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# Fatty Acid Profiling of the Main Tissues of Spanish Olive Fruit: Effect of the Oil Extraction Method

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**Abstract** The aims of the present study were as follows: (1) study of the extraction efficiency of the methods to obtain extra virgin olive oil and pomace oil, and the fatty oil composition provided by each tissue and the whole fruit using both methods; (2) characterisation of the oil obtained by each extraction method according to quality parameters; and (3) development of chemometric models able to explain the differences between the oils obtained from the different extraction procedures, olive varieties or fruit tissues. With these objectives in mind, eight fruit varieties (i.e. arbequina, frantoio, hojiblanca, lechín from Sevilla, manzanilla, ocal, picual and picudo) from the same geographical area, harvested when the maturation was complete and the fatty acids content was at a maximum, were used. Oils from the whole fruit and from each of its main tissues (i.e. ectocarp, mesocarp, endocarp, and kernel) were sequentially extracted by the two methods based on principles similar to those used in the oil industry: pressure + centrifugation for extra virgin oil extraction and organic solvents for pomace oil. The oils thus obtained were weighed to know the oil richness of the fruit and that of each separate tissue, and also to determine the efficiency of each extraction method. Then, the fatty acids were subject to derivatisation, individual separation of the products by gas chromatography and identification–quantitation by tandem-mass-spectrometry. The results thus obtained (fatty-acid profile of the oil from each tissue obtained with each oil-extraction method and from each olive-fruit variety) were subject to statistical studies to fulfill the aims of the research.

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## Introduction

Olive (*Olea europea* L.) is among the oldest cultivated trees in the world. Presently, about 98% of olive-tree cultivation in the world is in the Mediterranean area. The oval-shaped olive fruit can be divided into four main parts: ectocarp or skin, mesocarp or flesh, and bone, formed by endocarp and kernel. Olives are mainly composed of water and fatty acids, but also of sugars, proteins, pectins, tannins, inorganic constituents, phenols, etc. The composition of this fruit depends on the tree variety and state, external factors [1] and ripening degree [2].

Despite the enormous range of olive-tree varieties, olive oil consists of two fractions: a saponifiable fraction (triglycerides) and unsaponifiable fraction (minor components). The former represents 98–99% of the oil mass and is mainly composed by triglycerides (fatty-acid glycerol esters) and free fatty acids. The most abundant fatty acids in olive oil are monounsaturated oleic acid (55–83% of oil weight), palmitic acid (8–20%), and, to a lesser proportion, polyunsaturated acids such as linoleic acid (3–21%). Other less concentrated fatty acids are palmitoleic (0.3–3.5%), stearic (0.5–5.0%) and linolenic (0–1.5%) [3], and also epoxy fatty acids [4]. The unsaponifiable fraction represents a percentage lower than, or equal to 2% of oil composition, but it has a key importance from the point of view of its biological value.

Previous studies dealing with characterisation of the oil obtained from different varieties have focused on the analysis of acylglycerols and fatty-acid components of the pulp, kernel and whole olive fruit oils [5]. The

composition, including non-esterified fatty-acids of monovarietal oils obtained by pressure, has been used to establish a relationship between these compounds and thermal properties of the oil [6, 7]. Also the effect of the ripening degree of the fruit on the oxidative stability and organoleptic properties of oils has been studied as a function of the phenolic and fatty-acid composition [8–10].

The research here presented is a part of a wider study of the olive-tree metabolome. As a first step, the study of olive-fruit metabolome is intended, starting off by the lipidome (viz. the profile of esterified and non-esterified fatty acids—EFAs and NEFAs, respectively—in olive fruit) has been tackled in this research. The study has been extended to 8 varieties of olive trees grown in the same area to avoid differences caused by different climate, soil type and other environmental conditions.

## Materials and Methods

### Apparatus

A magnetic stirrer with temperature control was used to mix the olive paste during the extraction process. A vortex (Ika-Works, Wilmington, USA), a centrifuge (Selecta, Barcelona, Spain) and an analytical balance (Explore Ohaus 110 ± 0.0001 g, Switzerland) were used in the extraction and derivatisation steps.

A 50-mL Soxhlet extractor from Afora (Barcelona, Spain) was used in conventional Soxhlet extraction. A rotary evaporator Büchi R-200 with a thermostated water-bath B-490 (Switzerland) was also used.

### Instruments

A CP-3800 gas chromatograph Varian (Walnut Creek, CA, USA) equipped with a split/splitless SPI/1079 programmable-temperature injector and coupled to a Saturn 2200 ion trap mass spectrometer (Sunnyvale, TX, USA) was used for individual separation and detection of the target analytes. The chromatograph was furnished with a COP 8400 autosampler Varian and a SP$^{TM}$-2380 fused silica capillary column (60 m × 0.25 mm I.D., 0.2 µm film thickness) from Supelco (Bellefonte, PA, USA).

### Reagents

All reagents were analytical grade or higher. Methanol and *n*-hexane were provided by Panreac (Barcelona, Spain), anhydrous sodium sulfate from Sigma–Aldrich (Steinheim, Germany) was used as drying agent for the non-polar phase prior to derivatisation. Methanolic solutions of 0.4 M KOH and 5% $H_2SO_4$ were used for derivatisation of esterified and non-esterified fatty acids.

A multistandard containing the methyl esters of the 26 fatty acids listed in Table 1, and *c*10 nonadecenoic (19:1) as internal standard were used.

### Obtaining and Preservation of the Raw Material

The olive fruits used in this research were collected in the region of the Guadalquivir valley, in Encinarejo (Córdoba). Eight genetic varieties of olive fruit (i.e. arbequina, frantoio, hojiblanca, lechín from Sevilla, manzanilla, ocal, picual and picudo) were harvested in January 2008, when maturation is completed. At this time, the content in fatty acids of the fruit is maximum. All samples were taken from the same geographical area to avoid variations caused by soil characteristics and environmental factors, taking into account that the hydrological year was dry, the hydric stress was controlled by irrigation. For representativeness, the fruits of each variety were taken from four different trees and divided into two portions and kept at −20 °C until use.

