Characterisation of virgin olive oils from variety Benizal (Albacete, Spain)

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Spain is the leading country in olive oil production and Castilla-La Mancha is the second region with the highest production. This rural region has a huge number of local cultivars that need to be analysed to evaluate their potential for olive oil production. *Benizal* is an important variety in the Campos de Hellín area (Castilla-La Mancha, Spain), as this variety must be obligatory included in the oils from the recently created collective mark "Aceites Campos de Hellín". In this study, the physicochemical and sensory characterization of the oils from the *Benizal* variety has been done. As a first step, a genetic analysis was done by means of RAPDs technique to ensure the correct identification of *Benizal* trees. The analysis of the amplified fragments using 15 primers showed 9 different groups, corresponding to the 9 varieties grown in the Campos de Hellín area, which confirm the usefulness of this simple technique to identify varieties, even if they are morphologically similar. Chemometric characterization of the oils shows the outstanding quality of *Benizal* oils, as well as the potential of this cultivar to be used for olive oil production. Chemical composition showed a lower ratio of mono/polyunsaturated acids than in other commercial cultivars, a high content in campesterol and low content in total sterols, which are intrinsic characteristics of the variety. In addition, *Benizal* oils have differential properties regarding sensory parameters, especially high levels in the pungent attribute. Knowledge of chemical and sensory properties of oils from different varieties may contribute to elaborate more balanced oils, which fit all the consumers' preferences.

Keywords: Benizal, characterisation, quality, RAPDs, virgin olive oil.

INTRODUCTION

Olive oil production has a traditional importance in Spain, which is the country that currently leads the production and exportations of olive oil in the world. In fact, Spanish olive production accounts for around 48.5% of European total production [1], with an oil production of over 1.3 million of tons for the 2016/2017 harvest season [2].

Olive oil production in Spain is mostly located in the southern regions of the country. Andalucía is the Spanish region where olive oil production stands out, whereas Castilla-La Mancha is the second largest region in Spain as regards olive tree surface, with an area of 369,560 ha of olive groves [3].

Campos de Hellín is an arid area situated in the south eastern section of Castilla-La Mancha, producing about 5,000 tons of oil. The main olive cultivars grown in Campos de Hellín are *Arbequina, Benizal, Cornicabra* and *Picual*, although it is also possible to find minor varieties, as *Cuquillo*, *Injerta*, *Manzanilla de Sevilla*, *Manzanilla Local* and *Negrilla*. Recently, Campos de Hellín area has obtained the declaration of the collective mark "Aceites Campos de Hellín", a sign to certify the geographical origin, quality and characteristics provided to

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the oil by the varieties grown in that area. As a regulation of that collective mark [4], mills must include at least 20% of olive oils from *Benizal* cultivar in the virgin olive oils produced. Thus, *Benizal* variety is considered an important local variety with about 150,000 that produce 4,500,000 kg per year on average [5].
The maintenance of the variety *Benizal* has two important facts to consider: (i) a high environmental value, as *Benizal* is a highly specialised variety adapted to the area, preventing erosion and desertification of the soil and keeping the balance of ecosystems, and (ii) a great commercial value, as the oil obtained from *Benizal* differs from other olive oils in terms of physicochemical and sensory features [6, 7].
Sometimes, the correct identification of trees from a variety is difficult only considering the morphological parameter. In this aspect, the introduction of DNA (deoxyribonucleic acid) markers provides a good discriminatory system, independent of environmental conditions. The random amplified polymorphic DNA (RAPD) technique has been applied in several studies to successfully distinguish between olive cultivars [8-11].
Due to the lack of information about the characteristics of the *Benizal* variety and the nonexistence of a deep characterisation of this variety, the aim of this study is to characterise monovarietal virgin olive oils obtained from *Benizal* cultivars by analysing the regulated physicochemical and sensory parameters, as well as stability parameters and the fatty acids, sterols and triterpenic dialcohols composition during five consecutive harvest seasons. As a first step, a genetic study is made among the varieties grown in the Campos de Hellín area to ensure the correct identification of the *Benizal* trees.

