

# Crop year, harvest date and clone effects on fruit characteristics, chemical composition and olive oil stability from an Empeltre clonal selection grown in Aragon

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## Abstract

**BACKGROUND:** In this study, the effects of crop year, harvest date and clone on the fruit characteristics and chemical composition of Empeltre olive oils were evaluated. For this purpose, the weight and oil content of fruit and the fatty acid composition, polyphenol content and oxidative stability of the olive oil was analysed throughout ripening during three successive seasons.

**RESULTS:** The weight and moisture in the fruit, as well as the fatty acids and polyphenol content in the olive oil, were mainly affected by crop year. In contrast, the stability was strongly influenced by the harvest date. Both factors had an influence on the fruit's oil content. The clone was not a substantial component in terms of variability, although the interaction with crop year was notable for some of the characteristics. The oil content increased significantly along with the harvest date and reached maximum values in the last period (44.9%). Conversely, stability and polyphenols decreased significantly (depending on the year, by 30–70%) from October to December, reaching the highest mean values between 1 October and 10 November (15.5 h; 500 mg caffeic acid kg<sup>-1</sup>). Oleic acid and monounsaturated/polyunsaturated fatty acids (MUFA/PUFA) did not show significant differences depending on the harvest date, but between years, with 2018 having the highest percentage of oleic acid (72.72%) and MUFA/PUFA (8.38).

**CONCLUSION:** Early harvesting of Empeltre olives would provide considerably more stable olive oils, regardless of the clone selected, with higher phenolic content. It would not affect the MUFA/PUFA ratio, mainly influenced by the crop year.

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**Keywords:** olive oil; Empeltre clone; crop year; harvest date; chemical composition; oxidative stability

## INTRODUCTION

The olive tree (*Olea europaea* L.) is economically one of the most important crops in the Mediterranean area, especially in Spain, the world's leading producer and exporter of olive oil.<sup>1</sup> Health benefits of virgin olive oil consumption are attributed to its high content in monounsaturated fatty acids (MUFA), mainly oleic acid, as well as minor components such as phenolic compounds, squalene, tocopherols and sterols.<sup>2</sup> On the other hand, fatty acid composition and phenolic compounds are directly related to the chemical stability of olive oil in terms of shelf-life and resistance to lipid oxidation.<sup>3–6</sup> The quality and chemical composition of virgin olive oil depend on agronomic and environmental factors such as cultivar, growing area, seasonal conditions and fruit ripening, among others.<sup>7,8</sup> For example, as olives progressively ripen, a decrease in polyphenol content and an increase in polyunsaturated fatty acids (PUFA) can be observed, thereby reducing the oil's shelf life by reducing its stability.<sup>5,9,10</sup>

Empeltre is one of the main olive cultivars in Spain. It is mainly harvested in the northeast of Spain, with an area of 70 000 ha.<sup>7</sup>

In Aragon (the sixth-largest olive-oil-producing region in Spain), 67% of the olive surface area is planted with Empeltre, which makes it the Spanish region with the most hectares of this cultivar. Empeltre olive oils are protected for their uniqueness under six protected designations of origin (PDO), two of which are in Aragon.

Commercial interest in Empeltre cultivar is high because of its good agronomic behaviour, with a very early ripening pattern, good yield and high productivity.<sup>7,11,12</sup> Empeltre olives have a twofold potential commercial use: either as black table olives or as olive oils.<sup>13–16</sup> As a consequence, the fruits are usually harvested

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when they are very ripe, which means that Empeltre olive oils are known for ripe fruitiness, low phenolic content, low bitterness and medium–low oxidative stability.<sup>13,17,18</sup> Few studies have been carried out on the evolution of the chemical composition of Empeltre olive oils according to their ripeness during several crop years.<sup>19</sup> Most studies in this area have been conducted with very ripe olives. Currently, certain high-quality Empeltre olive oils are being obtained with olives with a low ripening index. Further information about the evolution of olive oil quality and chemical composition under such conditions would be necessary. These trials could be of interest due to the rise in sales of Empeltre olive oils obtained from low- to medium-ripe olives.

In Spain, during the 1998–2002 periods, a clonal pre-selection for the genetic improvement of Empeltre cultivar was carried out, with the participation of the six regions where Empeltre traditionally grows (Aragon, Catalonia, La Rioja, Navarra, Valencia and the Balearic Islands). Sixteen clones were selected,<sup>20,21</sup> then propagated by cuttings, and planted in two comparative trials in two different locations: Gandesa (Catalonia) and Alcañiz (Aragon).<sup>22,23</sup> Studies on the variability of fruit and olive oil characteristics were carried out, taking into account the influence of the region and year of production, and harvesting olives with ripening indices close to four. The effect of ripening was not evaluated in those clonal studies, although several of them reported higher variability for certain elaiotechnical characteristics including olive parameters and olive oil quality.<sup>5,10,24,25</sup>

The aim of the current study was to evaluate the influence of harvest date, crop year and clone, and of the interactions among them, on fruit characteristics and on fatty acid composition, phenolic content and oxidative stability in olive oil. For this purpose, a selection of Empeltre clones from Aragon was studied.

