Títol del treball: *Influence of the ripening stage and crushing temperature on polyphenols content of extra virgin olive oil*

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Article

Influence of the ripening stage and crushing temperature on polyphenols content of extra virgin olive oil

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Resum: L'oli d'oliva verge extra (OOVE) conté polifenols que protegeixen el colesterol LDL de l'oxidació. La seva concentració depèn de diferents factors agraris i tècnics, com ara l'estadi de maduració i la temperatura de trituració. L'objectiu d'aquest treball va ser avaluar l'impacte d'aquests dos factors en el perfil fenòlic de l'OOVE de "Corbella".

Els polifenols de l'OOVE es van mesurar a tres graus de maduració (índex de maduresa (IM) = 0,3; IM = 1,2; IM = 3,2) i tres temperatures de trituració (10 °C, 18 °C i 25 °C). Aquests compostos es van analitzar mitjançant cromatografia líquida d'alta eficàcia acoblada a un espectròmetre de masses en tàndem. Les conclusions a les que es podria arribar després de finalitzar el treball són que la suma de polifenols disminueix quan augmenta la temperatura de trituració, però augmenten polifenols individuals com l'oleocantal o l'oleaceïna. A més, els polifenols totals disminueixen amb la maduresa de la fruita. En canvi, augmenten les flavones, l'aglicona d'hidroxioleuropeïna, l'acetat d'hidroxitirosol i els lignans.

Abstract: Extra virgin olive oil (EVOO) contains polyphenols, which protect LDL cholesterol from oxidation. Its concentration depends on different agrological and technical factors, such as the ripening stage and the temperature of crushing. The aim of this study was to evaluate the impact of these two factors on the 'Corbella' EVOO phenolic profile.

The polyphenols were measured at three degrees of ripeness (maturity index (IM) = 0.3; IM = 1.2; IM = 3.2) and three crushing temperatures (10 °C, 18 °C and 25 °C) in EVOO. These compounds were analysed by ultra-high-performance liquid chromatography coupled to a tandem mass spectrometer. The conclusions that could be reached after completing the work are that the sum of polyphenols decrease when the crushing temperature is increased, but individual polyphenols like oleocanthal or oleacein increase. Further, total polyphenols decrease with fruit maturity. Instead, flavones, hydroxyoleuropein aglycone, hydroxytyrosol acetate and lignans increase.

Keywords: olive; oil; polyphenols; crushing; ripening; oleocanthal; oleacin

1. Introduction

Extra-virgin olive oil (EVOO) has a unique fatty acid composition. In addition, it has polyphenols, which are responsible of its functional properties, as prevention of cardiovascular diseases, and high oxidative stability during storage (1,2). According to the European Union they contribute to the protection of blood lipids from oxidative stress so they protect LDL cholesterol from

oxidation (3). Polyphenol concentration depends on different agrological and technical factors, such as the ripening stage and temperature of crushing, between others (4,5).

It has been proved that the content of phenolic compounds in virgin olive oil is affected by the malaxation temperature and mixing time (6), during the extraction the endogenous enzymes play a key role in the EVOO phenolic profile, as well as the non-enzymatic oxidation (7,8); literature indicates that most phenolic compounds decrease with the malaxation time, in the case of oleocanthal and oleacein increased instead (9). Others authors point out that there is a difference between olive oil varieties (10,11). Also, the cultivar, the ripening stage, and even the harvesting method have an influence on their content that affect the organoleptic and pysico-chemical profile (12–14). Furthermore, it is also important to take into account the partition coefficient of the polyphenols, mostly the hydrophilic, in the water-oil mixture (15).

For all these, in this study the effect of crushing temperature and ripening index on the phenolic content of EVOO was studied. The EVOO studied was 'Corbella' monovarietal oil a very aromatic virgin oil, with diverse connotations of ripe fruits, apples and herbs. This variety has prevalence in the Bages and the 'Vera' in the Vallès Oriental, but it can be find outside these regions scattered throughout the regions of La Segarra, La Noguera, Solsonès, Alt Urgell and Pallars Jussà, where it is called 'Llargueta' (16,17). Due to historical reasons, its cultivation was stopped but nowadays work is underway to recover the production of these olive trees and, consequently enhancing biodiversity. It is interesting in softening bitter oils, adding chromatic complexity. Its low stability makes it advisable to closely monitor the storage and packaging conditions (18), making it interesting to increase its polyphenolic content to enhance its stability. Its most common characteristics are:

Fruit weight (g)	Pulp/pine ratio	Oil content (% over dry)	C18:1 (%)	SAT (%)	MONOINSAT (%)	POLIINSAT (%)	Total polyphenols (mg / kg)	K235	Stability (h a 120 °C)
2.31	3.67	48.34	67.76	15.72	69.80	14.48	202.0	0.205	5.0

Table 1. Corbella fruit characteristics from Ninot et al. *(2015)* (16)

Due to the low polyphenols content and the high level of polyunsaturated fatty acids, it has low stability, which results in a short shelf life of the oils of these varieties. In order to keep evaluate the concentration of these compounds, it was determined the differences in EVOO polyphenol content between three types of three harvest days and three crushing temperatures.