MATERIALS AND METHODS

SAMPLING

Olive samples were taken at the beginning of the harvest season (first fortnight of November), when most of the fruits had a purple-black skin. The total number of *Benizal* samples was 30 (6 plots × 5 consecutive olive crops). In each plot, 15 kg of healthy olives were picked by hand for oil extraction.
To perform the genetic analysis, healthy leaves from 9 varieties grown in Campos de Hellín (*Arbequina*, *Benizal*, *Cornicabra*, *Cuquillo*, *Injerta*, *Manzanilla de Sevilla*, *Manzanilla Local*, *Negrilla* and *Picual*) were collected throughout Campos de Hellín area. The leaves were immediately frozen until DNA extraction.

OLIVE OIL EXTRACTION

About 2 l of oil were obtained from each sample using an experimental oil mill, *Oliomio TF-30* (Toscana Enologica Mori, Tavarnelle Val di Pesa - FI - Italy). Oil extraction was carried out under the best working conditions (without fustiness and with recommended temperature and processing times). The oil was decanted and placed in smaller bottles until further analysis.

ANALYTICAL DETERMINATIONS

Regulated physicochemical quality parameters (free acidity, peroxide value and UV absorption characteristic, K_{270} and K_{232}) and regulated sensory parameters (median of defects, median of fruity and panel test classification) were determined as reported in [12].
Stability parameters (content of total polyphenols and oxidative stability at 100°C) were analysed using different methodologies. Oxidative stability was evaluated by the Rancimat method [13]. Total polyphenol compounds were determined as indicated in Vázquez et al. (1973) [14], expressing values as milligram of caffeic acid per kg of oil.
Determination of fatty acids and sterols was done as reported in Rabadán et al. (2017) [15].
Analytical tests were performed in triplicate at least.

DEOXYRIBONUCLEIC ACID EXTRACTION

Genomic DNA was extracted from young leaves using G-spin™ IIp DNA extraction kit (Intron Biotechnology, Kyunggi-do, Korea). The concentration of the DNA was determined by electrophoresis on 1.5% agarose gels.

RAPD ANALYSIS

Twenty-five oligonucleotides (OPL 1-20, OPM 17-20) were used for PCR amplifications and those with higher polymorphism and reproducible patterns were selected. Amplification reactions were carried out in 25 µl volumes, with 30 cycles composed of 1 min at 95°C, 1 min at 40ºC and 1 min. at 72ºC. The amplification products were separated by electrophoresis on 2% agarose gels. The analysis of the amplification fragments was made with the Phylip 3.69 program (University of Washington, Seattle, WA, USA).

DATA ANALYSIS

Results were expressed as means ± standard deviations. Clusters were evaluated using the Ward's method, using the SPSS programme, release 23.0 for Windows.

RESULTS AND DISCUSSION

GENETIC ANALYSIS

To ensure the correct identification of the *Benizal* variety trees, a first genetic approach was made by

means of the RAPD technique. After an initial screening of 25 primers, the amplification products of 15 primers were selected for further analysis according to the generation of conspicuous and highly reproducible polymorphic amplification fragments. An example of the amplification pattern obtained with primer OPL-17 is shown in Figure 1. The used primers produced 97 bands, 51 of which resulted in polymorphic fragments. These polymorphic bands were useful to differentiate the olive varieties grown in Campos de Hellín area.

