



## Characterization of apple seed oil with Denomination of Origin from Asturias, Spain

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**SUMMARY:** The content and composition of lipids isolated from the seeds of seven apple species from Asturias (Spain) were characterized. The highest content in oil corresponded to Collao ( $22.73 \pm 0.81$  g·100 g<sup>-1</sup> of seed), followed by Raxao and Riega ( $20.19 \pm 0.74$  g·100 g<sup>-1</sup> of seed;  $19.67 \pm 0.85$  g·100 g<sup>-1</sup> of seed), respectively. The linoleic acid was found to be the main component in Limón Montés ( $60.78 \pm 3.07\%$ ) followed by Riega ( $60.01 \pm 3.41\%$ ). Solarina seed oil was the one with the highest content in total sterols ( $558.52 \pm 9.42$  mg·100 g<sup>-1</sup> of oil) while Blanquina was the one that presents the lowest amount ( $166.55 \pm 1.89$  mg·100 g<sup>-1</sup> of oil). Phosphatidylcholine ( $70.58 \pm 3.85\%$ ) was found to be the main constituent in Blanquina followed by Collao ( $55.55 \pm 2.96\%$ ). Raxao presented the highest content in  $\beta$ -tocopherol ( $125.29 \pm 12.62$ ) and  $\alpha$ -tocopherol was the most important tocopherol in Limón Montés ( $84.68 \pm 5.61$  mg·kg<sup>-1</sup> of oil). The main triglycerides were LLP ( $41.17 \pm 1.98$ – $39.32 \pm 1.66\%$ ) followed by LLL ( $27.12 \pm 1.32$ – $17.80 \pm 1.96\%$ ). Good separation among specie samples according to the statistical analysis of the principal component (PCs) and linear discriminant analysis (LDA) was obtained.

**KEYWORDS:** Apple; Fatty acids; Oils; Phospholipids; Sterols; Tocopherols; Triglycerides

**RESUMEN:** Caracterización de aceite de semilla de manzanas con Denominación de Origen de Asturias, España.

Se ha caracterizado el contenido y composición de los lípidos aislados de semillas de siete especies de manzanas de Asturias (España). El contenido más alto en aceite correspondió a Collao ( $22.73 \pm 0.81$  g·100 g<sup>-1</sup> de semilla), seguida de Raxao y Riega ( $20.19 \pm 0.74$  g·100 g<sup>-1</sup> de semilla y  $19.67 \pm 0.85$  g·100 g<sup>-1</sup> de semilla) respectivamente. El ácido linoleico fué el de mayor contenido en Limón Montés ( $60.78 \pm 3.07\%$ ) seguido por la Riega ( $60.01 \pm 3.41\%$ ). El contenido más alto en esteroides correspondió a Solarina ( $558.52 \pm 9.42$  mg·100 g<sup>-1</sup> de aceite) mientras que Blanquina fué el que presentó los valores más bajo ( $166.55 \pm 1.89$  mg·100 g<sup>-1</sup> de aceite). Fosfatidilcolina ( $70.58 \pm 3.85\%$  del total FL) fué el principal constituyente en Blanquina seguida por Collao ( $55.55 \pm 2.96\%$  del total FL). Raxao presentó el contenido más alto en  $\beta$ -tocoferol ( $125.29 \pm 12.62$  mg·Kg<sup>-1</sup> de aceite) y  $\alpha$ -tocoferol en Limón Montés ( $84.68 \pm 5.61$  mg·Kg<sup>-1</sup> de aceite). Los triglicéridos principales fueron LLP ( $41.17 \pm 1.98$ – $39.32 \pm 1.66\%$ ) seguido de LLL ( $27.12 \pm 1.32$ – $17.80 \pm 1.96\%$ ). Se obtuvo una buena separación entre las distintas variedades de manzana teniendo en cuenta el análisis estadístico de los principales componentes (PCs) y el análisis discriminante lineal (LDA).

