Evaluation of chemical components of debittered olives undergone preservation and polyphenol oxidation

Journal:	<i>International Journal of Food Science and Technology</i>
Manuscript ID	IJFST-2015-19289.R3
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	n/a
Complete List of Authors:	Brenes, Manuel; Instituto de la Grasa (CSIC), Food Biotechnology Ramírez, Eva M ^a ; Instituto de la Grasa (CSIC, Food Biotechnology Department Romero, Concepción; Instituto de la Grasa (CSIC), Food Biotechnology Garcia, Pedro; Instituto de la Grasa (CSIC, Food Biotechnology Department
Keywords:	Olives, Polyphenols, Processing Effects

SCHOLARONE™
Manuscripts

Graphical Abstract

Graphical Abstract
190x254mm (96 x 96 DPI)

**Evaluation of chemical components of debittered olives undergone preservation
and polyphenol oxidation**

Eva M^a Ramírez, Pedro García, Manuel Brenes* & Concepción Romero

Food Biotechnology Department, Instituto de la Grasa (IG-CSIC), Campus University

Pablo de Olavide, Ctra. Utrera km 1, 41013-Seville, Spain

*Corresponding author, Tel.: +34 954611550; fax: +34 954616790

e-mail address: brenes@cica.es

15 **Summary**

16 Olives can reduce their bitterness by oleuropein oxidation via an enzymatic reaction. In
17 this study, olives of the Manzanilla and Hojiblanca varieties preserved in acidified brine
18 were submitted to oxidation tests under overpressure of oxygen (0.3 bars) for 3 days. It
19 was found that the oxidation of fruit preserved for more than 3-4 months showed a
20 reduction in the concentration of oleuropein but with great variability (28-100 %). By
21 contrast, when olives were preserved for only one month the oxidation treatment was
22 able to decrease more than 90% of the content of oleuropein in the fruit. These oxidized
23 olives were stored again in the acidified medium for 6 months and no spoilage or
24 organoleptic problems were detected. Oxidation gave rise to darker olives (L^* parameter
25 diminished from 40 to 34). Moreover, the oxidized fruit was enriched with free phenols
26 such as hydroxytyrosol and tyrosol as a consequence of the acid hydrolysis of phenolic
27 glucosides during the preservation step.

29 **Keywords:** olive, bitter, oleuropein, phenolic, oxidation

41 Introduction

42 Table olives are highly appreciated for their sensory characteristics and nutritive value,
43 and they are used as an appetizer accompanying beverages or in salads, pizzas and
44 Mediterranean-style meals. The most significant production methods of table olives are
45 the Spanish and the Californian styles, both involving a debittering process with sodium
46 hydroxide solutions. Besides these two elaboration methods, alternative processes are
47 also used to debitter the olives without the alkaline treatment. Among them, the most
48 common method worldwide consists of putting the olives directly into a brine solution
49 where they undergo complete or partial fermentation but debittering is slow and takes
50 place for months or even years depending on the olive variety and the initial oleuropein
51 concentration in the fruit. According to “Trade standard applying to table olives” (IOC,
52 2004) these fruit are labelled as natural (green, turning colour or black), and they are
53 very appreciated in local markets. Besides, they are rich in bioactive substances such as
54 phenolic compounds and triterpenic acids (Romero *et al.*, 2004; 2010).

55 Oleuropein is formed by glucose, elenolic acid and hydroxytyrosol, the latter
56 compound being linked to the rest of the moiety by an ester bond. The cleavage of this
57 bond gives rise to non-bitter substances (Brenes, & De Castro, 1998). It has been
58 proposed that natural olives lose their bitterness due to the diffusion of the oleuropein
59 from the fruit into the surrounding brine and further acid hydrolysis of the glucoside
60 with time (Brenes *et al.*, 1993). Ciafardini *et al.* (1994) suggested that microorganisms,
61 particularly lactic acid bacteria, can contribute to the hydrolysis of oleuropein by means
62 of β -glucosidase production, although the growth of these microorganisms in brine may
63 be inhibited by products of the oleuropein hydrolysis (Medina *et al.*, 2010). Recently, it
64 has also been reported that the endogenous β -glucosidase of the fruit participate to a
65 large extent in the debittering process of the natural olives (Medina *et al.*, 2009).

