Accepted Manuscript

Waxy fraction containing long-chain aliphatic aldehydes in virgin olive oils

M.C. Pérez-Camino, R.B. Gómez-Coca, W. Moreda

PII: S0308-8146(11)01743-2
DOI: [10.1016/j.foodchem.2011.12.001](https://doi.org/10.1016/j.foodchem.2011.12.001)
Reference: FOCH 11857

To appear in: *Food Chemistry*

Received Date: 26 September 2011
Revised Date: 8 November 2011
Accepted Date: 2 December 2011

Please cite this article as: Pérez-Camino, M.C., Gómez-Coca, R.B., Moreda, W., Waxy fraction containing long-chain aliphatic aldehydes in virgin olive oils, *Food Chemistry* (2011), doi: [10.1016/j.foodchem.2011.12.001](https://doi.org/10.1016/j.foodchem.2011.12.001)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

1 **Waxy fraction containing long-chain aliphatic aldehydes in virgin olive oils**

2 M.C. Pérez-Camino*, R.B. Gómez-Coca and W. Moreda

3 Food Quality and Characterization Department. Instituto de la Grasa. Consejo Superior de
4 Investigaciones Científicas (CSIC). Avda. Padre García Tejero 4, 41012 Sevilla, Spain

5 Phone: +34-95-461-1550; Fax: +34-95-461-6790

6 *E-mail address: mcperezcamino@ig.csic.es

7

ABSTRACT

Long-chain aliphatic aldehydes are natural minor components occurring in the cuticle of numerous plant species and also evidenced in virgin olive oils. The fraction containing these compounds can be isolated from the oil samples by using a solid-phase extraction silica-gel cartridge and then directly analysed by GC on a 5% diphenyl–95% dimethylsiloxane capillary column, using an on column-injection system. The proposed methodology showed that extra virgin olive oils contain long-chain aliphatic aldehydes, with even carbon-atom numbers from C_{22} to C_{30} . Quantitative results, using the synthesised aldehyde C_{21} as internal standard, give concentrations of total long-chain aliphatic aldehydes in a variable range below 116 mg kg^{-1} , being hexacosanal ($C_{26\text{-al}}$) the most abundant aldehyde. The different experimental conditions utilized during olive oil extraction processes influence the total aldehydes concentration. Besides contribution to the knowledge of the minor-component composition present in olive oil, their interest and relationship with wax esters, aliphatic alcohols and *n*-alkanes are discussed.

Keywords: Aldehydes, aliphatic alcohols, GC-analysis, *n*-alkanes, olive oil, wax esters.

1. Introduction

Saturated and lineal aldehydes with 22 to 30 carbon atoms (C₂₂-al - C₃₀-al), also named long-chain aliphatic aldehydes (LCAAs), are a family of compounds almost never described or mentioned when minor components of edible oils are studied, in spite of having been found long time ago as naturally occurring compounds in virgin olive oil (VOO) (Pérez-Camino Moreda, Mateos & Cert, 2003). LCAAs have been described to be present in various parts of higher plants (Avato, Bianchi & Mariani, 1984), like cuticles of many species, (Nass, Markstädter, Hauke & Riedere, 1998), tobacco leaves (Kawasaki, Matsui, Akakabe, Itai & Kajiwara, 1998), waxy components of some dicotyledonous families (Tulloch, 1987), and aromatic plants. They have been found in the waxy material covering the surface of olive fruits too (Bianchi, 1987) and they were mentioned as components of distillates obtained during the oils refining process (Lanzani, Bondioli, Allievi, Baretteau & Bertini, 1988). In addition, Guillén and Manzanos (1998) reported quantitative results on the dichloromethane extracts of leaves, flowers and stems of *Thymus vulgaris*, where aldehydes with large carbon-atom number were included. Finally, Nass and co-workers (Nass, Markstädter, Hauke & Riedere, 1998) developed a comprehensive methodology to determine LCAAs from stems and leaves of *Euphorbia antisiphilitica* Zucc and *European beech*, assaying and selecting conditions for better and more reproducible quantitative determinations.

In relation to their origin, presence and functions, it has been suggested that they are intermediates in the biosynthesis of surface lipids or epicuticular waxes where very long-chain fatty acids (LCFAs) are converted into n-alkanes, alcohols and wax esters. These compounds form part of the fruit cuticle and have an active function as barrier, preventing excessive water loss and the entry of harmful substances and pathogens (Reina-Pinto & Yephremov, 2009).

Supporting the above expressed idea, different authors have reported that aldehydes are not simple intermediates in the pathway to other significant minor compounds. Thus,

Kubo and co-workers (Kubo, Lunde & Kubo, 1995) tested the important role of medium-chain aldehydes, considering from hexanal to 2,4-decadienal, against microorganisms and proved that increasing the length of the carbon tail seemed to result in an increased activity. Also, Battinelli and co-workers (Battinelli et al., 2006) demonstrated the antifungal action of aliphatic aldehydes from hexanal to 2-nonenal. These authors suggested that aldehydes coming from olive extracts could be useful agents in the topical treatment of fungal cutaneous infections acting in synergy with other components of olive oils, mainly oleuropein, secoroid derivatives, and hydroxytyrosol, potentiating their activities. In the case of leaf epicuticular waxes of wheat plants Reisinger and co-workers (Reisinger, Gorzelanny, Daniels & Moerschbacher, 2006) found octacosanal, a C28-aldehyde, to be the most active component.

