UNIVERSITÀ POLITECNICA DELLE MARCHE
Repository ISTITUZIONALE

Influence of pre-harvest calcium applications on table olive characteristics during Spanish-style elaboration process

This is the peer reviewd version of the followng article:

*Original*
Influence of pre-harvest calcium applications on table olive characteristics during Spanish-style elaboration process / Lodolini, E. M.; Fernandez, A.; Morales-Sillero, A.; Mendiano, A.; Martin-Vertedor, D.. - In: SCIENTIA HORTICULTURAE. - ISSN 0304-4238. - ELETTRONICO. - 308:(2023). [10.1016/j.scienta.2022.111577]

*Availability:*
This version is available at: 11566/329238 since: 2024-05-06T15:17:13Z

*Publisher:*

*Published*
DOI:10.1016/j.scienta.2022.111577

*Terms of use:*

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. The use of copyrighted works requires the consent of the rights' holder (author or publisher). Works made available under a Creative Commons license or a Publisher's custom-made license can be used according to the terms and conditions contained therein. See editor's website for further information and terms and conditions.
This item was downloaded from IRIS Università Politecnica delle Marche (https://iris.univpm.it). When citing, please refer to the published version.

(Article begins on next page)

08 January 2025

# Influence of pre-harvest calcium applications on table olive characteristics during Spanish-style elaboration process

Enrico Maria Lodolini[1], Antonio Fernández[2], Ana Morales-Sillero[3], Amparo Mendiano[2], Daniel Martín-Vertedor[2*]

[1]Research Centre for Olive, Fruit and Citrus Crops, Council for Agricultural Research and Economics, Via di Fioranello, 52, 00134, Rome, Italy.

[2]Technological Institute of Food and Agriculture. Junta of Extremadura. Avda. Adolfo Suárez S/N 06007 Badajoz, Spain.

[3]Department of Agronomy, ETSIA, University of Seville, Ctra. de Utrera S/N Km 1, 41013 Seville.

[*]Corresponding author: daniel.martin@juntaex.es

ORCID: http://orcid.org/0000-0002-8134-0618

**Abstract**

Calcium is largely used in agri-food industry due to its positive effects, such as, preserving activity or protection against pathogens. An increase in firmness and phenolic content has been spotted after post-harvest spraying of calcium chloride ($CaCl_2$) solutions. The aim of this research was to investigate the effects of pre-harvest applications of two $CaCl_2$ concentrations (Ca 0.5% and Ca 1.0%) during the Spanish-style elaboration process (beginning, middle and final of fermentation process). In particular, the Ca concentration in the whole fruit and in the different tissues (pulp, pit and seed), the firmness, the phenolic content, and the antioxidant activity were analysed in 'Ascolana tenera' table olive. An increase in Ca concentration in the pulp (20.2% and 55.1%) and in the seed (13.4% and 36.5%) was observed along monitoring period when 0.5% and 1.0% $CaCl_2$ concentrations were respectively compared with Ca 0% (water). No significant Ca increase was found in the pit. The fruit firmness was higher after the Ca-treatments (22.8% and 68.3%, respectively). The content of Hydroxytyrosol, Tyrosol and Verbascoside decreased during the elaboration process. The highest content found of these phenols were at the first date of monitoring for 1.0% $CaCl_2$ concentration. Hydroxytyrosol, Oleuropein and PB1 showed significant difference at different monitoring dates. During the fermentation period, the antioxidant activity presented an increase between 63.3 and 87.2% with respect to Control (Ca 0%). Similar increasing trend was also registered for the phenolic compounds. Our results are useful for the table olive sector to improve the handling in post-harvest processing and increase the final quality of the olives.

**Keywords**: 'Ascolana tenera'; post-harvest; firmness; phenolic compounds; antioxidant activity.

## Introduction

Farmers growing olive trees (*Olea europaea* L.) in the Mediterranean basin are improving the production processes to increase the quality of the table olives on the international markets (International Olive Council, 2020). At the industrial level, there are different production methods, and the Spanish-style is one of the most important used all over the world (Rallo et al., 2018). Olives are debittered with lye solutions and then washed and submitted to lactic fermentation in brine for several months. Different table olive varieties are processed according to the Spanish-style such as 'Manzanilla', 'Gordal', 'Hojiblanca', 'Carrasqueña' and 'Cacereña' (Cabrera-Bañegil et al., 2018). Also 'Ascolana tenera' is processed as the Spanish-style. This olive variety, grown mainly in the Marche Region in the central Italy under the Protected Origin Designation "Oliva Ascolana del Piceno", has a big-size fruit and a high pulp-to-pit ratio. However, it has a very sensitive pulp and the fruits can be subjected to damages during harvest, transport and processing, decreasing the final quality and value of the production (Lodolini et al., 2019; Morales-Sillero et al., 2021).

Calcium has an important role in the fruit quality (Conway et al., 2002; Morales-Sillero et al., 2021) by creating cross-bridges with pectic polymers in the cell wall strengthening it (Manganaris et al., 2005), and increasing its resistance to hydrolytic enzymes (Miceli et al., 1999). Calcium is also able to improve the resistance against pathogens, such as Anthracnose or Botrytis, affecting fruit tissues (Miceli et al., 1999; Langer et al., 2019; Madani et al., 2014). The decrease in calcium content leads to enzymatic browning of the tissues (Jemrić et al., 2016) , causing the degradation of the fruit and its quality decline. Ca-treatments has been used in agri-food industry to improve the quality of many fruits, such as, apples (Holb et al., 2012), olives (Gouvinhas and Barros, 2021; Tsantili et al., 2008), peaches (Manganaris et al., 2005) and table grapes (Ciccarese et al., 2013). Pre-harvest treatments applied to olives, using foliar