66 Consequently, processors must wait for months to market natural olives, and
67 researchers have explored new systems to accelerate the debittering step. Yeasts and
68 lactic acid bacteria are the predominant microorganisms in the brines of natural olives,
69 and species isolated from olive brines have shown the ability to produce β -glucosidase
70 (Ciafardini *et al.*, 1994; Psani & Kotzekidou, 2006; Tofalo *et al.*, 2013; Bleve *et al.*,
71 2015). Moreover, the use of lactic acid bacteria and yeasts starters to ferment natural
72 olives seems to shorten the debittering process (Servili *et al.*, 2006; Pistarino *et al.*,

2013; Bevilacqua *et al.*, 2013), although it depends on the olive variety (Servili *et al.*, 2008).

Olives can also lose their bitterness due to oleuropein oxidation, as reported for black dry-salted olives (Ramírez *et al.*, 2013). Oleuropein is oxidized to its quinone (Antolovich *et al.*, 2004), which rapidly polymerizes to non-bitter brown pigments, which can also affect the quality of the product (Sánchez *et al.*, 2013; Ramírez *et al.*, 2015). It has recently been discovered that natural fruit in brine can lose its bitterness when olives are oxidized under an overpressure of oxygen (García *et al.*, 2008). However, this method has failed with some batches of fruit in several seasons and no reasons were found for this phenomenon. The aim of this work was to study the debittering of olives by oleuropein oxidation in depth, and to optimize the promising new method.

Materials and methods

Industrial olive samples for oxidation assays

Olives of the Hojiblanca and Manzanilla varieties were obtained from two Table Olive Cooperatives located in the province of Seville (Spain) during the 2012/2013 and 2013/2014 seasons. On arrival to the factories, leaves and small branches were removed and the fruits with green-yellow colour on the surface were washed and put into fiberglass underground tanks which contained about 9500 kg of fruit and 5500 L of acidified brine (6% NaCl and 1 % acetic acid) (De Castro *et al.*, 2007). Samples of olives were taken from the tanks after 4, 6 and 7 months of brining during the 2012/2013 season and one month of brining during the 2013/2014 season. Subsequently, they were transported to the laboratory for oxidation experiments.

Oxidation experiments

Oxidation tests were performed with olives preserved for months during the two studied seasons. Five kilograms of fruit were put into a cylindrical stainless container (30 cm Ø x 80 cm length) that was hermetically sealed. Subsequently, oxygen was introduced through an inlet valve and expelled through an outlet valve for 5 min. The outlet valve

103 was closed, and an overpressure of 0.3 bars was obtained in the container. The drum
104 was rotated one revolution per hour. The oxygen was released after three days of
105 oxidation and the olives were analysed.

106

107 Pilot plant assays with Manzanilla olives

108 Fruit of the Manzanilla variety harvested during the season 2013/2014 with green-
109 yellow colour on the surface were put into 3 L PVC vessels and covered with a brine
110 (9% w/v, NaCl) acidified with acetic acid (0.3 % w/v). After one month of brining, the
111 olives were submitted to oxidation under an overpressure of 0.3 bars for 3 days.
112 Subsequently, the oxidized fruits were put again into the PVC vessels and covered with
113 the same preservation brine for 6 months (Figure 1). All experiments were run in
114 duplicate. Chemical compounds were purchased from Panreac (Barcelona, Spain).

115

116 Microbiological analyses

117 Samples of the preservation solutions from oxidized olives were taken with a sterile
118 pipette from the geometric centre of the PVC vessels. Solutions and appropriate decimal
119 dilution (in sterile 0.1 % peptone water) were plated using a Spiral System model DS
120 (Interscience, Saint Nom La Breteche, France). Enterobacteriaceae were counted on
121 crystal violet neutral-red bile dextrose agar (Merck, Darmstadt, Germany) incubated at
122 37 °C, lactobacilli were counted on MRS agar (Oxoid Ltd Basingstoke, UK) with azide
123 (0.02 %), and yeasts on GYE (Glucose Yeast Extract) (Oxoid). Except
124 Enterobacteriaceae, all media were incubated at 32 °C.