## MATERIALS AND METHODS

### Plant material

Eight of the 16 clones planted in 2004 as part of the Empeltre clonal selection comparative trials were selected. The olive trees were grown in an olive orchard belonging to the Government of Aragon, located in Alcañiz, Teruel (NW Spain; 41° 03' 27" N, 0° 08' 36" W), under identical agronomic and pedoclimatic conditions. The trial was randomly designed and arranged with an 8 × 6 m frame (three trees/clone), in clay loam soil with a drip irrigation system. The assays were conducted in the course of the 2017, 2018 and 2019 seasons. Table 1 shows the monthly temperature and rainfall data recorded for those 3 years.

Aragon was the region of origin of the eight clones used in the study, with the region's three provinces represented by different towns. Clone 3 (Valderrobres), clone 5 (Cretas) clone 6 (Calanda), clone 7 (Calaceite) and clone 8 (La Codoñera) were from the province of Teruel. Clones 1 and 2 (Barbastro) were from the province of Huesca, and clone 4 (Caspé) was from the province of Zaragoza. Clone 5 was identified as a standard in the clonal pre-selection trials. A previous microsatellite DNA study did not identify any differences among clones 1, 5 (standard) and 8,<sup>23</sup> as a consequence, olives of these three clones were grouped together and identified as clone Std.

Olives from clones 2, 3, 4, 6, 7 and Std (4 kg) were randomly hand-picked around the tree for each harvest date, at fortnightly intervals from October to December. After collection, the samples were immediately taken to the laboratory and processed.

### Olive fruit assays and olive oil extraction

One hundred olives from each sample were randomly selected to determine fresh fruit weight (FW) and the olive ripeness index (RI) based on colour changes of the skin and flesh.<sup>26</sup>

Fruit moisture content (M) and oil content of the fruit, the latter expressed as a percentage of the weight of fresh (OCFW) and dry olive paste (OCDW), were determined using a near-infrared (NIR) analyser (FoodScan Lab, type 78 800, Foss, Runcorn, UK).

Olive oil was extracted using the Abencor system (MC2, Ingeniería y Sistemas SL, Seville, Spain).<sup>27</sup> Olive fruits were milled at 3000 rpm by a 3 mm sieve stainless hammer mill, without addition of water or any other adjuvants, and the resulting olive paste was malaxed at 30 °C for 30 min. Olive oil was then separated by centrifugation at 3500 rpm for 1 min.

The oil obtained after decanting was filtered through cellulose paper and stored in amber glass bottles under nitrogen atmosphere at –20 °C until analysis.

### Olive oil analysis

#### Fatty acid composition

Fatty acid methyl esters (FAME) were determined according to the official EU method,<sup>28</sup> by cold transmethylation with sodium hydroxide in 2 mol L<sup>-1</sup> ethanol, followed by gas chromatographic analysis (GC). An Agilent chromatograph (7890 N, Agilent, Santa Clara, CA, USA) with an SP-2380 60 m × 0.25 mm inner diameter × 0.2 µm film thickness capillary column (Supelco, Bellefonte, PA, USA) was used with helium as a carrier gas (with a flow of 1.2 mL min<sup>-1</sup>). Oven temperature was 170 °C, for 30 min, increasing by 5 °C min<sup>-1</sup> up to 200 °C. The flame ionization detector (FID) and split/spitless injector temperatures were 260 and 250 °C, respectively.

#### Total phenol content

For extraction and quantification of polyphenols (TP), the method described by Vázquez Roncero<sup>29</sup> was used. Phenolic compounds were isolated from the oil (10 g) by triple extraction with 20 mL of a methanol–water mixture (60:40, v/v) after dissolving the oil in hexane (50 mL). The absorbance of the resulting solution after reaction of the hydroalcoholic extract with Folin–Ciocalteu reagent in basic medium was measured at 725 nm using a UV–visible spectrophotometer (Specord 205, Analytik, Jena, Germany). Total phenol content was expressed as mg caffeic acid kg<sup>-1</sup> oil.

#### Oxidative stability

The oils' resistance to rancidity, expressed as the oxidation induction time (h), was measured by the Rancimat method<sup>30</sup> using a Rancimat 743 apparatus (Metrohm AG, Herisau, Switzerland). The oil samples (3 g), subjected to forced oxidation, were heated to 120 °C and an air flow of 20 L h<sup>-1</sup> was passed through.

### Statistical analysis

First, a descriptive analysis was carried out to obtain information on all the results of the study generated in the Empeltre cultivar in a specific locality: Alcañiz (Aragon). All parameters were determined in duplicate.