64 application of calcium, demonstrated that a higher calcium content augmented the phenolic
65 compounds and increased the fruit firmness, slowing down the degradation of the cell wall
66 (Morales-Sillero et al., 2021). Post-harvest Ca-treatments may be useful as well to enhance the
67 firmness and elongate the shelf life of the olive (Rincón and Martínez, 2015). In the same way,
68 spraying calcium solution to the fruits after harvest may be useful to overcome calcium
69 deficiencies. The bibliography also reports that fruits, in this case the table olives, tend to form
70 cracks in the skin surface (membrane breaking) when they are mature, (Harker and Ferguson,
71 1988). These fractures can act like an easy-way access for the calcium into the fruit. However,
72 the fruit quality enhancement induced by calcium, as well as its absorption mechanisms,
73 transport and storing in the fruit itself, is not yet fully understood (Hocking et al., 2016).
74 Previous studies highlighted that there may be difference in the Ca concentration required to
75 increase its content in the fruit based on the tree species or cultivar (Lara, 2013). Tomatoes
76 increased the firmness when dipped in a $CaCl_2$ (2%) solution after harvest (Gharezi and
77 Gharezi, 2012), as well as papayas when treated with 2.5% concentration (Gao et al., 2020) or
78 fresh-cut cantaloupe melons when dipped in $CaCl_2$ (1-5%) concentration (Luna-Guzmán et al.,
79 1999). Post-harvest treatments with $CaCl_2$ increased the total phenolic content for storage
80 cherries, along with an improvement of the antioxidant activity (Aghdam et al., 2013). In
81 previous research studies, post-harvest Ca-treatment effects have been assessed in Californian-
82 style black and green table olives (Martín-Vertedor et al., 2021, 2020).
83 The effects of pre-harvest application of calcium chloride on the quality of Spanish-style table
84 olives have not been deeply investigated. The lack of literature about this topic can depend on
85 several factors, as for example the effective concentration or the frequency of application to be
86 applied in order to obtain significant changes in the fruit characteristics (Morales-Sillero et al.,
87 2021). For all the above, the proposed hypothesis states that pre-harvest Ca applications

88 increase table olives quality after the elaboration process. In order to contrast this, the aim of
89 this study was to deepen the knowledge about the effect of two $CaCl_2$ concentrations applied in
90 three different dates of the fruit development (Morales-Sillero et al., 2021), through the quality
91 of the olives during the Spanish-style fermentation process for table olives.
92
93 **Material and Methods**
94 **Chemical reagents**
95 To apply the foliar calcium treatments in the olive trees, $CaCl_2$ $2H_2O$, 99.0% (Labkem,
96 Barcelona, Spain) and a commercial adjuvant Tween-20, 0.5% (ThermoFisher Scientific, MO,
97 USA) were used. For the olive elaboration according to the Spanish-style, sodium hydroxide,
98 acetic acid and sodium chloride were purchased from Sigma Aldrich (Sigma-Aldrich, San Luis,
99 USA). Commercial grade nitric acid (Sigma-Aldrich, San Luis, USA) solutions were used for
100 calcium analysis. For phenol profile apigenin-7-O-glucoside, hydroxytyrosol, luteolin,
101 oleuropein, procyanidin B1 (PB1), and verbascoside were purchased by Extrasynthése (Genay,
102 France). Apigenin, epicatechin, luteolin-7-O-glucoside, quercetin-3-rutinoside and tyrosol (p-
103 HPEA) by Sigma-Aldrich Chemie (Steinheim, Germany). p-coumaric acid by Fluka Chemie
104 (Steinheim, Germany). Acetonitrile and methanol (HPLC grade) were supplied from Fisher
105 Scientific (Loughborough, UK), formic acid by PANREAC (Barcelona, Spain), and sodium
106 fluoride by Sigma-Aldrich Chemie (Steinheim, Germany).
107 **Plant material**
108 The study was carried out in 2017 on the cultivar 'Ascolana tenera' in a drip-irrigated olive
109 orchard planted in 2009 in Montalto delle Marche (Central Italy) with a planting density of 200
110 trees $ha^{-1}$ (7.0 m × 7.0 m) as reported in Morales-Sillero et al. (2021). The cultivar was drip-

111 irrigated orchard (~1500 $m^3 \cdot ha^{-1}$). The mean temperature and total annual rainfall were 13.4 °C
112 and 820 mm, respectively (Servizio Agrometeo ASSAM, Regione Marche).
113 This variety is characterized by a large size fruit (7-8 g), a high pulp-to-pit ratio, and a very soft
114 flesh. The high sensitivity of 'Ascolana tenera' can lead to the appearance of black spots on the
115 skin during the harvesting operations and the transport process. The fruit is typically picked at
116 the pale green ripening stage for small-scale production (local market) of table olives under the
117 Protected Designation of Origin 'Oliva Ascolana del Piceno' (Lodolini et al., 2019).
118 During our study, full bloom and harvest occurred on May 30$^{th}$ and September 14$^{th}$ 2017,
119 respectively. Olives were harvested at yellowish-green skin/pulp colour stage of maturation and
120 presented morphological characteristics as indicated in Morales-Sillero et al. (2021). The fruit
121 mass of 'Ascolana tenera' variety in all the experimental treatments was 5 g.
122
123 **Experimental Design**
124 Two different calcium concentrations, Ca 0.5% and Ca 1.0%, were administered in the morning
125 by foliar applications with a commercial adjuvant (Tween-20; 100 $mL \cdot 100\ L^{-1}$). The treatments
126 were repeated three times during the fruit development: i) at the end of fruit set; ii) at the end
127 of pit hardening; and iii) two weeks before harvesting. Water was used as Control (Ca 0%) and
128 sprayed the same days when calcium was applied. Each calcium concentration was applied to
129 three replicates of five homogeneous trees in a randomized block design with guard trees around
130 the trees, as reported in Morales-Sillero et al. (2021).
131 **Spanish-style elaboration process**
132 Immediately after harvest, the olives were kept separated according to the Ca-treatment,
133 transported to the processing facility and separately elaborated according to the Spanish-style
134 (Schaide et al., 2019). Each replicate of fermented fruits consisted of three 225L-fermentors