A portion of each variety was subject to manual separation of the 4 main tissues present in the olive fruit—

**Table 1** Identification and quantitation fragments for the target compounds

| Compound | Identification/quantitation fragments |
|---|---|
| C9:0 | 74, 87, 129, 143, 173 |
| C12:0 | 74, 87, 143, 171, 214 |
| C16:0 | 74, 87, 143, 227, 270 |
| C16:1-n7 n9 | 55, 81, 237, 269 |
| C17:0 | 74, 87, 143, 284 |
| C17:1-n10 | 55, 69, 96, 250, 283 |
| C18:0 | 74, 87, 143, 255, 298 |
| C18:1-n9 t | 55, 69, 83, 97, 110, 180, 222, 164, 197 |
| C18:1-n9 | 55, 69, 83, 97, 110, 180, 222, 264, 297 |
| C18:1-n7 | 55, 69, 83, 97, 110, 180, 222, 264, 297 |
| C18:2 t9, t12 | 67, 81, 95, 262, 294 |
| C18:2 c, t | 67, 81, 95, 262, 294 |
| C18:2 t, c | 67, 81, 95, 262, 294 |
| C18:2 c, c | 67, 81, 95, 262, 294 |
| C18:3 t, t, c y t, c, t | 55, 67, 69, 74, 79, 87, 95, 292, 326 |
| C18:3 c, t, t y c, c, t | 55, 67, 69, 74, 79, 87, 95, 292, 326 |
| C18:3 c, t, c | 55, 67, 69, 74, 79, 87, 95, 292, 326 |
| C18:3 t, c, c | 55, 67, 69, 74, 79, 87, 95, 292, 326 |
| C18:3 c, c, c | 55, 67, 69, 74, 79, 87, 95, 292, 326 |
| C20:3-n6 | 55, 67, 79, 81, 95, 150, 320, 354 |
| C20:4-n3 | 67, 79, 91, 119 |
| C20:5-n3 | 67, 79, 81, 91, 95, 105, 133, 201, 318, 350 |
| C26:0 | 74, 79, 87, 91, 119, 143, 199, 293, 371, 410 |

ectocarp, mesocarp, endocarp and kernel. The aim was the study of the individual tissues in order to know its contribution to the fatty-acid composition of commercial oils, obtained from the whole fruit. Other portion was used to obtain conventional oils from the whole fruit.

The separation process was as follows: the ectocarp or olive skin from each olive variety was manually separate with extreme care from the mesocarp by a scalpel; then, the mesocarp or pulp was obtained by separation from the bone also using a scalpel. After mesocarp removal, the bone was washed with water and the rest of pulp was removed by the scalpel; the bone was broken and the endocarp and kernel were manually separated and preserved, as the other fractions, at −20 °C.

## Extraction Methods

Two methods similar to those used in the industry were sequentially applied to the whole fruit and the different fruit tissues from each variety. The steps of the overall extraction process are shown in Fig. 1.

### *Extraction of Extra Virgin Olive Oil (VO)*

The sample (the whole olive fruit or each separated tissue) was weighed, ground in a mincer to break cells and release their oil content, and the paste thus formed was subject to agitation for 1 h to facilitate aggregation of oil drops. Then, the paste was centrifuged at 13.6 g (3,500 rpm) for 30 min to separate the two phases: a semisolid phase (pomace) and an oily phase. The latter was stored at −20 °C until use. The temperature during the whole process did not surpass 30 °C.

### *Extraction of Pomace Oil (PO)*

The semisolid phase from the previous step has a high oil content which was obtained by conventional Soxhlet extraction with 80 mL *n*-hexane for 24 h at 69 °C (boiling point of hexane). After extraction, the extractant was removed under vacuum and, the oil remaining in the flask was stored at −20 °C until use.

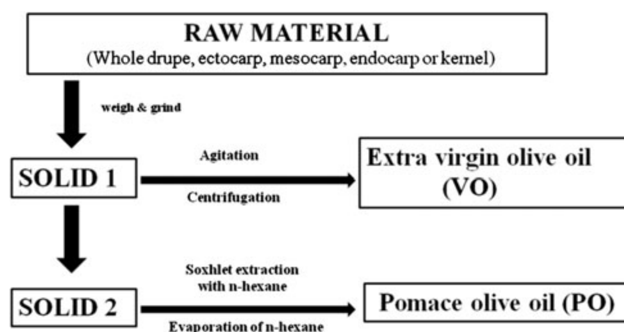


**Fig. 1** Flow diagram of the sequential extraction of oils from whole fruit and individual tissues

The solid fraction in the Soxhlet cartridge after extraction was stored at −20 °C as the matter to study other fruit metabolites.

Traces of water in the oils obtained by the two extraction methods were removed by using a gentle $N_2$ stream; then, the oil was weighed and subjected to the derivatisation step.

## Derivatisation Step

The fatty acids in the oil were derivatised using the method proposed by the International Olive Council (IOC) [11] for preparation of fatty acids methyl esters (FAMEs), more volatile compounds able to be separated individually by gas chromatography.

The whole derivatisation method involves two steps: (1) transesterification into methyl esters with subsequent extraction of the esterified fatty acids, and, (2) derivatisation and isolation of the non-esterified fatty acids fraction.

The analytical sample (0.05 g oil) was diluted with 2 mL *n*-hexane, mixed with 500 μL 0.4 M KOH in methanol and homogenised for 5 min in a vortex. The mixture was left for 5 min for reaction completion and the hexane phase containing most of the derivatised analytes, was transferred to a test tube. A second extraction of the oily phase was performed with 2 mL *n*-hexane, agitation in a vortex for 2 min, followed by phases separation. The two *n*-hexane extracts were mixed, 1:250 diluted with *n*-hexane containing 5 μg $mL^{-1}$ of *c*10 nonadecenoic acid as internal standard, and 10 μL of the mixture was injected into the gas chromatograph.

