Analysis and Characterization of Edible Oils by Chemometric Methods

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ABSTRACT: Chemometric techniques have been used to group samples with similar features as well as to discriminate among experimental data on edible oils. The objective of this study was to provide a simple method for differentiating vegetable oil types and to classify unknown samples using analytical techniques commonly used in the edible oil industry. We used principal component analysis to study the relationship between FA composition, tocopherol levels, CIE (Commission Internationale de l'Eclairage) parameters, and a photometric color index. The total variance in the original data matrix was established mainly by three principal components. Data processing allowed the oil samples to be identified and created a 2-D map as a fingerprint of the oil types. This analysis can be used successfully to differentiate vegetable oil types and classify them as crude or refined oils.

Paper no. J11145 in *JAOCS 83*, 303–308 (April 2006).

KEY WORDS: Chemometrics, CIE parameters, edible oils, fatty acids, photometric color index, principal component analysis, tocopherols.

Vegetable oils are mainly composed of TAG (95–98%) and complex mixtures of minor compounds (2–5%) of a wide range of chemical components (1). TAG are a combination of glycerol and FA. The composition and abundance of FA and minor constituents present in vegetable oils depend on the plant species from which they were obtained. Moreover, in the same species, their abundance and composition may vary depending on the agronomic and climatic conditions, fruit or seed quality, the oil extraction system, and refining procedures (2). Quantifying the FA and tocopherol composition, and measuring the color are essential for the analytical assessment of oil quality. This assessment allows the nutritional value to be preserved and avoids any possible alteration or adulteration of the vegetable oils (1).

Although aesthetic quality is an important commercial parameter, color and appearance need to be monitored to control the cost of the refining process, the quality of the finished product, and what the product looks like. Some crude oils can have unexpectedly high pigmentation caused by field damage; improper storage; or faulty handling during crushing, extraction, or rendering. During processing, the appearance of the product may indicate a problem, such as from improper bleaching, oxidation, or ineffective filtration (2).

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Many analytical methodologies have been proposed for the characterization, analysis, and authentication of vegetable oils based on their components, including GC, ^{1}H and ^{13}C NMR spectroscopy, MS, NIR spectroscopy, and Raman spectrometry (1,3–6).

The use of a spectrophotometer to obtain a photometric color index (PCI) can provide an objective color measurement of fats and oils. The use of the tristimulus colorimetry recommended by the Commission Internationale de l'Eclairage (CIE) allows a better discrimination than the red, yellow, and blue parameters normally used in the oil industry, so the use of CIE-1976 (L*a*b*) or the CIELAB system has been accepted in most industries (7,8).

However, information from the properties under study may be difficult to interpret if a high number of edible oils of different origins, crude or refined, are analyzed. The use of multivariate statistical methods or chemometric techniques offers an opportunity to look critically at both the quantity and quality of data.

Chemometric procedures such as principal component analysis (PCA), among others, have frequently been used to obtain the maximum information from retention data matrices of considerable dimensions (9), and have been notably improved in recent years with the assistance of computer science (10).

PCA allows the number of variables to be reduced while maintaining most of the information by simultaneously studying all of the variable relationships. Because of its simplicity, PCA has frequently been used in food science and technology to classify foodstuffs according to their chemical composition, to group samples with similar features, and to discriminate among different vegetable oils (11–19).

The aim of this work was to establish a methodology to differentiate vegetable oils using chemometric analysis and analytical techniques commonly used in the fats and edible oils industry. Experimental data were obtained in our laboratory from more than 50 samples; data processing allowed the oil sample to be identified and a 2-D map to be created as a fingerprint of the oil types.

Canola, grapeseed, industrial crude soybean oil (ICS), and edible commercial oil mixtures, such us sunflower–corn oil (S-C), sunflower–olive oil (S-O), and sunflower–soybean oil (S-S), were used to test the usefulness of the method. The goal of the methodology proposed here was to be capable of distinguishing different oil groups in a biplot of the principal components (PC) obtained using our experimental data.

