ELSEVIER

Food Chemistry 73 (2001) 93–101

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

HPLC analysis of tocopherols and triglycerides in coffee and their use as authentication parameters

A.G. González [a], F. Pablos [a], M.J. Martín [a,*], M. León-Camacho [b], M.S. Valdenebro [b]

[a] *Department of Analytical Chemistry, University of Seville, E-41012-Seville, Spain*
[b] *Instituto de la Grasa, CSIC, E-41012-Seville, Spain*

Received 2 May 2000; received in revised form 8 October 2000; accepted 8 October 2000

Abstract

The triglyceride and tocopherol contents of green and roasted coffee beans belonging to the *arabica* and *robusta* varieties were determined by reversed phase and normal phase high resolution liquid chromatography, respectively. Refractive index detector was used in the case of the triglycerides and fluorescence for tocopherols. Coffee oil was Soxhlet extracted with hexane. By considering the triglyceride and tocopherol profiles as chemical descriptors, a chemometric study with authentication purposes was performed to differentiate coffee varieties. Pattern recognition techniques like principal component analysis and linear discriminant analysis were carried out. Discrimination between *arabica* and *robusta* coffees was achieved with both profiles, but only tocopherols also allow the differentiation between green and roasted coffees.

Keywords: Coffee; Triglycerides; Tocopherols; High performance liquid chromatography; Pattern recognition; Chemometrics

1. Introduction

Coffee is one of the most consumed drinks in the world together with tea. There are two species of importance in the trade which are *Coffea arabica* and *Coffea canephora*, commonly known as *arabica* and *robusta*, respectively (Smith, 1985). The majority of the commercially available coffees consist of beans belonging to the *arabica* and *robusta* varieties or blends of these two. Nowadays, *arabica* coffees are more appreciated by the consumers because of their fine and aromatic flavour and their good full body. Actually, the 100% *arabica* coffees are the preferred by the coffee drinkers and they are considered of better quality (Briandet, Kemsley, & Wilson, 1996), so they are more expensive than *robusta* coffees or blends. Due to this, it is important to have methods to differentiate between these two varieties. Before the beans have been roasted, the visual criterion is very effective to distinguish the varieties. *Arabica* beans are green to pale green in colour and have an oval shape whereas *robusta* ones tends to be rounder and may be brownish rather than green. After roasting, this visual criterion cannot be used and it is necessary to apply other kind of methods to differentiate the varieties. Chemical analysis has been proved to be useful for these purposes (Bicchi, Ombretta, Pellegrino, & Vanni, 1997; Martín, Pablos, & González, 1996; Suchánek, Filipova, Volka, Delgadillo, & Davies, 1996). Some of the chemical parameters used to characterise the *arabica* and *robusta* coffees have been the metal content (Martín, Pablos, & González, 1998a), the volatile components (Bicchi et al., 1997), the chlorogenic acid and the caffeine (Martín et al., 1998). The lipid composition of the coffee seeds has also been analysed in coffee, in particular, the sterols fraction (Carrera, León-Camacho, Pablos, & González, 1998; Valdenebro, León-Camacho, Pablos, González, & Martín, 1999), fatty acids (Lercker et al., 1996; Muratore, Cataldi-Lupo, Fiorenza, & Asmundo, 1998), diterpenic alcohols (Frega, Bocci, & Lercker, 1995; Speer, Tewis, & Montag, 1991). Important components of the lipids present in the coffee oil are triglycerides, they represent around 75% of the coffee bean lipids (Nikolova-Damyanova, Velikova, & Jham, 1998). Tocopherols are also present

* Corresponding author. Tel. : +34-954-557173; fax: +34-954-557168.
E-mail address: m.martin@cica.es (M.J. Martín).

0308-8146/01/$ - see front matter
PII: S0308-8146(00)00282-X

in the coffee oil but as minor components (Fig. 1; Folstar, Van der Plas, Pilnik, & Heus, 1977); they have antioxidant properties (Abidi & Mounts, 1997) and together with the tocotrienols are the eight vitamers that constitute the vitamin E (Konings, Roomans, & Beljaars, 1996). High performance liquid chromatography (HPLC) is very adequate for the analysis of triglycerides and tocopherols in oil samples, in both cases, the analysis can be carried out in a very straightforward way diluting the oil and injecting it into the cromatograph. Refractive index detector (RI; Fedeli, Cortesi, & Rovellini, 1998; León-Camacho & Cert, 1994) can be used for triglycerides and ultraviolet (Jung, Yoon, & Min, 1989; Psomiadou & Tsimidou, 1998) or fluorescence (F; Abidi & Mounts, 1997; Chase, Akoh, & Eitenmiller, 1994) detectors for tocopherols.

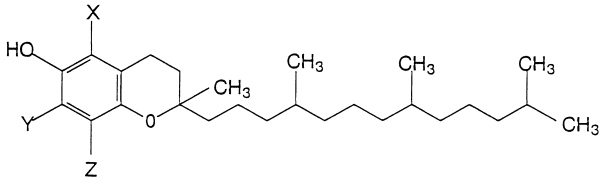
In the present paper the two profiles (triglycerides and tocopherols) of coffee samples have been investigated. Accordingly, triglycerides and tocopherols (α-, β- and γ-tocopherol) have been determined by using reversed phase (RP)-HPLC-RI and normal phase (NP)-HPLC-F, respectively. In each case, the analysed compounds were used as descriptors to apply pattern recognition (PR) methods in order to differentiate coffee varieties of green and roasted beans. A critical comparison between these two approaches is also given.

2. Experimental

2.1. Chromatographic systems

The triglycerides analysis were carried out by using an HPLC system consisting of a Hewlett Packard (Palo Alto, CA, USA) 1050 liquid chromatograph, a Rheodyne (Cotati, CA, USA) injection valve with a HP 1047A refractive index detector and a HP 3396 II integrator. The separation was performed in a 250×4 mm Superspher 100 RP-18 Merck (Darmstadt, Germany) 4-μm column. The temperatures of the column and detector were held, respectively, at 40 and 35°C. The mobile phase was acetonitrile/acetone (50:50, v/v) at a flow rate of 1 ml min^{-1}.