Focusing on the LCAAs presence in oils, an analytical methodology for their determination in olive oils has been developed, which included isolation of the corresponding waxy fraction by SPE and quantification by GC on a polar stationary phase using on-column injection (Pérez-Camino et al., 2003). The assay conditions allowed the obtaining of a chromatogram, where the peaks corresponding to LCAAs eluted before those corresponding to lineal waxes and could be quantified by using C21-al as internal standard. In respect to other results or references on LCAAs presence in edible oils, we are not aware if they have been reported so far. The present work focuses on the study of the waxy fraction of olive oils containing aldehydes, aliphatic hydrocarbons and wax esters, and also on the study of total aliphatic alcohols. In addition, their relationship and the influence of the oil obtaining procedure are discussed with the aim of contributing to both olive oil characterization and alternative way to detect admixtures of VOO with seed oils. Even more, we are confident that their study and knowledge will contribute, as other minor compounds from olive oils do (Montealegre, Marina Alegre & García-Ruiz, 2010), to be considered as traceability markers.

2. Materials and methods

2.1. Raw material

Two batches of VOO samples were prepared.

The first one (set 1) consisted of Arbequina extra VOOs from Seville. It was obtained from healthy fruits in a lab olive oil mill through the Abencor® analyser method. Eight different oil extraction processes were assayed varying the mixing time of the olive paste (10, 30, 60 and 90 min) and also the mesh size of the grids in the hammer crusher (5 and 6 mm). In all cases the rotation speed in the hammer crusher was 3000 rpm, the temperature was 25 °C and the malaxation time was established in 20 min plus 10 min after water addition. Finally the olive paste was centrifuged at 3500 rpm and the oil separated by decantation. The procedure was repeated three times.

The second set (set 2) included 25 extra VOO samples obtained from non-controlled varieties obtained from different local industries.

In every case mature fruits with a ripeness index higher than 5 according to Uceda & Frias (1975) were processed.

2.2. Reagents and solutions

All the reagents were of analytical grade otherwise specified. Pyridium chlorochromate, heneicosanol, 1-triacontanol, 1-docosanol, 1-hexacosanol, arachidyl laurate, and cholesteryl stearate, were purchased from Aldrich (Steinheim, Germany). The SPE cartridge (6 ml) packed with silica (1000 mg) was purchased from Varian (Middelburg, The Netherlands).

Standard solutions. Solutions of heneicosanal (C₂₁-al), eicosane (n-C₂₀) and arachidyl laurate

(C₃₂-W) in hexane were used as internal standards for the quantitative determination of aldehydes, hydrocarbons and wax esters, respectively. A solution of n-eicosanol (C_{20:0}OH) was used for quantitative determination of aliphatic alcohols.

2.3. Aldehyde synthesis

Long chain aldehydes (C₂₁-al to C₃₀-al) were synthesized starting from their corresponding alcohols since they were not available in the market. The synthesis were carried out by oxidation of the corresponding alcohols using pyridinium chlorochromate and later purification by silica-gel column chromatography, as described previously (Nass et al., 1998, Pérez-Camino et al., 2003).

2.4. Isolation of the waxy fraction from VOO by solid-phase extraction

The isolation procedure by silica SPE cartridge was previously established (Pérez-Camino et al., 2003), although modifications regarding the sample size and the first eluent were accomplished. Thus, the Si-SPE cartridge (1000 mg) was placed in a vacuum elution apparatus and conditioned with 6 ml hexane. The oil sample (100 mg) containing the standards (50 µl of 0.2 mg ml⁻¹ C₃₂-W and 0.1 mg ml⁻¹ C₂₁-al solutions) was dissolved in 1 ml hexane. The solution was then applied to the column and the solvent pulled through, leaving the sample and the standards on top of the sorbent. The sample container was washed with two 0.5-ml portions hexane, next, the column was eluted with 7 ml n-hexane, collecting this fraction. Then it is pass through 7 ml of an hexane:toluene (85:15, v/v) blend in order to eliminate the majority of squalene and this fraction was discarded. Finally, the column was eluted with 10 ml of the same admixture keeping this fraction. The first and third eluates were evaporated to dryness in a rotary evaporator, at room temperature, under vacuum. The residues were redissolved in 300 µl heptane and a 1-µl aliquot was directly injected into the gas chromatograph, as none of the compounds needed further derivatization to be determined.

2.5. Lineal alcohols determination

Lineal alcohol determination required the preparation of the unsaponifiable matter, its fractionation by TLC and derivatization before GC. Briefly: 1 g of the oil sample containing $C_{20}OH$ as internal standard (0.2 mg ml^{-1}) was saponified during 1 hour using KOH in ethanol. Once the unsaponifiable matter was isolated by liquid-liquid extraction it was taken through TLC and the corresponding band scraped. Next, it was derivatized (silylated) and injected onto the GC column (IUPAC, 1992).