**PALABRAS CLAVE:** Aceites; Ácidos grasos; Esteroides; Fosfolípidos; Manzana; Semilla; Tocoferoles; Triglicéridos

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## 1. INTRODUCTION

In the last few years, there has been a considerable interest in finding new food sources that will meet the health and nutritional needs of the world's population (Maazouzi *et al.*, 2007; Ohama *et al.*, 2006; Yukui *et al.*, 2009). In Japan, many of the new foods with health claims have been wild vegetables (Cai *et al.*, 2003), such as mustard leaf (Liang *et al.*, 2006) and elm fruit (Hu *et al.*, 2006). Such resources are limited, and more attention should be paid to existing waste resources such as apple seed oils. Denomination of origin (DO) is a special consideration for some foods and drinks to recognize that they have benefits and particular characteristics in many differentiated geographical areas, and the influence of human factor in their production (European Union, 2008). Spain is one of the most important countries of the European Union for the production of cider, producing about 585,000 tons of apples as fruit for human consumption and 75,000 tons of apples to make cider. Asturias is the region with the most important cultivation of apples for the production of cider and have 22 apple varieties included in the DO. Cider, in general, is produced with different varieties of apples, but the cider with DO from Asturias is produced only with apples recognized by the DO (BOE, 2003). In the process for producing cider, there are two important by-products, seeds and skins. The seeds have variable contents in oils. In the cider industry, this seed is not used for producing apple seed oil, and it is a waste product. Fruit seed oil has been widely applied in food, perfumes, toiletries and chemical additives (Stone and Kushner, 2000; Etherton and Etherton, 2003). Plant seed oil is one of the most interesting essential oils due to its properties (Anwar *et al.*, 2006; Lei Tian *et al.*, 2010). A few authors have published papers about apple seed oil and they describe some of its properties (Yu *et al.*, 2007; Yukui *et al.*, 2009; Lei Tian *et al.*, 2010).

The aim of this research was to study the oils of seven different apple seed oils from Asturias (Spain), Blanquina, Raxao, Collaos, Durena, Riega, Solarina and Limón Montés species pertaining to the DO cider from Asturias. The composition in fatty acids, sterols, phospholipids, tocopherols and triglycerides were characterized, as well as the total contents in oil and moisture.

## 2. MATERIAL AND METHODS

### 2.1. Samples

The apple seed species (Table 1) used in the present study came from different plantations in the region of Asturias (Spain), Serida (Principado de Asturias) and San Martín-Llanes (Asturias) collected during a period of suitable ripening. Once

prepared at room temperature, the solid matter (skin and pulp) was separated from the seeds.

### 2.2. Solvent oil extraction and water content

Samples were blanched and ground in an electrical grinder. The oil was extracted in a Soxhlet glass apparatus using hexane as solvent (IUPAC, 1987a). Moisture was determined by weight loss after heating in an oven at 105 °C in accordance with (IUPAC, 1987b).

### 2.3. Fatty acid analysis

Fatty acid methyl-esters (FAME) were analyzed by gas chromatography (GC). FAME were extracted with n-heptane after cold methylation with 2N KOH in methanol (IUPAC, 1987c). FAME analysis was performed on an HP-5890-II apparatus (Hewlett-Packard, Palo Alto, CA) using a fused silica capillary SP-2380 column (60m×0.25 mm, 0.2 µm film thickness) with a flame ionization detector (FID). The oven temperature was kept at 160 °C for 13 min and was then raised to 190 °C at a rate of 1.5 °C min<sup>-1</sup> and held isothermally for 20 min. The injector temperature was kept at 225 °C, while the detector temperature was 250 °C. Hydrogen (19 psi inlet pressure) was used as carrier gas, while the make-up gas was nitrogen. Standards of each fatty acid were used to identify the fatty acids. These were purchased from Sigma-Aldrich (St. Louis, MO): palmitic (P=C16:0), palmitoleic (Po=C16:1), stearic (S=C18:0), oleic (O=C18:1 *ω*9 *cis*), linoleic (L=C18:2) alpha and gamma, linolenic (Lo=C18:3). Fatty acids were identified in the samples by comparing the retention times for standards and those of the samples. The area was expressed as percentages of areas of the total fatty acids.

### 2.4. Sterol analysis

The unsaponifiable fraction was extracted as described (European Communities 1991). 0.5 mL 5- $\alpha$ -cholestanol (Fluka, Buchs, Switzerland) solution in chloroform was added to 5 g of oil as an internal standard. The mixture was saponified for 0.5 hour with 50 mL of 2N ethanolic potassium hydroxide. The solution was then passed to a 500 mL decanting funnel, 100 mL distilled water were added and the mixture was extracted twice with three 80 mL portions of diethyl ether. The diethyl ether extracts were combined in another funnel and were washed several times with 100 mL portions of water, until the wash reached a neutral pH. The diethyl ether solution was dried over anhydrous sodium sulphate and evaporated to dryness in a rotary evaporator at 30 °C under reduced pressure. After purification by thin-layer chromatography (IUPAC, 1987d), the sterol fraction was analyzed and quantified by gas chromatography in an HP 5890-II apparatus equipped with a