(olives of 5 trees) for ~110 kg of olives per replica. Thus, olives were treated with NaOH solution (2.5%, w/v at 25 °C) for debittering until 2/3 of the flesh was reached. Then the alkaline solution was removed and clean water was flown for 12 hours for the washing stage. Finally, to allow the fermentation process, olives were placed into NaCl brine solution at 10%, w/v that was stabilized with olives at 6% of NaCl. The total chloride concentration present in the brine was also monitored and controlled. The pH of the brine was continuously adjusted a 3.8 point in all the trials by adding lactic acid, in order to maintain adequate chemical condition in each fermenter. A spontaneous fermentation occurred between October 2017 and March 2018 (160 days). The final of the fermentation process was also determined by evaluating olive color by the tasting panel. According to official protocol of the Protected Designation of Origin 'Oliva Ascolana del Piceno', after the elaboration process and during the conservation period, NaCl concentration was maintained around 8% and the pH below 4.5. No enterobacteria, coliforms, *Bacillus*, and *Pseudomonas* were detected during the elaboration process. The elaboration process was done in three replicates for each treatment.

Fruit samples were collected at different dates of the fermentation process: 1) beginning of fermentation process; 2) middle of fermentation process; and 3) final of fermentation process.

The analysis for the calcium content, firmness, phenol profile and antioxidant activity were performed.

**Calcium analysis**

Fifty fruits per replicate were washed three times in distilled water and dried using a filtering paper. The olive pulp was removed from the pit with a manual stoner. The seeds were also separated by the pit with a commercial hammer. The vegetal material was crushed with a thermobeater and 2 g was dried at 100 ºC for 24 hours and ashed in a muffle furnace at 550 ºC (1º C $min^{-1}$). The ashes were dissolved in 20 mL of 4% $HNO_3$. The calcium content was

159 determined using an ICP-OES Perkin-Elmer 5300 DV spectrophotometer at 317.933 nm in a
160 radial mode, with a concentric nebulizer and a cyclonic nebulization chamber. Results were
161 expressed as mg $kg^{-1}$ on dry weight (d.w.).

162 **Sensory analysis of the fruits**

163 Sensory analysis was performed by eight panellists trained according to the standardized norm
164 of the International Olive Council [32]. The sensory properties of table olive, including
165 abnormal fermentation, other defects, salty, bitterness, acidity hardness, fibrousness and
166 crunchiness were assessed by the trained panel.

167 **Firmness of the fruits**

168 The method followed to determine the olive firmness was described by Morales-Sillero et al.
169 (2020). Forty fruits per replicate were used for firmness determination using a TA-TX2 texture
170 analysers (Stable Micro Systems, Godalming, UK) connected to a computer and fitted with a
171 30kg load cell. Each olive fruit was placed between a flat steel plate mounted on the machine.
172 Olive firmness was measured by compression test, applying a force (N) to crush the olive
173 through a 20 mm diameter probe to achieve a 6% deformation of the fruit diameter. The test
174 speed and the trigger force were 0.5 $mm \cdot s^{-1}$ and 0.04903 N, respectively. Values were expressed
175 as breaking force in Newton (N).

176 **Determination of phenolic compounds**

177 The phenolic profile analysis was carried out in 40 fruits per block with an Agilent 1100 model
178 HPLC system (Hewlett-Packard, Waldbronn, Germany) following the method described by
179 (Cabrera-Bañegil et al., 2017). 2g of homogenized samples was extracted in ultrasonic bath (P-
180 Selecta ultrasonic bath, mod 516, Barcelona, Spain) with 10 mL of methanol, containing NaF
181 2 mM, during 30 min. After centrifuged (Thermo Scientific Sorvall Legend XT/XF centrifuge,
182 with a F13-14x50c carbon fiber rotor, Thermo Fischer Scientific, USA) at 1677g at 4 ºC during

10 min, the extracts were filtered prior the injection into the HPLC system. Stock standard solutions of the phenols studied were accurately prepared and dissolved in methanol.

**Statistical analysis**

One-way ANOVA and Tukey's test were performed to determine significant differences between treatments. SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used to perform ANOVA analysis. Outcomes were expressed as mean values and corresponded to the calcium treatments applied in each experiment. Statistical significance was accepted at the level of $p < 0.05$. Data were expressed as mean ± standard deviation.

## Results

### Calcium content

During table olives Spanish-style elaboration process, olives were spontaneously fermented in their corresponding fermentation tanks at the same chemical conditions. No lactic acid bacteria, enterobacteria, coliforms, Bacillus, and Pseudomonas were detected in the fermented olives for each calcium treatments (data not shown). Furthermore, the calcium content in the pulp, the pit, the seed and the whole fruit was monitored during the fermentation process. The pulp calcium content increased with the Ca-treatments (Figure 1a). A significant difference was found between tested concentrations. The increase of calcium content was 55.1% and 20.2% for Ca 1.0% and Ca 0.5% concentrations, respectively. The pit showed a higher calcium content than the pulp when Ca 0% was applied (Figure 1b). Nonetheless, this tendency changed when the calcium was used but without significant differences between calcium treatments within each sampling date. The content of calcium in the seed (Figure 1c) was significantly higher than in other tissues of the fruit (three times compared to the pulp and pit). Nevertheless, the