2.6. Gas chromatography analysis

Chromatographic analysis was performed using an Agilent 6890 gas chromatograph fitted with flame ionization detection and on-column injection system. Separations were carried out on a SPB-5 capillary column (12 m x 0.25 mm I.D.), coated with 5%-diphenyl–95%-dimethylsiloxane (Cert, Moreda & Pérez-Camino, 2002; EEC, 2011). For hydrocarbon separation and quantification the following chromatographic conditions were used: Oven temperature: 50 °C for 0.2 min and then increased at 20 °C/min up to 160 °C; then increased at 4 °C/min up to 350 °C. Injector and detector temperatures were 50 °C and 365 °C, respectively. Hydrogen was used as carrier gas at flow-rate of 3.5 ml min^{-1} . Aldehydes and wax esters were determined also by on-column gas chromatography using the same chromatographic run and conditions, as those described above for hydrocarbons and with slight differences as previously reported by Pérez-Camino et al. 2003), and by the recent European normative on the simultaneously determination of waxes and alkyl esters (EEC, 2011)

For aliphatic alcohol determination a SPB-5 capillary column (25 m x 0.25i.d.) coated with 5% diphenyl–95% dimethylsiloxane was used, the oven temperature was set from 230 °C to

290 °C at 2.5 °C/min, and the injector and detector temperatures were 300 °C and 325 °C, respectively (EEC, 1991).

Data acquisition and processing were carried out using a HP Chemstation.

2.7. Quantitative analysis and statistical treatment

In the case of set 1 the experiences were carried out in triplicate and results were expressed as the mean values $\pm$ standard deviation of the three replicates. In the case of set 2 results are expressed as the mean values $\pm$ standard deviation of three determinations. The data were statistically analyzed by one-way analysis of variance (ANOVA). Means were compared by Scheffe's test; significance was accepted at the 5% level ($P \leq 0.05$).

3. Results and discussion

The developed analytical procedure allowed the collection of fractions containing oil minor compounds with increasing (but lower than triglycerides) polarities. The first eluate out of the three collected from the SPE procedure corresponded to the least polar fraction present in the olive oil samples and contained the aliphatic hydrocarbons. The second fraction encompassed the unsaturated hydrocarbons and contained the majority of the existent squalene, therefore it was important to eliminate it since it interfered on the determination of the components included in the third eluate. The third fraction contained aldehydes, wax esters and fatty acid alkyl esters. Since this study is focused on n-alkanes, aldehydes and wax esters, only the first and third fractions were taken into account.

Figure 1 shows a representative part of the gas chromatogram corresponding to the third fraction isolated from VOO and comprised the C₂₂-al to C₃₀-al LCAs (peaks 2, 3, 4, and 6), and the waxes: C₃₂-W to C₃₆-W (group of peaks 9) and the lineal ones, C₄₀-W to C₄₆-W (group of peaks 10).

Regarding the influence of the oil extraction conditions, all samples from the Arbequina olive oil set (set 1) showed the same LCAAs profile independently of the mixing time or the mesh size of the grids, as can be observed from the data reported in Table 1. However, extraction yields depended on both malaxation time and hole size of the crusher in a way that the longer the malaxation time, the higher the yield and the lower the fatty aldehydes content. These results made us envisage the great variability and quantities of aldehydes that could be found in oils from different varieties and collection systems, as the olive oil extraction procedures change a big deal from one factory to another. Table 2 includes the LCAAs distribution corresponding to set 2, which consisted of 25 different extra virgin olive oils collected directly from local industries. These oils are usually mixtures of a number of virgin olive oils from different varieties. Surprisingly, data from set 1 and set 2 do not differ significantly from each other. The main fatty aldehydes in all the olive oils studied are the even series from docosanal to triacontanal (C_{22} -al - C_{30} -al). Table 2 shows values ranging from 23.3 to 81.7 mg kg⁻¹, which are in agreement with the information enclosed in Table 1. In both sets there is a clear predominance of the aldehyde hexacosanal (C_{26} -al) followed by octacosanal (C_{28} -al) and tetracosanal (C_{24} -al). In some of the samples included in Table 2 the C_{22} -al aldehyde is not detected (VOO9). This matches with the data presented in Table 1, where the C_{22} -al aldehyde does not appear. Few other samples do not show the C_{30} -al aldehyde, which cannot be related to the variety or the maturation grade as all of the oils were obtained from olive fruits with a high maturity level.

Although it is not the scope of this study, fruit growth and development seem to influence slightly the LCAAs content as was reported by Kawasaki et al. (1998). These authors concluded that LCAAs presence in tobacco leaves increased as chlorophyll content decreased from the beginning of the planting to flowering time. The opposite trend was observed in the case of some VOO minor components, such as sterols (Salvador, Aranda &

Fregapane, 2001), tocopherols, phenols and major volatile compounds (Fregapane, Gómez-Rico & Salvador, 2010), whose content diminished slightly during ripening. However, regarding volatile compounds, contradictory results have been published (Angerosa, Mostallino, Basti & Vito, 2001).