125

126 Polyphenol analysis

127 Pitted fruits were crushed with an Ultraturrax homogenizer, and 3 g of the paste were
128 mixed with 10 mL of dimethyl sulfoxide (DMSO) (Sánchez *et al.*, 2013). After 1 min of
129 agitation by vortex, the mixture was centrifuged at 6000 g for 5 min, and the
130 supernatant was filtered through a 0.22 µm pore size nylon filter. An aliquot of 250 µL
131 was mixed with 250 µL of internal standard (0.2 mmol/L of syringic acid in DMSO)

132 and 500 μ L of DMSO. Finally, 20 μ L of the mixture were injected into the
133 chromatograph. A Spherisorb ODS-2 (5 μ m, 250 x 4.6 mm, Waters Inc.) column was
134 used. The HPLC system consisted of a Waters 2695 Alliance (Waters Inc., Mildford,
135 MA, USA) with a pump, column heater and auto sampler and the detection was
136 performed with a Waters 996 diode array detector at 280 nm. Separation was achieved
137 using an elution gradient with an initial composition of 90 mL of water (pH adjusted to
138 2.3 with phosphoric acid) and 10 mL of methanol. The concentration of the latter
139 solvent was increased to 30/70 mL (methanol/water) over 10 min and maintained for 20
140 min. Subsequently, the methanol concentration was raised to 40/60 (methanol/water)
141 over 10 min, maintained for 5 min and then increased to 50/50 (methanol/water).
142 Finally, the methanol concentration was increased to 60/40, 70/30 and 100% in 5 min
143 intervals. The flow rate of 1 mL/min and a temperature of 35 $^{\circ}$ C were used (Ramírez *et*
144 *al.*, 2015). DMSO and methanol were obtained from Panreac (Barcelona, Spain).

146 Colour analysis

147 Colorimetric measurements on olives were performed using a BYK-Gardner Model
148 9000 Colour-view spectrophotometer, equipped with computer software to calculate
149 CIE L^* (lightness), a^* (redness) and b^* (yellowness) parameters by scanning the surface
150 from 380 to 720 nm. The data from each measurement were the average of 20 olives.

152 Chemical parameters of brines

153 The concentration of NaCl was analysed by titration with a 0.1 N silver nitrate solution,
154 using a potassium chromate solution as indicator. The pH of brines was measured with a
155 Beckman model 45 pH-meter. Free acidity was measured by titration using a Metrohm
156 670 Titro processor (Herisau, Switzerland) up to pH 8.3 with 0.2 M NaOH and
157 expressed as % (w/v) of lactic acid (Garrido-Fernández, Fernández-Díez, & Adams,
158 1997).

159 Statistical analysis

1
2
3 160 Statistica software version 7.0 was used for data processing (Statistica for Windows,
4 161 Tulsa, OK, USA). A comparison among mean variables was made by Duncan's
5 162 multiple-range tests, and the differences were considered significant when $p < 0.05$.

6
7
8
9 163

10 11 164 **Results and discussion**

12
13 165 García *et al.* (2008) showed that olives preserved directly in brine and submitted to an
14 166 overpressure of oxygen of 0.3 bars for several days reduced their content in the bitter
15 167 compound oleuropein due to its enzymatic oxidation. However, oxidation tests carried
16 168 out in our lab under these conditions (0.3 bars for 5 days) with olives from different
17 169 varieties have disclosed that in some cases the concentration of oleuropein slightly
18 170 decreased. All the failed tests occurred with olives preserved for a long time, and it
19 171 suggested the importance of the preservation time on the successful of the tests. In this
20 172 study, the oxidation tests were performed on fruit of the Manzanilla and Hojiblanca
21 173 varieties, which are the two most employed varieties in Spain for processing as table
22 174 olives. They were preserved in acidified brine for several months before oxidation
23 175 during the 2012/2013 season. The results presented in Table 1 show that a complete
24 176 elimination of oleuropein was reached in fruits of the Hojiblanca variety preserved for
25 177 180 days due to the oxidation treatment. In contrast, a remaining concentration of this
26 178 substance was found in all oxidized Manzanilla olives regardless of the preservation
27 179 time. The initial concentration of the bitter compound was lower in preserved olives of
28 180 the Hojiblanca than Manzanilla variety, which can be related to the higher concentration
29 181 reported in raw olives of the latter variety than the former (Ramírez *et al.*, 2014). The
30 182 reduction in oleuropein concentration in oxidized Manzanilla olives ranged from 28 to
31 183 98% but it was not correlated with the time of preservation or the acidity conditions of
32 184 the brine. As can be observed in Table 2, the pH and acidity of the brines did not vary
33 185 greatly among samples thereby the variability in loss of oleuropein cannot be attributed
34 186 to the pH of the olives, which ranged from 3.6 to 3.9 units. It must be noted that there is
35 187 equilibrium between olive and brine so that the pH and acidity of the fruit is the same as
36 188 the brine.