207 contribution of the seed is not significant compared with the whole fruit due to its low weight
208 and the seed high calcium content was not reflected on the total calcium content. The calcium
209 content resulted significantly higher when the Calcium treatments were applied but no
210 significant differences were found between the tested Ca concentrations. A statistical difference
211 between Ca-treatments and Ca 0% was also registered in the whole fruit (Figure 1d). The
212 calcium content of the fruit increased of the 13.4% and 36.5% compared to Ca 0%, with Ca
213 0.5% and Ca 1.0% concentrations, respectively.
214 Regarding the evolution of the Ca content during the elaboration process, no significant
215 differences were found between sampling dates when the pulp, the pit, the seed or the entire
216 fruit were considered. This result indicates that the calcium applied during the fruit growth
217 development was not removed during the whole elaboration process.
218 **Sensory analysis**
219 A triangular test was carried out to verify if the tasters were able to differentiate not-treated
220 Spanish-style table olives with those treated with pre-harvest calcium applications (Table 1). It
221 can be said that the different samples were correctly identified in the different trials carried out.
222 Thus, it could be statistically affirmed that tasters were able to differentiate between Ca-treated
223 olives (0.5% and Ca 1%) and those without calcium addition. This is a very interesting result
224 since tasters are able to discriminate samples based on their olfactory-gustative senses.
225 However, it should be noted that the percentage of success for differentiation of the samples
226 subjected to calcium treatments in the triangle test was less than the 66%. Results of the sensory
227 analysis are shown in Table 2. Some significant differences were observed for each sensory
228 attribute. The highest differences were found for bitter, hardness, and crunchiness. Values
229 ranged from 2.1 to 3.5 for bitter and from 3.6 to 6.4 for hardness. Crunchiness also showed
230 slight significant differences. For these sensory attributes, table olives subjected to calcium

applications recorded the highest values. Nevertheless, the attributes related to negative sensations (i.e. salty, acidity, fibrousness, and color) did not show differences when Spanish-style table olives were compared to those subjected to pre-harvest calcium treatments.

**Fruit firmness**

The compression force (firmness) applied to the fruits significantly decreased during the elaboration process in Ca 0% and Ca 1.0%, while did not show variation in Ca 0.5% (Figure 2). Nevertheless, Ca-treatments increased the compression force in the fruit when compared with Ca 0% samples. On the first date of monitoring, a 22.8% and 43.8% increase of the compression force was registered for Ca 0.5% and Ca 1.0% concentrations, respectively. On the second date, the increase for Ca-treatments was 42.8% for Ca 0.5% and 68.5% for Ca 1.0%. For the third date, the tendency seen in the previous sampling dates changed: no significant differences were found between Ca concentrations recording an increase of 68.3% compared to Control.

The results showed that the compression force needed did not change significantly during the whole elaboration process for Ca 0.5% (Figure 2). However, the firmness decreased significantly at the second date for Ca 1.0% during the elaboration process. In addition, comparing the firmness of Ca-treatments on the second and third date, no significant difference on compression force were observed.

**Phenol composition**

During the elaboration process of table olives, the phenol composition was determined (Table 3). The most representative phenolic compounds were Hydroxytyrosol and Oleuropein, whereas phenols that showed the lowest concentration were Apigenin and Apigenin-7-O-

glucoside. Phenol profile tended to decrease during the elaboration for some compounds, such as Hydroxytyrosol, Tyrosol, Oleuropein, PB1 and Verbascoside decreased, whereas the composition during the whole elaboration process did not change for others like Luteolin-7-O-glucoside, Luteolin, Epicatechin, p-coumaric acid, Quercetin-3-rutinoside, Apigenin and Apigenin-7-O-glucoside. This information was applicable to Ca 0% samples and also to the Ca-treated ones (Table 1).

The content of phenol composition decreased during the elaboration process. The results showed that there were significant differences between Control and Ca-treatments within each sampling date for all analysed phenols. The first date of monitoring showed that the concentrations of Hydroxytyrosol, Tyrosol and Verbascoside were included between 16.5% and 65.7%, higher for the Ca treated table olives compared to Ca 0% ones. Ca-treatments showed higher concentrations than Control samples for the second and third date (between 16.0% and 64.1% and between 23.6% and 80.8%, respectively). The highest contents for these phenolic components were found at the first sampling date for Ca 1.0% concentration: 331.4, 41.6 and 82.0 mg $kg^{-1}$ for Hydroxytyrosol, Tyrosol and Verbascoside, respectively.

For Oleuropein and PB1, significant differences were found between the applied Ca concentrations and between Ca-treated fruits and Ca 0%. Thus, table olives submitted to Ca 0.5% and Ca 1.0% presented higher Oleuropein and PB1 concentrations than Control table olives (Table 3). At the first sampling date, the content of Oleuropein and PB1 was 29.8% and 12.7% higher than Ca 0% for Ca 0.5% treatment and 48.9% and 26.2% higher for Ca 1.0% treatment. At the second sampling date, the content of the same phenols was between 12.6% and 29.8% and between 26.1% and 49.0% higher than Ca 0% for Ca 0.5% and Ca 1.0% concentration, respectively. At the third sampling date the content was between 12.5% and 22.9% and between 26.3% and 37.7% higher than Ca 0% for 0.5 % and 1.0% Ca-treatments,

279 respectively. The highest content of Oleuropein and PB1 was registered for Ca 1.0% treatment
280 at the first sampling date (287.7 and 82.1 mg $kg^{-1}$, respectively).
281 The content of Luteolin-7-O-glucoside also decreased during the elaboration process. In
282 particular, at the first sampling date, no significant differences were found between Ca 0% and
283 the Ca 0.5%, whereas significant differences were seen for Ca 1.0% (the content was 31.1%
284 higher than Ca 0%). For Ca 0.5% and Ca 1.0% treatments, the Luteolin-7-O-glucoside
285 concentration at the second sampling date was 10.4% and 42.9% higher than Ca 0%,
286 respectively. At the third sampling date, no significant differences were found between the Ca
287 0% and the Ca 0.5% treatment, whereas significant differences were seen for Ca 1.0% (the
288 content was 43.0% higher compared to the Ca 0%). The highest concentration of Luteolin-7-
289 O-glucoside was registered for Ca 1.0% treatment at the first sampling date (39.1 mg $kg^{-1}$).
290 The remaining analysed phenolic compounds did not show any significant differences between
291 Ca 0% and the applied calcium treatments within each sampling date or between sampling dates
292 within the same treatment. However, the highest contents were found at the first sampling date
293 for Ca 1.0% concentration excepted for p-coumaric acid that showed the highest value for Ca
294 0.5% treatment. For some of the phenols, such as Hydroxytyrosol, Oleuropein and PB1 the
295 results showed a significant difference between the phenolic content at different monitoring
296 dates. The highest content appeared at the first date and decreased gradually. Tyrosol showed
297 a significant difference with the first date and the second and third date, this last two did not
298 present significant differences. The first date showed higher concentrations of Tyrosol.
299 Verbascoside, did not show significant difference between the first date and the second one, but
300 both dates showed a significant difference with the third one. This last monitoring date showed
301 the lowest concentration out of the three. For the rest of the phenols mentioned before, not