Tocopherols were determined in a Perkin-Elmer (Perkin-Elmer, Norwalk, CT, USA) 400 Liquid Chromatograph, a Rheodyne injection valve with a 20-μl sample loop, a Shimadzu (Kyoto, Japan) RF-535 fluorescence detector set at an excitation wavelength at 290 nm and an emission wavelength of 330 nm and a Hewlett-Packard 3390A integrator. A 25 cm×4 mm Lichrosphere Si-60 Merck, 5-μm column was used for the separation. The mobile phase was *n*-hexane/propan-2-ol (99:1, v/v) at a flow rate of 1 ml min^{-1}.



X = Y = Z = CH_3 α - tocopherol

X = Z = CH_3 ; Y = H β - tocopherol

X = H ; Y = Z = CH_3 γ - tocopherol

Fig. 1. Structures of tocopherols.

2.2. Chemicals and reagents

Acetone, acetonitrile, *n*-hexane and propan-2-ol (Romil, Cambridge, UK) were of HPLC grade. Anhydrous sodium sulphate (Fluka, Buchs, Switzerland) used for the analysis was of analytical reagent grade.

A tocopherol kit consisting of α-, β-, γ- and δ-tocopherol (>95% each) was obtained from Merck. They were stored at 4°C. Stock standard solutions (40 μg ml^{-1}) were prepared weekly in *n*-hexane and also stored at 4°C. Working standard solutions were prepared from the stock solutions by dilution with *n*-hexane.

2.3. Coffee samples

A set composed by 32 coffee samples from different geographic origins was selected for the analysis. Sixteen of them were green coffee beans (labelled as G) and the remained were the same samples but submitted to a roasting process (labelled as R). Eight of the samples belonged to the *robusta* variety (labelled as r) and 24 of them to the *arabica* variety (labelled as a). Table 1 includes a short description of the samples. They were ground and stored in polyethylene flasks till the analysis.

2.4. Oil extraction

The extraction of the coffee oil was carried out according to the current normative of the CEE (Directive 91/2568/CEE) for the analysis of fats and oils. Thus, 18.0 g, exactly weighed, of coffee sample were extracted with hexane in a Soxhlet for 8 h, siphoning six times per hour. The extract was dried over anhydrous sodium sulphate and placed in a 250-ml round-bottom flask. Using a vacuum rotary evaporator, the solvent was evaporated and the residue was dried at 105°C to obtain the coffee oil.

2.5. HPLC analysis of triglycerides

Coffee oil (0.1 g), exactly weighed, was dissolved in 2 ml of acetone. A portion of this solution was filtered through a disposable 0.45-μm filter unit. Aliquots of 20 μl of this

solution were injected for HPLC analysis. The contents of the main estimated triglycerides in the coffee samples expressed as relative percentage are shown in Table 2. Quantitation was performed by the internal normalisation method (Debbrecht, 1985).

2.6. HPLC analysis of tocopherols

About 10 mg, exactly weighed, of the coffee oil was dissolved in 10 ml of *n*-hexane. Aliquots of this solution were injected for HPLC analysis. The contents of α-, β- and γ-tocopherol in the coffee samples were obtained from calibration graphs and expressed in μg/g of coffee (dry base). The results are shown in Table 3.

2.7. Data analysis

Two separated data matrices were prepared and used for the chemometric calculations. In both, the rows are the cases (32 coffee samples) and the columns are the variables (10 triglycerides for one matrix and three tocopherols for the other). In this study, the applied PR procedures have been principal component analysis (PCA; Chatfield & Collins, 1980) and linear discriminant analysis (LDA; Coomans, Massart, & Kaufman, 1979). Chemometrics calculations were made using the statistical package STATISTICA 99 from Statsoft™ (Tulsa, OK, USA).

Table 1
Analysed coffee samples

Variety	Origin	Sample code[a]
Arabica	Brazil	1Ga
Arabica	Jamaica	2Ga
Arabica	Australia	3Ga
Arabica	Puerto Rico	4Ga
Arabica	Colombia	5Ga
Arabica	Guatemala	6Ga
Arabica	India	7Ga
Arabica	Costa Rica	8Ga
Arabica	Nicaragua	9Ga
Arabica	Ethiopia	10Ga
Arabica	Cuba	11Ga
Arabica	Mexico	12Ga
Robusta	Uganda	13Gr
Robusta	Cameroon	14Gr
Robusta	Ivory Coast	15Gr
Robusta	Vietnam	16Gr
Arabica	Brazil	17Ra
Arabica	Jamaica	18Ra
Arabica	Australia	19Ra
Arabica	Puerto Rico	20Ra
Arabica	Colombia	21Ra
Arabica	Guatemala	22Ra
Arabica	India	23Ra
Arabica	Costa Rica	24Ra
Arabica	Nicaragua	25Ra
Arabica	Ethiopia	26Ra
Arabica	Cuba	27Ra
Arabica	Mexico	28Ra
Robusta	Cameroon	29Rr
Robusta	Ivory Coast	30Rr
Robusta	Uganda	31Rr
Robusta	Vietnam	32Rr

[a] Ga, green *arabica*; Gr, green *robusta*; Ra, roasted *arabica*; Rr, roasted *robusta*.