Univariate factorial analysis of variance (three-way ANOVA) was used to evaluate the effect of clone, crop year and fruit ripeness (expressed as harvest date) on fruit characteristics, oil content and the oil's physicochemical characteristics. Interactions among the effects were also examined. To assess the effect of ripening, the six harvest dates were grouped into two clusters: early harvest

**Table 1.** Weather data in Alcañiz (Teruel) for 2017, 2018 and 2019 crop seasons. Monthly air temperatures (mean, maximum and minimum) and monthly and annual rainfall (mm)

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
<i>Crop year 2017</i>													
$T^a$ mean (°C)	5.5	8.9	12.3	14.4	19.6	24.7	25.4	24.8	19.1	17.1	9.3	6.6	
$T^a$ max (°C)	10.3	14.9	19.7	22.5	28.0	33.0	33.9	32.9	26.1	24.9	16.0	12.0	
$T^a$ min (°C)	1.3	3.7	5.3	6.0	11.2	16.7	17.5	17.4	12.4	10.2	3.7	1.5	
$P$ (mm)	12	30	29	9	24	52	20	28	9	4	3	4	223
<i>Crop year 2018</i>													
$T^a$ mean (°C)	8.5	6.3	10.4	13.8	17.0	22.1	26.3	25.5	22.6	15.4	10.5	8.1	
$T^a$ max (°C)	13.5	10.9	16.2	20.8	24.2	29.0	34.5	33.5	30.1	21.5	15.5	13.0	
$T^a$ min (°C)	3.8	1.8	5.0	7.1	10.7	15.5	18.6	18.3	15.6	9.7	6.3	3.7	
$P$ (mm)	42	32	30	75	67	35	27	24	18	102	43	14	508
<i>Crop year 2019</i>													
$T^a$ mean (°C)	6.1	8.8	12.2	13.4	17.2	23.6	26.7	25.9	21.1	17.0	10.9	8.6	
$T^a$ max (°C)	11.2	16.8	19.8	20.1	24.7	32.1	35.2	34.0	28.8	23.6	15.9	13.5	
$T^a$ min (°C)	1.6	2.0	4.9	7.4	9.8	14.0	18.3	17.9	13.8	10.8	6.3	4.5	
$P$ (mm)	15	3	7	28	28	4	14	8	14	38	33	25	216

(1 October to 10 November) and late harvest (11 November to 15 December). Duncan's test ( $P < 0.05$ ) was used to determine differences between the mean values for clones and crop years, while a  $t$ -test for independent groups was applied to each date cluster. Clones 4 and 7 were not taken into account in the three-way ANOVA, as for those varieties there was only 1 year of results, but they were included in the remainder of the statistical analysis.

To study the evolution of OCDW, C18:2, TP and OxStb according to harvest date in each crop year, one-way ANOVA and post hoc Duncan's test ( $P < 0.05$ ) were used. Graphs were constructed with Excel 2010.

Exploratory principal component analysis (PCA) and Pearson's correlation were used to examine the relationships between the parameters analysed in olive fruit and olive oils, and to determine which attributes provided the main contribution to the differences between groups (by clone, year or harvest date).

Statistical analyses were carried out using IBM SPSS Statistics 24.0 software (IBM Corp., Armonk, NY, USA).

## RESULTS AND DISCUSSION

### Descriptive analysis of results

A descriptive analysis of all the results obtained during the 3 years of study is shown in Table 2. In general, the main fatty acids were the parameters with the lowest variability. The high dispersion of the RI (40.5% coefficient of variation, CV) was due to the broad range of the degree of ripening (0.6–6.1) attained by the sampled fruits, with a mean value of 3.3. High OCDW contents were recorded, with a maximum of 54.0%, which are similar to those described by other authors<sup>20,21,23</sup> during the clonal selection of Empeltre. Regarding the chemical composition of the oils, specifically the main fatty acids, linoleic acid (C18:2) was the fatty acid with the highest variability (CV 23.03%). High values of linoleic acid (6.66–15.65%) in Empeltre were also reported by Gracia.<sup>17,19</sup> In contrast, oleic acid (C18:1) showed very low variability (CV 3.71%), with a mean content of 71.04%. Lower<sup>19,32,33</sup> and similar and/or higher contents than these have been reported in the literature.<sup>12,13,21</sup> TP presented a very wide concentration range, which produced the highest dispersion (CV 55%). The maximum value (987 mg kg<sup>-1</sup> caffeic acid) was measured in early autumn

harvest, while the minimum (85 mg kg<sup>-1</sup> caffeic acid) was measured in late harvest (December), when very low minimum temperatures were recorded (Table 1), including frost. Although Gracia and Marco<sup>19</sup> reported higher TP contents than most studies conducted on Empeltre,<sup>12,13,17,32,33</sup> the concentrations determined in our first samplings in 2017 and 2018 were even higher (Fig. 1). The harvesting of fruits with very high and very low ripening indices, in marked contrast to the referenced publications, could be the reason for the wide range of values observed. A similar phenomenon occurred with oxidative stability (OxStb), although featuring a lower dispersion (CV 25.6%). A minimum of 6.2 h was recorded for the last samples in December, and a maximum of 20.4 h at 120 °C. Harvesting the fruit at earlier dates, with lower ripening indices than those published in other studies for Empeltre cultivar,<sup>12,17,19,32,33</sup> could be the reason for the higher stability found in this study at the beginning of fruit ripening.<sup>8,10,30</sup>