37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54 189 It has been demonstrated that the oxidation of oleuropein in olives preserved in
55 190 brine is catalysed by the enzyme polyphenoloxidase (PPO) (García *et al.*, 2008). PPO
56 191 activity increases with olive maturation (Ortega-García *et al.*, 2008) but also decreases

192 largely with the time of the fruit in the acidified brine (Ramírez *et al.*, 2015).). A rapid
193 inactivation of PPO obtained from green olives (Toscano *et al.*, 2003) and in olive paste
194 during the malaxation step of the olive oil processing (Taticchi *et al.*, 2013) have been
195 reported. Pectinesterase and glycosidase activity are lost in cucumbers within the first
196 week of fermentation in brine (Maruvada, & McFeeters, 2009). All these data imply
197 that the activity of endogenous enzymes diminishes to a large extent in fruits preserved
198 in acidified brine with time, particularly that of PPO. Despite PPO activity has been
199 undetectable after one month of brining (Ramírez *et al.*, 2015); its effect has been
200 observed on olives submitted to oxidation (García *et al.*, 2008). Besides, the reduction
201 of the oleuropein concentration in olives of the Manzanilla variety preserved for 210
202 days after oxidation (Table 1) depended to a large extent on the batch tested, which is
203 likely due to the great variability of both phenolic compounds and PPO activity found in
204 raw olives (Ramírez *et al.*, 2014).

205 Taking into consideration the results reported in Table 1 and the previous
206 references on the loss of enzymatic activity with the time of fruit in brine, oxidation
207 tests were performed on 8 batches of olives preserved for only one month in acidified
208 brine during the 2013/2014 season. This time is necessary to reach salt and acidity
209 equilibrium between olive and surrounding liquid. The results presented in Figure 2
210 show that a great reduction in the oleuropein concentration was reached in all the fruits
211 regardless of the olive variety. Again, the Manzanilla fruit had a higher concentration of
212 oleuropein than Hojiblanca after one month of brine for all the batches. It must be
213 highlighted that less than 10% of oleuropein remained in all the olives submitted to
214 oxidation for several days (Table 3). Other phenolic compounds greatly affected by
215 oxidation were the *o*-phenolic hydroxytyrosol 1-glucoside and the dialdehydic form of
216 decarboxymethyl elenolic acid linked to hydroxytyrosol. In contrast, the non-
217 orthodiphenolic hydroxytyrosol 4-glucoside, salidroside and tyrosol reduced their
218 concentration in olives during the oxidation treatment to a lesser extent.

219 These results have demonstrated that the bitter glucoside oleuropein can be
220 eliminated to a large extent in green olives preserved directly in acidified brine but it
221 must be carried out soon after brining. From a practical point of view, the olives can be
222 oxidized just one month from brining and most bitterness would be lost. However, it
223 means that processors should oxidize all their olives in just 1-2 months from brining and
224 they must sell the product immediately. A new experiment was designed to explore the

possibility of preserving the oxidized olives for at least 6 months in the same brine used before the oxidation step. If the experiments were successful processors will have a period of 6 months to pack and sell their product. These assays were carried out with Manzanilla olives at pilot plant scale following the scheme reflected in Figure 1. The results indicated that the pH in brine decreased from 4.2 to 4.0 units during the 6 months of the post-oxidation period, whereas the free acidity increased from 0.4 to 0.7 % expressed as lactic acid, which means that a strong lactic acid fermentation did not occurred. In fact, yeasts were the predominant microbiota in the brines of the oxidized olives although lactobacilli were also detected. Besides, no off-odour or any other symptoms of spoilage were detected during the storage of oxidized fruit by three table olive judges, which are members of the Table Olives Sensory Panel of the Instituto de la Grasa with over 25 years of experience on this field.