EXPERIMENTAL PROCEDURES

Oil sample collection. The oil samples were crude and refined oils obtained from an industrial process or extracted in our laboratory from the following varieties: sunflower, corn, soybean, canola, grapeseed, and olive. Commercial oil samples were obtained in local supermarkets.

Both pure oils and mixtures were used to evaluate the chemometric analysis. Soybean oil was obtained from the beans by Soxhlet extraction with hexane for 6 h. Duplicate extractions were performed. Olive oil was extracted with a laboratory-scale system that simulated a standard olive mill (7).

FA profile. Oils were methylated and FAME were analyzed by GC using an HP 5890 Series II chromatograph with an FID detector and an HP model 3395 integrator. An HP-Innowax, 30 m, 025 mm, 025 μm silica capillary column (Hewlett-Packard, Palo Alto, CA) was used. Nitrogen was used as the carrier gas. FAME peaks were identified by comparison with retention times of the respective standards (Sigma Chemical Co., St. Louis, MO).

The peak areas were normalized using unity response factors. The amounts of palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids were calculated as one hundredths of a percent. Results are the mean value of three replicates.

Tocopherol analysis. Tocopherol content was analyzed by HPLC using a Varian 5000 liquid chromatograph, a 10-μL manual loop injector with a model 2550 UV-visible detector, and a Varian 4270/4290 integrator for output data processing. The mobile phase was hexane–isopropanol (99.5:0.5) (HPLC grade; Sintorgan, Buenos Aires, Argentina) with a flow rate of 1 mL/min.

A normal-phase Lichrospher Si-60 column (LiChroCART®-250-4; Hewlett-Packard) and an Si-5 guard column (Hewlett-Packard) were used at room temperature.

Two grams of sample was diluted in 25 mL of hexane and injected directly into the HPLC instrument. The tocopherol peaks were identified by comparison with the retention times of the corresponding standards (Sigma Chemical Co.). The purity and stability of standards were defined by extinction coefficient values measured in a Hewlett-Packard HP 8453 spectrophotometer.

The maximum absorption wavelengths used in the chromatographic runs were 294 nm. Results were expressed as the weight percentage of the oil. Each one is the mean value of three replicates.

Chromatic measurements. A PCI was used to measure the color of fats and oils with an automatic instrument. The color parameter was calculated from the edible oil visible spectrum using Equation 1:

$$PCI = 1.29(A_{460}) + 69.7(A_{550}) + 41.2(A_{620}) - 56.4(A_{670}) \quad [1]$$

where A is the absorbance measured at 460, 550, 620, and 670 nm, respectively (2,20).

Tristimulus colorimetry was used to improve the objective analysis of color in oils. CIELAB was used to describe the precise 3-D location of color. In this system, L* is used to describe relative lightness, whereas a* and b* represent relative redness–greenness and relative yellowness–blueness, respectively.

Oil spectra were obtained using a UV-vis diode spectrophotometer (Hewlett-Packard HP 8453) in 10-mm quartz cells. PCI values were calculated using Equation 1 and the spectral data at the corresponding absorption wavelengths (20).

The L*, a*, and b* parameters were measured by the PFX 190 Lovibond Tintometer®.

All chromatographic (GC and HPLC) and chromatic analyses were performed on the same oil samples.

Statistical analyses. Statistical analyses were carried out using Stata 3 (StataCorp LP, College Station, TX) and Statistica (StatSoft Inc., Tulsa, OK) software.

RESULTS AND DISCUSSION

FA profile and tocopherol quantification. The mean values obtained for FA composition as well as for α-, β-, γ-, and δ-tocopherol content of the vegetable oils analyzed are shown in Table 1 and are in accordance with normal values shown in published data (2).