2. Materials and Methods

2.1. Chemicals and standards

Acetonitrile, methanol (99.9%), formic acid, and acetic acid were purchased from AppliChem, Panreac Quimica SA (Barcelona, Spain). Hexane, *p*-coumaric acid, luteolin, oleuropein, oleocanthal, and pinoresinol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydroxytyrosol was acquired from Extrasynthese (Genay, France) and apigenin from Fluka (St. Louis, MO, USA). Ultrapure water was obtained using a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Extraction of the oil

2.2.1. Olives and oil

The fruit came from old olive trees (over 50 years old), grown in dry land in Oli Migjorn plantation from Bages. Oli Migjorn is a project addressed to the cultivation of olive trees and the production of organic extra virgin olive oil. The recovery of local varieties and synergy with the territory are one of its main objectives.

The oil is described as a fruity profile and a medium green colour with ripe touches. In the mouth it has an intense spicy and bitter balanced with sweet, and an appreciable astringency that is positive and does not interfere with the profile. Secondary aromas of vegetable type, with connotations of vegetable plants (artichoke, almond), as well as other ripe tomatoes, notes of almond and walnuts in the mouth.

2.2.2. Ripening study

The fruit was collected in three different moments (25/09/17, 9/10/17 and 25/10/17), the ripening index for each harvest was calculated according to the method described by Uceda and Frías (1975), explained before in another paper (19). The stages were: between 0-1, 1-2 and 2-3. The oil was extracted by the method of Abencor ("Ingeniría y Sistemas, Sevilla"), which reproduces the industrial process on a laboratory scale. The olives were crushed in a 5 mm diameter sieve and the olive paste was placed in a 28 ºC malaxator for 30 min. Then, it was centrifuged at 3,000 rpm for 3 min., without the addition of talcum powder or hot water. The oil obtained was separated by decantation, transferred to amber glass bottles and stored in the freezer (-20 °C) until further analysis. Three repetitions were carried out for each ripening stage.

2.2.3. Crushing temperature study

Other samples of healthy olives (at a maturation stage of 3.2) were washed and heated to three different temperatures: 10 ºC, 18 ºC and 25 ºC in a water bath. Once the desired temperature had been reached, they were crushed in a 5 mm diameter sieve and the obtained paste is placed in a malaxator at 18- 19 ºC for 30 min. It was then centrifuged at 3,000 rpm for 3 min., without the addition of talcum powder or hot water (3 repetitions were performed per temperature tested). The oil obtained was separated by decantation, transferred to amber glass jars and stored in a freezer (-20 °C) until further analysis.

2.3. Extraction and analysis of phenol compounds

The extraction of polyphenols from olive oil was carried out using the method of Capriotti et al. (2014) (20). In a centrifuge glass tube, 0.5 g of oil was weighed. It was added 1 mL of hexane and 2 mL of MeOH4:1H_2O, the dispersion was mixed in a stirrer for 30 seconds and then centrifugated for 3 minutes at 3,000 rpm at 4 °C. The two phases were separated, the methanol phase was defeated with 1 mL of hexane and the oil phase was reextracted with 2 mL of MeOH4:1H_2O. The methanolic extracts were combined and evaporated under a stream of nitrogen to dryness. It was then reconstituted with 800 μL of H_2O:Methanol (80:20 v/v). It was finally filtered with 0.2 μm PTFE syringe filters and transferred to an 2mL topaz vial and stored at -80 °C until analyses.

To quantify the olive oil polyphenols it was used the methodology proposed by Suárez et al. (2008) which consist on a UPLC-MS/MS (21). It was used an AcquityTM UPLC (Waters; Milord, MA, USA) with an API 3000 triple-quadruple mass spectrometer (PE Sciex, Framingham, MA, USA) coupled the source of iron spray was a turbo. Compound separation was accomplished using an Acquity UPLC® BEH C18 column ((2.1×50 mm, id, 1.7 μm particle size) (Waters Corporation®, Wexford, Ireland)) and an Acquity UPLC® BEH C18 Pre-column ((2.1×5 mm, id, 1.7 μm particle size) (Waters Corporation®, Wexford, Ireland)). Chromatographic conditions were detailed in previous work of the group (19).