### Variability factors in fruit characteristics

Significant effects and their influence on total variability in fruit characteristics can be seen in Table 3. The three-way ANOVA showed that crop year was the largest source of variability for OCFW (46.3%), M (64.9%), and FW (70.4%), followed, for the first two parameters, by harvest date. The 2017 crop showed the highest fat yield (OCFW and OCDW) and the lowest M. That year was characterized by an autumn with wider thermal oscillation, lower minimum temperatures and lower rainfall (Table 1), with meteorological conditions leading to moisture losses in the fruit.<sup>24</sup> In addition, average summer temperatures in 2017 were the lowest of the 3 years, thereby allowing for greater oil accumulation in the olives.<sup>24,38</sup> On the other hand, the FW was significantly higher in 2019. Perhaps this was due to the lower production in that year.<sup>36</sup>

The harvest date effect was the most pronounced in RI (55.7%), as in previous studies,<sup>25,39</sup> and the difference between crop years was less important (10.3%). In 2018, the RI was significantly lower than in other years. Perhaps lower average temperatures and higher rainfall in October 2018 could have slowed the fruit ripening process<sup>40</sup> during that year. On OCDW, harvest date (28.3%) was the most important effect, but other sources of variation also

**Table 2.** Descriptive statistics on the olive characteristics and oil physicochemical parameters analysed during the 3 years of the study ( $n = 78$ )

Parameter	Mean	SD	CV (%)	Min	Max
RI	3.3	1.3	40.5	0.6	6.1
OCFW (%)	19.2	4.5	23.6	11.4	33.2
M (%)	54.4	7.0	12.9	38.1	63.0
OCDW (%)	41.9	4.6	11.0	28.6	54.0
FW (g)	2.39	0.71	29.79	1.23	4.24
OxStb (h)	13.6	3.5	25.6	6.2	20.4
TP (mg kg <sup>-1</sup> caffeic acid)	388	214	55	85	987
C16:0 (%)	14.14	1.18	8.38	11.57	16.99
C18:0 (%)	1.66	0.20	12.05	1.33	2.20
C18:1 (%)	71.04	2.63	3.71	65.42	75.74
C18:2 (%)	9.74	2.24	23.03	6.66	15.65
C18:3 (%)	0.81	0.07	9.05	0.67	1.01
SFA	16.5	1.1	6.4	14.0	19.0
MUFA	73.0	2.5	3.5	67.3	77.6
PUFA	10.6	2.2	21.2	7.4	16.4
M/P	7.27	1.69	23.28	4.09	10.48

SFA, sum of saturated fatty acids; MUFA, sum of monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; M/P, MUFA/PUFA.

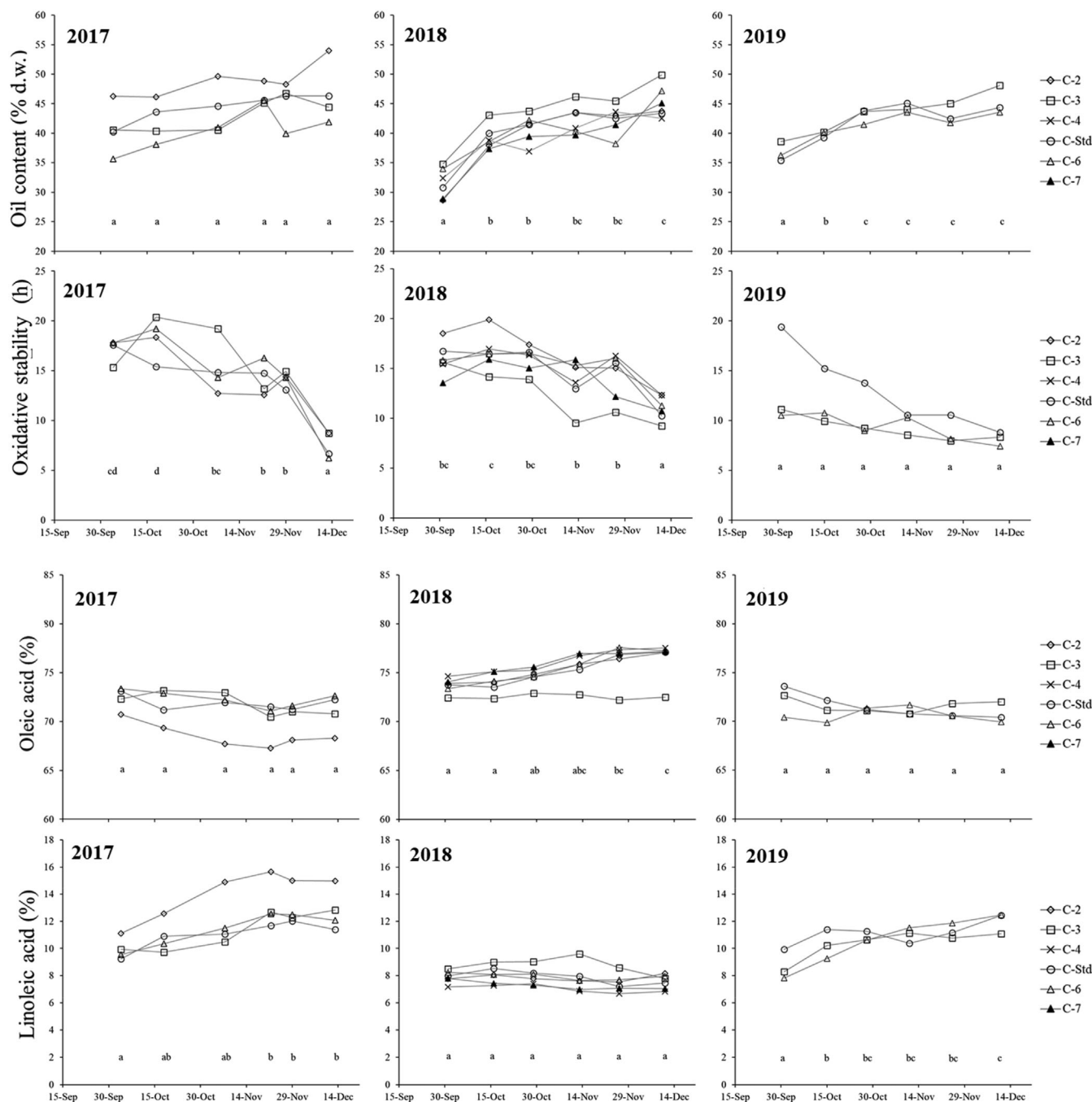
exerted a significant influence, such as the different behaviour of clones among different years (15.2%), clone (9.1%) and crop year (9.1%). Similar results were obtained by de la Rosa *et al.*<sup>25</sup> in different olives from breeding selections in which genotype was the third factor explaining variability, after date and crop year. Navas-López *et al.*<sup>34,35</sup> indicated that oil accumulation would mainly depend on abiotic factors; thus, different varieties in the same environment would reach their maximum oil content simultaneously. Although harvest date was not the main source of variability for most fruit characteristics, all differences among dates were significant. Fruit harvested in late autumn showed higher RI as well as higher yields (OCFW and OCDW) and FW, but lower M, in agreement with other authors.<sup>10,24,25,41</sup> Figure 1 shows the evolution of several parameters according to the sampling period for each crop. To determine the oil yield trend, OCDW was preferred to OCFW to avoid the effect of climatic conditions on the evolution of oil content during sampling.<sup>38</sup> A similar pattern can be observed in oil accumulation in each of the 3 years: OCDW increases as autumn progresses and slows down in the second half of November. Oil accumulation profiles are one of the criteria used to determine the optimal harvest date.<sup>19,34,35,38</sup> This pattern of OCDW accumulation was followed by each clone in 2017, although no significant differences among dates were observed in the set of clones. Perhaps the cause for this was the high dispersion of values among clones during that year.