PCI and CIELAB parameters. The UV-vis absorption spectra of extra virgin olive oil and refined edible oils are shown in Figure 1. Extra virgin olive oil spectra have two intense absorption bands, one in the region between 400 and 550 nm that corresponds to the absorption of carotenoid and chlorophyll pigments, and an isolated absorption band of chlorophyll pigments in the region of 620 to 670 nm (Fig. 1). In general, the UV-vis spectra of crude oils had more intense bands than refined oils, and the differences were strongly influenced by the method of oil processing used.

Table 2 shows the PCI mean values and L*a*b* parameters obtained for each oil. As one can observe, olive oil had a negative PCI value that coincided with the highest chlorophyll content (Fig. 1), and refined soybean oils had lower PCI values than crude soybean oil, since in the refining process the chlorophyll and carotenoid fractions were lowered.

The PCI values of commercial mixtures such as S-C and S-O, both with a declared proportion of 95% sunflower oil, were similar to other sunflower samples analyzed. As expected, the PCI values of S-S were similar to the PCI values of pure soybean oil, since the commercial mixture was 95% soybean oil.

L* values allowed us to distinguish crude from refined oils, since the refined oils showed L* values higher than 90 as a consequence of the bleaching process used. The positive b* values indicated that the yellow coloration of the oils was related to the carotene content. The a* parameter changed from positive values in soybean and corn oils to negative values in refined oils and olive oils. The more positive values of a* were related to the red color and darkness observed in soybean oils. The negative values of a* were related to the greenish cast usually shown in olive oils; these were lighter in refined oils.

Chemometric analysis. PCA was selected to provide an overview of the capacity to distinguish among vegetable oils

TABLE 1
Mean Values of FA and Tocopherol (T) Content of Different Oils: (A) Oils Used to Develop the Method, and (B) Oils Used to Test the Method

	Oil	N[a]	16:0	18:0	18:1	18:2	18:3	α-T	β-T	γ-T	δ-T
A	Corn	5	11.64	1.55	33.63	52.93	0.50	198.20	92.77	508.2	ND[b]
	Sunflower	10	4.03	2.04	29.73	63.02	0.02	453.50	100.40	96.44	ND
	Olive	10	13.42	1.49	73.02	10.93	0.63	53.27	ND	ND	ND
	Crude soybean[c]	17	10.42	4.03	19.78	56.45	9.19	255.43	200.54	300.08	252.21
	Soybean[d]	2	10.77	4.90	23.99	55.19	5.66	144.50	24.75	692.20	175.60
B	Canola	1	3.12	1.35	60.38	28.35	6.80	102.80	76.73	173.00	ND
	Grapeseed	1	7.17	24.23	62.26	6.22	0.12	102.80	ND	29.72	ND
	Crude soybean[e]	1	10.15	3.09	21.81	54.96	9.99	1335.92	13.28	22.03	1530.86
	Sunflower–corn	1	6.60	2.44	31.22	59.64	0.10	113.20	16.66	30.42	ND
	Sunflower–olive	1	6.80	2.72	35.19	55.08	0.21	355.20	39.6	32.19	ND
	Soybean–sunflower	1	10.43	4.69	25.20	53.70	5.98	101.30	24.75	424.40	137.10

[a]*N*, number of samples used.
[b]ND, not detected.
[c]Laboratory crude oil.
[d]Commercial refined oil.
[e]Industrial crude oil.

based on chromatographic results (FA composition and tocopherol content), the PCI, and CIELAB parameter data using different combinations of the PC obtained.

Results of the PCA are compiled in Table 3. In all cases, only the first three PC had eigenvalues greater than one, and they explained more than 80% of the total variance.

The score and loading plot of the first two PC (PC1 and PC2) obtained in the PCA of the FA composition data of all the oil samples used in this work shows four different oil groups that could clearly be distinguished from one another (Fig. 2). In the present case, the PC1 loading was high and positive for 18:2 and 18:3 and high and negative for 18:1. Likewise, PC2 showed a high positive influence and a high negative influence for 16:0 and 18:0, respectively.