TABLE 1. Oil (g·100 g<sup>-1</sup> seed), moisture (g·100 g<sup>-1</sup> seed) and seed yield (g·10 Kg<sup>-1</sup> fruit) from the different varieties of apples

Cultivar	Variety	Fruit (kg)	Seed yield (g)	Oil (%)	Moisture (%)
A	Blanquina	10±0.13	5.19±0.23*	18.82±0.62	9.26±0.12
B	Raxao	10±0.19	4.76±0.32*	20.19±0.74	8.79±0.18*
C	Collaos	10±0.14	5.56±0.19	22.73±0.81	8.19±0.13*
D	Durona	10±0.16	4.56±0.16*	17.71±0.65	8.53±0.11
E	Riega	10±0.12	6.43±0.19	19.67±0.85	7.62±0.10
F	Solarina	10±0.14	7.21±0.17*	16.87±0.61*	8.12±0.13
G	Limón Montés	10±0.18	7.49±0.21	18.67±0.66*	7.13±0.12*

Mean standard deviation (n=3).

\*P≤0.001.

split-splitless injector and a flame ionization detector. An HP-5 fused silica capillary column (30 m×0.32 mm i.d., 0.25 mm film thickness) was used with hydrogen (7 psi inlet pressure) as the carrier gas and nitrogen the make-up gas. The oven temperature was maintained isothermally at 265 °C for 30 min. The injector temperature was 280 °C, while the detector was kept at 300 °C. Previously, the sterol fraction was derivatized as trimethylsilyl ethers (TMS) according to the method proposed in (European Communities, 1991). The sterols were identified by comparison of the mass spectral data with those of authentic reference compounds and by comparing their retention times with sterols from olive, sunflower and soybean oils (León-Camacho and Morales, 2000).

## 2.5. Phospholipid and triglyceride analysis

Phospholipids were analyzed by reverse phase analysis of the lipids carried out on a Water Acquity Ultra Performance LC (UPLC) system using an Acquity UPLC-Bech C18 column (2.1×10 mm, 1.5 µm) at 40 °C. The solvent consisted of water/methanol (10/90) and the flow rate of 1 mL min<sup>-1</sup>, which was coupled to an evaporating light scattering detector (ELSD 2424-Waters). As the nebulizing gas, N<sub>2</sub> was used at a flow rate of 1.4 L min<sup>-1</sup>, and a nebulizing temperature of 80 °C. The injection volume was 10 µL (30 mg 10 mL<sup>-1</sup> oil sample). The assignment of chromatographic peaks was carried out by means of standards purchased from Sigma-Aldrich (St.Louis, MO). Triglycerides were performed on a capillary gas chromatography column (25×0.25 mm) coated with TAB-CB in an HP 5890-II apparatus equipped with a split-splitless injector and a flame ionization detector (Chrompack) according to the procedure described by Alonso, 1993.

## 2.6. Tocopherol analysis

Tocopherols were quantified by high performance liquid chromatography (HPLC). The HPLC system consisted of a low pressure quaternary pump

HP-1050, a Rheodyne injection valve (20 mL loop), a thermostatic furnace and a fluorescence detector RF-235 (Shimadzu, Kyoto, Japan). Separation was performed in a 250×4 mm particle size 5 µm Lichrospher Si-60 (Merck, Darmstadt, Germany) column. The column and detector were kept at a temperature of 40 °C. The mobile phase was n-hexane/2-propanol 99/1 (v/v). The flow rate was supported at 1 mL min<sup>-1</sup> isocratic elution. Quantification was carried out by a calibration system based on standards (IUPAC, 1987d).

## 2.7. Statistical analysis

In order to determine significant differences among species, the main effects analysis of variance (ANOVA) was performed according to the general lineal model procedure. The data were analyzed using the statistical package CSS: Statistica 8.0 software (StatSoft Inc., 1995). The compounds identified were considered as chemical descriptors. A data matrix was built where rows are the samples and columns are the variables. Each element of this matrix, expressed as x<sub>ij</sub>, corresponds to the content of compound j for the sample i. In order to obtain a better understanding of the data trends, pattern recognition methods such as principal component analysis (PCA) and linear discrimination analysis (LDA) were applied.

