.78	3.89	9.14
Sample L (Soybean and sunflower oil 4)				
Myristic $C_{14:0}$		5.636		0.33 ± 0.02
Palmitic $C_{16:0}$		6.779		11.45 ± 0.55
Stearic $C_{18:0}$		8.722		5.23 ± 0.08
Oleic $C_{18:1}$ (ω-9)		9.092		24.29 ± 0.37
Linoleic $C_{18:2}$ (ω-6)		9.877		53.26 ± 1.19
γ-linolenic $C_{18:3}$ (ω-6)		10.409		0.51 ± 0.02
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.796		5.09 ± 0.43
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
17.01	24.29	58.8	3.46	10.57
Sample N (Sunflower and corn oil 1)				
Myristic $C_{14:0}$		5.593		0.70 ± 0.07
Palmitic $C_{16:0}$		6.742		14.48 ± 10.99
Stearic $C_{18:0}$		8.648		3.54 ± 0.36
Oleic $C_{18:1}$ (ω-9)		9.037		33.06 ± 2.64
Linoleic $C_{18:2}$ (ω-6)		9.759		47.99 ± 15.11
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.704		2.06 ± 0.21
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
18.72	33.06	50.05	2.67	23.33
Sample P (Sunflower, corn, and canola oil)				
Myristic $C_{14:0}$		5.621		0.30 ± 0.14
Palmitic $C_{16:0}$		6.787		13.44 ± 6.82
Palmitoleic $C_{16:1}$		7.014		0.15 ± 0.08
Stearic $C_{18:0}$		8.729		3.96 ± 0.54
Oleic $C_{18:1}$ (ω-9)		9.127		36.01 ± 1.38
Linoleic $C_{18:2}$ (ω-6)		9.872		44.28 ± 8.18
α-linolenic $C_{18:3}$ (ALA, ω-3)		10.787		1.64 ± 0.74
Tricosanoic $C_{23:0}$		17.861		1.21 ± 0.61
ΣSFA	ΣMUFA	ΣPUFA	ΣPUFA/ΣSFA	ω-6/ω-3
18.90	36.16	45.93	2.43	26.95

[a]Values expressed as the mean and standard error of the mean (SE_x) of three independent replicates. [b]Σ represents the summation of SFA: Saturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, and PUFA: Polyunsaturated Fatty Acids.

In both cases, $C_{16:0}$ and $C_{18:2}$ are the discriminatory fatty acids. Hence, it stands to reason that as long as sunflower oil does not exceed 10 g/100 g the ternary blend can be treated as a two oil blend. The impact of incorporating sunflower oil in higher proportions into the ternary blend should be examined further. A similar scenario can be observed with the sunflower, corn, and canola oil (Figure 3C). In this case, according to the order of ingredients, sunflower should be the predominant oil (Table 3). It would seem that some departure in behavior is observed in $C_{18:2}$ when compared to the binary mixture of sunflower: corn oil (Figure 3D, sample P and Figure 4).

Our data clearly indicates that the graphical expression of the results can also aid in the assessment of samples suspected to be spurious. For example, it is easy to see that the profile of the analytical mixtures for sunflower and soybean oil (Figure 3A) are almost a perfect match to the commercial sunflower and soybean oil blends (Figure 3B). In contrast, when commercial sunflower/corn oil blends are compared to the mixtures prepared with analytical standards (Figure 3D), sample M can easily be out of the trend for sunflower and corn oil blends and hence can be ruled out as an outlier ($p < 0.05$, Figure 3D). A similar scenario occurs with samples B and H (Figure 2B) that significantly ($p < 0.05$) differ from, both, the rest of the samples and the behavior of analytical mixtures (Figure 2A). These differences between pure standard oil blends and commercial blends might be explained by the differences in oil nature and refinement process. As the composition of crude oil is highly variable, depending upon the plant species, geographical location of the source and method

of oil extraction [28]. Additionally, unintentional or intentional use of cheaper (i.e., lower quality), less pure, or mislabeled raw materials can occur during the oil blending or extraction process [29, 30]. Addition of unwanted additives or mislabeling (especially if a manufacturer produce several type of oil blends in parallel) could also occur within the final product [29, 30]. This might also explain why some trace fatty acids (i.e, < 1 g/100 g) are lost when the standard mixture profile (*n* = 22 fatty acids) is compared with those profiles obtained in commercial samples (*n* = 7 fatty acids).