With regards to the phenolic compounds, the concentration of hydroxytyrosol increased in the olive pulp with time of preservation, which is probably related to the decrease in hydroxytyrosol 4-glucoside as a consequence of its acid hydrolysis (Figure 3). A similar statement can be made for tyrosol and salidroside, the latter substance being tyrosol glucoside. Luteolin 7-glucoside and rutin concentration also decreased during the post-oxidation step of the fruit in brine. Moreover, the low content of oleuropein in oxidized olives disappeared after 6 months of storage because of the acid conditions (data not shown). Therefore, the olives reduced their concentration in phenolic compounds during the oxidation step but a high concentration of these substances still remained in the oxidized and preserved fruit.

As it has been previously observed (García et al., 2008), the oxidation treatment gave rise to darker olives (Table 4). The lightness (L^*) and yellowness (b^*) parameters were reduced for all olives, regardless of the variety, although the final values were higher for the olives of the Hojiblanca than the Manzanilla variety. Consequently, the fruit changed its colour from yellow-brown to brown. It must also be noted that the colour of the oxidized olives after one month of preservation slightly lightened during the second storage period for 6 months.

Conclusion

256 The bitterness of olives preserved in acidified brine can be reduced by maintaining the
257 fruit under an overpressure of oxygen for several days. This phenomenon is based on
258 the enzymatic oxidation of the bitter glucoside oleuropein. However, the activity of the
259 enzyme PPO is decreasing with time of brining, and therefore the olives must be
260 submitted to the oxidation treatment as soon as possible from brining. In fact, it was
261 found that olives of the Manzanilla and Hojiblanca varieties lost most of their
262 oleuropein concentration when they were oxidized after one month of preservation.
263 These oxidized olives were preserved again in the acidified brine for 6 months and no
264 sensory defects were detected. As it was expected, the colour of the olives darkened due
265 to the oxidation treatment, and a hydrolysis of the phenolic glucosides occurred during
266 the storage period. This new process must be assayed on many other olive varieties
267 from many countries but the results are very promising to accelerate the debittering step
268 of natural olives non-treated with alkali.

270 **Acknowledgements**

271 This work was supported by the Project AGL-2013-42739-R and the European Union
272 FEDER funds.

274 **References**

275 Antolovich, M., Bedgood, D., Bishop, A. G., Jardine, D., Prenzler, P. D., & Robards, K.
276 (2004). LC-MS investigation to oxidation products of phenolic antioxidants.
277 *Journal of Agricultural and Food Chemistry*, **52**, 962-971.

278 Bevilacqua, A., Beneduce, L., Sinigaglia, M., & Corbo, M. R. (2013). Selection of
279 yeasts as starter cultures for table olives. *Journal of Food Science*, **78**, M742-
280 M751.

281 Bleve, G., Tufariello, M., Durante, M., Grieco, F., Ramires, F. A., Mita, G., Tasioula-
282 Margari, M., & Logrieco, A. F. (2015). Physico-chemical characterization of
283 natural fermentation process of Conservolea and Kalamata table olives and
284 development of a protocol for the pre-selection of fermentation starters. *Food*
285 *Microbiology*, **46**, 368-382.