In a second PCA, the score and loading plot of PC1 and PC2 that resulted when using the FA composition and PCI data (Fig. 3) showed four oil groups according to their agronomic origin as in Figure 2, but this PCA allowed us to discriminate among crude (IIIa) and refined (IIIb) soybean oils in group III.

The score and loading plot of PCA based on the FA composition and CIELAB parameter data is shown in Figure 4. A better discrimination between refined and crude soybean oils was observed. The CIE parameters (a* and b*) and 18:3 tended to pull samples to high values for PC1, whereas L* tended to pull samples down along the PC1 axis. These results confirm that tristimulus colorimetry allows a better discrimination between the oils than was obtained with FA composition and PCI data (Figs. 2, 3).

Because tocopherols are natural antioxidant components present in oils whose concentration can vary significantly during the refining process, tocopherols were included in the PCA to evaluate their influence on the discrimination of edible oils. When this was done, a strong correlation between α- and δ-tocopherol was observed, and the PCA results using β-, γ-, and δ-tocopherol showed that a single eigenvalue explained 57% of the original variance. No significant differences were observed when using only tocopherol or when using the tocopherol and FA composition as variables for the PCA. Similarly, the PCA with tocopherols and the PCI did not result in good discrimination between the oil groups.

Figure 5 shows the score and loading plot of the PCA when tocopherol levels and CIELAB parameters were used. A good classification was obtained between crude soybean oil collected in different years in our laboratory ($III_{a\text{-}1}$, $III_{a\text{-}2}$) and refined oils. Olive oil (group I) constitutes a particular case among the analyzed oils since it is a well-differentiated group close to the refined oils.

The PC1 loading values of a* and b* were high and positive, but the contribution of L* was high and negative. The PC2 axis was influenced by β-tocopherol in a high and positive direction.

The efficiency of the method was tested using canola, grapeseed, and some commercial mixtures such as S-O, S-S, and S-C purchased from local supermarkets. PCA with the analytical results obtained were used to characterize these oils and to evaluate the capacity for differentiation.

Canola and grapeseed oils have an appearance that is similar to other edible oils usually found in local supermarkets, but when PCA were used with the analytical results, canola and grapeseed oils were separated from the other refined oil groups previously identified, as one can see in Figures 2–5.

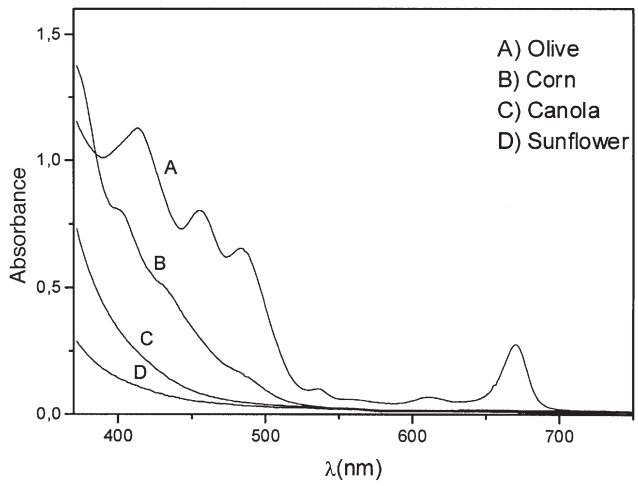

FIG. 1. Absorption spectra of extra virgin olive oil and refined edible oils.

TABLE 2
Mean Values for the Photometric Color Index (PCI) and CIELAB Parameters of Different Oils: (A) Oils Used to Develop the Method, and (B) Oils Used to Test the Method

	Oil	N[a]	PCI	L*	a*	b*
A	Corn	5	2.04	97.64	7.37	25.16
	Sunflower	10	1.41	99.76	–2.06	5.41
	Olive	10	–7.75	94.62	–6.81	58.12
	Crude soybean[b]	17	17.99	67.64	13.92	102.96
	Soybean[c]	2	0.385	95.87	–7.64	33.19
B	Canola	1	1.62	99.71	–4.45	10.40
	Grapeseed	1	16.72	89.47	–19.33	32.81
	Crude soybean[d]	1	4.43	88.38	7.40	133.3
	Sunflower–olive	1	1.60	98.81	–5.11	19.07
	Soybean–sunflower	1	0.46	95.00	–7.50	32.20
	Sunflower–corn	1	0.66	99.82	–3.14	7.00

[a]N, number of oil samples used.
[b]Laboratory crude oil.
[c]Commercial refined oil.
[d]Industrial crude oil.