Albeit, the capacity of discrimination for the individual fatty acids, in blends were sunflower oil is involved, is somewhat limited when compared, for example, to palm oil blends (Figure 3A). Interestingly, similar fatty acid makers have been previously used to distinguish among pure palm, blend palm, and packet oils [31]. As MUFA are more stable in terms of oxidation [32], considerable oil degradation would restrict the model scope as when PUFA are used as markers (e.g., $C_{18:2}$, see below).

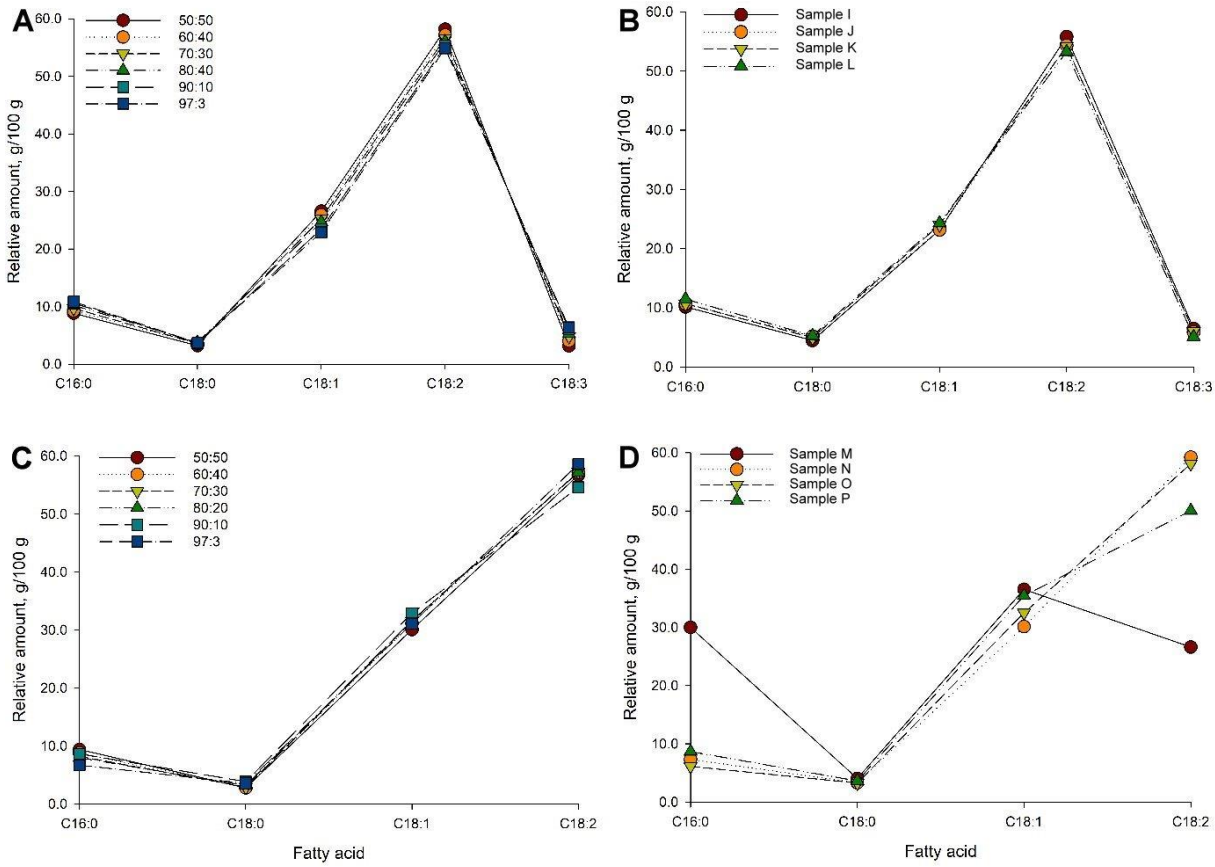

Figure 3. Fatty acid profile for A. Soybean and sunflower oil blends using pure standards. B. Commercial oil samples labeled as soybean mixtures with sunflower oil. Key: Sample I, Soybean:Sunflower oil 1; Sample J, Soybean:Sunflower oil 2, Sample K, Soybean:Sunflower oil 3, Sample L, Soybean:Sunflower oil 4. C. Sunflower and corn oil blends using pure standards. D. Commercial oil samples labeled as sunflower mixtures with corn oil and a sunflower, corn, and canola oil. Key: Sample M, Sunflower:Corn oil 1; Sample N, Sunflower: Corn oil 2, Sample O, Sunflower:Corn oil 3, Sample P, Sunflower:Corn:Canola oil.