3. Results and discussion

3.1. Triglycerides profile

The triglycerides profiles of the coffee samples have been studied. Fig. 2 shows the chromatogram corresponding to the triglycerides fraction of a coffee oil. A tentative identification of the peaks could be achieved by comparing the retention times of the triglyceride peaks with those obtained from the chromatography of vegetable oils (soy and olive) of known triglyceride

Table 2
Triglycerides contents (%) of the coffee samples[a]

Case[b]	LLL	PLLn	OLL	PLL	OLO	PLO + SLL	PLP	POP	SOS
1Ga	6.11	2.10	3.85	24.51	1.35	14.73	21.98	4.81	0.85
2Ga	6.33	2.13	3.71	24.00	1.06	14.29	21.03	5.34	1.43
3Ga	6.68	2.02	4.03	24.32	1.29	14.83	20.11	3.00	1.56
4Ga	6.20	2.04	3.60	25.52	1.02	13.75	23.17	4.09	1.06
5Ga	5.95	1.94	3.26	26.69	0.82	12.84	24.94	3.95	0.62
6Ga	6.41	2.09	3.40	27.80	0.94	12.89	24.79	2.89	0.98
7Ga	5.68	2.19	2.95	26.22	0.85	12.42	25.83	3.91	0.61
8Ga	6.34	1.94	3.51	26.05	1.01	13.59	23.11	4.02	1.29
9Ga	7.13	1.99	3.59	28.82	0.91	12.79	24.76	3.04	0.77
10Ga	4.17	1.97	3.36	23.03	1.59	14.34	25.21	4.81	1.61
11Ga	7.16	2.06	3.44	26.18	1.05	13.21	22.27	3.99	1.50
12Ga	6.56	1.94	3.23	24.31	0.92	12.44	20.58	3.91	2.08
13Gr	2.62	1.89	3.85	18.80	2.03	17.68	23.95	5.15	1.91
14Gr	3.33	2.00	3.82	20.01	1.98	15.90	23.96	4.49	1.38
15Gr	3.74	2.18	5.90	18.04	1.50	12.30	17.75	3.31	1.08
16Gr	2.24	3.36	4.57	17.94	1.28	12.43	23.23	4.27	1.47
17Ra	4.36	1.35	4.28	20.14	0.98	10.83	15.81	3.08	0.68
18Ra	6.84	2.04	3.90	23.80	1.40	14.59	19.27	4.42	0.99
19Ra	6.92	1.77	4.24	26.75	1.76	19.26	23.74	6.49	3.11
20Ra	6.47	2.15	3.65	25.54	1.15	13.91	21.52	4.03	0.73
21Ra	6.59	1.80	3.50	27.24	0.90	12.87	23.57	3.29	0.73
22Ra	6.72	2.11	4.66	31.44	1.00	15.50	26.74	3.95	1.32
23Ra	5.49	1.93	3.61	31.53	0.85	14.24	28.89	4.75	1.19
24Ra	6.64	2.01	3.76	25.33	0.88	10.90	21.90	3.71	1.29
25Ra	7.64	1.56	4.16	30.20	1.36	15.25	26.71	4.25	1.77
26Ra	4.00	1.84	3.64	23.42	1.80	14.83	25.42	4.92	3.44
27Ra	4.87	1.61	2.75	23.40	0.69	15.19	25.31	4.81	1.81
28Ra	7.23	2.00	3.48	26.56	0.87	13.37	21.97	2.68	1.19
29Rr	3.77	1.56	4.10	20.65	2.24	16.14	23.65	4.29	1.32
30Rr	2.63	1.34	3.92	20.54	2.41	16.37	24.35	4.87	1.30
31Rr	2.90	1.25	3.86	19.02	2.76	16.88	24.57	5.16	1.21
32Rr	5.34	1.94	4.17	18.88	2.34	16.38	22.16	3.89	1.17

[a] L, linoleic acid; Ln, linolenic acid; O, oleic acid; P, palmitic acid; S, stearic acid.

[b] Ga, green *arabica*; Gr, green *robusta*; Ra, roasted *arabica*; Rr, roasted *robusta*.

composition . The elution order can be determined by calculating the equivalent carbon numbers (AOCS, 1997a). Also considering the fatty acid composition of the coffee oil and the 1,3-random-2-random distribution hypothesis (Folstar, 1985; Lercker et al., 1996), a possible identification of the triglycerides present in the coffee samples could be as follows: LLL, PLLn, OLL, PLL, OLO, PLO, SLL, PLP, POP and SOS, though due to the lack of resolution of the PLO and SLL peaks, they were considered as one peak for quantification purposes. There are some peaks in the chromatogram that could correspond to other triglycerides present in the coffee oil whose identification has not been achieved. The triglycerides percentage of the corresponding profiles of the 32 analysed samples are presented in Table 2. Considering the average values it can be indicated that the principal triglycerides present in the coffee oil are: PLL (23.8%) and PLP (22.9%), followed by PLO + SLL (14.6%), in the case of LLL, OLL, OLO, POP and SOS, their contents vary between 7.6% and 0.7%. At a glance, it can be observed that the mean percentage for the *arabica* samples is higher for LLL (6.1%) than for *robustas* (3.7%). By the contrary, *arabica* coffees have lower contents in OLO (1.1%) with respect to *robustas* (2.3%). The same trends apply for PLO + SLL, *arabica* coffees having lesser contents (13.8%) than *robustas* (16.4%). For the remaining analysed triglycerides, there are no apparent differences between the coffee varieties.