For derivatisation of NEFAs into their fatty acids methyl esters, a small amount of anhydrous sodium sulfate was added to the remaining fraction from the previous step to remove residual water; then, 500 μL $H_2SO_4$ was added and the tube was plunged for 30 min into a water bath thermostated at 70 °C. After cooling, 1 mL *n*-hexane was added, agitated for 2 min in a vortex and, after phase separation, the top *n*-hexane phase containing the derivatised NEFAs was transferred to a test tube. Liquid–liquid extraction was repeated to ensure total recovery of NEFAs, the organic phase containing the NEFAs led to dryness under an $N_2$ stream, and the residue dissolved in 500 μL *n*-hexane and shaken for 2 min. Finally, after 1:75 dilution with *n*-hexane containing the internal standard, 10 μL of this solution was injected into the gas chromatograph.

## Individual Separation and Identification–Determination

The individual separation of FAMEs by GC and identification by MS were carried out using the GC–MS method

developed by Sánchez-Ávila et al. [12]. Briefly, high-purity helium (99.9%) at 1.0 mL min$^{-1}$ was used as the carrier gas in the chromatographic step. The injection volume was 10 μL. The temperature program of the injector was as follows: start at 70 °C, held for 0.5 min, increase at 100 °C min$^{-1}$ to 250 °C, and then kept for 78 min. The injection was in the split–splitless mode. The splitter was open (100:1) for 0.5 min, closed for 3.5 min and then open at 100:1 split ratio for 10 min.

The samples were analyzed using the following oven temperature program: initial temperature 70 °C (held for 1.2 min), increased at 25 °C min$^{-1}$ to 120 °C, followed by a second gradient of 2 °C min$^{-1}$ to 243 °C and, finally, increased by 40 °C min$^{-1}$ to 270 °C and held at this temperature for 5 min.

The mass spectrometer operating in the EI mode was used for full scan and $\mu$_Selected Ion Storage ($\mu$_SIS, similar to Selected Ion Monitoring) experiments. The manifold, trap and transfer line temperatures were set at 60, 170 and 200 °C, respectively. The analyses were performed with a filament–multiplier delay of 11 min. Full scan acquisition, used for the identification of the FAMEs, was performed in the range $m/z$ 40–650, with a background mass of $m/z$ 45. Quantitation was developed using the $\mu$_SIS mode. The fragments used for the individual identification and determination of the FAMEs are listed in Table 1.

The limit of detection (LOD) for each analyte was expressed as the mass which gives a signal that is 3$\sigma$ above the mean blank signal (where $\sigma$ is the standard deviation of the blank signal). The LODs obtained ranged between 0.2 and 20 μg Kg$^{-1}$. The limits of quantitation, expressed as the mass of analyte which gives a signal 10$\sigma$ above the mean blank signal, ranged from 0.66 to 66 μg Kg$^{-1}$.

### Chemometric Analysis

Models based on principal component analysis (PCA) were developed in order to study the influence of the extraction methods, the fruit variety and the type of tissue in the oil extracted from the fruit. Esterified and non-esterified fatty acids, expressed as μg g$^{-1}$ of raw material, were used as variables for development of the models. Unscrambler 9.0 from CAMO (Oslo, Norway) was used as the statistical software.

## Results and Discussion

The results of the research are presented and discussed in the following order: first, the efficiency of the extraction methods, total oil content of each tissue and that of the whole fruit were studied; second, olive fatty acids, as their main fatty acid families were characterised and quantitated; and, finally, statistical studies were performed using three methodologies in order to check the influence of: (1) the extraction methods on the nature of the oils obtained; (2) the fruit variety on the oil obtained from the different tissues; and, (3) the tissue on the oil obtained from each variety.

### Efficiency of the Extraction Methods, Total Oil Content of Each Tissue and That of the Whole Fruit

The extraction efficiency of the two extraction methods sequentially used for removal of the oil was studied for the whole fruit, each of the tissues, and all varieties under study. With this aim, the oil obtained from each sample was accurately weighed after removal of water traces. The results obtained, summarised in Table 2, show that the VO

**Table 2** Efficiency of the extraction methods and total oil content of each tissue and whole fruit

| A* | | B* | | C* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Extraction efficiency | | Whole fruit | | VO | | | | PO | | | |
| | | VO | PO | Ectocarp | Mesocarp | Endocarp | Kernel | Ectocarp | Mesocarp | Endocarp | Kernel |
| Arbequina | 12.89 | 55.58 | 44.42 | 0.00 | 39.00 | 4.47 | 12.11 | 0.00 | 1.08 | 23.37 | 19.97 |
| Frantoio | 13.84 | 90.14 | 9.86 | 0.00 | 66.68 | 21.77 | 1.68 | 1.10 | 0.79 | 3.00 | 4.96 |
| Hojiblanca | 11.21 | 77.34 | 22.66 | 0.00 | 52.19 | 17.93 | 7.23 | 7.18 | 0.15 | 4.32 | 11.01 |
| Ocal | 21.04 | 72.62 | 27.39 | 3.04 | 60.02 | 5.73 | 3.83 | 10.42 | 0.84 | 6.50 | 9.63 |
| Manzanilla | 4.68 | 69.77 | 30.24 | 0.00 | 51.77 | 12.41 | 5.59 | 0.00 | 0.95 | 18.31 | 10.98 |
| Lechín from Sevilla | 9.17 | 64.08 | 35.92 | 3.11 | 55.07 | 1.05 | 4.85 | 3.75 | 5.16 | 7.68 | 19.33 |
| Picual | 10.74 | 84.30 | 15.70 | 0.00 | 71.48 | 2.96 | 9.87 | 2.23 | 0.18 | 9.44 | 3.85 |
| Picudo | 12.40 | 53.30 | 46.70 | 0.00 | 18.09 | 7.97 | 27.24 | 10.66 | 0.31 | 5.03 | 30.70 |

A*: percentage of oil in the fruit of each variety referred to the sample weight

B*: percentage of total oil obtained by each extraction method

C*: contribution of each tissue to the total oil obtained by each extraction method

method provided a higher extraction efficiency than PO for almost all the varieties (frantoio and picual being the varieties which the highest extraction efficiencies—90 and 84%, respectively, of the total extracted oil). The efficiency of the other varieties (except for arbequina and picudo, which yielded the same amount of oil by both methods) ranged between 64 and 77%, for lechín from Sevilla and hojiblanca, respectively.