# 3. RESULTS AND DISCUSION

## 3.1. Oil composition analysis

Table 1 shows the content in oil, moisture and seed yield for Blanquina, Raxao, Collaos, Durona, Riega, Solarina and Limón Montés apple seeds. The highest oil content corresponded to Collaos apples at 22.73±0.81 g 100 g<sup>-1</sup> seed, followed by Raxao and Riega at 20.19±0.74 g 100 g<sup>-1</sup> seed and 19.67±0.85 g 100 g<sup>-1</sup> seed, respectively. These values are slightly higher than those reported by Tian *et al.* (2010) in a study of antioxidant and antimicrobial

TABLE 2. Fatty acid composition (%) of lipid fractions extracted from apple seed oils

Cultivar	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0
A	8.49±0.98	0.08±0.01*	1.90±0.13	32.71±2.65	53.98±3.16	0.30±0.06	1.53±0.19	0.51±0.08	0.28±0.03
B	8.67±0.67	0.08±0.01	1.98±0.11	30.53±1.98	56.31±3.25	0.25±0.05	1.34±0.11	0.40±0.07	0.25±0.02
C	8.07±0.71*	0.07±0.02	2.30±0.15*	36.57±2.08	50.34±2.91	0.25±0.04*	1.48±1.15	0.45±0.09	0.25±0.04
D	8.28±0.84	0.08±0.01	1.90±0.18	34.32±2.15	52.97±3.29	0.28±0.06	1.27±0.09	0.48±0.08	0.25±0.03
E	9.18±0.92*	0.12±0.03	1.75±0.12*	27.02±1.69	60.01±3.41	0.34±0.06	1.14±0.12*	0.36±0.06	0.17±0.02
F	9.01±0.85	0.09±0.02	1.97±0.13	31.85±2.07	54.49±2.91	0.40±0.04*	1.28±1.11	0.46±0.08	0.23±0.03
G	8.89±0.93*	0.12±0.02*	1.75±0.12*	27.02±1.91	60.01±3.07	0.34±0.03	1.14±0.13	0.36±0.07	0.17±0.02

Mean standard deviation (n=3).

\* $P \leq 0.001$ .

activities of the oil in apple seeds. The moisture contents ranged between  $7.13 \pm 0.12$  g 100 g<sup>-1</sup> seed and  $9.26 \pm 0.12$  g 100 g<sup>-1</sup> seed in Limón Montés and Blanquina. The highest seed yields were for Limón Montés ( $7.49 \pm 0.21$  g of seed 10 Kg<sup>-1</sup> of fruit) and Solarina ( $7.21 \pm 0.17$  g of seed 10 Kg<sup>-1</sup> of fruit). Table 2 shows the fatty acid compositions of the apple seed oil. Linoleic acid was found to be the main component in the oil of the Limón Montés species ( $60.78 \pm 3.07$  g 100 g<sup>-1</sup> of oil) followed by the Riega species ( $60.01 \pm 3.41$  g 100 g<sup>-1</sup> of oil) these contents

are higher than those found by other authors (Yukui *et al.*, 2009; Tian *et al.*, 2010). The highest concentrations of oleic ( $36.57 \pm 2.08$  g 100 g<sup>-1</sup> of oil) and stearic acids ( $2.30 \pm 0.15$  g 100 g<sup>-1</sup> of oil) were found for the Collaos species. These data are somewhat in agreement with those found by Yu *et al.* (2007) in a study of the proximate composition of the apple seed and the characterization of its oil. Almost all phospholipid (PL) classes were identified in the phospholipid fraction (Table 3). Phosphatidylcholine ( $70.58 \pm 3.85\%$  of the total PL) was found to be the

TABLE 3. Phospholipid (% of total PL) compositions from apple seed oils

Cultivar	Total PL	LPC	PC	PE	PI
A	0.38±0.06	3.75±0.31	70.58±3.85	8.71±0.52*	17.16±0.21*
B	0.45±0.08	4.88±0.39	35.78±0.31*	27.53±0.22	31.80±0.25
C	0.41±0.06	5.18±0.57	55.55±2.96	16.95±1.52*	22.32±1.91
E	0.52±0.07	3.10±0.27*	40.65±2.61	31.39±1.69	24.85±1.51
F	0.49±0.08	3.01±0.19*	42.59±2.85	30.80±1.54	23.59±1.32

Men standard deviation (n=3).

\* $P \leq 0.001$ .