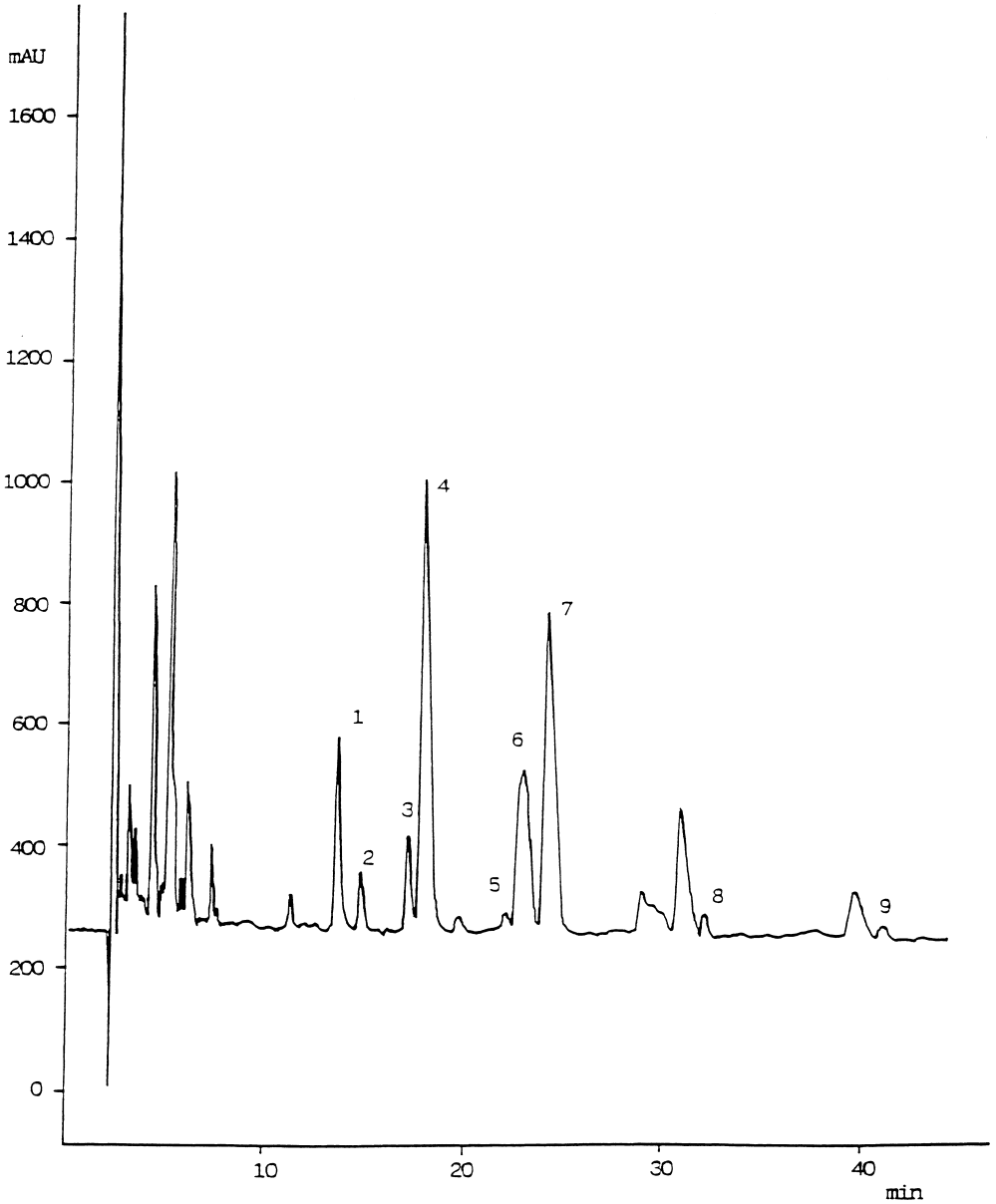


Fig. 2. High performance liquid chromatograph chromatogram of triglycerides fraction of coffee oil. Conditions: column, 250×4 mm, 4 μm Superspher 100 RP-18 (at 40°C); mobile phase: acetonitrile/acetone (50:50, v/v) at 1 ml min^{-1}; 20 μl injection volume; RID (at 35°C). (1) LLL; (2) PLLn; (3) OLL; (4) PLL; (5) OLO; (6) PLO + SLL; (7) PLP; (8) POP; (9) SOS.

3.2. Tocopherol profile

α-, β- and γ-Tocopherols were identified in the coffee samples by comparing their retention times with those of the standard solutions. The accuracy of the procedure was checked by performing recovery assays from spiked samples. Three additions of standard mixtures of the studied tocopherols were spiked on samples of coffee beans, and then the tocopherols were determined by the proposed procedure. Average recoveries (in %) for each tocopherol were calculated: α-tocopherol (89.3%), β-tocopherol (94.1%) and γ-tocopherol (90.6%). These results show that the analytical procedure can be considered accurate according to the AOAC guidelines (AOAC, 1993). Fig. 3 shows chromatograms corresponding to green *arabica* and *robusta* coffee samples and the respective roasted ones. As can be seen, the peaks of the three tocopherols appear in all the cases, but the profile of the chromatograms is quite distinct. There are differences between the chromatograms of the *arabica* and *robusta* green samples. Peak 2 (β-tocopherol) is higher in the chromatograms corresponding to *arabica* samples. A major difference is appreciated for peak 3 (γ-tocopherol) which is higher for the roasted coffee samples, both *arabica* and *robusta*.

The concentrations of tocopherols in the analysed samples are depicted in Table 3. These results were obtained from triplicate measurements and the relative standard deviations are less than 3% for the three tocopherols. Some general trends of the data can be

Table 3
Contents (μg/g coffee, dry base) of tocopherols in coffee samples

Case[a]	α-Tocopherol	β-Tocopherol	γ-Tocopherol
1Ga	16.76	88.05	2.56
2Ga	8.60	47.65	1.51
3Ga	13.23	59.96	1.57
4Ga	9.74	60.21	1.87
5Ga	6.20	42.12	1.29
6Ga	14.59	55.16	1.74
7Ga	14.13	49.41	1.54
8Ga	2.02	38.84	0.90
9Ga	16.14	87.46	3.73
10Ga	10.44	49.74	3.55
11Ga	14.89	58.14	1.46
12Ga	14.82	64.78	4.77
13Gr	6.57	15.75	5.59
14Gr	3.06	9.68	3.66
15Gr	4.58	14.01	2.28
16Gr	6.56	12.96	3.99
17Ra	31.76	161.14	68.82
18Ra	24.73	148.48	74.16
19Ra	31.35	133.69	73.26
20Ra	30.74	137.35	94.76
21Ra	28.40	144.89	65.92
22Ra	25.68	137.78	83.48
23Ra	30.61	107.45	58.80
24Ra	29.11	131.33	78.30
25Ra	33.54	159.16	70.57
26Ra	20.64	93.99	77.34
27Ra	28.24	120.30	87.12
28Ra	30.60	138.47	71.84
29Rr	2.64	16.04	46.49
30Rr	10.32	21.19	69.55
31Rr	7.55	16.46	56.76
32Rr	8.28	37.87	58.64

[a] Ga, green *arabica*; Gr, green *robusta*; Ra, roasted *arabica*; Rr, roasted *robusta*.