The study of the contribution from each tissue to the overall extraction efficiency showed that the application of the VO method did not allow extraction of oil from the ectocarp in most varieties. Small amounts of oil (about 3% of the total oil extracted) were from this tissue in ocal and lechín varieties. Taking into account that total isolation of the ectocarp from the mesocarp is difficult, the oil extracted from the former tissue could come from rest of mesocarp tissue remaining with the ectocarp (this possibility is discussed after chemometric analysis of the fatty acid profile obtained from the different oils).

Mesocarp was the tissue with the highest oil content for all the varieties with the exception of the picudo variety. In this case, the amount of oil present in the kernel was twice the oil in the mesocarp. The energy applied in the VO procedure (grinding plus agitation) is high enough to collapse the vesicles where the oil is stored and most of the oil is removed during centrifugation. Therefore, the amount of oil which remained in this tissue to be removed in the next extraction step was small—only the oil which could not be removed by centrifugation.

The oil content of the endocarp, the woody tissue of the fruit, was small for most varieties with the exception of frantoio, manzanilla and hojiblanca. The contribution of endocarp to the total oil content of these varieties is significant (see Table 2). This behavior can be explained similarly to the anomalous values provided by the ectocarp: it is easy to remove the mesocarp from the endocarp but it is not so easy to achieve total isolation of the kernel from the endocarp. Therefore, the oil from this tissue could come from some rest of kernel remaining with the endocarp (this aspect is clarified by the chemometric analysis of the results).

The contribution of kernel to the total amount of oil extracted is also high except for varieties with high oil contribution from the endocarp.

The subsequent extraction step—pomace extracted with organic solvent (PO)—allowed extraction of a high amount of oil from tissues which made a small contribution to the previous extraction method—ectocarp, endocarp and kernel—as discussed previously. The contribution of the mesocarp was small because the oil obtained from this tissue was that not removed by centrifugation in the VO method.

Concerning ectocarp, this tissue provided the main contribution to the oil obtained from ocal by the PO method.

The use of high temperatures and an organic solvent allows the extraction of a high amount of oil from the endocarp, a minor contributor to the oil from the total fruit by the VO method. The varieties with the highest contribution from this tissue were manzanilla and arbequina. For the rest of varieties, the kernel was the tissue which made the main contribution to the oil obtained by the PO method (similarly to mesocarp in the VO method).

The amounts of oil obtained from the whole fruit ranged between 4.68% of the raw material (manzanilla variety) and 21.04% (ocal variety); the percent for the other varieties being within 9 and 13%—for lechín from Sevilla and frantoio, respectively. The high difference in oil content between manzanilla and the other varieties was foreseeable taking into account that manzanilla fruits are not used for oil production, but they are almost exclusively used as table olives.

## Characterisation and Quantitation of Olive Fatty Acids

The complexity and number of data obtained from the fatty acid profile made it unfeasible to use the raw data for characterisation and comparison of the oils obtained by both extraction methods. In order to overcome this problem, oil quality parameters such as content of *trans*, polyunsaturated, monounsaturated, $\omega 6$ and $\omega 3$, and free fatty acids were used (Table 3). The quality of the oils obtained by the VO and PO methods was based on these parameters.

The unhealthy properties of *trans* fatty acids are well known, however, they stabilise the oil quality. The concentration of *trans* fatty acids in the oil obtained by PO was twice that in the oil obtained by the VO method. This fact is explained by the relationship between the presence of these fatty acids and thermal degradation of the oils [13]. The use of an organic solvent at high temperature during the PO method favours *cis*/*trans* isomerisation. The concentration of these fatty acids is a function of the olive variety, the given tissue and extraction method.

The study of the oils obtained from the whole fruit showed that arbequina and lechín were the varieties which yielded the highest *trans*-fatty acid concentrations in the oils obtained by the VO and PO methods, respectively. As can be seen in Table 3, the concentration of these fatty acids was not the same in all tissues. Thus, the external tissues—ectocarp and mesocarp—provided oils with higher concentrations of these acids than the internal ones—endocarp and kernel—by both extraction methods. This behavior can be explained by the different strength of

**Table 3** Concentration range, average concentration and standard deviation of fatty acid families extracted by the two methods from different olive fruit tissues