LPC: Lisophosphatidilcolina; PC: Phosphatidilcolina; PE: Phosphatidylethanolamina; PI: Phosphatidylinositol.

TABLE 4. Total sterol (mg·100 g<sup>-1</sup> of oil) of lipid fractions extracted from apple seed oils

Cultivar	Cholesterol	Campesterol	Stigmasterol	Chlerosterol	β-Sitosterol	Sitostanol	Δ5-Avenasterol	Δ5,24-Stigmastadienol	Δ7-Stigmastenol
A	1.39±8.22	17.70±0.22	1.61±0.12	1.26±0.02	166.55±1.89	5.21±0.16	14.37±0.43	1.31±0.12	9.85±1.17
B	3.03±2.80	28.16±0.75	1.78±0.21	1.76±0.10	225.13±1.89	1.34±0.25	20.05±0.20	2.05±0.22	5.05±1.53*
C	2.94±2.39	22.94±1.78	1.58±0.31	1.52±0.39	231.6±12.39	7.9±0.39	22.69±1.40	2.43±0.79	26.65±2.75
D	1.74±8.19	21.93±0.65	1.98±0.23	1.86±0.28	254.04±7.64	1.93±0.89*	14.42±1.11	6.29±0.70*	32.22±2.04
E	2.40±0.16	39.76±0.62	3.19±0.29	1.66±0.05	392.48±8.92	21.58±0.69	28.35±1.19	2.42±0.27	67.51±2.93
F	2.97±0.24	48.63±2.52	4.05±0.37*	2.44±0.15	558.52±9.42*	40.64±0.86	56.59±1.94	4.94±0.63	103.70±2.13
G	2.64±0.29	49.25±1.16	2.56±0.21	2.06±0.45	410.75±5.34	13.30±0.62	31.22±1.12	1.97±0.45	30.00±2.74

Mean standard deviation (n=3).

\* $P \leq 0.001$ .

TABLE 5. Tocopherol contents (mg·Kg<sup>-1</sup> of oil) of lipid fractions extracted from apple seed oil samples

Cultivar	$\alpha_{TF}$	$\beta_{TF}$	$\gamma_{TF}$	$\delta_{TF}$
A	54.83±6.23	99.15±11.64	ND	0.12±0.02
B	57.52±6.87	125.29±12.62	0.28±0.03	0.69±0.09*
C	55.27±4.88	85.22±6.12	0.41±0.06	1.78±0.15
D	52.43±3.06	84.57±5.93	0.31±0.05*	0.10±0.02
E	79.65±4.15	79.31±3.66	0.46±0.05	1.33±0.08
F	84.76±5.02	89.27±4.89	0.77±0.12	2.13±0.17
G	84.68±5.61	79.21±4.25*	4.33±0.21	7.55±0.58

Mean standard deviation (n=3).

\*P≤0.001.

ND: Not Detected.

TABLE 6. Triglyceride composition (%) of lipid fractions extracted from apple seed oils

Cultivar	LLL	LLO	LLP	OOL	SOL	SOL	OOO	OOP	PPO
A	22.57±1.18	6.10±0.58	41.17±1.98	5.60±0.35	20.42±1.38	0.57±0.07	1.77±0.23	1.38±0.26	0.23±0.08
B	27.12±1.32	7.90±0.72*	39.79±1.35	5.75±0.21*	16.85±1.29	0.50±0.08	1.44±0.13*	0.51±0.18	0.17±0.04
C	17.80±1.96	4.49±0.18*	40.69±2.04	5.67±0.06	24.71±1.86	0.96±0.08	3.96±0.15	1.76±0.04	0.28±0.03
D	23.15±1.96	7.61±0.61	39.32±1.66	6.67±0.51	19.09±1.38	0.81±0.13*	2.04±0.16	1.18±0.14	0.22±0.05
E	22.11±1.64	7.20±0.49	39.88±1.47	7.29±0.38*	19.35±1.24	0.76±0.16*	1.98±0.19	1.09±0.11	0.31±0.06

Mean standard deviation (n=3).