Table 3 shows total quantities of aldehydes, aliphatic hydrocarbons, lineal waxes and total lineal alcohols from the Arbequina olive oil set of samples (set 1). These results prove that when the mixing time increases there is also an increment in the total fatty aldehydes quantity, which is in accordance with the tendency observed previously with individual quantities. However, the corresponding concentrations of total aliphatic hydrocarbons, lineal waxes or lineal alcohols are not affected. This is in agreement with the analogous data shown in Table 4 for oils coming from unknown varieties (set 2), where correlations between aldehydes and hydrocarbons ($r^2 = 0.09$), waxes ($r^2 = 0.05$) or alcohols ($r^2 = 0.21$) could be calculated.

Figure 2 displays the distribution values of hydrocarbons, aldehydes and lineal aliphatic alcohols for all the analysed samples. The most abundant hydrocarbons were those belonging to the odd series with predominance of 23 to 31 carbon-atom compounds, mainly C25-, C27-, and C29- hydrocarbons. Total quantities range from 70.5 to 100.6 mg kg⁻¹. Both quantity and distribution values are in agreement with published data from where their relationship with the geographical origin and the olive varieties are also established (Guinda, Lanzón & Albi, 1996; Koprivnjak, Moret, Populin, Lagazio & Conte, 2005; Moreda, Pérez-Camino & Cert, 2001). In the case of lineal aliphatic alcohols, there is a predominance of the even series with 22 to 30 carbon-atom compounds, mainly C24-, C26, and C-28 alcohols, with abundance of hexadocosanol (C₂₆-OH). Although there is not an Official Regulation on the limits for total quantities of aliphatic alcohols in VOO, data here obtained are in accordance with those published for VOO of extra category (Rivera del Álamo, Fregapane,

Aranda, Gómez-Alonso & Salvador, 2004; Salvador & Fregapane, 2010). Finally, alcohol distribution matches with that of aliphatic aldehydes where also the 26 carbon-atom counterpart is the most abundant one.

LCAs are reported to come from LCFAs with even carbon number which suffer an α -oxidation and are firstly transformed into n-aldehydes with one carbon-atom less; subsequently there is a decarboxilation process to convert the n-aldehydes into their corresponding hydrocarbons (Iyer et al., 1998; Riendeau & Meighen, 1985; Galliard & Matthew, 1976). This information fits with some of our findings, as the majority of hydrocarbons are those with 25 carbon atoms, which could come from the abundant C26-aldehyde. Besides, the C26-aliphatic hydrocarbon is also the major component in the case of olive oils coming from some specific olive varieties such as *Empeltre* (Guinda et al. 1996), *Buza* or *Leccino* (Koprivnjak et al., 2005). The well known metabolic pathway that converts LCAs into aliphatic hydrocarbons and the number of papers describing hydrocarbons, compared with the absence of those where LCAs are determined induces to think of the aldehydes as very labile and/or active compounds, and disagrees with the fact that there are not chemical reactions to transform aldehydes into hydrocarbons. Nevertheless, the presence of fatty aldehydes in marine sediments (Sánchez-García, de Andres, Martin-Rubi, González-Vila & Polvillo, 2008) demonstrates the opposite; LCAs should be considered as very stable compounds. In higher plants, the biological process where the aldehydes are implicated is explained by the presence in the plant cuticular waxes of a membrane-bound decarbonylase enzyme. This enzyme, categorized as long-chain aldehyde dehydrogenase, catalyses the decarboxilation of n-aldehydes yielding alkanes and CO (Ishige, Tani, Sakai & Kato, 2000). On the other hand, the presence of a long-chain aldehyde dehydrogenase that transforms the aldehyde into a fatty acid and this into R-CoA has been demonstrated. At the same time, it has been proven the existence of an aldehyde reductase that transforms aldehydes into alcohols.

Alcohols esterify to R-CoA and subsequently are transformed into wax esters by the action of an acyl-CoA:alcohol transacylase (Ishige et al., 2000).

4. Conclusions

There is an undoubted absence of quantitative correlation among the compounds encompassed in the waxy fraction of VOO (aldehydes, hydrocarbons, waxes and alcohols), pointing out that the complexity of their relationships extends far beyond our current understanding. Nevertheless, this non-correlation does not exclude a precursor-product relationship, neither it invalidates the mechanism above described according to which LCFAs

Apart from being an integral part of the waxy fraction and from its role as a protective agent against pathogens, we move forward to propose a new role that aldehydes could play in the field of olive oil characterization, as has been previously reported for other minor unsaponifiable compounds, such as sterols or hydrocarbons (Montealegre et al., 2010, Moreda, Pérez-Camino & Cert 2004). Besides, LCAAs could contribute to the studies on traceability of olive oils as they have been proven to be very stable in adverse conditions (Sánchez-García et al., 2008). Finally, since LCAAs figures in VOO moves within a narrow range, LCAAs mere presence and quantification could be used to detect blends since the have not been found in refined seed edible oils.

Acknowledgement

This study was funded by the Spanish Ministry of Science and Innovation within the framework of Project AGL2009-07618. The authors would like to thank Mrs R. González Cordones and Mr M. Rodríguez Aguilar for technical assistance.