302 significant differences were recorded for the concentration between the different dates of
303 monitoring.
304
305 **Discussion**
306 Table olives pre-harvest Ca-treatments implies an assimilation of this macronutrient in pulp, pit
307 and seed. This absorption is beneficial in terms of firmness, bioactive compounds synthesis and
308 antioxidant capability. Agreeing with other researchers, calcium is an important macronutrient
309 that can be used at different stages of the elaboration process of table olives or in pre-harvest
310 treatments in different cultivars. Previous studies (García-Serrano et al., 2020; Gouvinhas and
311 Barros, 2021; Tsantili et al., 2008) reported pre-harvest foliar applications of an organic calcium
312 compound to three different cultivars. The application in different moments of the fruit
313 development enhanced the final Ca content, similarly to the data obtained in our research. Other
314 studies indicated this enhancement of calcium content in the pulp of several fruits after Ca-
315 treatments. For example, Manganaris et al. (2005) observed this increase for peach when Ca
316 sprays were applied once a week during 10 or 6 weeks before harvest until commercial ripening
317 of the fruit. In olive, Tsantili et al. (2008) reported an accumulation of calcium after spraying
318 three times a $CaCl_2$ solution (0.65% w/v) throughout the month previous to harvest. More
319 recently, (Morales-Sillero et al., 2021) demonstrated that foliar applications of Ca were also
320 effective when applied during fruit development, at three different stages and suggested a
321 potential positive influence along the Spanish-style elaboration process to be demonstrated with
322 further analysis.
323 The results of our study showed a significant increase of calcium content in the pulp and the
324 seed of the fruit when three Ca foliar applications were applied during the fruit development.
325 Other studies also found an enrichment in calcium content in the seed of fruits after Ca-

326 treatments. Ciccarese et al. (2013) observed an increase in berries when $CaCl_2$ was sprayed
327 twice, from fruit set to veraison and from veraison to harvest. Our study showed that the calcium
328 content on the seed did not change during the different stages of the elaboration process as table
329 olives. Our results also highlighted a significant difference of Ca content between the seed and
330 the other tissues of the fruit, showing values that were quite the double comparing the seed with
331 the pulp. Ciccarese et al. (2013) concluded that the difference could be due to the translocation
332 of the calcium from the skin to the seed, even though this was not confirmed, and further
333 investigations are required. Harker & Ferguson (1988) explained that Ca-treatments are highly
334 successful in the fruit, being this sink able to absorb $Ca^{+2}$ better than the other portions of the
335 tree with a later re-translocation of calcium. This study also mentioned than in mature fruits the
336 absorption of calcium take place because of the cuticle cracking due to the growth of the fruit.
337 According to this, cracking makes easier the absorption of $Ca^{+2}$, but to increase the fruit content,
338 Ca-treatments should be applied.
339 Our study confirmed that the calcium content does not decrease through the elaboration process
340 of table olives when Ca was applied to developing fruits on the tree (pre-harvest), so that no
341 further applications were required in post-harvest. This positive effect can be attributed to the
342 accumulation of calcium in the lamella region of the cell wall with the following stabilization
343 (Tobias et al., 1992). Calcium is a phloem immobile nutrient; it only can be transported with
344 normal water flow for its accumulation on the fruit. The cell wall permeability to water can be
345 modify by calcium, so it can decide its own transport (Hocking et al., 2016). Calcium connects
346 to the cell walls of the fruit once it is applied to the fruits in the field. During the elaboration
347 process, the Ca included inside the fruit cell increases the rigidity of the membrane, strongly
348 reducing the diffusion of calcium to the brine and immobilizing it through stable bonds,
349 although this inhibitory effect depends on the pH of the brine solution during Spanish-style

fermentation process (Brenes et al., 1994). In our study, the physico-chemical parameters have been artificially controlled to maintain all the experimental treatments under the same chemical conditions during the fermentation process. This was confirmed by our results: average Ca content in the pulp did not decrease during the elaboration process for table olives (Figure 1). Same trend was observed for the Ca content in the pit and in the seed. The high calcium content of the olives subjected to pre-harvest calcium treatments did not affect the microbial development and activity during the fermentation process, excluding a possible toxic effect of the Ca studied concentrations.

The sensory profile of the olives subjected to pre-harvest calcium applications showed appreciable differences between them (Table 1 and Table 2). It is known that the post-harvest calcium applied during Spanish-style table olives can contribute to a higher bitter taste in the final product (Martín-Vertedor et al., 2020; 2021). In fact, pre-harvest calcium application doses should not be excessively high as this could affect the sensory quality of the processed table olives. However, with the concentrations applied in this study, it can be concluded that the bitterness values were included in the normal range for this kind of olive processing protocol (Schaide et al., 2019). The application of Ca-treatments helped to maintain the fruit firmness even after the alkaline stage of the elaboration process, contributing to increase the quality of the olive by improving the fruit texture (Table 2; Figure 2). This aspect, together with the crunchiness, was also positively evaluated by the tasters in the olives subjected to pre-harvest calcium applications. Such result can provide an extra-quality value to this type of olives, indicating a potential market for pre-harvest Ca-treated Spanish-style table olives. Schaide et al. (2019) reported no bitterness and high acceptability of table olives elaborated with olive leaf extract according to the Spanish-style. The firmness of the fruit is also important to contrast disease attacks and prevent physiological disorders as reported by Rincón and Martínez (2015).