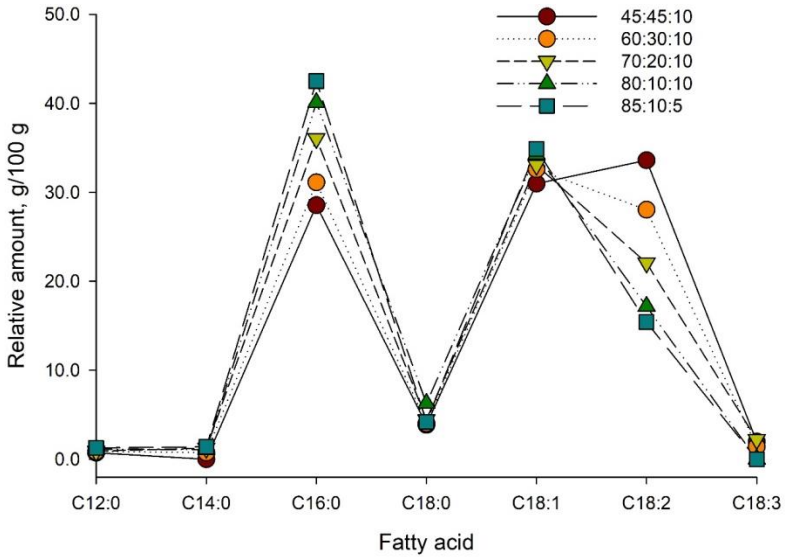

Figure 4. Fatty acid profile for ternary blends constructed with pure standards of palm, soybean, and sunflower oils.