References

- Angerosa, F., Mostallino, R., Basti, C., & Vito, R., (2001). Influence of malaxation temperature and time on the quality of virgin olive oils. *Food Chemistry*, 72, 17-28.
- Avato, P, Bianchi, G., & Mariani, G., (1984). Epicuticular waxes of Sorghum and some compositional changes with plant age. *Phytochemistry*, 23, 2843-2846.
- Bianchi, G., (1987). Chemical genetics of plant surface lipid biothynthesis. *Gazzetta Chimica Italiana*. 117, 707-716.
- Battinelli, L., Daniele, C. Cristiani, M., Bisignano, G., Saija, A. and Mazzanti, G. (2006). In vitro antifungal and anti-elastase activity of some aliphatic aldehydes from *Olea europaea* L. fruit. *Phytomedicine*. 13, 558-563
- Cert, A., Moreda, W. & Pérez-Camino, M.C. (2002). Chromatographic analysis of minor constituents in vegetable oils. *Journal of Chromatography A*. 881, 131-148.
- European Union Commission (2011). Regulation EEU/61/2011. Modification to the Regulation EEC/2568/91 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. *Official Journal of Eurpean Community*. L 23, 1-14.
- European Union Commission (1991). Regulation EEC/2568/91 on the characteristics of olive oil and olive residue oil and on the relevant methods of analysis. *Official Journal of European Community*, L248, 1-83.
- Fregapane, G., Gómez-Rico, A., & Salvador, M.D. (2010). Influence of the irrigation management and ripening on virgin olive oil quality and composition. In *Olives and olive oil in health and disease prevention*. (pp 239-247) Elsevier Inc.
- Galliard, T. & Matthew, J.A. (1976). The enzymic formation of long chain aldehydes and alcohols by –oxidation of fatty acids in extracts of cucumber fruit (*Cucumis sativus*). *Biochimica et Biophysica Acta*, 424, 26-35.
- Guillén, M.D. Manzanos, M.J (1998). Sudy of the composition of the diferent parts of a Spanish *Thymus vulgaris* L. plant. *Food Chemistry*, 63, 373-383
- Guinda, A., Lanzón A. & Albi T. (1996). Differences in hydrocarbons of virgin olive oils obtained from several olive varieties. *Journal of Agricultural and Food Chemistry*, 44, 1723-1726
- IUPAC. (1992). Determination of the unsaponifiable matter. Method 2401. In Paquot, A Hautfenne (Eds.) *IUPAC Standard Method for the Analysis of Oils, Fats and Derivatives*, 7th ed, Oxford: Blackwell

- 1 Ishige, T., Tani, A., Sakai Y. & Kato N. (2000). Long-chain aldehydes dehydrogenase that
2 participates in n-alkane utilization and wax esters synthesis in *Acinetobacter* strain M-
3 1. *Applied and Environmental Microbiology*, 66, 3481-3486
- 4 Iyer, S., Millar, T., Clems, S., Zachgo, S., Giblin, M., Taylor, D. & Kunst, L. (1998)
5 in: J. Sánchez, E. Cerdá-Olmedo, E. Martínez- Force (Eds.) *Advances in Plant Lipid*
6 *Research*, (p. 87) Sevilla: Publicaciones U. de Sevilla.
- 7 Kawasaki, W., Matsui, K., Akakabe, Y., Itai, N., & Kajiwarra, T. (1998). Long-chain
8 aldehyde-forming activity in tobacco leaves. *Phytochemistry*, 49, 1565-1568
- 9 Koprivnjak, O., Moret, S., Populin T., Lagazio C., & Conte, L.S. (2005). Variety
10 differentiation of virgin olive oil based on n-alkane profile. *Food Chemistry*, 90, 603-
11 608
- 12 Kubo, A., Lunde, C.S. & Kubo, I. (1995). Antimicrobial activity of the oil flavour
13 compounds. *Journal of Agricultural and Food Chemistry*, 43, 1629-1633
- 14 Lanzani, A., Bondioli, P., Allievi, C., Baretteau, P., Bertini G.C. (1988). Recovery
15 Technologies of components of practical and economical interest from condensation
16 products of soybean oil industrial deodorization. *Rivista Italiana delle Sostanze*
17 *Grasse*, 65, 607-611.
- 18 Montealegre C, Marina Alegre, M.L. and García-Ruiz, C. (2010). Traceability markers to the
19 botanical origin in olive oils. *Journal of Agricultural and Food Chemistry*. 58, 28-38
- 20 Moreda, W., Pérez-Camino, M.C., & Cert A. (2001). Gas and liquid chromatography of
21 hydrocarbons in edible vegetable oils. *Journal of Chromatography A*, 936, 159-171
- 22 Moreda, W., Pérez-Camino, M.C. & Cert, A. (2004). Analysis of Neutral Lipids:
23 Unsaponifiable Matter. In Nollet LML (Eds.) *Handbook of Food Analysis*, 2nd
24 Edition. vol 1. (pp 313–347) New York: Marcel Dekker, Inc
- 25 Nass R, Markstädter, C. Hauke, V. Riedere M, 1998 Quantitative gas chromatographic
26 analysis of plant cuticular waxes containing long-chain aldehydes. *Phytochemical*.
27 *Analysis*, 9, 112-118
- 28 Pérez-Camino, M.C., Moreda, W., Mateos, R., & Cert, A. (2003). Simultaneous
29 determination of long-chain aliphatic aldehydes and waxes in olive oils. *Journal*
30 *Chromatography A*, 983, 283-288
- 31 Reina-Pinto, J.J. & Yephremov, A. (2009) Surface lipids and plant defenses. *Plant*
32 *Physiology and Biochemistry*, 47, 540-549.
- 33 Reisinger K., Gorzelanny Ch., Daniels U. and Moerschbacher B. M. (2006). The C28
34 aldehyde octacosanal is a morphogenetically active component involved in host