\*P≤0.001.

main constituent in the blanquina species followed by the Collaos species (55.55±2.96% of the total PL). Phosphatidylethanolamine (30.80±1.54% of the total PL) and phosphatidylinositol (31.80±0.25% of the total PL) showed a higher value for Limón Montés and Raxao species than the other species. These values were somewhat higher than those obtained by Zlatanov *et al.* (1997) in a study of the phospholipid composition of Rosaceae seed oil. Table 4 shows the content in sterols in Blanquina, Raxao, Collaos, Durona, Riega, Solarina and Limón Montés apple seed oils.  $\beta$ -sitosterol was the major sterol in all apple seed varieties. Solarina seed oil was the one with the highest content at 558.52±9.42 mg 100 g<sup>-1</sup> of oil while Blanquina seed oil was the one that presents the lowest values at 166.55±1.89 mg 100 g<sup>-1</sup> of oil. Likewise, Limón Montés seeds had the highest content in campesterol at 49.25±1.16 mg 100 g<sup>-1</sup> of oil and Blanquina the least at 17.70±0.22 mg 100 g<sup>-1</sup> of oil. The stigmaterol content in Solarina was worth noting at 4.05±0.37 mg 100 g<sup>-1</sup> of oil. With regard to  $\Delta$ -5 avenasterol, Solarina seed contained 56.59±1.94 mg 100 g<sup>-1</sup> of oil, while Blanquina had only 14.37±0.43 mg 100 g<sup>-1</sup> of oil. Table 5 shows the content in tocopherols in the studied apple seed oils. Raxao presented the highest content in total  $\beta$ -tocopherol at 125.29±12.62 mg Kg<sup>-1</sup> of oil and Limón Montés seed oil the lowest content at

79.21±4.25 mg Kg<sup>-1</sup> of oil.  $\alpha$ -tocopherol was the prominent tocopherol in Limón Montés, with a content of 84.68±5.61 mg Kg<sup>-1</sup> of oil followed by the Solarina species with 84.76±5.02 mg 100 Kg<sup>-1</sup> of oil. The main triglyceride composition of apple seeds (Table 6) was made up of LLP (41.17–39.32%) followed by LLL (27.12–17.80%). The triglycerides present in small amounts were SOL (0.81–0.50%) and PPO (0.31–0.17%). Triglycerides with three

TABLE 7. Eigenvalues of correlation matrix, and related statistics. Active variables only

Compound classes	PC	Eigenvalue	% Total-variance
Fatty acids	1	5.665	62.95
	2	1.464	16.27
Triacylglycerols	1	5.120	56.89
	2	2.058	22.88
Phospholipids	1	3.347	66.95
	2	1.296	25.91
Sterols	1	7.686	69.88
	2	1.442	13.11
Tocopherols	1	2.171	54.27
	2	0.847	21.17