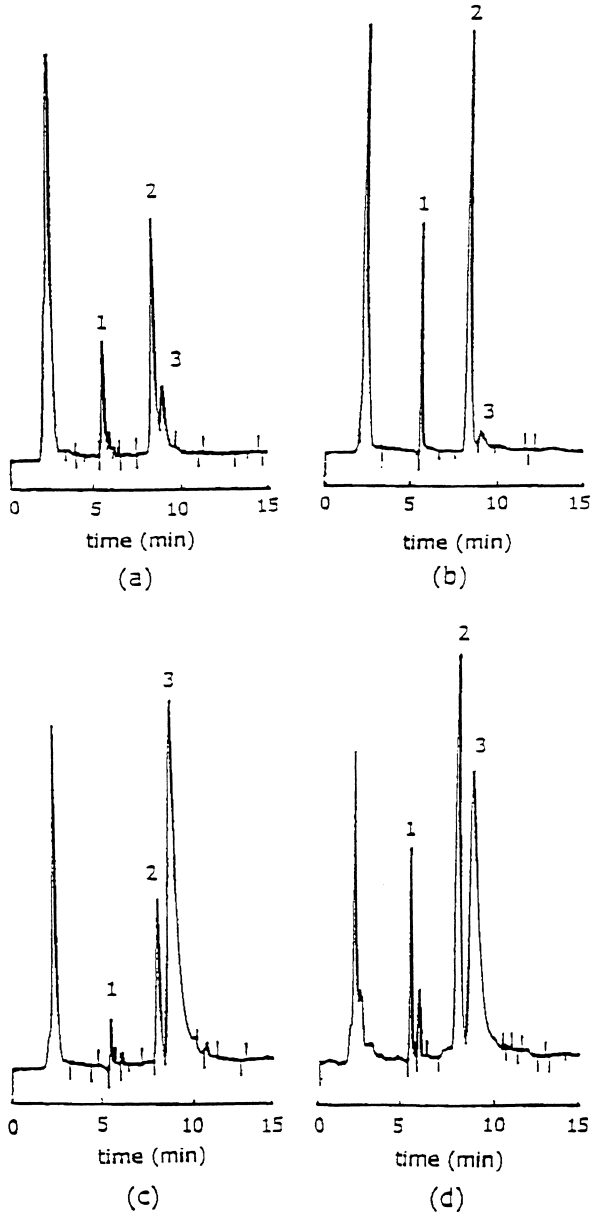


Fig. 3. High performance liquid chromatograph chromatograms of tocopherol fraction of coffee oil. Conditions: column, 5 μm Lichrosphere Si 60, 25 cm×4 mm; mobile phase: *n*-hexane/propan-2-ol (99:1, v/v) at 1 ml min^{-1}; 20 μl injection volume; fluorescence detection at 330 nm (excitation at 290 nm). (a) Green *robusta*; (b) green *arabica*; (c) roasted *robusta*; (d) roasted *arabica*. (1) α-Tocopherol; (2) β-tocopherol; (3) γ-tocopherol.

inferred. The amount of α-tocopherol in roasted coffees ranges between 7.55 and 33.54 μg/g, whereas in the green ones lays into the interval 2.02 and 16.76 μg/g. In the case of β- and γ-tocopherol remarkable differences between green and roasted samples are observed, being their contents higher in the roasted coffees. Thus, the mean value of β-tocopherol for green coffees is 47.12 and 106.60 μg/g for roasted ones. In the case of γ-tocopherol its content varies from 2.63 μg/g for green samples to 70.99 μg/g for roasted coffee beans. This increasing of the tocopherols amount during the roasting process has been already described for some oilseeds (Yen, 1990), and it could due to the liberation of the combined tocopherols during the roasting process.

3.3. Discrimination of samples

By considering the triglycerides profile differences between *arabica* and *robusta* coffees can be observed. Using the analysed triglycerides as chemical descriptors, a possible differentiation of the coffee samples by applying chemometric procedures was considered. To obtain a first evaluation of the discriminant efficiency of the selected parameters, as well as the visualisation of the samples trends, PCA method was performed. Thus, the two first PCs were calculated explaining up to 56.1% of the total variance. PC1 explained 35.6% and PC2 explained 20.5% of the total information. Fig. 4 shows the corresponding scores plot. It can be seen that all the green and roasted *arabica* coffees are situated at the left and upper side of the plot being well separated from the *robusta* samples. A detailed examination of the variable loadings of PC1 shows that the descriptors with more contribution to this PC are OLO, PLO + SLL and POP. This is in accord with the differences found for the mean values of these variables in each coffee variety, cited in the previous paragraph.

In the case of the tocopherol profile, as it was indicated there were big differences in the contents of β- and γ-tocopherol between the two species. Accordingly, it is possible to visualise the data trends in an easier way drawing the corresponding variable–variable plot. The scatter plot of the samples, using as axis these variables is depicted in Fig. 5. The separation of green and roasted coffee samples is fair. Green samples appear at low values of γ-tocopherol whereas roasted samples present higher values of this variable. It is also remarkable a differentiation between the coffee varieties, being *arabica* coffee samples situated at higher values of β-tocopherol whereas *robusta* ones have low values of this tocopherol.