Furthermore, in the experiment, the following polyphenols were also quantified with a different method: oleochantal, oleacein, oleuropein aglycone and ligstroside aglycone. It was carried out with an adjustment of the method described by Sánchez de Medina et al. (2017) (22). The chromatographic and mass equipment was the same used for the rest of phenolic compounds and the exact analytical conditions are explained elsewhere (19).

The quantification of the compounds was done with a curve made in refined olive oil. The stardards used for the quantitation were apigenin for apigenin; oleocanthal for oleocanthal; oleuropein for hydroxytyrosol acetate, elenolic acid, hydroxyoleuropein aglycone, methyloleuropein aglycone and hydroxyelenolic acid; oleacein for oleacein; oleuropein aglycone for oleuropein aglycone and ligstroside aglycone; hydroxytyrosol for hydroxytyrosol; luteolin for luteolin; *p*-coumaric acid for *p*-coumaric acid and *m*-coumaric acid; and pinoresinol for pinoresinol.

2.4. Statistical analyses

The normality of the data was tested by the Q-Q plot diagnostic graphic. After viewing the results, all subsequent tests performed were non-parametric. To assess differences between groups Kruskal-Wallis test was performed and the post-hoc test was the pairwise Mann–Whitney U-test. All the statistics were performed with R Project for Statistical Computing 3.6.0.

3. Results

3.1. Ripening study

The results of the maturity assay are showed in **Table 2**. Total polyphenols decrease with fruit maturity. However, in the case of flavones and lignans their concentration increases with the maturity index (IM). Phenolic alcohols increase at the beginning of the maturation but then their concentration decreases.

Group/Compound	Maturity Index (mg/kg)		
	25/09/2017 (IM=0.3)	09/10/2017 (IM=1.2)	25/10/2017 (IM=3.2)
Total Polyphenols	566±51 (a)	484±27 (b)	319±17 (c)
Secoiridoids	556±51 (a)	473±26 (b)	306±17 (c)
Elenolic acid	272±10 (a)	229±16 (b)	125±10 (c)
Hydroxyelenolic acid	8.68±0.4 (a)	8.79±0.3 (a)	4.32±0.2 (b)
Hydroxyoleuropein aglycone	1.11±0.09 (a)	1.17±0.05 (a)	2.03±0.07 (b)
Ligstroside aglycone	15.1±0.8 (a)	11.9±0.5 (b)	9.00±0.5 (c)
Methyloleuropein aglycone	0.926±0.04 (a)	0.876±0.02 (a)	0.866±0.007 (a)
Oleacein	94.2±6 (a)	78.7±4 (b)	34.1±2 (c)
Oleocanthal	9.14±1 (a)	6.84±0.3 (b)	2.36±0.2 (c)
Oleuropein aglycone	155±6 (a)	135±3 (a)	98±4 (a)
Phenolic alcohols	1.34±0.2 (a)	2.16±0.1 (b)	0.715±0.04 (c)
Hydroxytyrosol	1.27±0.08 (a)	0.676±0.06 (b)	0.458±0.05 (c)
Hydroxytyrosol acetate	0.0763±0.02 (a)	0.107±0.01 (a)	0.205±0.02 (b)
Flavones	0.903±0.09 (a)	1.15±0.08 (b)	2.73±0.4 (c)
Apigenin	0.488±0.01 (a)	0.824±0.06 (b)	2.23±0.2 (c)
Luteolin	0.414±0.02 (a)	0.324±0.04 (a)	0.695±0.04 (a)
Phenolic acids	2.12±0.3 (ab)	2.16±0.06 (b)	1.90±0.1 (a)
m-coumaric acid	1.53±0.04 (a)	1.47±0.03 (a)	1.42±0.03 (b)

p-coumaric acid	0.590±0.05 (ab)	0.687±0.05 (b)	0.503±0.04 (a)
Lignans	5.57±0.8 (a)	6.56±0.7 (a)	7.84±0.8 (b)
Pinoresinol	5.57±0.8 (a)	6.56±0.7 (a)	7.84±0.8 (b)

Table 2. Different letters within the same row mean significant differences (p-value < 0.05) according to the Kruskal-Wallis test.

3.2. Crushing temperature study

The results of the study of the phenolic profile at different crushing temperatures can be seen in **Table 3**. Total polyphenols decrease with crushing temperature, a significant difference is observed between 10 °C and 18 °C. The groups follow the same trend although there are compounds, such as oleocanthal, which concentration increases.