Although the effect of clone was not important in the total variability of oil content (OCFW and OCDW) and FW, it was significant (Table 3). In contrast, it was not significant in RI and M. Clone 2 had the highest percentage of OCFW and OCDW, and clone 6 the lowest, both results similar to these described by Tous *et al.*<sup>20</sup> in a study carried out *in situ* during 1999–2002. This was not the case in the trial in Catalonia,<sup>21</sup> where the same clones did not display any differences. The low clone influence on certain fruit characteristics of the Empeltre cultivar is similar to that found in the Arbequina clonal selection carried out between 1995 and 1998.<sup>37</sup>

#### Variability factors in the chemical composition and oxidative stability of olive oils

Table 4 shows the variation in fatty acid composition, polyphenol content and oxidative stability in the olive oils. The variability observed in the main fatty acids, except for C18:3 and SFA, is mainly due to the crop year, followed by the clone  $\times$  year interaction. This interaction, important for palmitic (C16:0) (21.4%) and oleic acid (C18:1) (21.6%), is due to interannual oscillations in the acidic composition in some clones.<sup>33</sup> Further significant interactions were observed, although their effects were not as pronounced. Crop year was also the main effect on TP variation (40.7%), followed by harvest date (19.8%). Olive oils obtained from fruit harvested in 2017 displayed the highest TP (590 mg kg<sup>-1</sup> caffeic acid) and C18:2 (11.96%), but the lowest C16:0 (13.53%). M/P ratio was the lowest of the three crops (5.58). The highest percentage of C18:1 (72.72%) and the lowest of C18:2 (8.10%) were obtained in olive oils from 2018, in which the highest M/P was likewise observed (8.38). Finally, the composition of olive oils from 2019 significantly displayed the lowest TP (232 mg kg<sup>-1</sup> caffeic acid) and C18:1 (68.84%), as well as the highest C16:0 (15.22%) and C18:3 (0.86%) contents. The maximum and minimum temperature effect (Table 1) observed on C18:1 during the 3 years under study is in line with several other authors.<sup>35,42,43</sup> The high maximum and minimum temperatures recorded in 2019 (especially in summer when the fruit was developing) negatively affected the C18:1 content. On the other hand, lower temperature amplitudes in 2018 increased the relative percentage of C18:1. No relation was found between the effects of rainfall and temperature on acidic composition as described by Beltrán *et al.*<sup>24</sup> Fruit moisture in 2019 was much higher than in 2017 (Table 3). This could be the reason for the lower TP content in 2019 compared to 2017, which was the highest. Several hypotheses have been advanced regarding the influence of fruit water content on oil phenol content during crushing and malaxation of the pulp.<sup>18,45,46</sup> The 2019 crop year displayed the lowest OxStb and the lowest polyphenol content, and those compounds are directly correlated





**Figure 1.** Evolution of oil content (% dry weight, d.w.), linolenic acid (%), total polyphenols (mg kg<sup>-1</sup> caffeic acid) and oxidative stability by harvest date for the clones (C-2, clone 2; C-3, clone 3; C-4, clone 4; C-Std, clone standard; C-6, clone 6; C-7, clone 7) in the three crop years studied. Different letters indicate significant differences by sampling date for each parameter (Duncan's test, *P* < 0.05).

with stability.<sup>30,44,47</sup> In contrast, the OxStb in 2017 was not different from 2018 despite the differing amounts of TP in the oil. Perhaps the high C18:2 value in the 2017 oils could have compensated for the effect of polyphenols on OxStd<sup>17,47</sup> in that year.

