Short note

Effect of ripening and olive cold storage on oil yield and some olive oil characteristics

M.J. Petrón*
M.L. Timón
A.I. Carrapiso
A.I. Andrés

Systems increasing the processing capacity are of special interest in the olive oil production. Additionally, virgin olive oil quality is directly related to the physiological stage of the fruit when processed. This paper reports how the duration (0, 1, 2, 3 and 4 weeks) of cold storage (5 ± 1°C) of olive fruits affects oil yield, some quality parameters (free acidity, peroxide value, (K_{232} and K_{270})) and phenol content of oil ('Picual' olives) according to the stage of ripeness of the fruits (green mature, veraison and black). Results showed that when the stage of ripeness was green, the value of free acidity of the oil was maintained below 0.8% for 4 weeks of cold storage, whereas this level was exceeded within 4 and 3 weeks, for veraison and black olives respectively. A significant decrease in the total phenol content in olives was revealed after the second week of cold storage for the three stages of ripeness. These results suggest that even if the free acidity stays at low levels in olive oil extracted from green mature olives stored in refrigeration for 4 weeks, a decrease in phenol content of olives after 2 weeks could limit the duration of storage at 5 ± 1°C for the three stages of ripening.

Keywords: acidity, phenols, virgin olive oil, ripening, cold storage.

1. INTRODUCTION

Extra virgin olive oil (EVOO) is a precious vegetable oil widely used in the Mediterranean areas. This oil can only be obtained by mechanical processes [1] that usually include the olive fruit going through physical procedures, such as washing, grinding, beating, pressing, decantation, centrifugation or filtration, and oil not being mixed with oils of any other nature. The quality of virgin olive oil is directly related to the quality of the fruit it is extracted from.

EVOO from 'Picual' olives is highly acclaimed on the international market due to its excellent sensorial quality, these variety olives are the most widely cultivated in Spain. However, this appreciated quality depends on the maturity of the fruit [2]. In fact, as ripening advances, the oil from this cultivar results in a reduction of sensory positive attributes and phenol content [3].

The rise in olive fruit production, due to the increasing use of intensive cultivation and the need for a rapid fruit processing will presumably force the industry to increase its processing capacity [4]. In general, the postharvest period of a fruit comprises all the processes that the olive is subjected to, from harvesting to its industrial transformation. The degree of excellence of virgin olive oil is directly related to the physiological stage of the fruit when processed, and this is the most important factor determining its level of quality. Cold storage may be an alternative to the increase in the milling capacity to preserve the soundness of the fruit prior to its processing. In this sense, García et al., [5] and Canet and García [6] demonstrated the viability of cold storage (5°C) of

(*) CORRESPONDING AUTHOR:
M.J. Petrón
Food Technology
University of Extremadura
Badajoz, Spain
Avda. Adolfo Suárez s/n. 06007 Badajoz
mjpetron@unex.es
phone/fax: 0034 924 286 200/0034 286 210

olive fruit 'Blanqueta' and 'Villalonga' on an industrial scale. Studies carried out with 'Picual' olives demonstrated that refrigeration at 5°C in an air atmosphere is effective to maintain chemical quality for 45 days, and to keep the values of free acidity, peroxide and ultraviolet absorbance within the limits admitted for extra quality [7]. Dag et al. [8], tested the effect of the temperature and storage time of mechanically harvested 'Picual' olives from an intensive density of cultivation on the EVOO quality parameters. They concluded that 'Picual' olives can be stored at 10°C or even at room temperature for 9 days without much reduction in the oil quality, in terms of phenol content and free fatty acid content. Yousfi et at. [9] studied the EVOO quality from mechanically harvested 'Arbequina' olives under cold storage, and they found that storage at 2°C maintained the best level of EVOO quality for a period of 12 days. Other authors reported that olive oil extracted from handpicked green 'Picual' olives had acceptable acidity after 6 weeks under storage at 5ºC [10].

This work aims to study how the cold storage of olives affects the quality of the extracted oil depending on the stage of ripeness, to use olive storage as a procedure to increase the processing capacity of the mill.

2. EXPERIMENTAL PART

2.1. PLANT MATERIAL

Olive fruits (approximately 90 kg) from 'Picual' cultivars were harvested on 2015/2016 season from a local mill in Badajoz (South western Spain). The olives were randomly picked from November to January and were distributed according to their average ripeness index (RI) in three ranges of 30 kg each (green mature: 2.4; veraison: 3.4 and black: 5.5, respectively). Sampling was carried out with two replicates for each stage. The 'Picual' olive fruits were manually harvested and randomly placed in 15 perforated plastic boxes with a capacity of 6 kg of olives each (five boxes for each stage).