	Crushing temperatura (mg/kg)		
Group/Compound	10 °C	18 °C	25 °C
Total Polyphenols	361±15 (a)	337±17 (b)	331±20 (b)
Secoiridoids	346±14 (a)	325±17 (b)	321±19 (b)
Elenolic acid	191±8 (a)	175±10 (a)	171±4 (b)
Hydroxyelenolic acid	7.44±0.5 (a)	6.06±0.5 (b)	6.13±0.3 (b)
Hydroxyoleuropein aglycone	2.14±0.08 (a)	1.72±0.1 (b)	1.74±0.08 (b)
Ligstroside aglycone	10.0±0.2 (a)	8.96±0.5 (b)	8.47±0.1 (c)
Methyloleuropein aglycone	0.865±0.009 (a)	0.867±0.006 (a)	0.867±0.004 (a)
Oleacein	16.5±0.6 (a)	29.7±2 (b)	35.2±1 (c)
Oleocanthal	0.953±0.09 (a)	2.05±0.1 (b)	2.42±0.2 (c)
Oleuropein aglycone	117±2.96 (a)	97.2±5 (b)	95.1±2 (b)
Phenolic alcohols	0.789±0.1(a)	0.450±0.05 (b)	0.393±0.04 (b)
Hydroxytyrosol	0.585±0.04 (a)	0.279±0.03 (b)	0.288±0.04 (b)
Hydroxytyrosol acetate	0.204±0.02 (a)	0.157±0.01 (b)	0.104±0.01 (c)
Flavones	3.74±0.2 (a)	3.04±0.2 (b)	1.91±0.09 (c)
Apigenin	2.85±0.2 (a)	2.28±0.1 (b)	1.43±0.07 (c)
Luteolin	0.894±0.09 (a)	0.666±0.04 (a)	0.486±0.05 (c)
Phenolic acids	2.08±0.09 (a)	1.79±0.2 (b)	1.65±0.009 (b)
m-coumaric acid	1.49±0.04 (a)	1.43±0.04 (ab)	1.38±0.03 (b)
p-coumaric acid	0.596±0.06 (a)	0.325±0.02 (b)	0.270±0.04 (b)
Lignans	8.06±1 (a)	6.99±0.8 (ab)	5.99±0.8 (b)
Pinoresinol	8.06±1 (a)	6.99±0.8 (ab)	5.99±0.8 (b)

Table 3. Different letters within the same row mean significant differences (p-value <0.05) according to the Kruskal-Wallis test.

4. Discussion

4.1. Ripening study

In the sense of the effect of ripening stage to the amount of phenols, there is a decrease in phenolic concentration (the final value corresponds to 44% less than the initial one) with the ripening index. This could be caused by the endogenous enzymes present in the olive drupes, such as esterases, polyphenol oxidases and β-glucosidases, which degrade the phenols during the maturation process (7,8,23). These results are in agreement with other papers which have seen the

same effect (23–26). One of these works with Frantoio and Manzanilla EVOO shows a gradual decrease from the first ripening index to the fifth (24). Although, others indicate that this effect does not begin until the 2.5-3 ripening index (23).

The concentration of secoiridoids is greatly affected with the harvest time (7,27,28). All of them decrease except in the case of the hydroxyoleuropein aglycone that increases, that may be due to the fact that the enzymes degrade the oleuropein and the ligstroside increasing the concentration of the aglycone. This group is the most present in the EVOO and many benefits are attributed to it. The role of oleocanthal on Alzehimer's disease should be emphasized, it has been shown that prevents the accumulation of β-amyloid proteins and facilitate their elimination both *in vitro* and *in vivo* (29,30).

Speaking of phenolic alcohols, the concentration of hydroxytyrosol decreases (28), while that of hydroxytyrosol acetate increases. This can be explained by the fact that the acetate form can be found as a part of more complex compounds like oleacein, oleuropein, and verbascoside and when they are degraded the free acetate form increases (31). In the case of hydroxytyrosol, which is also a degradation product, its decrease in its concentration could be because it is more labile than acetate. This fact is interesting since the EFSA consider that to bear the claim (for protection of LDL particles from oxidative damage) people must consume at least 5 mg of hydroxytyrosol and its derivatives (12). Therefore, it would not be wise to increase the maturity index since it decreases hydroxytyrosol much more than acetate increases.

In the case of flavones, an upward trend is observed that is comparable with other studies such as that of Bengana et al. (2013) (32). In the droplet we find the glycosidic forms and during the maturation β-glucosidase breaks down the bond with the sugar, making the compounds more soluble in oil. Then, during the extraction the flavones are more retained in the oil than the ones that are in the water, compared to the previous maturity stage. Similar results are found in other studies (33,34). Although looking specifically at the compounds other studies found that luteolin increased and apigenin was maintained with Hojiblanca, Picual and Picudo (35) or even decreased with Arbequina variety (36). The differences in behavior during maturation can be attributed to the fact that they are different varieties, therefore, the composition and the endogenous enzymes may also change (13,37).