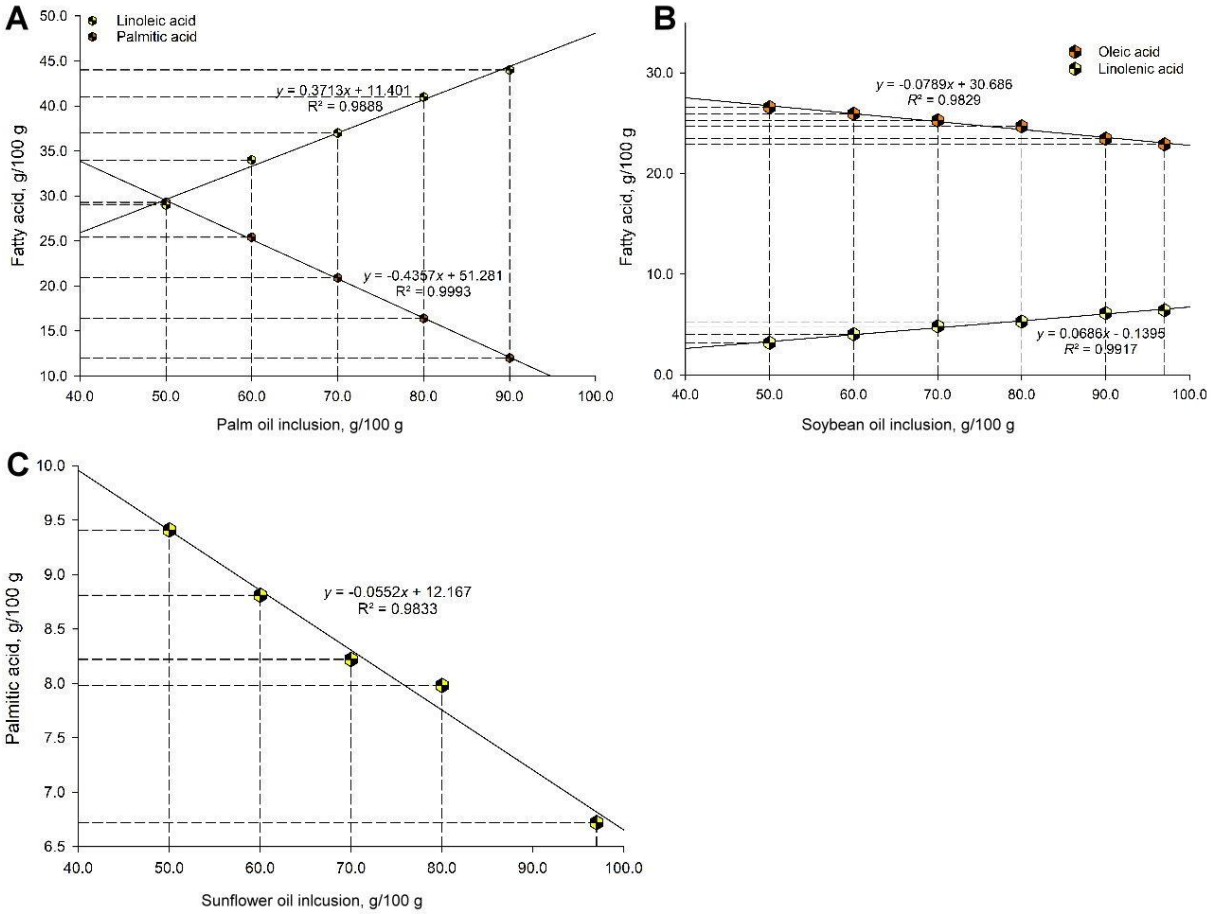

Figure 5. Calibration curves constructed based on selected fatty acids for each blend oil A. Palm: Soybean (linoleic and palmitic acids), B. Soybean: Sunflower (oleic and linolenic acids), and C. Sunflower: Corn (palmitic acid). Slopes and intercepts of the mathematical equations rendered are expressed as median values of n = 5 individual measurements.

Construction of Regression Model to Calculate Blend Fractions

As the mixtures were built and the fatty acid profiles were plotted, a clear trend arises, and the proportion of some fatty acids can be single out as they associate directly with the amount of oil fraction. If a relationship is evident, linear models can be constructed. All three models have been built with either negative or positive slopes that exhibited formidable goodness of fit with a coefficient of determination > 0.98 (Figure 5 A, B, and C). Three different linear equations resulted from the analysis. For palm oil-based blends the resulting equivalences were $y = (0.3713 \pm 0.0217)x + (11.401 \pm 0.68)$ for C18:2 and $(0.4357 \pm 0.0254)x + (51.281 \pm 2.90)$ for C16:0 (Figure 5A). For soybean oil-based blends another two fatty acids were used to construct the regression $y = (-0.0789 \pm 0.0046)x + (30.686 \pm 1.71)$ for C18:1 and $(0.0686 \pm 0.0040)x - (0.1395 \pm 0.0081)$ for C18:3 (Figure 5B). Finally, for sunflower oil-based blends $y = (-0.0552 \pm 0.0032)x + (12.167 \pm 0.6105)$ for C16:0, provided the best fit (Figure 5C).. With additional data, a more robust model can be attained, and modeling using techniques such as linear discriminant analysis can be developed. Such approaches have already been applied in milk speciation [33].

It should be noted that as a food, labeling indicates that the first ingredient to be reported to be of most abundance within the formulation. Hence, an oil labeled as "Soybean, palm oil blend" should have a higher proportion of soybean than palm oil. This is true for the samples tested with no declared ratio in the label (Table 4). Additionally, those brands that report the oil proportions are well within reasonable specification (Table 4). Other compounds present in oils can be used to detect oil adulteration [34]. However, some of these indicators are found in lower concentrations and can be more challenging to assess. Thus, considering fat a macro quality parameter, the fatty acid profile is by excellence a convenient, relatively fast, and amicable tool to determine oil blend composition and thus less inclined to be altered by the oil origin or processing.

Table 4. Results obtained in the prediction of the composition of commercial vegetable oil mixtures with respect to what is declared on the label, from the fatty acid profile.

Sample	Proportion declared in label, g/100 g	Proportion calculated from profile, g/100 g[a]
C	Non declared	40-46 palm olein
I	90:10	96:4
N	97:3	98:2
F	Non declared	46-56 palm olein
J	94:6	95:5
E	Non declared	50-62 palm oil
D	Non declared	55-67 palm olein
K	94:6	85:15
L	94:6	81:19
P	Non declared	63 sunflower oil

[a]: Data predicted using equations obtained above.