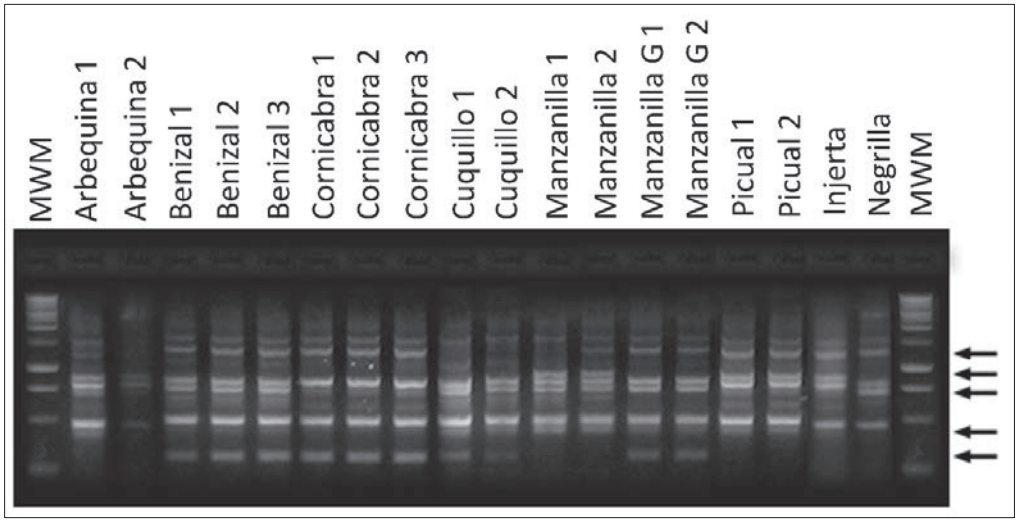

Figure 1 - RAPD pattern from the varieties grown in the Campos de Hellín with the primer OPL-17. MWM: Molecular Weight Marker. Arrows indicate polymorphic bands.

This study confirms the usefulness of RAPD markers as a powerful tool for olive varieties identification since all the cultivars could be uniquely characterised with a relatively low number of primers, as occurring in previous studies [8, 16]. This simple technique clearly discriminates the varieties, even when they are morphologically quite similar.

Cluster analysis (Fig. 2) suggested 9 different groups, corresponding to each studied variety. All the *Benizal* samples studied were included into a single group. Therefore, our results show that the *Benizal* variety can be clearly differentiated from the rest of the varieties.

The identification of a variety may be important when the special characteristics of this variety need to be used to elaborate monovarietal oils.

PHYSICOCHEMICAL AND STABILITY PARAMETERS AND CHEMICAL COMPOSITION

Regulated physicochemical and stability parameters and fatty acid, sterol and triterpenic dialcohol composition obtained for the *Benizal* olive oil samples were analysed (Tab. I). All the samples showed very low values for the regulated physicochemical parameters evaluated (free acidity ≤ 0.8%; peroxide value ≤ 20 mEq O_2/kg; K_{270} ≤ 0.22; K_{232} ≤ 2.5), with all of them being classified within the "extra virgin" category, as stated by European Regulation [17]. The low values for these parameters indicate higher quality of *Benizal* oils. According to the values found throughout olive oil studies in Castilla – La Mancha [12, 18], *Benizal* oils do not have the highest or the lowest values for physicochemical parameters in relation to other olive varieties.

About stability parameters, *Benizal* oils presented a high content of total polyphenols. This high total polyphenol content (456.39 mg/kg) may be related with the sensory profile of this variety, especially the high pungent and bitter taste [19]. Although it has been said that the natural polyphenols improve the resistance to oxidation [12, 18, 20-23], the oxidative stability values found in *Benizal* oils were lower than expected (47.57 h). The low stability may be due to the low content of tocopherols, and the ratio mono/polyunsaturated acids [22-25].

The distribution of fatty acid composition of the *Benizal* oils covered the normal range expected for virgin olive oil according to the current regulation [26]. However, *Benizal* oils showed a low ratio of mono/polyunsaturated acids caused by the low content of oleic acid and high content of linoleic acid, which may contribute to its low oxidative stability [27, 28]. A similar low ratio has been reported for the oils from other Spanish varieties such as *Verdial* [29], *Arbequina* [18, 30] or *Onil de Povedilla* [12].