Observing the scores plot obtained with the triglycerides profile (Fig. 4), a virtual line could be traced to separate the *arabica* samples from the *robusta* ones. Due to this quasi-linear separation of the coffee samples, the LDA supervised pattern recognition method was applied to the data set. With this method, adequate classification rules can be obtained to separate the classes according to a minimisation of the ratio of within-class and between-class sum of squares. As there are two classes (*arabica* and *robusta*), there is only one discriminant function, calculated as linear combinations of the chemical descriptors. LDA was performed by

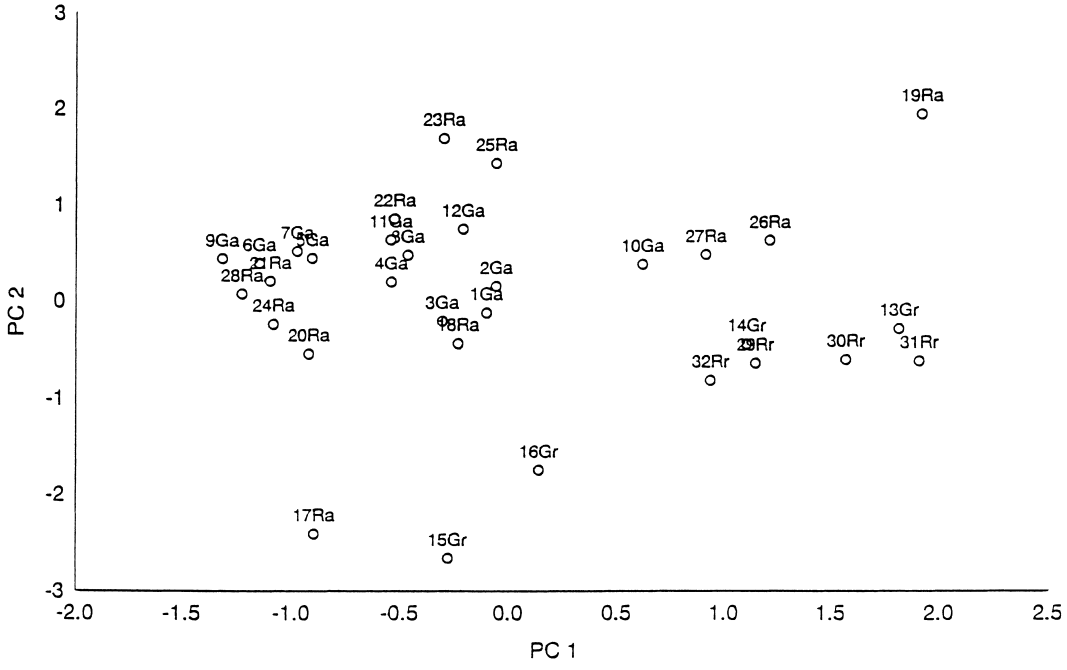


Fig. 4. Scores plot for the first PCs considering the triglycerides profile.

choosing the standard approach (Powers & Keith, 1968): all the variables were included in the model, the equation of the discriminant function (DF) being as follows:

$$DF = -0.75\ LLL + 0.56\ PLLn + 0.85\ OLL - 0.29\ PLL + 1.34\ OLO + 0.11\ PLO + SLL + 0.50\ PLP - 0.69\ POP + 0.73\ SOO - 0.97\ SOS$$

with a recognition ability of 100% for both *arabica* and *robusta* classes.

In the tocopherol profile besides the separation of the two varieties of coffee, it can also be observed a quasi linear separation between green and roasted coffee samples (Fig. 5). Due to this fact, LDA was applied to the data set by considering now four classes (Ga, Gr, Ra and Rr, as indicated above) in order to obtain proper classification rules. The corresponding discriminant functions were calculated as linear combinations of the chemical descriptors (α-, β- and γ-tocopherol). In order to check the discriminant power of β- and γ-tocopherol already shown by the variable-variable plot, a LDA with feature selection was carried out by choosing the backward stepwise approach. In a first run, all the variables are present in the model, in each step the variable with less discriminant power, according to the Wilk's λ statistic test (Gardiner, 1997), is rejected. In our case, the selected variables were β- and γ-tocopherol, in perfect agreement with the variable-variable plot, obtaining classification rules with a recognition ability of 100%. Fig. 6 shows the scatter plot for the two first discriminant functions. As can be seen, a complete separation of the four classes of samples was accomplished. In the case of *arabica* coffee samples, their profile of distribution changes from a lineal one, for the green samples, to a rounded distribution for the roasted samples. In the case of *robusta* coffees, their profile hardly changes, although certain dispersion is appreciable for the roasted samples whereas the green ones appear to be more grouped.

3.4. Comparison of triglycerides and tocopherols profiles

After this chemometric study, a comparison between both profiles, triglycerides and tocopherols, can be carried out. Although the chemical analysis of both kind of compounds is performed in a quite similar way, namely: extraction of the coffee oil, dilution of an adequate aliquot and injection in the chromatographic system, from an authentication point of view, there are differences between the performance of the triglycerides and tocopherols. In the case of tocopherols there are several advantages. Between them we can emphasise the shorter time of analysis, minor amount of sample, lesser number of analysed parameters, the possibility of obtaining standards and, consequently, a more accurate identification of the peaks in the chromatograms. Also, the contents of β- and γ-tocopherols can be used for discrimination between coffee varieties even after roasting process whereas the tryglicerides profiles can separate coffees belonging to different varieties but cannot distinguish between green and roasted samples.