- 286 Brenes, M., García, P., Durán, M. C., & Garrido, A. (1993). Concentration of phenolic
287 compounds change in storage brines of ripe olives. *Journal of Food Science*, **58**,
288 347-350.
- 289 Brenes, M., & De Castro, A. (1998). Transformation of oleuropein and its hydrolysis
290 products during Spanish-style green olive processing. *Journal of the Science and*
291 *the Food Agriculture*, **77**, 353-358.
- 292 Ciafardini, G., Marsilio, V., Lanza, B., & Pozzi, N. (1994). Hydrolysis of oleuropein by
293 *Lactobacillus plantarum* strains associated with olive fermentation. *Applied and*
294 *Environmental Microbiology*, **60**, 4142-4147.
- 295 De Castro, A., García, P., Romero, C., Brenes, M., & Garrido, A. (2007). Industrial
296 implementation of black ripe olive storage under acidic conditions. *Journal of*
297 *Food Engineering*, **80**, 1206-1212.
- 298 García, A., Romero, C., Medina, E., García, P., De Castro, A., & Brenes, M. (2008).
299 Debittering of olives by polyphenol oxidation. *Journal of Agricultural and*
300 *Food Chemistry*, **56**, 11862-11867.
- 301 Garrido-Fernández, A., Fernández-Díez, M. J., & Adams, M. R. (1997). *Table olives.*
302 *Production and processing*. Chapter 12. Chapman & Hall, 1997.
- 303 International Olive Council (IOC). 2004. Trade Standard Applying to Table Olives.
304 COI/OT/NC N° 1 December. Madrid. Spain.
- 305 Maruvada, R., & McFeeters, R. F. (2009). Evaluation of enzymatic and non-enzymatic
306 softening in low salt cucumbers fermentation. *International Journal of Food*
307 *Science and Technology*, **44**, 1108-1117.
- 308 Medina, E., García, A., Romero, C., De Castro, A., & Brenes, M. (2009). Study of the
309 anti-lactic acid bacteria in table olives. *International Journal of Food Science*
310 *and Technology*, **44**, 1286-1291.
- 311 Medina, E., Gori, C., Servili, M., de Castro, A., Romero, C., & Brenes, M. (2010). Main
312 variables affecting the lactic acid fermentation of table olives. *International*
313 *Journal of Food Science and Technology*, **45**, 1291-1296.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

314 Ortega-García, F., Blanco, S., Peinado, M. A., & Peragón, J. (2008). Polyphenol oxidase
315 and its relationship with oleuropein concentration in fruits and leaves of olive
316 (*Olea europaea*) cv. “Picual” trees during fruit ripening. *Tree Physiology*, **28**,
317 45-54.

318 Pistarino, E., Aliakbarian, B., Casazza, A., Paini, M., Cosulich, M. E., & Perego, P.
319 (2013). Combined effect of starter culture and temperature on phenolic
320 compounds during fermentation of Taggiasca black olives. *Food Chemistry*,
321 **138**, 2043-2049.

322 Psani, M., & Kotzekidou, P. (2006) Technological characteristics of yeast strains and
323 their potential as starter adjuncts in Greek-style black olive fermentation. *World*
324 *Journal of Microbiology and Biotechnology*, **22**, 1329-1336.

325 Ramírez, E., García, P., De Castro, A., Romero, C., & Brenes, M. (2013). Debittering of
326 black dry-salted olives. *European Journal of Lipid Science and Technology*, **115**,
327 1319-1324.

328 Ramírez, E., Medina, E., Brenes, M., & Romero, C. (2014). Endogenous enzymes
329 involved in the transformation of oleuropein in Spanish table olive varieties.
330 *Journal of Agricultural and Food Chemistry*, **62**, 9569-9575.

331 Ramírez, E., Gandul-Rojas, B., Romero, C., Brenes, M., & Gallardo-Guerrero, L.
332 (2015). Composition of pigments and colour changes in green table olives
333 related to processing type. *Food Chemistry*, **166**, 115-124.

334 Romero, C., Brenes, M., Yousfi, K., García, P., García, A., & Garrido, A. (2004). Effect
335 of cultivar and processing method on the contents of polyphenols in table olives.
336 *Journal of Agricultural and Food Chemistry*, **54**, 2096-2102.

337 Romero, C., García, A., Medina, E., Ruíz-Méndez, M^a. V., de Castro, A., & Brenes, M.
338 (2010). Triterpenic acids in table olives. *Food Chemistry*, **118**, 670-674.