The group of phenolic acids shows a decrease between the first and second stage; although the concentration decreases, it varies very little. This effect has already been observed in other evaluations (34). Against, there are studies indicating that there could be an increase in these compounds which would be explained by hydrolytic enzymes on the droplet (28,38).

In the case of lignans if the first stage is compared with the last pinoresinol increases a 41% approximately. There are two different compounds: acetoxypinoresinol and pinoresinol in oil (39), it is possible that acetoxy degrades and forms pinoresinol and therefore it increases. Acetoxypynoresinol is not within the limits of detection, so it is not in the results, but it may be degraded and in doing so pinoresinol is formed, which is quantified. It is a very stable compound, because of this it is not lost during ripening. This group seems to have an inhibitory activity in front of the growth of cancer cells, in addition to having antiestrogenic effects (40,41).

4.2. Crushing temperature study

The amount of total polyphenols decreases when the temperature is increased (specifically about 8% between 10 °C and 25 °C), fact that has already been observed previously in a different study (42). It has been observed that the greatest change occurs between 10 °C and 18 °C, while from this to 25 °C there is no significant difference. Instead, Caponio et al. (2002) evaluated olives of Cima di Bitonto cultivar and obtain that with three crusher temperature (12 °C, 16 °C and 20 °C) gradually decreased the phenol content. If the temperature to which the paste is subjected during crushing varies, in the

next step, which is malaxation, the paste will have a certain temperature. Depending on whether it is colder or hotter, it can trigger a series of variations such as changing the phenolic profile or having more or less enzymatic activity (like polyphenol oxidase and peroxidase) and chemical activity during the process (43). As seen in other studies in which the temperature of the malaxation stage is controlled the greatest losses in experiments were found when temperature was raised from 25 °C to 35 °C (44). Furthermore, it cannot be ignored that the quality of the oil depends on the composition, its oxidative stability and its sensorial and chemical characteristics, lower malaxation temperatures avoided any negative organoleptic attributes and undesired chemical compounds formation (45).

In the case of secoiridoids, at high temperatures the oleuropein aglycone and ligstroside aglycone are degraded, resulting in oleacein and oleocanthal possibly due that during crushing and malaxation, β-glucosidases and esterases are released from the flesh and the seed in the pit and they can interact easily with the phenols, depending on the temperature they are more active and stable. Hence, oleacein and oleacanthal increase at higher temperatures because they are formed by the degradation of oleuropein and ligstroside aglycones, thanks to the increase in temperature the kinetics of the reaction increases (46). It would be important to find an equilibrium since high concentration of oleocanthal and oleacein might be desirable due to their health properties (47); however other polyphenols may be degraded.

The group of phenolic alcohols, both hydroxytyrosol and hydroxytyrosol acetate, decreased by 51% and 49% respectively between 10 °C and 25 °C. While hydroxytyrosol undergoes a great change between temperatures of 10 °C and 18 °C, the acetate form does it progressively. EFSA has recognized a claim on hydroxytyrosol and its derivatives. Therefore, by decreasing the concentration of these, is also decreasing the probability that the final consumer can reach the established dose and that a protection of LDL particles from oxidative damage (12).

In the case of flavones, there is a difference of 49% between the first and last crushing temperature EVOOs. Being the lowest temperature where more quantity is obtained. If we compare it with another study that works with Ayvalik oils those who are obtained at 27 °C have a higher concentration in luteolin compared to oils obtained at malaxation temperatures of 37 °C and 47 °C (48).

The effect of the group of phenolic acids closely resembles that of total polyphenols since the decrease is observed between the temperature of 10 °C and 18 °C. This fact is due to the fact that at such low temperature phenolic compounds do not degrade or degrade very little, on the other hand the degradation increases substantially at 18 °C and that between 18 °C and 25 °C this difference is no longer observed.

Finally, although a less change can be observed lignans also decrease with temperature, which could be compared with another study that indicates that the concentration of this group is maintained with the change in temperature during malaxation (49).

5. Conclusions

Total polyphenols when the crushing temperature is increased, they decrease, but individual polyphenols like oleocanthal or oleacein increase. Total polyphenols decrease with fruit maturity. Instead, flavones, hydroxyoleuropein aglycone, hydroxytyrosol acetate and lignans increase. Then for obtaining a "Corbella" EVOO rich in phenolic compounds early harvest and low crushing temperature are necessary. On the contrary, if the concentration of some individuals phenols as oleocanthal or oleacein is desired, due to their health properties, a moderate crushing temperature would be required.

In consideration of these results, the aim of future research could be to study the enzymatic activity according to maturity index or how the temperature affects fruits collected at different times.