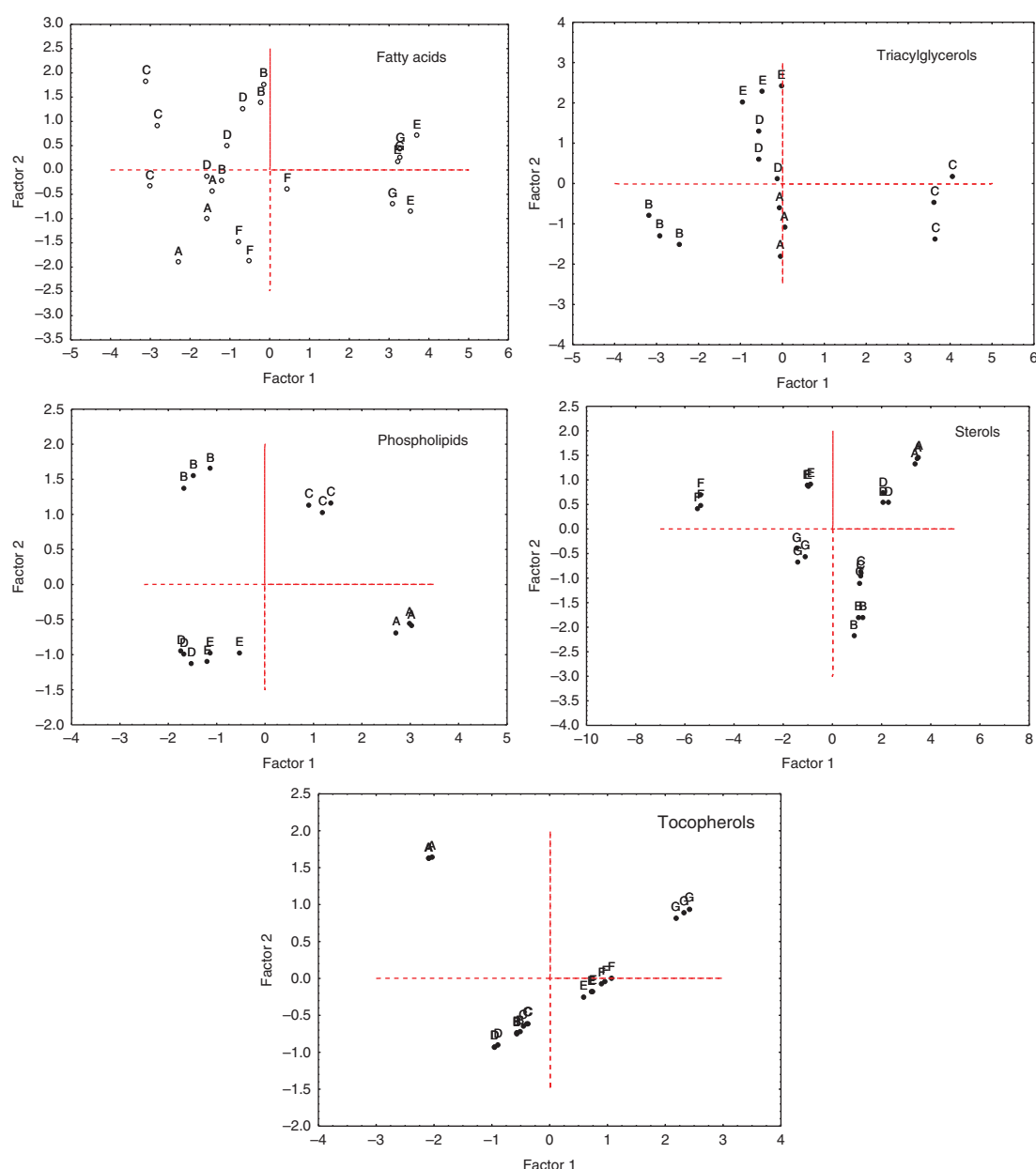


FIGURE 1. Score plot in the plane of the two first PCs. Letters A, B, C, D, E, F, G, I corresponding to the different compound groups that are gathered in tables 1 to 7.

unsaturated acids presented the highest proportions, followed by those that have two unsaturated acids.

### 3.2. Principal component analysis

A PCA was applied to the data set to obtain linear combinations of the variables called principal components (PCs). The first principal component (PC1) expresses the largest variability and each successive PC represents as much of the residual variability as possible. The results of this analysis are included in

Table 7. As can be seen, the two first PCs obtained present eigenvalues greater than 1, the explanation of the original variance for each PC is shown in this table. For fatty acids PC1 is highly influenced by C16:1, C18:0, C18:1, C18:2, C20:0, C20:1 and C22:0. In the case of PC2, the most contributing variable is C18:3. In the case of triacylglycerols, PC1 is highly influenced by LLL, LLO, SOL, SOLn, OOO and OOP. The most contributing variables to PC2 are OOL and PPO. For phospholipids PC1 is highly influenced by the total content, PC, PE and PI.