About sterol profile, the oils from *Benizal* are characterised by a high content of campesterol, with most of the samples exceeding the maximum level (4%)

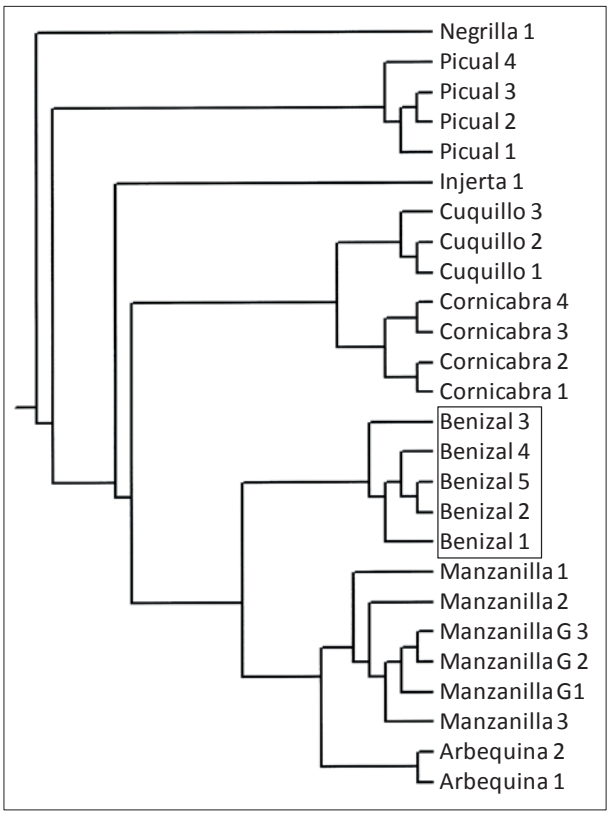

Figure 2 - Phylogenetic tree of 8 varieties grown in Campos de Hellín area (Castilla – La Mancha, Spain).

allowed by current regulation [26]. The high campesterol content seems to be an intrinsic characteristic of this variety, as occurs in other Protected Designations of Origin from Castilla-La Mancha with *Cornicabra* variety [20], or in several regions of Argentina with *Arbequina* [29, 31].

With respect to stigmasterol, the *Benizal* oils showed a low concentration (0.72%), which may be indicative of the healthy fruits used for the oil extraction, avoiding any damage during collection. High levels of stigmasterol content may correlate with high acidity and low organoleptic quality [32, 33]. In addition, *Benizal* oils showed low values of apparent β-sitosterol and stigmasterol compared to those found in Sierra de Alcaraz [12] and a high apparent β-sitosterol content compared to Campos the Montiel [18].

With respect to the level of erythrodiol + uvaol, *Benizal* stood out with its high content (2.82%). Similar values have been reported in the case of oils produced in the nearby Protected Designations of Origin Aceite Campo de Montiel [18], and some varieties of Extremadura (Spain), as *Corniche, Verdial Badajoz* and *Carrasqueña* [29].

Another characteristic of the *Benizal* oils is the low content in total sterols, which values are below the minimum allowed by current regulations (1,000 mg/kg). Figure 3 shows a comparison of total sterols with other Spanish varieties. There is a wide variation in the total sterols content, ranging from the lowest values from

Table I - Average values ± standard deviations for the regulated physicochemical and stability parameters, fatty acids, sterols and triterpenic dialcohols composition evaluated in the *Benizal* virgin olive oil samples analyzed for 5 consecutive crop years.

Physicochemical parameters	Mean ± SD
Free acidity (g oleic acid/100g oil)	0.14 ± 0.05
Peroxide value (mEq O_2/kg)	5.62 ± 1.12
K_{270}	0.13 ± 0.03
K_{232}	1.67 ± 0.08
Stability parameters	
Total polyphenols (mg caffeic acid/kg oil)	456.39 ± 128.96
Oxidative stability at 100ºC (h)	47.57 ± 14.15
Fatty acids (%)	
C14:0	0.01 ± 0.00
C16:0	11.55 ± 0.85
C16:1	0.75 ± 0.12
C17:0	0.06 ± 0.06
C17:1	0.10 ± 0.05
C18:0	2.21 ± 0.39
C18:1	75.50 ± 2.19
C18:2	8.02 ± 2.08
C18:3	0.80 ± 0.09
C20:0	0.37 ± 0.05
C20:1	0.34 ± 0.05
C22:0	0.10 ± 0.01
C24:0	0.08 ± 0.04
Sterols (% of total sterols)	
Cholesterol	0.18 ± 0.07
Campesterol	3.93 ± 0.46
Stigmasterol	0.72 ± 0.18
Apparent β-sitosterol	94.17 ± 0.73
Δ7-stigmastenol	0.24 ± 0.08
Total sterols (mg/kg)	962 ± 159
Triterpenic dialcohols (%)	
Erythrodiol + uvaol	2.82 ± 0.67