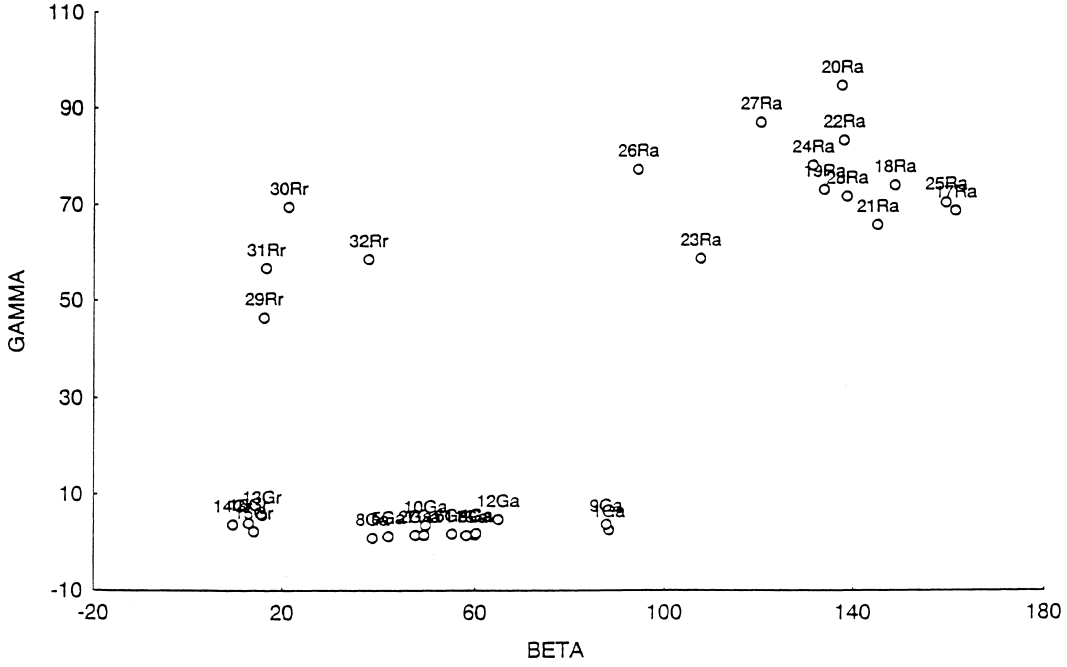


Fig. 5. Variable–variable plot. β-Tocopherol–γ-tocopherol.

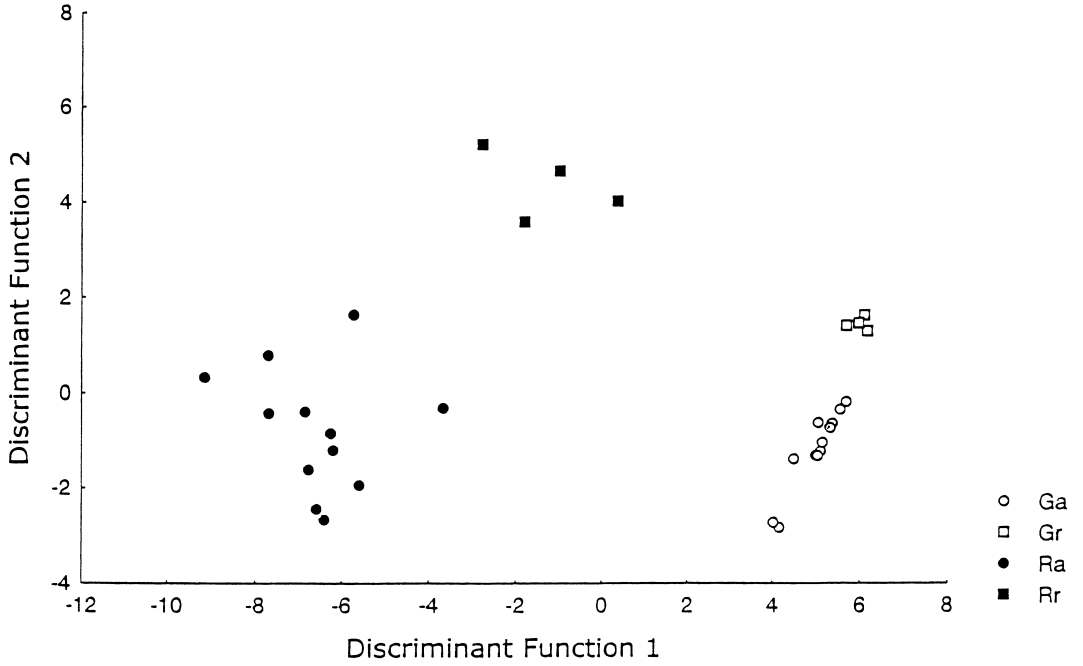


Fig. 6. Plot of the two discriminant functions. Ga, green *arabica*; Gr, green *robusta*; Ra, roasted *arabica*; Rr, roasted *robusta*.

4. Conclusions

The contents of triglycerides and tocopherols fraction present in *arabica* and *robusta* coffee samples, both green and roasted, have been determined. These parameters have been considered as chemical descriptors and used to differentiate coffee varieties. Using the triglycerides contents and applying PCA and LDA, a complete separation of *arabica* and *robusta* coffees was achieved. In the case of tocopherols, the differences in the amounts of tocopherols are significant and can be used in an easier way to differentiate coffee varieties, even after roasting process. Considering that the determination of tocopherols offers several analytical advantages, it can be conclude that these components of the coffee seeds constitute better parameters for coffee authentication purposes.

References

Abidi, S. L., & Mounts, T. L. (1997). Reversed-phase high-performance liquid chromatographic separations of tocopherols. *Journal of Chromatography A, 782*, 25–32.

AOAC. (1993). Peer verified method program, manual on policies and procedures, Arlington, VA.

AOCS (1997a). Sampling and Analysis of Commercial Fats and Oils. AOCS Official Method Ce 5b-89. Triglycerides in vegetable oils by HPLC.

AOCS (1997b). Sampling and Analysis of Commercial Fats and Oils. AOCS Official Method Ce 5c-93. Individual triglycerides in oils and fats by HPLC.

Bicchi, C. P., Ombretta, M. P., Pellegrino, G., & Vanni, A. C. (1997). Characterization of roasted coffee and coffee beverages by solid phase microextraction–gas chromatography and principal component analysis. *Journal of Agricultural and Food Chemistry, 45*, 4680–4686.