Acknowledgments: This research was funded by CICYT [AGL2016- 75329-R]. The author wants to thank Semen Cardona company for the olives and IRTA Mas Bové for the processing of the EVOO.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Rotondi A, Bendini A, Cerretani L, Mari M, Lercker G, Toschi TG. Effect of olive ripening degree on the oxidative stability and organoleptic properties of cv. Nostrana di Brisighella extra virgin olive oil. J Agric Food Chem. 2004;52(11):3649–54.
2. Kalogeropoulos N, Kaliora AC. Effect of Fruit Maturity on Olive Oil Phenolic Composition and Antioxidant Capacity [Internet]. Olive and Olive Oil Bioactive Constituents. AOCS Press; 2015. 123–145 p. Available from: http://dx.doi.org/10.1016/B978-1-63067-041-2.50011-2
3. European Commission. European Commission. European Commission Regulation EC No. 432/2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health. Off. J. Eur. Union L 20. Off J Eur Union L 282. 2012;56(1924):43–5.
4. Boneza MM, Niemeyer ED. Cultivar affects the phenolic composition and antioxidant properties of commercially available lemon balm (Melissa officinalis L.) varieties. Ind Crops Prod [Internet]. 2018;112(January):783–9. Available from: https://doi.org/10.1016/j.indcrop.2018.01.003
5. Correa Fuentes JDP, Martín Gordo D, Pinzón Reyes SP, Cárdenas González OE. Efecto del estado de maduración sobre el contenido de polifenoles totales en frutos de Solanum marginatum. I3+. 2017;3(2):86.
6. GóMez-Rico A, Inarejos-García AM, Salvador MD, Fregapane G. Effect of malaxation conditions on phenol and volatile profiles in olive paste and the corresponding virgin olive oils (olea europaea L. Cv. cornicabra). J Agric Food Chem. 2009;57(9):3587–95.
7. Ben Brahim S, Kelebek H, Ammar S, Abichou M, Bouaziz M. LC–MS phenolic profiling combined with multivariate analysis as an approach for the characterization of extra virgin olive oils of four rare Tunisian cultivars during ripening. Food Chem [Internet]. 2017;229:9–19. Available from: http://dx.doi.org/10.1016/j.foodchem.2017.02.025
8. Clodoveo ML, Hbaieb RH, Kotti F, Mugnozza GS, Gargouri M. Mechanical strategies to increase nutritional and sensory quality of virgin olive oil by modulating the endogenous enzyme activities. Compr Rev Food Sci Food Saf. 2014;13(2):135–54.
9. Miho H, Moral J, López-González MA, Díez CM, Priego-Capote F. The phenolic profile of virgin olive oil is influenced by malaxation conditions and determines the oxidative stability. Food Chem [Internet]. 2020;314(August 2019):126183. Available from: https://doi.org/10.1016/j.foodchem.2020.126183
10. Ceci LN, Mattar SB, Carelli AA. Chemical quality and oxidative stability of extra virgin olive oils from San Juan province (Argentina). Food Res Int [Internet]. 2017;100(June):764–70. Available from: http://dx.doi.org/10.1016/j.foodres.2017.07.074
11. Dag A, Kerem Z, Yogev N, Zipori I, Lavee S, Ben-David E. Influence of time of harvest and maturity index on olive oil yield and quality. Sci Hortic (Amsterdam) [Internet]. 2011;127(3):358–66. Available from: http://dx.doi.org/10.1016/j.scienta.2010.11.008
12. Panel E, Nda A. Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage (ID 1333, 1638, 1639, 1696, 2865), maintenance of normal blood HDL cholesterol concentrations (ID 1639), mainte. EFSA J. 2011;9(4):1–25.
13. JH Y, C W, M S. Effects of Agronomic and Oil Processing Conditions on Natural Antioxidative Phenolics in Olive (Oleaeuropaea L .). Austin J Nutr Food Sci. 2014;2(9).
14. Rallo L, Díez CM, Morales-Sillero A, Miho H, Priego-Capote F, Rallo P. Quality of olives: A focus on