| Extraction method | Tissue | *trans* Fatty acids | | | Polyunsaturated fatty acids | | | Monounsaturated fatty acids | | | ω6 Fatty acids | | | ω3 Fatty acids | | | Non esterified fatty acids | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Range | X | S | Range | X | S | Range | X | S | Range | X | S | Range | X | S | Range | X | S |
| VO | Ectocarp | 0.00(-)–69.31(L) | 9.19 | 24.34 | 0(-)–0.79(O) | 0.15 | 0.29 | 0(-)–82.34(L) | 20.29 | 37.58 | 0(-)–0.28(L) | 0.05 | 0.10 | | | | 0(-)–6.02(L) | 1.18 | 2.30 |
| | Mesocarp | 0.71(P)–13.92(Pd) | 8.99 | 4.52 | 0.22(P)–18.37(Pd) | 10.13 | 6.49 | 14.60(P)–84.84(O) | 62.45 | 21.56 | 0.02(P)–1.75(M) | 0.51 | 0.67 | 0(-)–0.02(-) | 0.01 | 0.01 | 2.48(Pd)–7.76(L) | 3.98 | 2.25 |
| | Endocarp | 0.00(-)–28.84(O) | 5.82 | 9.57 | 0(H)–95.76(P) | 54.07 | 44.79 | 0(H)–99.06(O) | 33.17 | 41.41 | 0(-)–35.11(F) | 4.42 | 12.40 | 0(-)–34.54(F) | 7.44 | 11.63 | 2.34(P)–10.92(O) | 6.13 | 3.69 |
| | Kernel | 0.05(Pd)–21.64(A) | 4.78 | 7.54 | 13.81(Pd)–95.67(A) | 32.48 | 31.69 | 0.05(Pd)–25.04(O) | 5.09 | 8.33 | 0(-)–5.25(A) | 0.80 | 1.82 | 0.05(M)–7.47(A) | 1.36 | 2.50 | 0.34(Pd)–7.71(O) | 3.50 | 2.32 |
| | Fruit | 0.7(P)–12.77(A) | 7.42 | 3.94 | 0.3(P)–21.69(A) | 10.72 | 6.84 | 14.95(P)–84.20(O) | 53.13 | 23.39 | 0.02(P)–1.68(M) | 0.57 | 0.65 | 0.02(P)–0.62(Pd) | 0.20 | 0.23 | 1.96(M)–7.11(H) | 4.01 | 1.80 |
| PO | Ectocarp | 0(-)–87.73(P) | 20.08 | 32.60 | 0(-)–26.42(O) | 6.91 | 11.64 | 0(-)–77.61(L) | 22.92 | 33.66 | 0(-)–10.03(O) | 2.18 | 3.95 | | | | 0(-)–100(O) | 21.93 | 35.07 |
| | Mesocarp | 1.81(P)–46.01(M) | 16.38 | 17.61 | 0.87(P)–39.98(O) | 15.80 | 15.34 | 26.97(P)–78.25(H) | 59.45 | 15.72 | 0.09(Pd)–13.27(H) | 1.99 | 4.57 | 0(-)–0.02(M) | 0.00 | 0.01 | 0.33(O)–54.09(H) | 18.66 | 20.77 |
| | Endocarp | 0(-)–64.09(M) | 9.07 | 22.27 | 0(F)–99.74(L) | 34.60 | 40.89 | 0(F)–82.89(O) | 37.23 | 38.70 | 0(-)–14.28(M) | 2.80 | 5.31 | 0(-)–17.90(M) | 5.73 | 6.79 | 0(F)–67.02(A) | 25.59 | 29.27 |
| | Kernel | 1.07(-)35.26(L) | 13.54 | 14.54 | 1.72(F)40.86(L) | 21.10 | 13.11 | 31.98(P)–95.36(F) | 67.59 | 23.87 | 0(-)–1.06(F) | 0.20 | 0.39 | 0(Pd)–0.51(A) | 0.19 | 0.21 | 3.49(A)–11.82(H) | 8.40 | 3.01 |
| | Fruit | 1.17(Pd)–34.05(L) | 12.90 | 13.94 | 1.71(F)–39.66(L) | 18.90 | 13.05 | 28.25(P)–93.21(F) | 67.51 | 24.53 | 0(-)–1.00(F) | 0.20 | 0.36 | 0.01(O)–0.59(A) | 0.24 | 0.21 | 8.9(P)–13.72(O) | 10.03 | 2.80 |

Concentration range expressed as percentage

In parenthesis, the variety that provided the minimum and maximum values: *A* arbequina, *F* frantoio, *H* hojiblanca, *L* lechín from Sevilla, *M* manzanilla, *O* ocal, *P* picual, *Pd* picudo, (-) is used when several varieties provided the same result

*S* standard deviation, *X* average, *VO* extra virgin olive oil, *PO* pomace oil extracted with hot organic solvent

fatty acid–matrix interactions, which makes necessary different working conditions for oil extraction.

The concentration of polyunsaturated fatty acids was also higher in the case of the oil extracted by PO than in that by VO. A higher concentration of these fatty acids favours thermal degradation of oils as they can be oxidised easier than those with low content in polyunsaturated fatty acids. Once again aberquina and lechín were the varieties with a higher concentration of these fatty acids. It is worth noting that most of fatty acids present in the oil obtained from the endocarp by VO were polyunsaturated (Table 3). As the contribution of the endocarp to total oil is low (Table 2), this behaviour does not significantly affect the quality of the oil extracted by VO.

It is well known that one of the main characteristics of olive oil is the presence of a high amount of monounsaturated fatty acids, endowed with healthy properties; therefore, their presence is characteristic of high-quality oil. Monounsaturated fatty-acid concentration obtained by both extraction methods was quite similar—slightly higher in the case of PO, as shows Table 3. The composition of oils obtained from the whole fruit showed that ocal and frantoio were the varieties with oils with the highest concentration of monounsaturated fatty acids by VO and PO methods, respectively. Concerning tissues, mesocarp and kernel provided the oils with the highest concentration of monounsaturated fatty acids when obtained by the VO and PO methods, respectively.

The concentration of $\omega$6 and $\omega$3 fatty acids in olive oils is low as compared with that of other polyunsaturated fatty acids. The concentration of $\omega$3 in the oils obtained by both extraction methods was similar; that of $\omega$6 was higher in the oil obtained by VO than in that by PO.

Finally, the concentration of free fatty acids—high concentrations of which are related with low-quality oils—was higher in the oil obtained by PO than in that by VO. The oil extracted from whole hojiblanca and ocal fruits had the highest content of free fatty acids; meanwhile the tissue which makes the main contribution of these acids was endocarp in both cases—VO and PO methods. A special case was the ocal ectocarp, which provided oil with only free fatty acids when obtained by PO.