2.2. STORAGE TREATMENTS AND MEASUREMENTS OF FRUIT CHARACTERISTICS

The boxes were stored under cold storage conditions at 5 ± 1ºC (RH 95%) for 4 weeks. Sampling days were programmed at 0, 7, 14, 21 and 28 days. The olive fruits were characterised for the maturity index, the flesh/stone ratio, moisture and oil content at day 0. The maturity index was based on the colour evaluation of the olive skin and flesh of 100 randomly picked fruits on a 0 (green skin) to 7 (purple skin and flesh) scale, according to Uceda and Frías [11]. Three replicates were made for each sample. The flesh/stone ratio was also measured on 100 fruits randomly taken. Three replicates were made per sampling day. Moisture content of crushed olive fruits was determined by desiccation according to the Commission Regulation ECC 2568/91 [12], five replicates per sampling day being analysed. Oil content was determined in triplicate by Soxhlet extraction with hexane (ECC 2568/91) [12].

2.3. OLIVE OIL EXTRACTION

Oil was extracted using an Abencor laboratory scale system (Mc2 Ingenieria y sistemas, Seville, Spain) and following the procedure described by Dag et al. [13]. The extraction process consisted of the following steps: fruits were crushed with a hammer mill equipped with a 4mm sieve. Seven hundred gram paste was taken and stirred for 60 min at 35 ±1°C in the Abencor malaxer after an addition of 40 g talc (Talcoliva, Boñar León, Spain). After malaxation, the samples were centrifuged for 60 s at 3000 rpm. After centrifugation, oils were decanted in graduated cylinders and the oil volume obtained was measured.

Yield (%) was calculated by multiplying the oil volume (cm^3) by 0.915 and 100 and dividing it by the weight (g) of the paste [13]. Oil extractability was calculated by multiplying oil yield by 100 and dividing it by the oil content.

The oil samples were filtered, transferred into amber glass bottles, and stored in the dark at 3ºC until further analyses performed within the next 2 days.

2.4. OLIVE OIL CHEMICAL ANALYSES

Free acidity, peroxide value, and K_{232} and K_{270} extinction coefficients were determined according to the Commission Regulation ECC 2568/91 [12] and subsequent amendments.

The total phenol content of the oils was determined colorimetrically using the Folin–Cicalteau reagent as described by Carrapiso et al. [14] based on the extraction method described by Gutfinger [15]. The extinction was measured at 725 nm. Results were given as mg/kg of caffeic acid.

2.5. STATISTICAL ANALYSIS

Analysis of variance (ANOVA) and Tukey test were applied to establish significant differences among groups by using SPSS statistical package 15.0 for Windows (SPSS Institute Inc., Chicago, USA). Differences were considered statistically significant when the probability was higher than 95% ($p < 0.05$).

3. RESULTS AND DISCUSSION

Table I shows the results for the characterization of the olive fruits. The average ripening index (RI) was

Table I - Mean, standard deviation (SD) and statistical significance (p-value) for the determinations performed on Picual olives at three ripening stages.

	Stage of ripening			
	Green mature	Veraison	Black	p-value
Ripening Index	$2.39^{a} \pm 0.01$	$3.41^{b} \pm 0.05$	$5.47^{c} \pm 0.15$	<0.001
Flesh/Stone	5.07 ± 0.44	4.73 ± 0.58	5.00 ± 0.12	0.846
Moisture (%)	60.91 ± 2.14	60.00 ± 3.15	57.25 ± 3.85	0.087
Oil content (% d.w.)	35.75 ± 2.85	34.33 ± 1.66	39.15 ± 4.50	0.061
Oil content (% f.w.)	15.75 ± 1.65	16.33 ± 1.53	18.15 ± 3.51	0.062

Each value is the mean±SD of four replicates. $^{a\text{-}c}$ Means in a column with different letters are significantly different (p <0.05). Percentage of fresh and dry olive paste weight (oil content, % f.w., % d.w)

2.39 for the green mature group, 3.41 for the veraison group and 5.47 for the black group. The values of flesh/stone ratio were within the usual range and it was similar for fruits of the three different stages. Water content at harvesting was irrespective of the stage of ripeness whereas oil content tended to be higher in the black batch, which averaged 39.2% (on dry matter basis). These values were similar to those described by other authors in olive fruit from 'Picual' cultivar [14, 16].