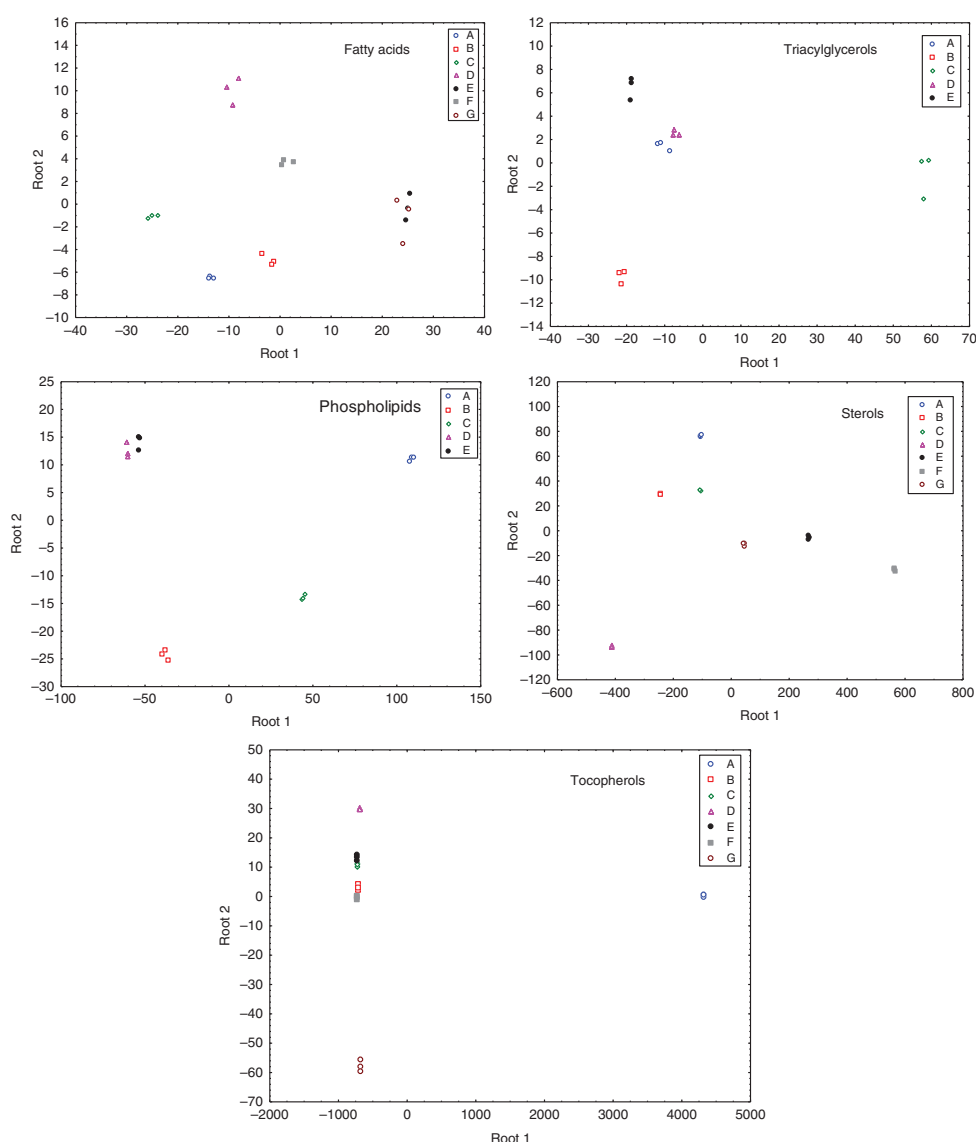


FIGURE 2. Scatterplot of the canonical scores corresponding to different species samples. Letters A, B, C, D, E, F, G, I corresponding to the different compound groups that are gathered in tables 1 to 7.

The most contributing variable to PC2 is LPC. In the case of unsaponifiable compounds, brasicasterol, campesterol, stigmasterol, sitosterol, sitostanol, avenasterol, stigmasterol, avenasterol, and tocopherol exert a great influence on PC1. The most contributing variables to PC2 are cholesterol and  $\gamma$ -tocopherol.

The two-dimensional plots of the PCs can be used to reveal the internal structure of the data and visualize data trends Jolliffe (2002). Figure 1 shows the distribution of the samples in the plane formed by the two first PCs, in this figure a good separation between species samples can be observed, along with their remarkable distribution of positive or negative values according to the scores of PC1 and PC2. Taking into account these results, linear discriminant analysis was applied to obtain an adequate classification model.

### 3.3. Linear discriminant analysis

The following step in our analysis was to apply the procedures of supervised pattern recognition in order to achieve a better separation. Accordingly, LDA (Coomans, *et al.*, 1979) was applied to the data set to obtain suitable classification rules for the samples. The corresponding discriminant functions were calculated as linear combinations of the chemical descriptors. The criterion used for feature selection was the backward stepwise approach. In a first run, all the variables are present in the model; in each step the variable with least discriminant power, according to the Wilks'  $\lambda$  statistic test (Gardine, 1997), is rejected. Figure 2 shows the sample distribution in the space of the different

obtained discriminant functions. The seven oil samples appear completely separated.

#### 4. CONCLUSIONS

On the basis of the present study, it may be concluded that the nutritional properties of the studied apple seed oils possess a high added value. The composition in lipids of these seeds presents high contents in polyunsaturated fatty acids C18:2, sterols ( $\beta$ -sitosterol), phospholipids (phosphatidilcoline), and tocopherols (alfa-tocopherol). The extraction of these apple seed oils is an option to obtain high added value oils and an additional channel for their use as rich in bioactive nutraceutical compounds to assess the potential use of the oils in foodstuffs. From the point of view of authenticity, it could be a useful tool to know the trazability of apple seed oil with DO compared to others in order to avoid adulteration through the study of the minor oil components.

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