## Chemometric Study

Prior to development of chemometric models, detection of potential outliers is mandatory. Principal component analysis (PCA) was applied to the data matrix corresponding to the concentration of EFAs and that of NEFAs for each sample analysed to detect the presence of outliers, which were absent. Two sample groups, see Fig. 2, can be associated to the oil obtained by the VO and PO methods.

### *Influence of the Extraction Step*

The oils obtained from the whole fruit of the different varieties can be divided into two groups as a function of the extraction method. Figure 2 shows the oils obtained by the VO method grouped on the left side, and those obtained by the PO method on the right.

Five principal components were necessary to obtain an explained variance over 97% in all cases. The influential variables were EFAs—C18:1c n9, C18:1c n7, C18:2 t c.

### *Influence of the Fruit Variety on the Oil Obtained from the Different Tissues*

Eight models based on PCA were developed—one for each extraction method and tissue (Fig. 3a–d for VO and Fig. 3e–h for PO, submitted as electronic information).

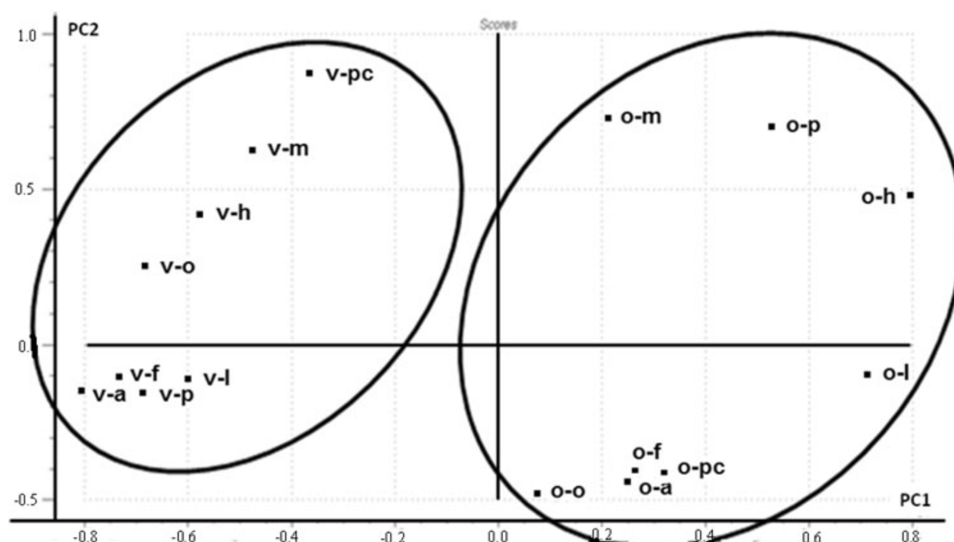


**Fig. 2** Samples in the space determined by the first two principal components (*v* extra virgin olive oil, *o* pomace oil, *m* manzanilla, *pc* picudo, *h* hojiblanca, *o* ocal, *f* frantoio, *a* arbequina, *p* picual, and *l* lechín from Sevilla)