- 1 plant recognition and infection structure differentiation in the wheat stem rust
- 2 fungus. *Physiology and Molecular Plant Pathology*, 68, 33–40.
- 3 Riendeau D, Meighen E. (1985). Enzymatic reduction of fatty acids and acyl-CoAs to long
- 4 chain aldehydes and alcohols. *Experientia*. 41, 707–713.
- 5 Rivera del Álamo, R.M., Fregapane, G., Aranda F., Gómez-Alonso S. and Salvador M.D.
- 6 (2004) Sterol and alcohol composition of Cornicabra virgin olive oil: the campesterol
- 7 content exceeds the upper limit of 4% established by EU regulations. *Food*
- 8 *Chemistry*, 84, 533-537.
- 9 Salvador, M.D, Aranda, F. & Fregapane, G. (2001). Influence of fruit ripening on
- 10 “Cornicabra” virgin olive oil quality. A study of four successive crop seasons. *Food*
- 11 *Chemistry*, 73, 45-53.
- 12 Salvador, M.D., and Fregapane, G. (2010). Major and minor lipid constituents of cornicabra
- 13 virgin olive oil and the influence of crop season changes. In *Olives and olive oil in*
- 14 *health and disease prevention*. (pp 51-58) Elsevier Inc.
- 15 Sánchez-García L, de Andres JR, Martin-Rubi JA, Gonzalez-Vila FJ, and Polvillo, O. (2008).
- 16 Use of lipid biomarker patterns as a proxy of environmental variability in the coastal
- 17 sedimentary record from the Gulf of Cadiz (SW Spain). *Organic Geochemistry*. 39,
- 18 958-964.
- 19 Tulloch, A.P. (1987). Epicuticular waxes of abies balsame and picea glauca: Occurrence of
- 20 long-chain methyl esters. *Phytochemistry*, 26, 1041-1043.
- 21 Uceda, M., Frias, L. (1975). Harvest dates. Evolution of the fruit oil content, oil composition
- 22 and oil quality. *Proceeding of II Seminario Oleícola Internacional*, pp. 125-130.
- 23 International Olive Oil Council: Cordoba, Spain

Figure 1. GC fraction from virgin olive oil collected by Si-SPE, with polarity between the aliphatic hydrocarbons and the triglycerides. 1: Internal standard (C21-al), 2: C22-al, 3: C24-al, 4: C26-al, 5: Squalene, 6: C28-al, 7: C30-al, 8: Internal Standard (CW32), 9: C32-W to C36-W waxes, 10: Lineal waxes C40-W to C46-W.

Table 1. Quantitative determination (mg/kg) of Long Chain Aliphatic Aldehydes in Arbequina olive oils obtained by different extraction conditions.

Mesh size	Beating Time (min)	Long Chain Aliphatic Aldehydes (mg/kg)				
		C ₂₂ -al	C ₂₄ -al	C ₂₆ -al	C ₂₈ -al	C ₃₀ -al
5 mm	10	n.d.	10.0 ± 0.2	56.1 ± 1.0	32.0 ± 0.7	7.5 ± 0.2
	30	n.d.	11.5 ± 0.3	52.2 ± 1.1	38.4 ± 0.8	5.9 ± 0.1
	60	n.d.	8.2 ± 0.1	57.2 ± 1.1	32.1 ± 0.7	10.2 ± 0.3
	90	n.d.	9.7 ± 0.2	74.8 ± 1.5	26.5 ± 0.6	5.0 ± 0.1
6 mm	10	n.d.	9.8 ± 0.2	43.9 ± 1.0	21.9 ± 0.4	5.7 ± 0.1
	30	n.d.	9.6 ± 0.1	52.3 ± 1.1	19.9 ± 0.3	5.0 ± 0.1
	60	n.d.	7.9 ± 0.3	47.1 ± 0.9	27.8 ± 0.5	7.0 ± 0.1
	90	n.d.	11.8 ± 0.5	58.1 ± 1.3	31.3 ± 0.6	6.5 ± 0.1

Data are the mean value of three replicates. n.d.: not detected

Table 2. Long Chain Aliphatic Aldehydes content of different virgin olive oil mixtures (unspecified varieties) obtained from local factories.