TABLE 3
Results of the Principal Component Analysis of Different Oil Samples Based on: (A) FA Composition, (B) FA Composition and the Photometric Color Index, (C) FA Composition and CIELAB Parameters, and (D) Tocopherol and CIELAB Parameters

	No. of component	Eigenvalue	Variance explained (%)	Sum of variance explained (%)
A	1	2.38070	47.74	47.74
	2	1.30992	26.20	73.94
	3	1.06193	19.24	95.18
	4	0.23922	5.78	99.96
	5	0.00191	0.04	100
B	1	2.56058	42.68	42.68
	2	1.48333	24.72	67.40
	3	1.15296	19.22	86.61
	4	0.57985	9.66	96.28
	5	0.22187	3.72	100
C	1	3.52653	44.08	44.08
	2	1.97581	24.70	68.78
	3	1.19155	14.89	83.67
	4	0.69996	8.75	92.42
	5	0.32598	4.07	96.50
	6	0.21468	2.68	99.18
	7	0.06424	0.82	100
D	1	2.23932	37.32	37.32
	2	1.70624	28.44	65.76
	3	1.01882	19.98	82.74
	4	0.50268	8.38	91.12
	5	0.34267	5.71	96.83
	6	0.19026	3.17	100

Applying the PCA, the commercial mixtures S-O and S-C, with a declared proportion of 95% sunflower oil, were plotted near the sunflower group (IV) instead of near the ICS, and S-S, with a declared proportion of 95% soybean oil, was plotted near group III comprising crude and refined soybean oils, respectively (see Figs. 2–5).

The results of this work demonstrate that the combination of chemometric techniques and experimental data obtained from GC, HPLC, and color measurements can be used to distinguish oils of different origins and to establish the crude or refined condition of the oil from a complex group of vegetable oils.

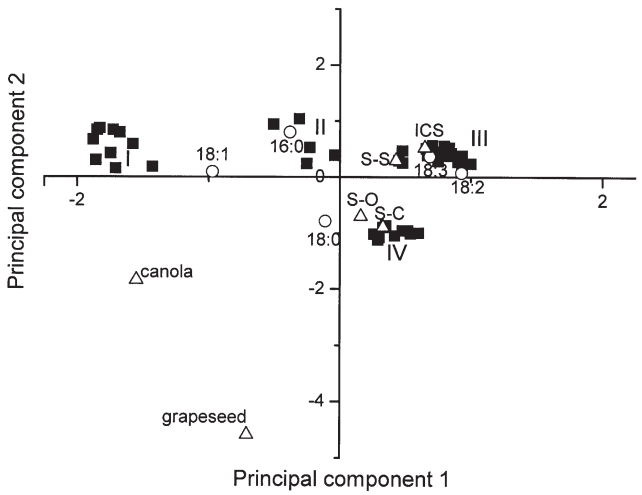

FIG. 2. Score and loading plot of a principal component analysis (PCA) based on the FA composition data of different oil samples. (■) Oils used to develop the method: (I) olive oil; (II) corn oil; (III) crude and refined soybean oils; (IV) sunflower oil. (△) Oils used to test the method: commercial mixtures of sunflower–olive oil (S-O, 95:5); sunflower–corn oil (S-C, 95:5); sunflower–soybean oil (S-S, 5:95); industrial crude soybean oil (ICS); canola oil; and grapeseed oil.