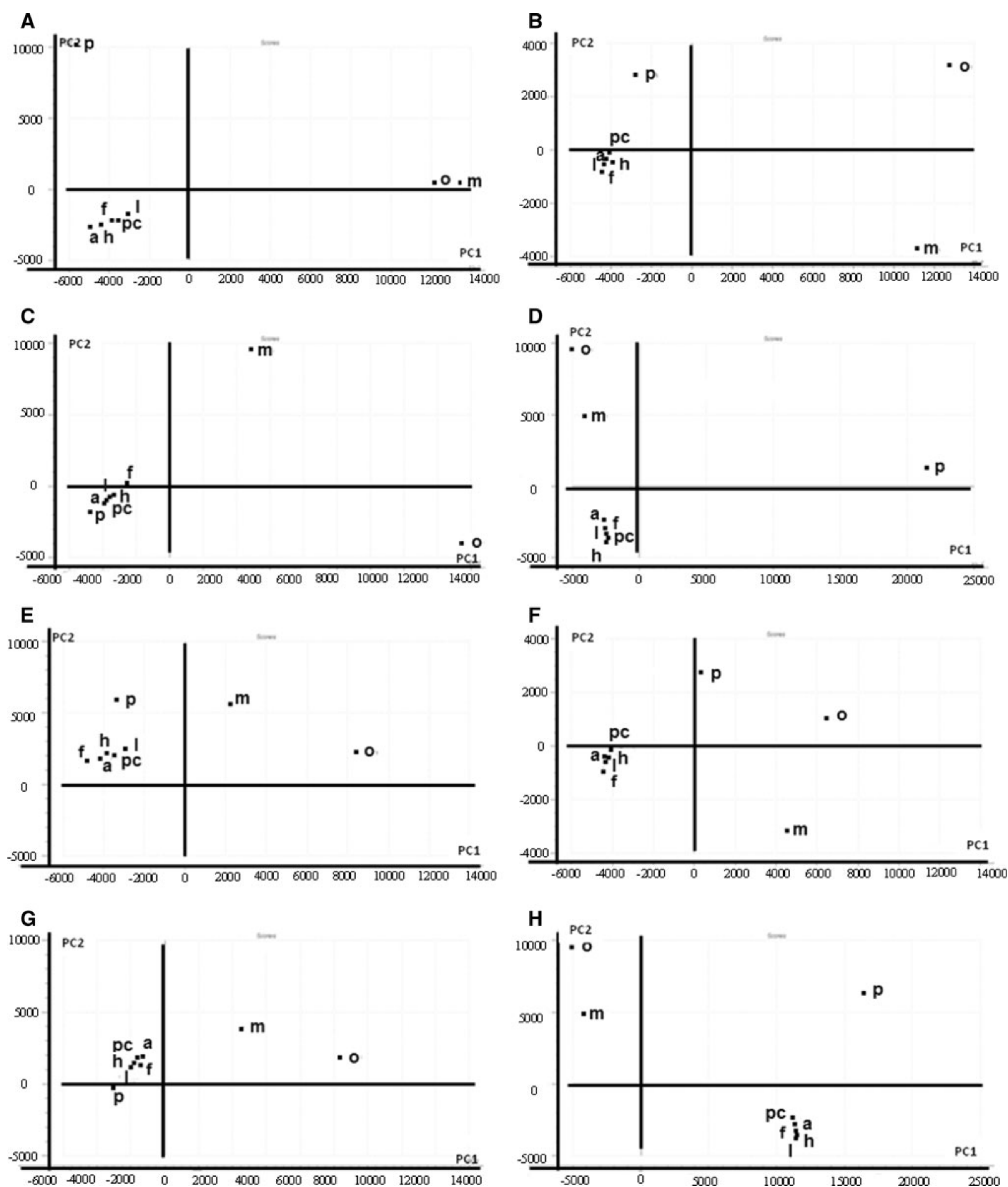


**Fig. 3** **a–d** PCA diagram of the influence of the fruit variety on the oil provided by the VO method from the different tissues (**a** ectocarp, **b** mesocarp, **c** endocarp, **d** kernel). **e–j** PCA diagram of the influence of the fruit variety on the oil provided by the PO method from the different tissues (**e** ectocarp, **f** mesocarp, **g** endocarp, **h** kernel). Abbreviations of varieties as in legend of Fig. 2

As can be seen in this figure, the results obtained were similar for both extraction methods and tissues, the oil obtained from one given tissue is similar for all the varieties with the exception of that obtained from manzanilla, ocal and picual, which also differed among themselves. Figure 3 shows the similitude between the oils obtained by the two extraction

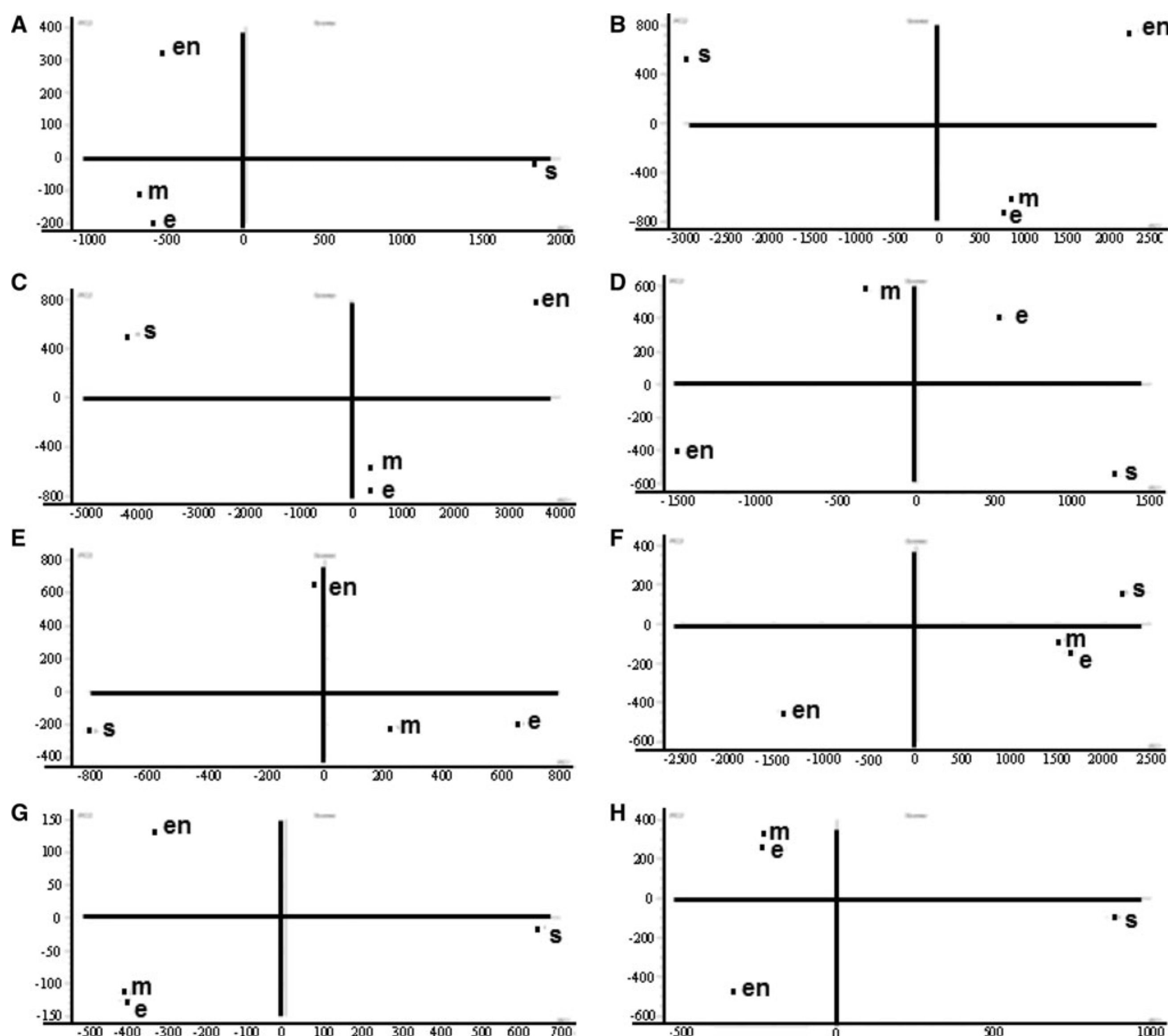


**Fig. 4** **a**–**h** PCA diagram of the influence of the tissue on the oil obtained by the VO method from each variety (**a** arbequine, **b** frantoio, **c** hojiblanca, **d** lechín from Sevilla, **e** manzanilla, **f** ocal, **g** picudo, **h** picual). **i**–**p** PCA diagram of the influence of the tissue on the oil obtained by the PO method from each variety (**i** arbequine, **j** frantoio, **k** hojiblanca, **l** lechín from Sevilla, **m** manzanilla, **n** ocal, **o** picudo, **p** picual). Abbreviations of tissues: *e* ectocarp, *m* mesocarp, *en* endocarp, *s* kernel

procedures and also between each tissue of the different varieties, with the exception of manzanilla, ocal and picual.