Harvest date exerted a greater or lesser influence than clone type, depending on the fatty acid under study. Its influence was not significant in the case of C18:1 and M/P, but it was the main effect on the variability of linoleic acid (C18:3) (37.1%) and SFA (23.5%), in contrast to the effect observed by other authors<sup>25,40</sup>

on other varieties. The results of Deiana *et al.*<sup>40</sup> disagreed with those of other authors, although they were comparing the same varieties, and that author indicated different pedoclimatic conditions as the cause. Harvest date was also the main factor of variability of OxStab (30.5%) compared to crop year (21.1%). Differing behaviour of clones according to crop year (C × Y) was the third source of variability for OxStab as well as for TP content. This influence can be observed in Fig. 1, where TP and OxStab parameters decreased as harvest progressed in the 2017 and 2018 crop years, but not in 2019. The absence of significant

**Table 3.** Sum of squares percentages for each source of variation in the analysis of variance (three-way ANOVA) with significance levels and comparison of means by clones, crop years and cluster harvest dates for the fruit characteristics evaluated

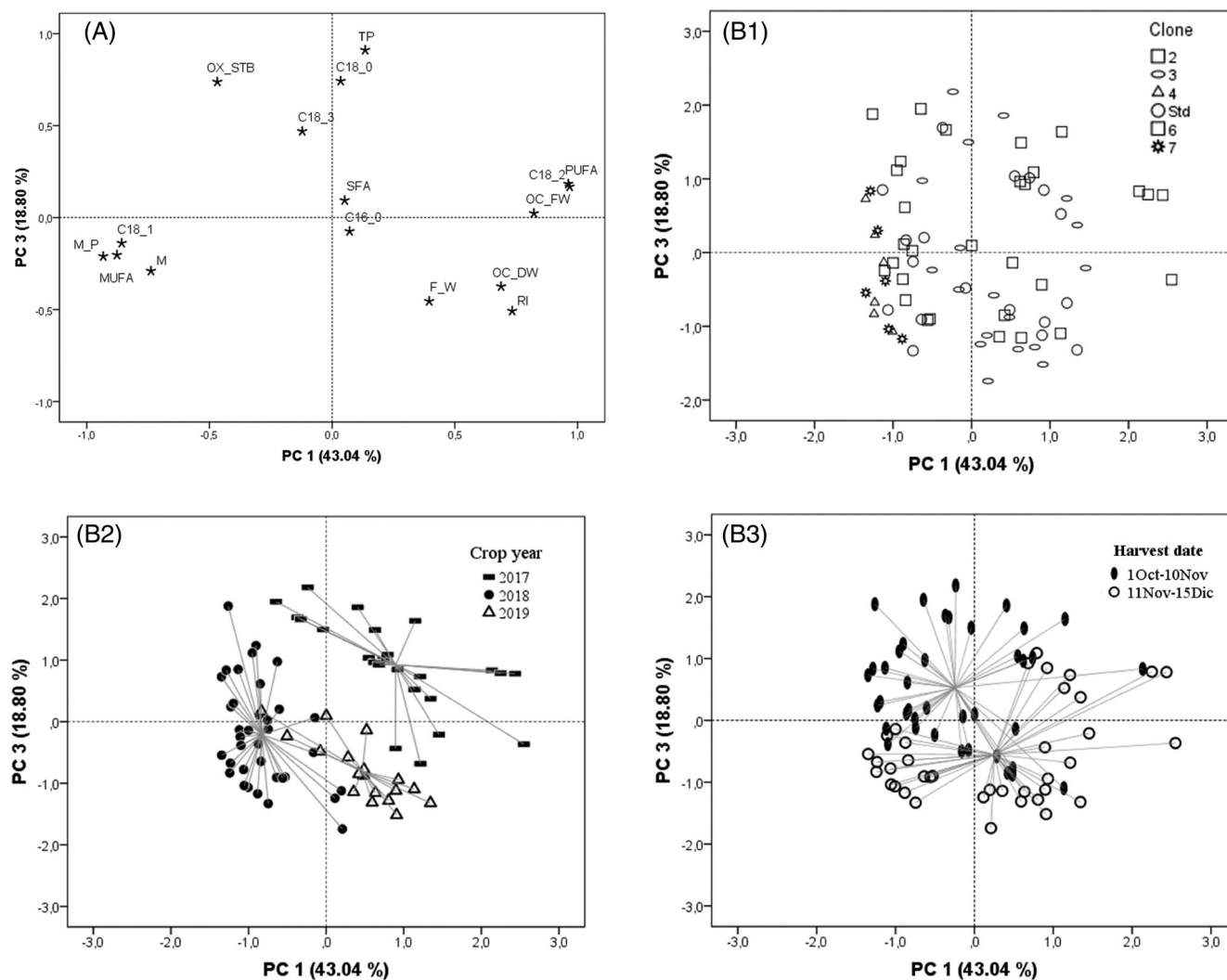
Source	RI	OCFW	OCDW	M	FW
Clone (C)	2.1 ns	8.4**	9.1*	3.1 ns	7.1***
Crop year (Y)	10.3***	46.3***	9.1**	64.9***	70.4***
Harvest date (D)	55.7***	21.1***	28.3***	8.2***	1.0*
C × Y	4.1 ns	6.3*	15.2**	2.1 ns	10.2***
C × D	0.0 ns	1.1 ns	0.0 ns	1.0 ns	0.0 ns
Y × D	1.0 ns	0.0 ns	1.0 ns	1.0 ns	1.0 ns
C × Y × D	1.0 ns	0.0 ns	1.0 ns	1.0 ns	0.0 ns
Error	25.8	16.8	36.4	18.6	10.2
<i>Clone (C)</i>					
2	3.5	22.5c	44.3a	50.1	2.2a
3	3.6	20.2b	43.4a	53.4	2.6b
6	3.1	18.0a	40.6b	55.7	2.2a
SD	3.5	19.7b	42.2ab	53.8	2.7b
<i>Crop year (Y)</i>					
2017	3.8a	24.0a	44.2a	45.8a	2.1a
2018	2.9b	17.4b	41.1b	57.8b	2.1a
2019	3.7a	17.8b	42.1b	58.1b	3.5b
<i>Harvest date (D)</i>					
1st Oct–10th Nov	2.4a	17.9a	40.2a	55.6a	2.4a
11th Nov–15th Dec	4.4b	21.9b	44.9b	51.5b	2.5b