Oil sample	Long Chain Aliphatic Aldehydes (mg/kg)				
	C ₂₂ -al	C ₂₄ -al	C ₂₆ -al	C ₂₈ -al	C ₃₀ -al
VOO-1	1.6 ± 0.1	3.8 ± 0.1	21.4 ± 0.5	10.5 ± 0.2	2.3 ± 0.1
VOO-2	2.8 ± 0.2	6.9 ± 0.3	32.8 ± 0.8	20.0 ± 0.5	2.9 ± 0.3
VOO -3	2.8 ± 0.1	6.8 ± 0.3	30.2 ± 0.7	17.1 ± 0.8	2.6 ± 0.2
VOO -4	2.3 ± 0.1	6.9 ± 0.3	41.7 ± 1.0	24.6 ± 1.0	6.1 ± 0.3
VOO -5	1.8 ± 0.1	1.9 ± 0.3	7.9 ± 0.5	2.9 ± 0.1	8.7 ± 0.5
VOO -6	3.7 ± 0.2	6.9 ± 0.3	21.5 ± 0.5	7.9 ± 0.3	10.6 ± 0.6
VOO -7	nd	4.8 ± 0.3	16.5 ± 0.3	6.8 ± 0.3	7.2 ± 0.5
VOO -8	2.3 ± 0.1	8.4 ± 0.3	30.1 ± 0.3	13.2 ± 0.7	2.1 ± 0.1
VOO -9	nd	7.6 ± 0.3	25.7 ± 0.5	16.2 ± 0.6	3.3 ± 0.1
VOO -10	3.0 ± 0.1	6.8 ± 0.3	27.7 ± 0.6	12.3 ± 0.5	23.4 ± 1.3
VOO -11	1.9 ± 0.1	7.3 ± 0.3	26.4 ± 0.5	18.3 ± 0.9	10.0 ± 0.7
VOO -12	1.3 ± 0.1	2.2 ± 0.3	9.3 ± 0.3	6.9 ± 0.5	13.5 ± 0.8
VOO -13	nd	7.1 ± 0.3	26.5 ± 0.5	8.8 ± 0.3	4.0 ± 0.4
VOO -14	0.9 ± 0.1	3.4 ± 0.3	9.8 ± 0.3	3.5 ± 0.3	2.5 ± 0.3
VOO -15	3.3 ± 0.1	11.6 ± 0.3	37.6 ± 1.0	10.6 ± 0.3	2.6 ± 0.1
VOO -16	4.0 ± 0.1	13.5 ± 0.4	36.5 ± 1.0	10.3 ± 0.5	2.7 ± 0.2
VOO -17	3.3 ± 0.2	11.0 ± 0.3	32.8 ± 1.0	9.0 ± 0.4	2.5 ± 0.3
VOO -18	2.4 ± 0.1	9.5 ± 0.2	21.5 ± 1.1	6.8 ± 0.3	6.4 ± 0.4
VOO -19	13.5 ± 0.3	16.4 ± 0.3	37.9 ± 1.7	10.4 ± 0.5	n.d.
VOO -20	13.5 ± 0.4	17.4 ± 0.7	35.3 ± 1.5	10.9 ± 0.6	n.d.
VOO -21	11.5 ± 0.1	14.9 ± 0.5	30.2 ± 1.2	9.3 ± 0.4	n.d.
VOO -22	14.5 ± 0.2	15.0 ± 0.6	33.6 ± 1.3	11.0 ± 0.5	n.d.
VOO -23	14.2 ± 0.5	15.6 ± 0.6	34.8 ± 1.1	10.8 ± 0.3	n.d.
VOO -24	13.7 ± 0.1	14.3 ± 0.5	29.7 ± 1.0	9.3 ± 0.2	n.d.
VOO -25	13.0 ± 0.3	14.5 ± 0.3	31.9 ± 1.3	9.6 ± 0.3	n.d.

Data are the mean value of three replicates. n.d.: not detected

Table 3. Total quantities of n-alkanes, long chain aliphatic aldehydes, aliphatic alcohols and lineal waxes in Arbequina olive oils obtained by different extraction conditions.

Mesh size	Beating Time (min)	n-Alkanes ($\Sigma C_{22}-C_{35}$) (mg/kg)	n-Aldehydes ($\Sigma C_{22}-C_{30}$) (mg/kg)	n-Alcohols ($\Sigma C_{22}-C_{30}$) (mg/kg)	Waxes ($\Sigma C_{40}-C_{46}$) (mg/kg)
5 mm	10	100.2 $\pm$ 2.0	105.7 $\pm$ 1.9	65.8 $\pm$ 0.9	103.3 $\pm$ 0.9
	30	91.5 $\pm$ 1.2	108.0 $\pm$ 2.0	66.4 $\pm$ 0.5	78.1 $\pm$ 0.7
	60	95.0 $\pm$ 1.3	107.7 $\pm$ 1.7	68.7 $\pm$ 0.8	74.3 $\pm$ 0.9
	90	98.0 $\pm$ 1.1	116.0 $\pm$ 1.8	71.5 $\pm$ 0.7	70.9 $\pm$ 0.8
6 mm	10	93.0 $\pm$ 1.2	81.3 $\pm$ 1.5	55.0 $\pm$ 0.7	121.0 $\pm$ 0.9
	30	88.5 $\pm$ 1.1	86.8 $\pm$ 1.6	56.2 $\pm$ 0.9	102.9 $\pm$ 0.8
	60	90.2 $\pm$ 1.3	89.8 $\pm$ 1.4	55.9 $\pm$ 0.8	92.9 $\pm$ 0.9
	90	91.5 $\pm$ 1.5	107.7 $\pm$ 1.7	57.3 $\pm$ 0.7	92.0 $\pm$ 0.5

Data are the mean value of three replicates

Table 4. Total quantities of n-alkanes, long chain aldehydes, aliphatic alcohols and lineal waxes of different virgin olive oil mixtures (unspecified varieties) obtained from local factories.