Benizal (the only variety with a value of sterols below 1,000 mg/kg) to values over 2,000 mg/kg reported by [29] for the variety *Corniche*. However, this is another intrinsic characteristic of this variety, and low levels in total sterols from *Benizal* oils must not be considered to detect fraudulent oils.

SENSORY ANALYSIS

Six *Benizal* oil samples were analysed to evaluate sensory characteristics. A panel of 12 trained judges was used to perform the sensory analysis. According to the panellists, all the samples were classified into the "extra virgin" category, since the median of the fruity attribute was above 0 and the median of defects was equal to 0. *Benizal* oils were characterised by its high intensities (above 5) in the bitter and pungent attributes, as 50% of the samples evaluated showed values >5 for bitterness, and 83.3% of the samples showed values >5 for pungent. When both attributes

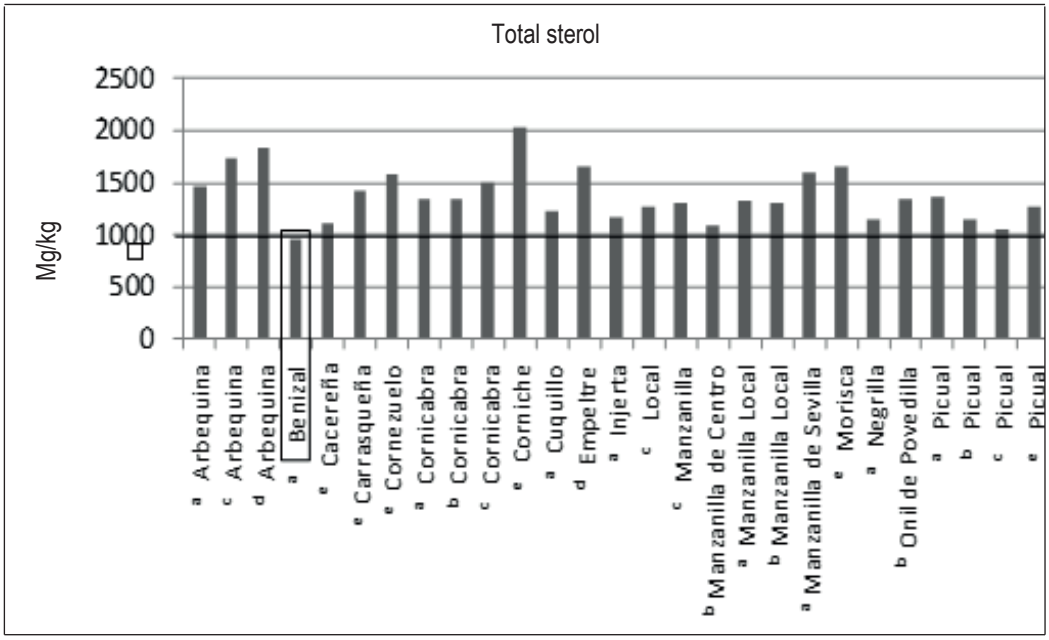

Figure 3 - Total sterol content in olive oils from *Benizal* variety compared with other Spanish olive varieties.
[a] Data from Campos de Hellin (Albacete, Spain); [b] Data reported by Pardo et al. (2011); [c] Data reported by Pardo et al (2007); [d] Data reported by Gracia et al. (2009); [e] Data reported by Sánchez et al. (2006).

were considered together, 33.3% of the oils showed values >5 for both attributes, bitterness and pungent, while 16.7% of the oils showed values <5 for both. Moreover, *Benizal* oils stand out in their unmistakable smell of Mediterranean scrubland with a predominant touch of mint that differentiates them from similar varieties in terms of bitter and pungent as *Picual* and *Cornicabra*.