374 Calcium is the second most important mineral involved in the cell walls in plants and its main
375 role is to maintain the structure of the wall (Jemrić et al., 2016). Tobias et al. (1992) indicated
376 that the extra rigidity on the cell walls could be because the creation of calcium cross-linkage
377 between pectic polymers. This study also mentioned that the calcium penetrating in the fruit
378 was accumulated in the middle of the lamella region of the cell wall and then stabilized. This
379 stabilization was caused by the formation of ionic bridges between pectic polysaccharides.
380 The effect of the Ca-treatments could be detected since the first date of sampling with the higher
381 breaking force values registered for the Ca-treated fruits. Tsantili et al. (2008) also found an
382 increase in firmness for table grapes after Ca-treatments were applied. Rincón & Martínez
383 (2015) also addressed the importance of the quality preservation of fruits for the
384 commercialization, characteristic that improved with the increased firmness after the
385 application of Ca-treatments.
386 Despite the Ca content in the pulp did not decrease, during the elaboration process a rigidity
387 decrease was observed. This could be because the elaboration process of Spanish-style uses
388 lactic bacteria and other microorganism that can metabolise and break the vegetable fibres
389 causing a decrease of the rigidity (Lanza, 2013). As said before, firmness decreased during the
390 elaboration process for table olives. Thus, olives went through an alkaline treatment that
391 modified the composition of the cell wall causing the olive softening. With the softening of the
392 fruit, the quality of the olive decreases (García-Serrano et al., 2020). Therefore, there is a need
393 of studying different strategies to avoid this process that cause a loss of rigidity in olives for
394 ensure high quality products.
395 The application of pre-harvest Ca-treatments also increased the content of some phenolic
396 compounds during Spanish-style elaboration process (Table 3). The highest content was
397 achieved with the highest Ca concentration. The obtained results are consistent with previous

398 studies where an increase of phenolic compounds was registered for sweet cherry fruits when
399 trees were sprayed with calcium solution once a week from flowering until two weeks before
400 harvest (Vangdal et al., 2008). As said before, Ca-treatments increased the concentration of
401 calcium in the olives stimulating the synthesis of the phenolic compounds (Miceli et al., 1999),
402 being this the possible explanation for our results. Charoenprasert & Mitchell (2014) reported
403 that several factors may have a huge effect on the increment of phenolic compounds in olive
404 fruits, existing differences between olive cultivars. The Ca content in the fruit can directly
405 contribute to the phenol concentration along the elaboration process. Since this macronutrient
406 gives rigidity and permeability to the sample, it is likely that phenols, that are functional water-
407 soluble compounds, do not diffuse as easily to the brine when calcium is present in high
408 concentration in the matrix. The increase in firmness due to an industrial scale application of
409 $CaCl_2$ can lead to an increase on hydrophilic molecules concentration in black olives, as
410 reported by Martín-Vertedor et al. (2021). This was seen also for green olives but with less
411 intensity (Martín-Vertedor et al., 2020). Both reported studies underlined the importance of
412 controlling the addiction of $CaCl_2$ to regulate the increase of acrylamide and protect consumer's
413 health.
414 As said before, the increase in phenol compounds in Ca-treated olives was higher even after
415 fermentation in brine, resulting in a healthier functional food for the consumer. In our study,
416 Hydroxytyrosol and Tyrosol were found as the main phenolic compounds. Hydroxytyrosol has
417 a wide range of health benefits, such as, cardioprotective, anticancer, neuroprotective,
418 antimicrobial, and other effects. Tyrosol is an effective cellular antioxidant but is also effective
419 against hypertension, atherosclerosis, coronary heart disease, chronic heart failure, insulin
420 resistance and obesity (Marković et al., 2019). According to these findings, the consumption of
421 Ca-treated table olives should result more beneficial than not-treated ones.

Moreover, the content of phenolic compounds increased with increasing Ca applied concentration treatments and consequently the antioxidant activity, being the factors highly correlated. Other studies also confirmed this correlation Giménez et al. (2014) in sweet cherries after the application of acetylsalicylic acid. Ozturk et al. (2014) indicated the increasing of antioxidant activity in plum fruits by applying calcium treatments. These authors indicated that this fact contributes to provided anti-mutagenic and anti-carcinogenic benefits for humans. Other researchers (Martínez-Esplá et al., 2014) related the increase of antioxidant activity with the activity of certain enzymes such as superoxide dismutase, catalase, peroxidase, ascorbate peroxidase, and polyphenol oxidase. When these enzymes and the phenols are together, they are able to collect free radicals and reactive oxygen species that provoke the increasing of the antioxidant activity. The increase in antioxidant activity caused by the increase in phenol profile due to pre-harvest Ca-treated provides a final product with a health benefit for human. This fact also provides the possibility of having a final product on the market with longer shelf-life or by reducing the degradation process of the table olives, especially when they are marketed with some quality mark, such as organic farming.

## Conclusion

The application of Ca-treatments during the fruit development (pre-harvest) by spraying 0.5% and 1.0% $CaCl_2$ concentrations enhanced the quality of the Spanish-style table olives by increasing the Ca content on the fruit, the firmness and the phenolic content. The fermentation process of the olives subjected to pre-harvest calcium treatments occurred regularly, without registering toxic effects of the calcium (no inhibition of the microorganisms responsible of the fermentation process). Further and deep studies about the effect of pre-harvest calcium treatments on the inhibition of spoilage bacteria are required.

An important result highlighted in our study was that pre-harvest Ca-treatments provided a better firmness to the olives throughout the fermentation process. This aspect is particularly important for those varieties, as 'Ascolana tenera', that are characterized by a soft pulp and are particularly sensible to damages during harvest or post-harvest manipulation. An increased pulp firmness could allow the mechanization of the fruit handling during harvest and post-harvest. Moreover, the effect of additional post-harvest Ca applications should be studied to verify if an improvement of the organoleptic characteristics (hardness and crunchiness) of the fruit is possible in order to give a better sensory experience to the consumers. Our results also demonstrated that fruit quality parameters can be increased by Ca-treatments during the fruit development. In fact, the accumulation of phenolic compounds was stimulated and their content did not decrease during the elaboration process. This result has an important consequence resulting the table olives with higher phenols and antioxidant activity an outstanding functional food for the consumers.