339 Sánchez, A. H., Romero, C., Ramírez, E., & Brenes, M. (2013). Storage of
340 mechanically harvested Manzanilla olives under controlled atmospheres.
341 *Postharvest Biology and Biotechnology*, **81**, 60-65.

- 342 Servili, M., Settanni, L., Veneziani, G., Esposto, S., Massitti, O., Taticchi, A., Urbani,
343 S., Montedoro, G. F., & Corsetti, A. (2006). The use of *Lactobacillus pentosus*
344 IMO to shorten the debittering process time to black table olives (cv. Itrana and
345 Leccino): a pilot-scale application. *Journal of the Agricultural and Food*
346 *Chemistry*, **54**, 3869-3875.
- 347 Servili, M., Minnocci, A., Veneziani, G., Taticchi, A., Urbani, S., Esposto, S.,
348 Sebastiani, L., Valmorri, S., & Corsetti, A. (2008). Compositional and tissue
349 modifications induced by the natural fermentation process in table olives.
350 *Journal of Agricultural and Food Chemistry*, **56**, 6389-6396.
- 351 Taticchi, A., Esposto, S., Veneziani, G., Urbani, S., Selvaggini, R., & Servili, M.
352 (2013). The influence of the malaxation temperature on the activity of
353 polyphenoloxidase and peroxidase and on the phenolic composition of virgin
354 olive oil. *Food Chemistry*, **136**, 975-983.
- 355 Tofalo, R., Perpetuini, G., Schirone, M., Suzzi, G., & Corsetti, A. (2013). Yeast biota
356 associated to naturally fermented table olives from different Italian cultivars.
357 *International Journal of Food Microbiology*, **161**, 203-208.
- 358 Toscano, G., Colarieti, M. L., & Greco, G. (2003). Oxidative polymerisation of phenols
359 by a phenol oxidase from green olives. *Enzyme and Microbial Technology*, **33**,
360 47-54.

361

Table 1

Concentration of oleuropein (mmol/kg) in olives oxidized under an overpressure of oxygen. Fruits of the Manzanilla and Hojiblanca varieties were obtained from industrial tanks where they had been preserved in acidified brine during the 2012/2013 season.

Sample	Time in brine (days)	Before oxidation (mmol/kg)	After oxidation (mmol/kg)	% reduction
Manzanilla 1	120	11.35 (4.48)a ^x	8.13 (0.74)a	28.4
Manzanilla 2	120	7.45 (0.87)a	4.73 (0.16)b	36.5
Hojiblanca 1	180	0.89 (0.11)a	0b	100
Hojiblanca 2	180	0.40 (0.03)a	0b	100
Hojiblanca 3	180	0.27 (0.02)a	0b	100
Manzanilla3	210	0.98 (0.06)a	0.61 (0.13)b	37.8
Manzanilla 4	210	2.18 (0.53)a	1.10 (0.09)b	49.5
Manzanilla 5	210	2.22 (0.13)a	0.05 (0.03)b	97.8
Manzanilla 6	210	3.12 (1.26)a	0.78 (0.41)b	75.0
Manzanilla 7	210	1.10 (0.22)a	0.52 (0.01)b	52.7

^xStandard deviation is shown in brackets. Different letters in the same mean value row indicates significant differences according to a Duncan’s multiple-range test ($p < 0.05$).

Table 2

Chemical characteristics of the brines containing the olives of the 2012/2013 season submitted to the oxidation treatment. Fruits were obtained from industrial tanks.

Sample	pH	acidity (expressed as % of lactic acid)
Manzanilla 1	3.71	0.40
Manzanilla 2	3.70	0.37
Hojiblanca 1	3.84	0.31
Hojiblanca 2	3.92	0.35
Hojiblanca 3	3.87	0.32
Manzanilla 3	3.70	0.26
Manzanilla 4	3.67	0.32
Manzanilla 5	3.92	0.52
Manzanilla 6	3.75	0.59
Manzanilla 7	3.58	0.46

Table 3

Percentage (%) of phenolic compounds remained in olives after the oxidation test. They were obtained from industrial tanks after one month of brining during the 2013/2014 season.