Table II reports the results for oil yield and the chemical analyses of olive oils from fruits stored at 5 ± 1ºC for 0 to 4 weeks. The cold storage did not greatly affect oil yield, so it would be a feasible option for the olive oil industry. Only the green mature batch showed free acidity values not larger than 0.8% (which is the limit set for extra virgin olive oil as determined by the International Olive Oil Council) all throughout 4-week storage. However, veraison and black groups exceeded this limit after 3 and 2 weeks, respectively. Free acidity on day 0 ranged between 0.2% and 0.4% with significant differences between green mature and black stage of ripeness (p <0.01). These results agree with those from other authors [17] and it could be ascribed to the progressive activation of the lipolytic activity during the olive ripening [2, 3, 18]. In this sense, Pereira et al. [18] using 'Cobrançosa' (RI=4), 'Madural' (RI=5.7) and 'Verdeal Tranmontana' (RI=3.1) drupes stored at 5°C for 14 days found that the storage of fruits produced a significant increase in oil acidity, which led to the loss of the 'extra' category. Recently, Yousfi et al. [10] found that olive oil extracted from 'Picual' green olives (RI=0.6) and stored at 5ºC for 6 weeks, had an acceptable acidity. In this

Table II - Mean, standard deviation (SD) and statistical significance (p-value) from a two-way ANOVA for the determinations performed on oils from Picual olives.

Ripening Stage	Storage days	Oil yield (%)	Free acidity (% oleic acid)	Peroxide value (mEq O_2/kg)	K_{232}	K_{270}	Phenol content (mg/kg)
Green	0	15.6 ± 0.31^{ab}	0.2 ± 0.14^{a}	4.2 ± 1.4^{ab}	0.95 ± 0.20	0.08 ± 0.00^{a}	268.60 ± 44.83^{c}
Mature	7	16.7 ± 0.31^{b}	0.3 ± 0.04^{a}	4.5 ± 1.0^{ab}	1.01 ± 0.24	0.08 ± 0.00^{a}	189.88 ± 18.37^{b}
	14	15.5 ± 0.29^{a}	0.3 ± 0.05^{a}	3.7 ± 0.6^{a}	0.87 ± 0.01	0.12 ± 0.00^{b}	128.37 ± 14.21^{a}
	21	15.6 ± 0.37^{a}	0.6 ± 0.14^{b}	5.9 ± 0.5^{b}	1.07 ± 0.08	0.11 ± 0.00^{b}	123.33 ± 11.37^{a}
	28	15.9 ± 0.78^{ab}	0.8 ± 0.18^{c}	8.7 ± 0.8^{c}	0.95 ± 0.24	0.11 ± 0.00^{b}	86.00 ± 6.48^{a}
	p	0.018	0.000	0.000	0.699	0.000	0.00
Veraison	0	16.4 ± 0.86	0.3 ± 0.06^{a}	7.3 ± 1.1	0.55 ± 0.18^{a}	0.10 ± 0.05	245.48 ± 33.97^{b}
	7	16.6 ± 0.28	0.4 ± 0.02^{a}	4.8 ± 2.0	0.94 ± 0.00^{bc}	0.12 ± 0.00	204.28 ± 13.90^{b}
	14	16.4 ± 0.39	0.3 ± 0.03^{a}	3.9 ± 0.5	1.01 ± 0.12^{c}	0.11 ±0.00	155.65 ± 9.38^{a}
	21	16.6 ± 0.28	0.7 ± 0.17^{b}	6.9 ± 0.5	0.66 ± 0.13^{ab}	0.11 ± 0.00	143.80 ± 5.95^{a}
	28	15.9 ± 0.77	2.0 ± 0.09^{c}	–	–	–	–
	p	0.069	0.000	0.060	0.001	0.862	0.000
Black	0	16.7 ± 0.41^{c}	0.4 ± 0.06^{a}	7.5 ± 1.2	0.60 ± 0.16	0.09 ± 0.06	194.33 ± 31.89^{b}
	7	15.6 ± 0.18^{a}	0.4 ± 0.06^{a}	5.5 ± 2.4	0.79 ± 0.30	0.13 ± 0.01	161.17 ± 40.20^{b}
	14	17.4 ± 0.39^{bc}	0.7 ± 0.06^{b}	4.9 ± 1.6	0.95 ± 0.03	0.11 ± 0.01	95.65 ± 9.38^{a}
	21	17.0 ± 1.54^{ab}	2.3 ± 0.35^{c}	–	–	–	–
	28	–	–	–	–	–	–
	p	0.005	0.000	0.156	0.093	0.443	0.004