**Acknowledgements**

The authors wish to thank "Olive Gregori" farm in the person of Luca Gregori for the support provided during this study.

**Funding:** This work has been supported by Junta de Extremadura (GR21121) and the "Garantía Juvenil" contract (TE-0078-19).

**Conflicts of Interest**: The authors state that they have no conflicts of interest.

**References**

Aghdam, M.S., Dokhanieh, A.Y., Hassanpour, H., Rezapour Fard, J., 2013. Enhancement of antioxidant capacity of cornelian cherry (Cornus mas) fruit by postharvest calcium treatment. Sci. Hortic. (Amsterdam). 161, 160–164. https://doi.org/10.1016/j.scienta.2013.07.006

Brenes, M., García, P., Garrido, A. 1994. Influence of salts and pH on the firmness of olives in acid conditions. J. Food Qual. 17(4), 335-346. https://doi.org/10.1111/j.1745-4557.1994.tb00155.x

Cabrera-Bañegil, M., Martín-Vertedor, D., Boselli, E., Durán-Merás, I., 2018. Control of olive cultivar irrigation by front-face fluorescence excitation-emission matrices in combination with PARAFAC. J. Food Compos. Anal. 69, 189–196. https://doi.org/10.1016/j.jfca.2018.01.021

Cabrera-Bañegil, M., Schaide, T., Manzano, R., Delgado-Adámez, J., Durán-Merás, I., Martín-Vertedor, D., 2017. Optimization and validation of a rapid liquid chromatography method for determination of the main polyphenolic compounds in table olives and in olive paste. Food Chem. 233, 164–173. https://doi.org/10.1016/j.foodchem.2017.04.052

Charoenprasert, S., Mitchell, A., 2014. Influence of California-style black ripe olive processing on the formation of acrylamide. J. Agric. Food Chem. 62, 8716–8721. https://doi.org/10.1021/jf5022829

Ciccarese, A., Stellacci, A.M., Gentilesco, G., Rubino, P., 2013. Effectiveness of pre- and post-veraison calcium applications to control decay and maintain table grape fruit quality during storage. Postharvest Biol. Technol. 75, 135–141. https://doi.org/10.1016/j.postharvbio.2012.08.010

Conway, W.S., Sams, C.E., Hickey, K.D., 2002. Pre- and postharvest calcium treatment of

493 apple fruit and its effect on quality, in: Acta Horticulturae. pp. 413–419.
494 https://doi.org/10.17660/ActaHortic.2002.594.53

495 Gao, Q., Tan, Q., Song, Z., Chen, W., Li, X., Zhu, X., 2020. Calcium chloride postharvest
496 treatment delays the ripening and softening of papaya fruit. J. Food Process. Preserv. 44.
497 https://doi.org/10.1111/jfpp.14604

498 García-Serrano, P., Romero, C., Medina, E., García-García, P., de Castro, A., Brenes, M.,
499 2020. Effect of calcium on the preservation of green olives intended for black ripe olive
500 processing under free-sodium chloride conditions. Lwt 118.
501 https://doi.org/10.1016/j.lwt.2019.108870

502 Gharezi, M., Gharezi, M., 2012. Effect of Post Harvest Treatment on Stored Cherry
503 Tomatoes. J. Nutr. Food Sci. 02. https://doi.org/10.4172/2155-9600.1000157

504 Giménez, M.J., Valverde, J.M., Valero, D., Guillén, F., Martínez-Romero, D., Serrano, M.,
505 Castillo, S., 2014. Quality and antioxidant properties on sweet cherries as affected by
506 preharvest salicylic and acetylsalicylic acids treatments. Food Chem. 160, 226–232.
507 https://doi.org/10.1016/j.foodchem.2014.03.107

508 Gouvinhas, I., Barros, A.I., 2021. Effect of Foliar Pre-Harvest Calcium Application on the
509 Mineral and Phytochemical Composition of Olive Oils 66.
510 https://doi.org/10.3390/foods_2020-07662

511 Harker, F.R., Ferguson, I.B., 1988. Transport of calcium across cuticles isolated from apple
512 fruit. Sci. Hortic. (Amsterdam). 36, 205–217. https://doi.org/10.1016/0304-
513 4238(88)90055-6

514 Hocking, B., Tyerman, S.D., Burton, R.A., Gilliham, M., 2016. Fruit calcium: Transport and
515 physiology. Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.00569

516 Holb, I. J., Balla, B., Vámos, A., Gáll, J. M. 2012. Influence of preharvest calcium applications,
517 fruit injury, and storage atmospheres on postharvest brown rot of apple. Postharvest Biol.
518 Technol., 67, 29-36. https://doi.org/10.1016/j.postharvbio.2011.12.008

519 International Olive Council [WWW Document], 2020. URL
520 https://www.internationaloliveoil.org/?lang=es_ES.

521 Jemrić, T., Fruk, I., Fruk, M., Radman, S., Sinkovič, L., Fruk, G., 2016. Bitter pit in apples:
522 Pre- and postharvest factors: A review. Spanish J. Agric. Res. 14.
523 https://doi.org/10.5424/sjar/2016144-8491

524 Langer, S. E., Marina, M., Burgos, J. L., Martínez, G. A., Civello, P. M., Villarreal, N. M. 2019.
525 Calcium chloride treatment modifies cell wall metabolism and activates defense responses
526 in strawberry fruit (*Fragaria ananassa*, Duch). J. Sci. Food and Agric. 99(8), 4003-4010.
527 https://doi.org/10.1002/jsfa.9626

528 Lanza, B. 2013. Abnormal fermentations in table-olive processing: microbial origin and
529 sensory evaluation. Front. Microbiol., 4, 91. https://doi.org/10.3389/fmicb.2013.00091