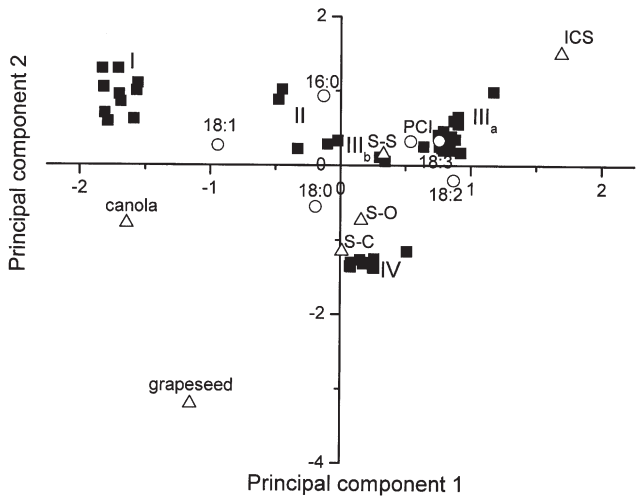

FIG. 3. Score and loading plot of PCA based on the FA composition and photometric color index of different oil samples: III_a, crude soybean oil; III_b, refined soybean oil. See Figure 2 for abbreviations.

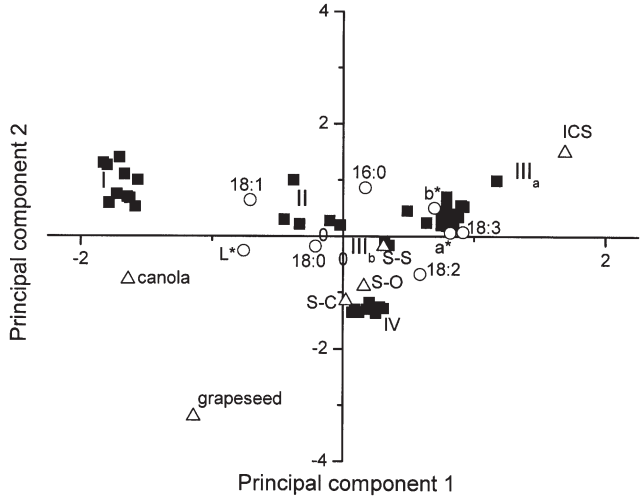

FIG. 4. Score and loading plot of PCA based on the FA composition and CIELAB parameter data of different oil samples. See Figures 2 and 3 for abbreviations.

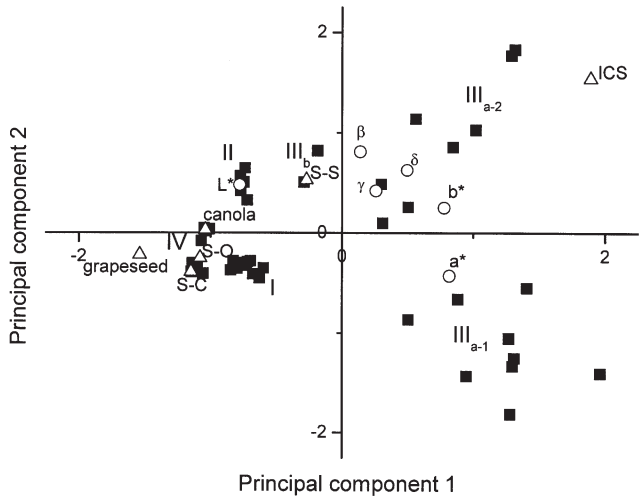

FIG. 5. Score and loading plot of PCA based on tocopherol and CIELAB parameter data of different oil samples. $III_{a\text{-}1,2}$, crude soybean oil. See Figures 2 and 3 for abbreviations.

ACKNOWLEDGMENTS

The authors thank Agencia Córdoba Ciencia, Fondo Nacional de Promoción Científica y Tecnológica (FONCYT-Argentina), Secretaría de Ciencia y Técnica de la Universidad Nacional de Río Cuarto (SECYT-UNRC), for financial support and the Department of Chemistry (UNRC) for permitting the HPLC measurements

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[Received May 31, 2005; accepted January 19, 2006]