agricultural preharvest factors. Sci Hortic (Amsterdam) [Internet]. 2018;233(July 2017):491–509. Available from: https://doi.org/10.1016/j.scienta.2017.12.034
15. Bejaoui MA, Beltrán G, Sánchez-Ortiz A, Sánchez S, Jiménez A. Continuous high power ultrasound treatment before malaxation, a laboratory scale approach: Effect on virgin olive oil quality criteria and yield. Eur J Lipid Sci Technol. 2016;118(2):332–6.
16. Ninot, A.; Howad A. RA. Varietats locals d'olivera Estudi a la Catalunya Central, Barcelona i el Penedès. Biodiversitat [Internet]. 2015;4. Available from: http://www.agrocultura.org/wp-content/uploads/2017/06/olivera.pdf
17. A Catalunya G de C. L'oli d'oliva a catalunya. RuralCat. 2015;80:28.
18. Tous, J.; Romero A. Variedades del olivo. Fundación. B 'La Caixa'-AEDOS., editor. 1993. 172 p.
19. López-Yerena A, Lozano-Castellón J, Olmo-Cunillera A, Tresserra-Rimbau A, Quifer-Rada P, Jiménez B, et al. Effects of organic and conventional growing systems on the phenolic profile of extra-virgin olive oil. Molecules. 2019;24(10).
20. Capriotti AL, Cavaliere C, Crescenzi C, Foglia P, Nescatelli R, Samperi R, et al. Comparison of extraction methods for the identification and quantification of polyphenols in virgin olive oil by ultra-HPLC-QToF mass spectrometry. Food Chem [Internet]. 2014;158:392–400. Available from: http://dx.doi.org/10.1016/j.foodchem.2014.02.130
21. Suárez M, Macià A, Romero MP, Motilva MJ. Improved liquid chromatography tandem mass spectrometry method for the determination of phenolic compounds in virgin olive oil. J Chromatogr A. 2008;1214(1–2):90–9.
22. Sánchez de Medina V, Miho H, Melliou E, Magiatis P, Priego-Capote F, Luque de Castro MD. Quantitative method for determination of oleocanthal and oleacein in virgin olive oils by liquid chromatography–tandem mass spectrometry. Talanta [Internet]. 2017;162(September 2016):24–31. Available from: http://dx.doi.org/10.1016/j.talanta.2016.09.056
23. Peres F, Martins LL, Ferreira-Dias S. Influence of enzymes and technology on virgin olive oil composition. Crit Rev Food Sci Nutr [Internet]. 2017;57(14):3104–26. Available from: https://doi.org/10.1080/10408398.2015.1092107
24. Alowaiesh B, Singh Z, Fang Z, Kailis SG. Harvest time impacts the fatty acid compositions, phenolic compounds and sensory attributes of Frantoio and Manzanilla olive oil. Sci Hortic (Amsterdam) [Internet]. 2018;234(November 2017):74–80. Available from: https://doi.org/10.1016/j.scienta.2018.02.017
25. Matos LC, Pereira JA, Andrade PB, Seabra RM, Oliveira MBPP. Evaluation of a numerical method to predict the polyphenols content in monovarietal olive oils. Food Chem. 2007;102(3):976–83.
26. Gómez-Rico A, Salvador MD, La Greca M, Fregapane G. Phenolic and volatile compounds of extra virgin olive oil (Olea europaea L. Cv. Cornicabra) with regard to fruit ripening and irrigation management. J Agric Food Chem. 2006;54(19):7130–6.
27. Lukić I, Krapac M, Horvat I, Godena S, Kosić U, Brkić Bubola K. Three-factor approach for balancing the concentrations of phenols and volatiles in virgin olive oil from a late-ripening olive cultivar. LWT - Food Sci Technol. 2018;87:194–202.
28. Amanpour A, Kelebek H, Selli S. LC-DAD-ESI-MS/MS–based phenolic profiling and antioxidant activity in Turkish cv. Nizip Yaglik olive oils from different maturity olives. J Mass Spectrom. 2019;54(3):227–38.
29. Abuznait AH, Qosa H, Busnena BA, El Sayed KA, Kaddoumi A. Olive-oil-derived oleocanthal enhances β-amyloid clearance as a potential neuroprotective mechanism against Alzheimer's disease: In vitro and in vivo studies. ACS Chem Neurosci. 2013;4(6):973–82.
30. Batarseh YS, Mohamed LA, Al Rihani SB, Mousa YM, Siddique AB, El Sayed KA, et al. Oleocanthal ameliorates amyloid-β oligomers' toxicity on astrocytes and neuronal cells: In vitro studies. Neuroscience [Internet]. 2017;352:204–15. Available from: http://dx.doi.org/10.1016/j.neuroscience.2017.03.059
31. Vilaplana-Pérez C, Auñón D, García-Flores LA, Gil-Izquierdo A. Hydroxytyrosol and Potential Uses in Cardiovascular Diseases, Cancer, and AIDS. Front Nutr. 2014;1(October):1–11.
32. Bengana M, Bakhouche A, Lozano-Sánchez J, Amir Y, Youyou A, Segura-Carretero A, et al. Influence of olive ripeness on chemical properties and phenolic composition of Chemlal extra-virgin olive oil. Food Res Int [Internet]. 2013;54(2):1868–75. Available from:

http://dx.doi.org/10.1016/j.foodres.2013.08.037
33. de Torres A, Espínola F, Moya M, Alcalá S, Vidal AM, Castro E. Assessment of phenolic compounds in virgin olive oil by response surface methodology with particular focus on flavonoids and lignans. LWT - Food Sci Technol [Internet]. 2018;90(December 2017):22–30. Available from: https://doi.org/10.1016/j.lwt.2017.12.003
34. Jiménez B, Sánchez-Ortiz A, Lorenzo ML, Rivas A. Influence of fruit ripening on agronomic parameters, quality indices, sensory attributes and phenolic compounds of Picudo olive oils. Food Res Int [Internet]. 2013;54(2):1860–7. Available from: http://dx.doi.org/10.1016/j.foodres.2013.08.016
35. Brenes M, García A, García P, Rios JJ, Garrido A. Phenolic compounds in Spanish olive oils. J Agric Food Chem. 1999;47(9):3535–40.
36. Artajo LS, Romero MP, Motilva MJ. Transfer of phenolic compounds during olive oil extraction in relation to ripening stage of the fruit. J Sci Food Agric. 2006;86(4):518–27.
37. Bilušić T, Žanetić M, Ljubenkov I, Generalić Mekinić I, Štambuk S, Bojović V, et al. Molecular characterization of Dalmatian cultivars and the influence of the olive fruit harvest period on chemical profile, sensory characteristics and oil oxidative stability. Eur Food Res Technol. 2018;244(2):281–9.
38. Baccouri O, Guerfel M, Baccouri B, Cerretani L, Bendini A, Lercker G, et al. Chemical composition and oxidative stability of Tunisian monovarietal virgin olive oils with regard to fruit ripening. Food Chem. 2008;109(4):743–54.
39. Olmo-Cunillera A, López-Yerena A, Lozano-Castellón J, Tresserra-Rimbau A, Vallverdú-Queralt A, Pérez M. NMR spectroscopy: a powerful tool for the analysis of polyphenols in extra virgin olive oil. J Sci Food Agric. 2020;100(5):1842–51.
40. Fini L, Hotchkiss E, Fogliano V, Graziani G, Romano M, De Vol EB, et al. Chemopreventive properties of pinoresinol-rich olive oil involve a selective activation of the ATM-p53 cascade in colon cancer cell lines. Carcinogenesis. 2008;29(1):139–46.
41. Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H. Identification of lignans as major components in the phenolic fraction of olive oil. Clin Chem. 2000;46(7):976–88.
42. Caponio F, Pasqualone A, Gomes T, Catalano P. Use of HPSEC analysis of polar compounds to assess the influence of crushing temperature on virgin olive oil's quality. Eur Food Res Technol. 2002;215(6):534–7.
43. Taticchi A, Esposto S, Veneziani G, Urbani S, Selvaggini R, Servili M. The influence of the malaxation temperature on the activity of polyphenoloxidase and peroxidase and on the phenolic composition of virgin olive oil. Food Chem [Internet]. 2013;136(2):975–83. Available from: http://dx.doi.org/10.1016/j.foodchem.2012.08.071
44. Angerosa F, Mostallino R, Basti C, Vito R. Influence of malaxation temperature and time on the quality of virgin olive oils. Food Chem. 2001;72(1):19–28.
45. Boselli E, Di Lecce G, Strabbioli R, Pieralisi G, Frega NG. Are virgin olive oils obtained below 27 °C better than those produced at higher temperatures? LWT - Food Sci Technol [Internet]. 2009;42(3):748–57. Available from: http://dx.doi.org/10.1016/j.lwt.2008.09.018
46. Abbattista R, Losito I, De Ceglie C, Basile G, Calvano CD, Palmisano F, et al. Structural characterization of the ligstroside aglycone isoforms in virgin olive oils by liquid chromatography–high-resolution Fourier-transform mass spectrometry and H/Dexchange. J Mass Spectrom. 2019;54(10):843–55.
47. Lozano-Castellón J, López-Yerena A, Rinaldi de Alvarenga JF, Romero del Castillo-Alba J, Vallverdú-Queralt A, Escribano-Ferrer E, et al. Health-promoting properties of oleocanthal and oleacein: Two secoiridoids from extra-virgin olive oil. Crit Rev Food Sci Nutr [Internet]. 2019;0(0):1–17. Available from: https://doi.org/10.1080/10408398.2019.1650715
48. Jolayemi OS, Tokatli F, Ozen B. Effects of malaxation temperature and harvest time on the chemical characteristics of olive oils. Food Chem [Internet]. 2016;211:776–83. Available from: http://dx.doi.org/10.1016/j.foodchem.2016.05.134
49. Parenti A, Spugnoli P, Masella P, Calamai L. The effect of malaxation temperature on the virgin olive oil phenolic profile under laboratory-scale conditions. Eur J Lipid Sci Technol. 2008;110(8):735–41.