<sup>†</sup>Significance levels: \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . Not significant: ns. Different letters in means indicate significant differences of three-way ANOVA (Duncan's test,  $P < 0.05$ ) for each parameter. Absence of letters: no significant differences between means were found.

**Table 4.** Partial sum of squares percentages for each source of variation in the analysis of variance (three-way ANOVA) with significance levels<sup>†</sup> and comparison of means by clones, crop years and cluster harvest dates for the chemical composition and oxidative stability

Source	OxStb	TP	C16:0	C18:0	C18:1	C18:2	C18:3	SFA	MUFA	PUFA	M/P
Clone (C)	4.2 <sup>ns</sup>	5.5**	10.2***	5.1***	5.9***	4.9***	14.4***	9.2***	5.9***	4.9***	3.0**
Crop year (Y)	21.1***	40.7***	29.6***	75.5***	54.9***	63.7***	9.3**	16.3***	55.4***	64.7***	69.3***
Harvest date (D)	30.5**	19.8***	14.3***	2.0***	0.0ns	5.9***	37.1***	23.5***	0.0 <sup>ns</sup>	4.9***	1.0 <sup>ns</sup>
C × Y	10.5*	8.8**	21.4***	11.2***	21.6***	8.8***	4.1ns	19.4***	19.8***	8.8***	6.9***
C × D	1.1 <sup>ns</sup>	1.1 <sup>ns</sup>	3.1*	1.0*	1.0 <sup>ns</sup>	0.0 <sup>ns</sup>	1.0 <sup>ns</sup>	4.1*	1.0 <sup>ns</sup>	0.0 <sup>ns</sup>	0.0 <sup>ns</sup>
Y × D	1.1 <sup>ns</sup>	3.3*	2.0 <sup>ns</sup>	0.0 <sup>ns</sup>	7.8***	5.9***	1.0 <sup>ns</sup>	2.0 <sup>ns</sup>	8.9***	5.9***	8.9***
C × Y × D	2.1 <sup>ns</sup>	1.1 <sup>ns</sup>	5.1*	1.0*	2.0*	2.0 <sup>ns</sup>	1.0 <sup>ns</sup>	6.1*	2.0*	2.0 <sup>ns</sup>	1.0 <sup>ns</sup>
Error	29.5	19.8	14.3	4.1	6.9	8.8	32.0	19.4	6.9	8.8	9.9
<i>Clone (C)</i>											
2	15.3	568a	13.92 ac	1.73a	70.08a	10.93a	0.80b	16.32b	71.97a	11.73a	6.58a
3	12.2	381b	14.81b	1.62b	69.99a	10.15b	0.82b	17.07a	71.97a	10.97b	6.61a
6	13.1	355b	13.77a	1.72a	70.78b	10.16b	0.86a	16.20b	72.80b	11.02b	6.72ab
SD	14.0	363b	14.29c	1.62b	70.88b	9.84b	0.79b	16.54b	72.83b	10.63b	7.06b
<i>Crop year (Y)</i>											
2017	14.4a	590a	13.53a	1.88a	69.42a	11.96a	0.81a	16.11a	71.13b	12.77a	5.58a
2018	14.8a	344b	14.16b	1.64b	72.72b	8.10b	0.80a	16.49b	74.63a	8.91b	8.38b
2019	10.6b	232c	15.22c	1.41c	68.84c	10.68c	0.86b	17.22c	71.23b	11.55c	6.17c
<i>Harvest date (D)</i>											
1 Oct–10 Nov	15.5a	500a	14.68a	1.70a	70.40	9.70a	0.86a	17.06a	72.40	10.57a	6.88
11 Nov–15 Dec	11.5b	306b	13.77b	1.63b	70.52	10.72b	0.77b	16.05b	72.47	11.49b	6.64

Significance levels: \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; ns, not significant. Different letters in means indicate significant differences of three-way ANOVA (Duncan's test,  $P < 0.05$ ) for each parameter. Absence of letters: no significant differences between means were found.