Each value is the mean±SD of four replicates. $^{a\text{-}c}$ Means in a column with different letters are significantly different (p <0.05).

sense, black olives are more sensitive to pathogenic infections and mechanical damage, which results in oils with higher acidity values [19].
The peroxide values of the oil obtained from veraison and black olives were significantly higher than the ones obtained from green mature fruits ($p < 0.01$, data not showed) in the samples immediately processed after the harvesting (day 0). In the green mature batch, peroxide values remained stable until week 2, when a significant increase was reported. Among the other parameters used to evaluate oil oxidation, only K_{270} showed significant changes due to the time of storage. After the first week of cold storage, the oils from green mature fruits exhibited significantly higher values for this parameter, whereas no significant changes due to cold storage time were found in the oil from veraison and black olives. These results agree with those obtained previously in oils from olives kept at 5ºC, revealing increased peroxide values after one week, and significantly higher values for K_{270} after 15 days of storage while K_{232} values remained constant for 30 days of fruit storage [7]. In addition, measurements of absorbance at specific wavelengths in the UV region are currently used to provide information on the quality of olive oil. EVOO is required to have extinction coefficients lower than 2.50 and 0.22, at 232 and 270 nm, respectively. Table II indicates that our results are within these mentioned limits and agree with results from Fuentes et al. [17] who did not find a clear trend during ripening for peroxide values, K_{270} and K_{232}. Consistently, storage of olives for 30 days did not lead to significant increases of K_{232} and K_{270} in the olive oils subsequently extracted from cool stored (5ºC) olives [5, 20-21]. However, in previous studies, oils obtained from olives at more advanced ripening stages showed a slight reduction in the content of conjugated fatty acids, according by their K_{232} [22-23].
The values of total phenol content (mg caffeic acid/kg oil) found in the analysed oils are shown in Table II. The maximum value was found in the olive oil extracted from green mature olives on day 0 (268.6 mg caffeic/kg oil), and significant differences were found between this green group and the black group, the last one showing the lowest values (194.3 mg caffeic/kg oil at day 0). According to Aguilera et al. [24], the total polyphenol content of olive oils varies between 50 and 1000mg/kg, depending on factors such as cultivar and degree of ripeness, as well as some agronomic and technological aspects of production [25]. Other authors found an increase in total phenol content until the olives ('Chetoui' variety) reached a red or black pigmentation stage (ripening index between 3 and 4) followed by a decline of this content, as ripening progressed [26]. In this experiment, the changes in the concentration of total phenol content did not vary during the storage period according to the ripeness stage. However, a significant decrease in the total phenol content was reported after the second week of storage, maintaining the initial value only for 14 days of storage at 5ºC and irrespective of the ripening stage. Previously, Yousfi et al. [4] had observed that total phenol content of EVOO from 'Arbequina' olive stored at 3°C decreased significantly after 7 days of storage. However, Dag et al. [8] reported that during the fruit storage at 4ºC, the total phenols content of VOO from green 'Picual' was relatively stable, with values ranging between 110 and 140 mg caffeic/kg oil for 23 days. Although our results for phenol content remained steady only for 2 weeks, the initial phenol content in our samples was higher than those reported in the mentioned study [8] (268,6 mg caffeic/kg oil in green olives) and the values remained higher than 123 mg caffeic/kg oil in green olives stored for 3 weeks. This fact could suggest that with a more phenol content at the beginning of storage, there is less stability the during cold storage of olives. However, different authors suggested that storing olive fruit at low temperatures could be beneficial for the sensory quality of virgin olive oils, despite a reduction in total phenol compounds [10, 27]. In this sense, further carefully designed studies considering the sensory quality of oil are necessary to establish how low temperatures might limit the duration of storage of 'Picual' olives at 5ºC.

4. CONCLUSIONS

Our results suggest that cold storage does not decrease oil yield and that oil free acidity remains below 0.8% longer (4 weeks) in olive oil from 'Picual' variety when the ripening index is lower (green mature olives). However, the significant decrease after the second week of cold storage reported for the total phenol content for the three stages of ripeness, could limit the duration of storage at 5ºC for 'Picual' olives. Further studies would be necessary to clarify how these findings could finally affect sensory characteristics of virgin olive oil.

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