CONCLUSION

Benizal oils are characterised by a high content in polyphenols, and a low ratio of mono/polyunsaturated acids resulting in oils with valuable health-promoting characteristics. These oils show low levels of total sterols and high levels of campesterol that do not answer the levels required by European regulations, and that can be considered intrinsic characteristics of this variety. From the sensory point of view, *Benizal* oils show differentiated characteristics, with a peculiar smell and high bitter and pungent levels related to its high content in polyphenols. The knowledge of the chemical and sensory characteristics of monovarietal oils may help elaborate balanced oils that answer all consumers' preferences.

REFERENCES

[1] FAO, http://www.fao.org/statistics/en (accessed Apr. 2017).
[2] EUC. Market situation in the Olive oil and Table olives sectors. Off. J. Eur. Commun. (2017).
[3] MAGRAMA. Anuario de estadística. Avance 2015. MAGRAMA, 695-708 (2016).
[4] Agriculture and Environment Department of the Castilla - La Mancha Autonomous Government, Off. J. of Castilla – La Mancha. Num.250, 59212-59222 (2010).
[5] J.E. Pardo, E. López, J.M. Núñez, A. Alvarruiz, A. Alfaro, E. Fernández *et al*. Caracterización del aceite de oliva virgen obtenido de la variedad Benizal. Fruticultura Profesional 170, 7-32 (2007).
[6] J.E. Pardo, E.Sena, M.A. Cuesta, J.D. Granell, J. Valiente, M. Álvarez-Ortí. Evaluation of potential and real quality of virgin olive oil from "Campos de Hellín" (Albacete, Spain). J. Am. Oil Chem. Soc. 90(6), 62-851 (2013).
[7] E. Sena, M. Álvarez-Ortí, D.C. Zied, A. Pardo, J.E. Pardo. Olive oils from Campos de Hellín (Spain) exhibit significant varietal differences in fatty acid composition, sterol fraction, and oxidative stability. Eur. J. Lip. Sc. And Tec. 117(7), 75-967 (2015).
[8] A. Fabbri, J.I. Hormaza, V.S. Polito. Random amplified polymorphic DNA analysis of olive (Olea europaea L.) cultivars. J. Am. Soc. for Hort. Sc. 120(3), 42-538 (1995).
[9] G.T. Mekuria, G.G. Collins, M. Sedgley. Genetic variability between different accessions of some common commercial olive cultivars. The J. of Hort. Sc. And Biotec. 74(3), 14-309 (1999).
[10] J.R. Guerin, S.M. Sweeney, G.G. Collins, M. Sedgley. The development of a genetic data-