CONCLUSIONS

Fatty acids can be a powerful and convenient tool for oil blend discrimination and label guarantee assessment, primarily since a GC/FID system can be found in most food analysis laboratories. The proposed approach to discriminate oil blends based on a few fatty acids helps to focus on a few fatty acids instead of the full-blown profile. As edible oil blends are frequently available in worldwide markets, regulations should contemplate at least the most relevant fatty acids for edible oil blends. Similar tests should include mixtures with other common edible oils (e.g., canola, rice bran, safflower, and flaxseed). An additional number of repetitions can generate mathematical models with improved robustness, accuracy, and statistical significance. For each type of oil mixture, it is necessary to define the indicator fatty acid; which in general terms seem to hint toward the most abundant fatty acids with the most probability of being those selected for discrimination. The fatty acids that do not present significant differences at different proportions may be omitted as markers for monitoring. The implementation or strengthening of continuous surveillance programs of oil blends available in markets could help improve edible oil quality and countermeasure potential fraud.

DECLARATION OF COMPETING INTERESTS

The authors declare no conflicts of interest.

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REFERENCES

[1] Choudhury, R.A., Costa, M.C. (2012). Impact of government law on edible oil supply chain in

Bangladesh perspective. *International Journal Supply Chain Management*, 1(1), 33-38.

[2] Mielke, T. (2017). World Markets for vegetable oils: Status and perspectives. In Encyclopedia of Sustainability Science and Technology, Edited by R.A. Meyers, Switzerland, Springer Science+Business Media LLC., 578p.

[3] Pilorgé, E. (2020). Sunflower in the global vegetable oil system: Situation, specificities, and perspectives. *OCL*, 27, 34.

[4] Dahimi, O., Sukri Hassan, M., Abdul Rahim, A., Abdulkarim, S.M., Mashitoh, S. (2014). Differentiation of lard from other edible fats by gas chromatography-flame ionisation detector (GC-FID) and chemometrics. *Journal of Food and Pharmaceutical Sciences,* 2, 27–31.

[5] He, M., Qin, C-X., Wang, X., Ding, N-Z. (2020). Plant unsaturated fatty acids: Biosynthesis and regulation. *Frontiers in Plant Science*, 11, 390.

[6] Orsavova, J., Misurcova, L., Ambrozova, J.V., Vicha, R., Mleck, J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. *International Journal of Molecular Sciences*, 16(6), 12871-12890.

[7] Mihai, A.L., Negoiţă, M., Adascălului, A.C., Ionescu, V., Belc, N. (2018). Evaluation of fatty acids composition of some food samples by using GC-MS and NMR techniques. *Agriculture for Life Life for Agriculture Conference* Proceedings 1(1), 548-554.

[8] Mihai, A.L., Negoiţă, M., Belc, N. (2019). Evaluation of fatty acid profile of oils/fats by GC/MS through two quantification approaches. *Romanian Biotechnological Letters*, 24(6), 973-985.

[9] Rueda, A., Seiquer, I., Olalla, M., Giménez, R., Lara, L., Cabreara-Vique, C. (2014). Characterization of fatty acid profile of argan oil and other edible vegetable oils by Gas Chromatography and discriminant analysis. *Journal of Chemistry*, ID 843908.

[10] Covaciu, F-D., Berghian-Grosan, C., Feher, I., Magdas, D.A. (2020). Edible oils differentiation based on the determination of fatty acids profile and Raman spectroscopy. *Applied Science*, 10(23), 8347.

[11] Zhang, L., Li, P., Sun, X., Wang, X., Xu, B., Wang, X., Ma, F., Zhang, X., Ding, X. (2014). Classification and adulteration detection of vegetable oils based on fatty acid profiles. *Journal of Agriculture and Food Chemistry,* 62(34), 8745-8751.

[12] Manchanda, S.C. (2016). Selecting healthy edible oil in the Indian context. *Indian Heart Journal*, 68(4), 447-449.

[13] Sun, Y., Neelakantan, N., Wu, Y., Lote-Ote, R., Pan, A., van Dam, R.M. (2015). Palm oil consumption increases LDL cholesterol compared with vegetable oils low in saturated fat in a meta-analysis of clinical trials. *The Journal of Nutrition*, 145(7), 1549-1558.

[14] Larbi Ayisi, C., Zhao, J., Wu, J-W. (2018). Replacement of fish oil with palm oil: Effects on growth performance, innate immune response, antioxidant capacity and disease resistance in Nile Tilapia (*Oreochromis niloticus*). *PLoS One,* 13(4): e0196100.

[15] Sudibyo, H., Pradana·Budhijanto, Y.S., Budjijanto, W. (2018). Bio-synthesis of Eicosapentaenoic acid (EPA) from palm oil mil effluent using anaerobic process. *Defect and Diffussion Forum*, 382, 286-291.