Three principal components were sufficient to obtain an explained variance over 95% in all cases. The influential variables were the same as commented under "influence of the extraction step". In addition, C12:0 was also influential when obtained from the ectocarp.

### *Influence of the Tissue on the Oil Obtained from Each Variety*

Sixteen PCA models were developed—one for each extraction method and variety (Fig. 4a–h for VO and Fig. 4i–p for PO, submitted as electronic information). The results were similar within a method and within a variety. The oils obtained from the ectocarp and mesocarp by VO were also similar and they showed small differences when extracted by PO. These results corroborate the theory proposed under "Efficiency of the extraction methods and total oil content of each tissue and the whole fruit": the oil obtained from the ectocarp by the VO method can come from rests of mesocarp non removed in sample preparation.

In addition, there are differences in the oil obtained from the endocarp and kernel. Therefore, the theory proposed under "Efficiency of the extraction methods and total oil

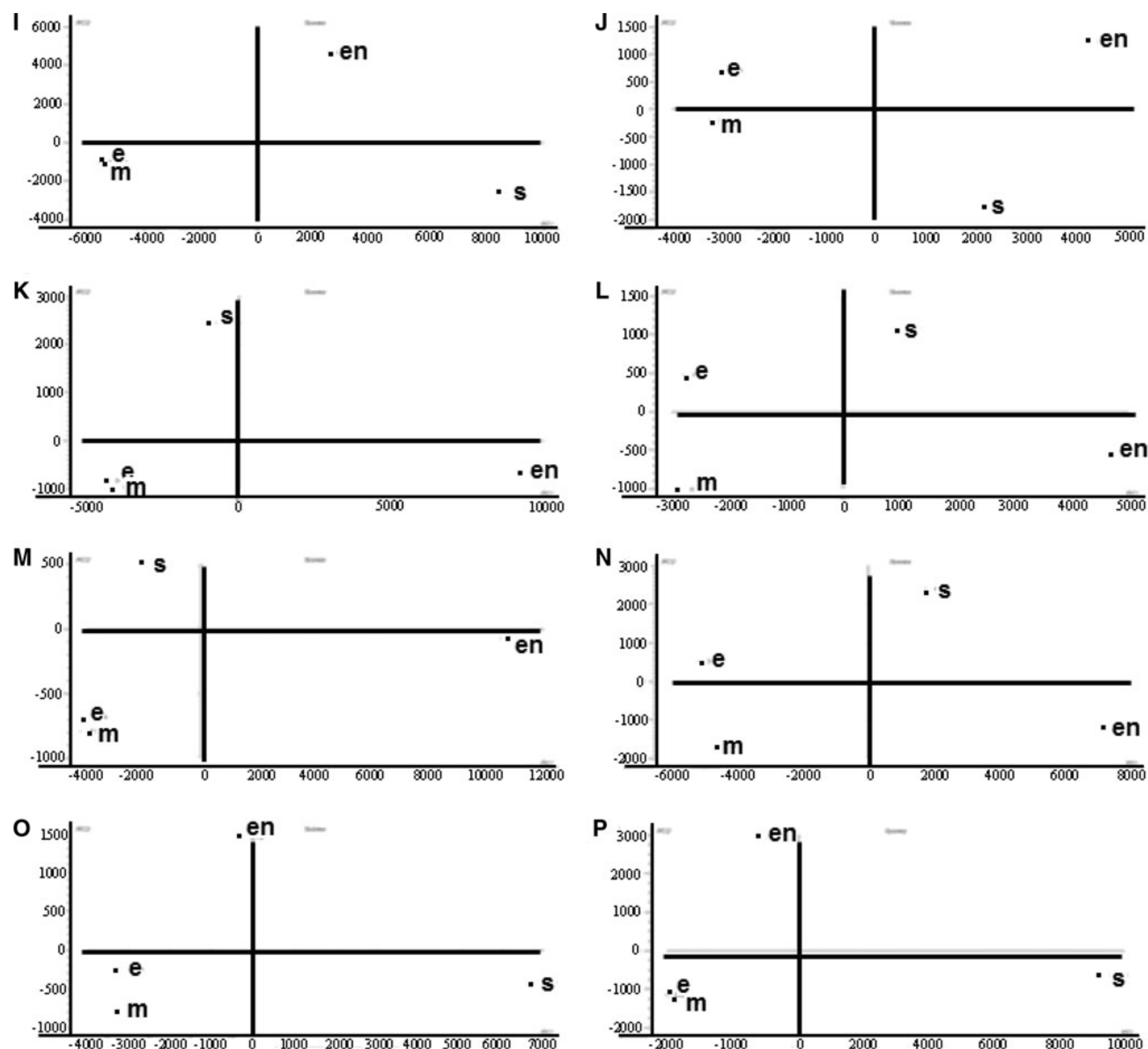


**Fig. 4** continued

content of each tissue and the whole fruit” is not acceptable and the oil obtained from the endocarp has no contribution from the kernel.

## Conclusions

The efficiency of extraction from the oils is a function of the variety and extraction method, but the oil obtained from a tissue from different varieties is similar in most cases (only manzanilla, ocal and picual varieties behave differently). The oil obtained by different extraction methods has different fatty-acid composition; thus, the oil obtained by the PO method has lower fatty-acid quality than that from VO. Mesocarp and kernel are the tissues with a higher contribution to the oil obtained by VO and PO, respectively.

The contribution of the ectocarp to the VO oil can be linked to the presence of a residue of mesocarp not removed in sample preparation.

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