base to identify olive cultivars. J. Am. Soc. for Hort. Sc. 127(6), 83-977 (2002).
[11] A. Belaj, I. Trujillo, R. de la Rosa, L. Rallo, M.J. Gimenez. Polymorphism and discrimination capacity of randomly amplified polymorphic markers in an olive germplasm bank. J. Am. Soc. for Hort. Sc. 126(1), 64-71 (2001).
[12] J.E. Pardo, M.A. Cuesta, A. Alvarruiz, J.D. Granell, M. Álvarez-Ortí. Evaluation of potential and real qualities of virgin olive oil from the designation of origin (DO) "Aceite Montes de Alcaraz" (Albacete, Spain). Food Chem. 124(4), 90-1684 (2011).
[13] A. Rabadán, M. Álvarez-Ortí, R. Gómez, A. Alvarruiz, J.E. Pardo. Optimization of pistachio oil extraction regarding processing parameters of screw and hydraulic presses. LWT - Food Sc. and Tech. 83, 79-85 (2017).
[14] A. Vázquez, C. Janer, M.L. Janer. Determination of total polyphenols in olive oils. Grasas y Aceites 24(35), 357 (1973).
[15] A. Rabadán, M. Álvarez-Ortí, R. Gómez, C. de Miguel, J.E. Pardo. Influence of genotype and crop year in the chemometrics of almond and pistachio oils. J. of the Sc. of Food and Agric. (2017).
[16] M. Talal, D.S. Hassawi. Genetic Analysis of Jordanian Olive (Olea europea L.) Using Random Amplified Polymorphic DNA (RAPD). Jordan J. of Agric. Sc. 2(2), (2010).
[17] EC, Off. J. Eur. Commun. L178, 11-16 (2008).
[18] J.E. Pardo, M.A. Cuesta, A. Alvarruiz. Evaluation of potential and real quality of virgin olive oil from the designation of origin "Aceite Campo de Montiel" (Ciudad Real, Spain). Food Chem. 100(3), 86-977 (2007).
[19] M. Benito, R. Oria, A.C. Sánchez-Gimeno. Characterization of the olive oil from three potentially interesting varieties from Aragon (Spain). Food Sc. and Tech. Int. 16(6), 30-523 (2010).
[20] M.D. Salvador, F. Aranda, G. Fregapane. Influence of fruit ripening on 'Cornicabra' virgin olive oil quality: A study of four successive crop seasons. Food Chem. 73(1), 45-53 (2001).
[21] J. Salas, M. Pastor, J. Castro, V. Vega. Influencia del riego sobre la composición y características organolépticas del aceite de oliva. Grasas y aceites 48(2), 5-74 (1997).
[22] J. Touss, A. Romero, J. Plana, L. Guerrero, I. Díaz, J.F. Hermoso. Características químico-sensoriales de los aceites de oliva «Arbequina» obtenidos en distintas zonas de España. Grasas y aceites 48(6), 415-424 (1997).
[23] M.S. Gracia, A. Royo, M. Guillén. Composición química de aceites de las variedades Arbequina y Empeltre cultivadas en regadío. Grasas y aceites 60(4), 321-329 (2009).
[24] R. Aparicio, L. Roda, M.A. Albi, F. Gutiérrez. Effect of various compounds on virgin olive oil stability measured by Rancimat. J. of Agric. and Food and Chem. 47(10), 5-4150 (1999).
[25] M. Poiana, A. Mincione. Fatty acids evolution and composition of olive oils extracted from different olive cultivars grown in Calabrian area. Grasas y Aceites 55(3), 90-253 (2004).
[26] EC, Off. J. Eur. Commun. L295, 57-77 (2003).
[27] G. Beltrán, A. Jiménez, M.P. Aguilera, M. Uceda. Análisis mediante HPLC de la fracción fenólica del aceite de oliva virgen de la variedad Arbequina. Relación con la medida del amargor K_{225} y la estabilidad. Grasas Aceites 51(5), 4-320 (2000).
[28] A. Rabadán, M. Álvarez-Ortí, R. Gómez, A. Pardo-Giménez, J.E. Pardo. Suitability of Spanish almond cultivars for the industrial production of almond oil and defatted flour. Scientia Horticulturae 225, 46-539 (2017).
[29] J. Sánchez-Casas, C. de Miguel, E. Osorio, J.M. Expósito, L. Gallardo, M. Martínez. Calidad sensorial de aceites de oliva virgen procedentes de variedades de aceitunas producidas en Extremadura. Grasas y Aceites 57(3), 313-318 (2006).
[30] A. Alvarruiz, M. Álvarez-Ortí, B. Mateos, E. Sena, J.E. Pardo. Quality and composition of virgin olive oil from varietties grown in Castilla-La Mancha (Spain). J. of Oleo Sc. 64(10), 82-1075 (2015).
[31] DIA. Hojas de Olivo. Dirección de Industria Alimentaria (4), 1-5 (2003).
[32] G. Gómez, M. Soledad. Composición química de distintas calidades de aceites de oliva virgen de la variedad "Empeltre" en el bajo Aragón. Grasas y Aceites 52(1), 52-58 (2001).
[33] L.N. Ceci, M. Melgarejo, A.A. Carelli. Caracterización de aceites de oliva varietales argentinos cosecha 2005, Parte II: Polifenoles, ceras, esteroles y eritrodiol-uvaol. Grasas y Aceites 62, 73-566 (2007).

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