[16] Almeida Schneider, V.A., Carbonera, F., Lopes, A.P., Santos, O.O., Oliveira, C., Souza, N.E., Visentainer, J.V. (2015). Effect of dietary replacement of soybean oil with different sources of gamma-linoleic acid fatty acid composition of Nile Tilapia. *Journal of the American Oil Chemists' Society*, 92, 225-231.

[17] EFSA, (2016). Erucic acid in feed and food. *EFSA Journal*, 14(11), e04593.

[18] Prada, F., Ayala-Díaz, I.M., Delgado, W., Ruiz-Romero, R., Romero, H.M. (2011). Effect of fruit ripening on content and chemical composition of oil from three oil palm cultivars (*Elaeis guineensis* Jacq.) grown in Colombia. *Journal of Agriculture and Food Chemistry*, 59(18), 10136-10142.

[19] Siew, W.L. (2002). Palm oil. In Vegetable Oils In Food Technology: Composition, Properties and Uses, Edited by F.D. Gunstone, Boca Raton, FLC, CRC Press, 356p.

[20] Akkaya, M.R. (2018). Prediction of fatty acid composition of sunflower seeds by near-infrared reflectance spectroscopy. *Journal of Food Science and Technology,* 55, 2318-2325.

[21] Ayyildiz, H.F., Topkafa, M., Kara, H., Sherazi, S.T.H. (2015). Evaluation of fatty acid composition, tocols profile, and oxidative stability of some fully refined edible oils. *International Journal of Food Properties*, 18(9), 2064-2076.

[22] Clemente, T.E., Cahoon, E.B. (2009). Soybean oil: Genetic approaches for modification of functionality and total content. *Plant Physiology*, 151(3), 1030-1040.

[23] Dorni, C., Sharma, P., Saikia, G., Longvah, T. (2018). Fatty acid profile of edible oils and fats consumed in India. *Food Chemistry*, 238, 9-15.

[24] Cert, A., Moreda, W., Pérez-Camino, M.C. (2000). Chromatographic analysis of minor constituents in vegetable oils. *Journal of Chromatography A*, 881(1-2), 131-148.

[25] Mahesar, S.A., Sherazi, S.T.H., Khaskheli, A.R., Kandhro, A.A., Uddin, S. (2014). Analytical approaches for the assessment of free fatty acids in oils and fats. *Analytical Methods,* 14.

[26] Al-Majidi, M.I.H., Bader, A.T. (2015). Physicochemical characteristics of some imported

edible vegetable oils in Iraq. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 6(5), 488-494.

[27] Mengistie, T., Alemu, A., Mekonnen, A. (2018). Comparison of physicochemical properties of edible vegetable oils commercially available in Bahir Dar, Ethiopia. *Chemistry International*, 4(2), 130-135.

[28] Vaisali, C., Charanyaa, S., Belur, P.D., Regupathi, I. (2015). Refining of edible oils: a critical appraisal of current and potential technologies. *International Journal of Food Science & Technology*, 50(1), 13-23.

[29] Casadei, E., Valli, E., Panni, F., Donarski, J., Gubern, J.F., Lucci, P., Conte, L., Lacoste, F., Maquet, A., Bendini, A., Toschi, T.G., 2021. Emerging trends in olive oil fraud and possible countermeasures. *Food Control*, 124, 107902.

[30] Lim, K., Pan, K., Yu, Z., Xiao, R.H., 2020. Pattern recognition based on machine learning identifies oil adulteration and edible oil mixtures. *Nature Communications*, 11, 5353.

[31] Kohr, Y.P., Sim, B.I., Abas, F., Lai, O.M., Wang, Y., Wang, Y., Tan, C.P., 2019. Quality profile determination of palm olein: potential markers for the detection of recycled cooking oils. *International Journal of Food Properties*, 22(1), 1172-1182.

[32] Maszewska, M., Florowska, A., Dłużewska, E., Wroniak, M., Marciniak-Lukasiak, K., Żbikowska, A. (2018). Oxidative stability of selected edible oils. *Molecules*, 23(7), 1746.

[33] Blasi, F., Lombardi, G., Damiani, P., Simonetti, M.S., Giua, L., Cossignani, L. (2013). Triacylglicerol stereospecific analysis and linear discriminant analysis for milk speciation. *Journal of Dairy Science*, 80(2), 144-151.

[34] Azadmard-Damirchi, S., Torbati, M. (2015). Adulterations in some edible oils and fats and their detection methods. *Journal of Food Quality and Hazards Control*, 2, 38-44.