530 Lara, I., 2013. Preharvest sprays and their effects on the postharvest quality of fruit. Stewart
531 Postharvest Rev. 9. https://doi.org/10.2212/spr.2013.3.5

532 Lodolini, E.M., Cabrera-Bañegil, M., Fernández, A., Delgado-Adámez, J., Ramírez, R.,
533 Martín-Vertedor, D., 2019. Monitoring of acrylamide and phenolic compounds in table
534 olive after high hydrostatic pressure and cooking treatments. Food Chem.
535 https://doi.org/10.1016/j.foodchem.2019.01.191

536 Luna-Guzmán, I., Cantwell, M., Barrett, D.M., 1999. Fresh-cut cantaloupe: Effects of CaCl2
537 dips and heat treatments on firmness and metabolic activity. Postharvest Biol. Technol.
538 17, 201–213. https://doi.org/10.1016/S0925-5214(99)00048-4

539 Madani, B., Mohamed, M. T. M., Biggs, A. R., Kadir, J., Awang, Y., Tayebimeigooni, A.,
540 Shojaei, T. R. 2014. Effect of pre-harvest calcium chloride applications on fruit calcium
541 level and post-harvest anthracnose disease of papaya. Crop Prot., 55, 55-60.

542 Manganaris, G.A., Vasilakakis, M., Mignani, I., Diamantidis, G., Tzavella-Klonari, K., 2005.
543 The effect of preharvest calcium sprays on quality attributes, physicochemical aspects of
544 cell wall components and susceptibility to brown rot of peach fruits (Prunus persica L.
545 cv. Andross). Sci. Hortic. (Amsterdam). 107, 43–50.
546 https://doi.org/10.1016/j.scienta.2005.06.005

547 Marković, A.K., Torić, J., Barbarić, M., Brala, C.J., 2019. Hydroxytyrosol, tyrosol and
548 derivatives and their potential effects on human health. Molecules 24.
549 https://doi.org/10.3390/molecules24102001

550 Martín-Vertedor, D., Fernández, A., Mesías, M., Martínez, M., Díaz, M., Martín-Tornero, E.,
551 Martínez, Manuel, Díaz, M., Martín-Tornero, E., 2020. Industrial strategies to reduce
552 acrylamide formation in californian-style green ripe olives. Foods 9, 1202.
553 https://doi.org/10.3390/foods9091202

554 Martín-Vertedor, D., Fernández, A., Mesías, M., Martínez, M., Martín-Tornero, E., 2021.
555 Identification of mitigation strategies to reduce acrylamide levels during the production
556 of black olives. J. Food Compos. Anal. 102. https://doi.org/10.1016/j.jfca.2021.104009

557 Martínez-Esplá, A., Zapata, P.J., Valero, D., García-Viguera, C., Castillo, S., Serrano, M.,
558 2014. Preharvest application of oxalic acid increased fruit size, bioactive compounds,
559 and antioxidant capacity in sweet cherry cultivars (prunus avium L.). J. Agric. Food
560 Chem. 62, 3432–3437. https://doi.org/10.1021/jf500224g

561 Miceli, A., Ippolito, A., Linsalata, V., Nigro, F., 1999. Effect of preharvest calcium treatments

562 on decay and biochemical changes in table grape during storage. Phytopathol. Mediterr.
563 47–53.

564 Morales-Sillero, A., Lodolini, E.M., Suárez, M.P., Navarrete, V., Jiménez, M.R., Casanova,
565 L., Gregori, L., Rallo, P., Martín-Vertedor, D., 2021. Calcium applications throughout
566 fruit development enhance olive quality, oil yield, and antioxidant compounds' content.
567 J. Sci. Food Agric. 101, 1944–1952. https://doi.org/10.1002/jsfa.10810

568 Ozturk, B., Yildiz, K., Kucuker, E., 2014. Effect of pre-harvest methyl jasmonate treatments
569 on ethylene production, water-soluble phenolic compounds and fruit quality of Japanese
570 plums. J. Sci. Food Agric. 95, 583–591. https://doi.org/10.1002/jsfa.6787

571 Rallo, L., Díez, C. M., Morales-Sillero, A., Miho, H., Priego-Capote, F., Rallo, P. 2018.
572 Quality of olives: A focus on agricultural preharvest factors. Sci. Hortic., 233, 491-509.
573 https://doi.org/10.1016/j.scienta.2017.12.034

574 Rincón, A., Martínez, E., 2015. Funciones del calcio en la calidad poscosecha de frutas y
575 hortalizas. Aliment. Hoy Rev. Asoc. Colomb. y Tecnol. Aliment. 24, 13–25.

576 Schaide, T., Cabrera-Bañegil, M., Pérez-Nevado, F., Esperilla, A., Martín-Vertedor, D., 2019.
577 Effect of olive leaf extract combined with Saccharomyces cerevisiae in the fermentation
578 process of table olives. J. Food Sci. Technol. 56, 3001–3013.
579 https://doi.org/10.1007/s13197-019-03782-x

580 Tobias, R.B., Conway, W.S., Sams, C.E., Gross, K.C., Whitaker, B.D., 1992. Cell wall
581 composition of calcium-treated apples inoculated with Botrytis cinerea. Phytochemistry
582 32, 35–39. https://doi.org/10.1016/0031-9422(92)80102-K

583 Tsantili, E., Christopoulos, M. V., Pontikis, C.A., Kaltsikes, P., Kallianou, C., Komaitis, M.,
584 2008. Texture and other quality attributes in olives and leaf characteristics after

preharvest calcium chloride sprays. HortScience 43, 1852–1856. https://doi.org/10.21273/hortsci.43.6.1852

Vangdal, E., Hovland, K.L., Børve, J., Sekse, L., Slimestad, R., 2008. Foliar application of calcium reduces postharvest decay in sweet cherry fruit by various mechanisms. Acta Hortic. 768, 143–148. https://doi.org/10.17660/ActaHortic.2008.768.16