**Figure 2.** PCA biplots of correlated loadings (A) and scores for the dataset labelled with respect to clone (B.1), crop year (B.2) and harvest date (B.3).

differences in TP and OxStb between the harvest dates during 2019 may be due to the high dispersion observed among clones for each sampled point. On the other hand, the olive oils obtained from less ripe olives had the highest OxStb (15.5 h), i.e., higher values than those described by Tous *et al.*<sup>37</sup> in Empeltre, compared to those from riper olives (11.5 h). Throughout the period sampled, from 1 October to 15 December, only C18:1 maintained a constant mean content, as did its indices (MUFA and M/P), as indicated by the lack of influence of harvest date on its variability. The remainder of the analysed chemical compounds decreased as the harvest date progressed (such as TP<sup>31,38,40,46</sup> and C16:0, C18:0 and C18:3), or increased (such as C18:2). Different patterns of fatty acid evolution have been described in different cultivars throughout the ripening period<sup>24,25,31,40,46</sup> so that the ripening effect is not completely clear. For example, the evolution of C18:2 (Fig. 1) was different in 2018, when no difference among harvest dates was observed, compared to 2017 and 2019, where there was an increase in tandem with ripening.

### Olive oil differentiation

Exploratory PCA showed that 86.8% of the total variability can be explained by the first three components. The first component (43.04% of variance) was positively related to RI, oil content, C18:2

and PUFA, but negatively related to M, C18:1, M/P and MUFA. C16:0 and C18:3 fatty acids were the main chemical compounds that correlated positively with the second component, explaining 24.9% of its variability. The third component (18.8%) was explained by the parameters TP and OxStab, both correlating positively. Figure 2(A) shows the biplot of the first and third principal component scores, to better visualize the relationships between TP and OxStab with the samples.

RI correlated significantly and positively with oil content (OCDW,  $r = 0.812$ ; OCFW,  $r = 0.769$ ) and with C18:2 ( $r = 0.606$ ), but negatively with OxStab ( $r = -0.659$ ). Other authors did not find such a correlation.<sup>25</sup> OCDW showed significant correlation with M ( $r = -0.918$ ) as ripening progressed (as expected), as well as with C18:2 ( $r = 0.744$ ). FW did not correlate with oil content as indicated by certain authors,<sup>19,35,37</sup> nor with any other parameter. As expected, C18:1 and C18:2 correlated negatively ( $r = -0.875$ ) in agreement with the literature,<sup>25,35</sup> although no correlation was found between C16:0 and C18:1. Finally, OxStab correlated negatively with RI, as already indicated, and positively with TP ( $r = 0.733$ ), but not with M/P, as certain other authors have found.<sup>47</sup> The correlation between stability and polyphenols has been described in many previous studies.<sup>17,19,31,47</sup>

No differentiation between olive oils from the different clones was observed (Fig. 2B.1) as there was overlap among them due

to variability associated with the crop year and date of harvest. On the other hand, crop year (Fig. 2B.2) and harvest date (Fig. 2B.3) did differentiate among the olive oils. In Fig. 2(B.2), PC 1 shows olive oils from 2018 on the left due to higher C18:1, M/P ratio and OxStab. PC 3 differentiates the 2017 oils from the 2019 oils by their higher TP content and OxStab, placing the former in the upper zone. Biplot 2.B.3 shows the separation of early-harvested oils from the rest, as they are located in the upper part of PC 3, the area of oils with greater stability and high polyphenol content. On the other hand, fruit harvested in early autumn tends to contain less OCDW than fruit harvested in the second half of November or December.

## CONCLUSIONS

The oil content of the fruit of Empeltre clones depends on the crop year and the date of harvest. In olive oils, our results show that fatty acids such as oleic and linoleic acid, along with polyphenol content, are principally affected by the crop year and secondly by the harvest date. The opposite occurs in the case of oxidative stability. In this sense, a substantial and positive effect of early harvesting on oil quality parameters can be observed in that it increases oil stability and phenolic content.

Selection of the clone in this study, grown under the same conditions allowed to observe the evolution of the fruit's oil content, mainly its oleic acid, although this is always subject to a joint effect with the crop year. Therefore, it is the factor with the least influence on the physicochemical parameters of the fruit and oil in the Empeltre cultivar.

Knowing the influence of these factors (clone, season and harvest date) on the Empeltre olive variety allows us to select the most suitable conditions for obtaining better quality olive oils, particularly regarding the harvest date, which is commercially relevant.

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