Briandet, R., Kemsley, E. K., & Wilson, R. H. (1996). Discrimination of *arabica* and *robusta* in instant coffee by fourier transform infrared spectroscopy and chemometrics. *Journal of Agricultural and Food Chemistry, 44*, 170–174.

Carrera, F., León-Camacho, M., Pablos, F., & González, A. G. (1998). Authentication of green coffee varieties according to their sterolic profile. *Analytica Chimica Acta, 370*, 131–139.

Coomans, D., Massart, D. L., & Kaufman, L. (1979). Optimization by statistical linear discriminant analysis in analytical chemistry. *Analytica Chimica Acta, 112*, 97–122.

Chase, G. W., Akoh, C. C., & Eitenmiller, R. R. (1994). Analysis of tocopherols in vegetable oils by high-performance liquid chromatography: comparison of fluorescence and evaporative light-scattering detection. *Journal of the American Oil Chemist's Society, 71*, 877–880.

Chatfield, C., & Collins, A. J. (1980). *Introduction to multivariate analysis*. London: Chapman and Hall.

Debbrecht, F. J. (1985). In R. L. Grob, Modern practice of gas chromatography. New York: John Wiley & Sons.

Directive 91/2568/CEE, Official Journal L-248/15, September 1991.

Fedeli, E., Cortesi, N., & Rovellini, P. (1998). L'analisi trigliceridica mediante HPLC nell'individuazione di commistioni di oli di seme ad alto contenuto in oleico con oli di oliva. *La rivista italiana delle sostanze grasse, 75*, 483–490.

Folstar, P., Van der Plas, H. C., Pilnik, W., & Heus, J. G. (1977). Tocopherols in the unsaponifiable matter of coffee bean oil. *Journal of Agricultural and Food Chemistry, 25*, 283–285.

Folstar, P. (1985). In R. J. Clarke, & R. Macrae, *Coffee* (Vol. I), Ch. 8, Qualitative and Quantative Analysis by Gas Chromatography. London: Elsevier.

Frega, N., Bocci, F., & Lercker, G. (1995). Determinazione del caffè robusta nelle miscele commerciali con l'arabica. *Industrie Alimentari, 34*, 705–708.

Gardiner, W. P. (1997). *Statistical analysis methods for chemists*. Cambridge: Royal Society of Chemistry.

Jung, M. Y., Yoon, S. H., & Min, D. B. (1989). Effects of processing steps on the contents of minor compounds and oxidation of soybean oil. *Journal of the American Oil Chemist's Society, 66*, 118–120.

Konings, E. J. M., Roomans, H. H. S., & Beljaars, P. R. (1996). Liquid chromatographic determination of tocopherols and tocotrienols in margarine, infant foods and vegetables. *International Journal of the Association of Official Analytical Chemists International, 79*, 902–906.

León-Camacho, M., & Cert, A. (1994). Recomendaciones para la aplicación de algunos métodos analíticos incluidos en el reglamento CEE 2568/91 relativo a las características de los aceites de oliva y de orujo de oliva. *Grasas y aceites, 45*(6), 395–402.

Lercker, G., Carboni, M. F., Bertacco, G., Turchetto, E., Lucci, A., Bortolomeazzi, R., Pagani, E., Frega, N., & Bocci, F. (1996). La frazione lipidica del caffè. 2: su alcuni parametri di qualificazione. *Industrie Alimentari, 35*, 1186–1193.

Martín, M. J., Pablos, F., & González, A. G. (1996). Application of pattern recognition to the discrimination of roasted coffees. *Analytica Chimica Acta, 320*, 191–197.

Martín, M. J., Pablos, F., & González, A. G. (1998a). Characterization of arabica and robusta roasted coffee varieties and mixture resolution according to their metal content. *Food Chemistry, 66*, 365–370.

Martín, M. J., Pablos, F., & González, A. G. (1998b). Discrimination between arabica and robusta green coffee varieties according to their chemical composition. *Talanta, 46*, 1259–1264.

Muratore, G., Cataldi-Lupo, M. C., Fiorenza, F., & Asmundo, C. N. (1998). La frazione lipidica del caffè in relazione al processo di tostadura. *Industrie Alimentari, 37*, 161–164.

Nikolova-Damyakova, B., Velikova, R., & Jham, G. N. (1998). Lipid classes, fatty acid composition and triacylglycerol molecular species in crude coffee beans harvested in Brazil. *Food Research International, 31*, 479–486.

Powers, J. J., & Keith, E. S. (1968). Stepwise discriminant analysis of gas chromatographic data as an aid in classifying the flavour quality of foods. *Journal of Food Sciences, 33*, 207–213.

Psomiadou, E., & Tsimidou, M. (1998). Simultaneous HPLC determination of tocopherols, carotenoids and chlorophylls for monitoring their effect on virgin olive oil oxidation. *Journal of Agricultural and Food Chemistry, 46*, 5132–5138.

Smith, A. W. (1985). In Clarke, R. J. & Macrae, R. *Coffee* (Vol. 1), Ch. 1, Introduction, London: Elsevier.

Speer, K., Tewis, R., & Montag, A.(1991).16-O-methylcafestol-ein neues diterpen im kaffee (1991). *Zeitschrift für Lebensmittel-Untersuchung und-Forschung, 192,* 451–454.

Suchánek, M., Filipova, H., Volka, K., Delgadillo, I., & Davies, A. N. (1996). Qualitative analysis of green coffee by infrared spectrometry. *Fresenius Journal of Analytical Chemistry, 354*, 327–332.

Valdenebro, M. S., León-Camacho, M., Pablos, F., González, A. G., & Martín, M. J. (1999). Determination of the arabica/robusta composition of roasted coffee according to their sterolic content. *The analyst, 124*, 999–1002.

Yen, G. C. (1990). Influence of seed roasting process on the changes in composition and quality of sesame (*Sesame indicum*) oil. *Journal of the Science of Food and Agriculture, 50*, 563–570.