Oil sample	n-Alkanes ($\Sigma C_{22}-C_{35}$) (mg/kg)	n-Aldehydes ($\Sigma C_{22}-C_{30}$) (mg/kg)	n-Alcohols ($\Sigma C_{22}-C_{30}$) (mg/kg)	Waxes ($\Sigma C_{40}-C_{46}$) (mg/kg)
VOO-1	99.5 $\pm$ 0.6	39.7 $\pm$ 0.5	200.5 $\pm$ 1.5	199.5 $\pm$ 1.5
VOO-2	100.6 $\pm$ 0.8	65.6 $\pm$ 0.6	225.6 $\pm$ 1.8	150.6 $\pm$ 1.5
VOO -3	85.7 $\pm$ 0.5	59.7 $\pm$ 0.6	95.6 $\pm$ 1.0	125.3 $\pm$ 1.2
VOO -4	76.9 $\pm$ 0.5	81.7 $\pm$ 1.0	20.2 $\pm$ 0.3	45.4 $\pm$ 0.5
VOO -5	100.2 $\pm$ 1.0	23.3 $\pm$ 0.5	45.6 $\pm$ 0.5	67.8 $\pm$ 0.5
VOO -6	75.9 $\pm$ 0.6	50.8 $\pm$ 0.6	56.8 $\pm$ 0.	79.5 $\pm$ 0.6
VOO -7	94.4 $\pm$ 1.1	35.4 $\pm$ 0.6	47.6 $\pm$ 0.6	66.3 $\pm$ 0.6
VOO -8	96.5 $\pm$ 0.7	56.1 $\pm$ 0.7	80.2 $\pm$ 0.7	100.5 $\pm$ 1.2
VOO -9	88.5 $\pm$ 0.7	52.8 $\pm$ 0.5	76.1 $\pm$ 0.9	98.6 $\pm$ 0.7
VOO -10	99.2 $\pm$ 0.9	73.3 $\pm$ 0.7	65.6 $\pm$ 0.8	70.2 $\pm$ 0.7
VOO -11	100.2 $\pm$ 1.0	64.0 $\pm$ 0.6	160.5 $\pm$ 1.5	200.2 $\pm$ 1.9
VOO -12	88.6 $\pm$ 0.5	33.3 $\pm$ 0.6	50.5 $\pm$ 0.3	54.6 $\pm$ 0.6
VOO -13	70.5 $\pm$ 0.4	46.6 $\pm$ 0.5	65.6 $\pm$ 0.6	70.8 $\pm$ 0.7
VOO -14	88.6 $\pm$ 0.8	20.2 $\pm$ 0.6	55 $\pm$ 0.5	76.6 $\pm$ 0.6
VOO -15	95.7 $\pm$ 0.5	67.2 $\pm$ 0.8	89.0 $\pm$ 0.8	125.7 $\pm$ 1.0
VOO -16	87.4 $\pm$ 0.9	67.3 $\pm$ 0.6	78.0 $\pm$ 0.6	99.6 $\pm$ 1.0
VOO -17	82.1 $\pm$ 0.8	58.8 $\pm$ 0.5	60.0 $\pm$ 0.5	79.5 $\pm$ 0.7
VOO -18	83.6 $\pm$ 0.9	46.7 $\pm$ 0.6	70.7 $\pm$ 0.6	85.6 $\pm$ 0.6
VOO -19	91.0 $\pm$ 1.0	78.2 $\pm$ 0.8	99.7 $\pm$ 1.0	122.5 $\pm$ 1.1
VOO -20	92.2 $\pm$ 1.0	77.1 $\pm$ 0.6	100.6 $\pm$ 1.2	140.6 $\pm$ 1.2
VOO -21	85.1 $\pm$ 0.8	65.9 $\pm$ 0.6	78.9 $\pm$ 0.7	87.7 $\pm$ 0.8
VOO -22	87.6 $\pm$ 0.9	74.1 $\pm$ 0.9	77.7 $\pm$ 0.7	89.6 $\pm$ 0.8
VOO -23	80.0 $\pm$ 0.6	75.3 $\pm$ 0.7	67.9 $\pm$ 0.6	79.6 $\pm$ 0.7
VOO -24	91.0 $\pm$ 1.0	67.0 $\pm$ 0.5	125.5 $\pm$ 1.0	130.5 $\pm$ 1.5
VOO -25	92.2 $\pm$ 0.9	68.9 $\pm$ 0.6	120.3 $\pm$ 1.1	145.7 $\pm$ 1.1

Data are the mean value of three replicates

Figure 2. Distribution of: A) total aliphatic hydrocarbons, B) total aliphatic aldehydes, and C) total aliphatic alcohols, in virgin olive oils. Data expressed as percentages of their total quantities.

Aldehydes with even carbon-atom from C_{22} to C_{30} are present in olive oils >
Concentrations of total long-chain aliphatic aldehydes in olive oils are below 116
 mg kg^{-1} > Hexacosanal ($C_{26\text{-al}}$) is the most abundant aldehyde in olive oils > Long-
chain aliphatic aldehydes is a good traceability markers for olive oils