Compound	Manzanilla	Hojiblanca
Hydroxytyrosol 1-glucoside	6.5 (2.3) ^a	10.1 (1.3)
Hydroxytyrosol 4-glucoside	62.7 (12.1)	70.6 (7.9)
Salidroside	103.2 (9.0)	107.5 (7.3)
Tyrosol	70.2 (5.1)	1.0 (0.1)
HyEDA ^b	ND ^c	15.4 (9.2)
Oleuropein	7.4 (3.1)	10.1 (6.5)

^aStandard deviation of 4 replicates (Manzanilla 1-4; Hojiblanca 1-4) is shown in brackets; ^bDialdehydic form of decarboxymethyl elenolic acid linked to hydroxytyrosol; ^cNot detected.

Table 4

Changes in the colour parameters of olives submitted to the oxidation treatment. Fruit were obtained from industrial tanks during the season 2013/2014. The fruit were submitted to oxidation after one month of brining.

Sample	L^*		a^*		b^*		ΔE_{ab}^*
	Before	After	Before	After	Before	After	
Manzanilla 1	41.2 (1.7) ^a	32.0 (0.1) ^b	6.6 (0.5) ^a	6.3 (0.3) ^a	23.2 (1.9) ^a	9.1 (0.3) ^b	16.8 (2.3)
Manzanilla 2	36.9 (0.3) ^a	31.7 (0.3) ^b	7.0 (0.0) ^a	6.5 (0.2) ^b	16.8 (0.3) ^a	8.6 (0.3) ^b	9.7 (0.8)
Manzanilla 3	36.9 (0.1) ^a	31.6 (0.7) ^b	7.5 (0.3) ^a	6.4 (0.1) ^b	16.2 (0.5) ^a	8.2 (0.4) ^b	9.6 (1.2)
Manzanilla 4	34.6 (0.2) ^a	31.1 (0.8) ^b	7.5 (0.6) ^a	6.7 (0.4) ^b	14.3 (0.1) ^a	8.3 (0.2) ^b	7.0 (0.6)
Hojiblanca 1	40.9 (0.5) ^a	35.8 (1.0) ^b	5.6 (0.1) ^a	5.7 (0.6) ^a	19.3 (0.0) ^a	13.9 (0.9) ^b	7.4 (0.4)
Hojiblanca 2	42.1 (0.6) ^a	37.0 (0.4) ^b	5.4 (0.1) ^a	5.9 (0.2) ^b	19.7 (1.9) ^a	14.6 (0.9) ^b	7.3 (0.9)
Hojiblanca 3	45.4 (0.5) ^a	36.4 (0.3) ^b	4.8 (0.0) ^a	5.5 (0.2) ^b	23.4 (0.2) ^a	14.3 (0.2) ^b	12.7 (0.3)
Hojiblanca 4	42.8 (0.6) ^a	37.4 (0.3) ^b	5.3 (0.3) ^a	5.5 (0.0) ^a	22.4 (0.7) ^a	14.1 (0.2) ^b	10.0 (0.3)

^xStandard deviation of duplicates is shown in brackets. Different letters in the same mean value row for each colour parameter indicates significant differences according to a Duncan's multiple-range test ($p < 0.05$). $\Delta E_{ab}^* = \sqrt{(L_b^* - L_a^*)^2 + (a_b^* - a_a^*)^2 + (b_b^* - b_a^*)^2}$

Figure legends

Figure 1. Flowchart of the oxidation and preservation experiment.

Figure 2. Content of oleuropein in olives preserved for one month in industrial tanks during the season 2013/2014, and submitted to the oxidation test. Bars mean the standard deviation of duplicates. Different letters on the bars mean significant differences for the same batch according to a Duncan’s multiple-range test ($p < 0.05$).

Figure 3. Evolution of phenolic compounds in the pulp of Hojiblanca olives after one month of brining which were oxidized and put back into their brine for another 6 months. After oxidation; After oxidation and 1 month of brining; After oxidation and 3 months of brining; After oxidation and 6 months of brining. Bars mean the standard deviation of duplicates. Different letters on the bars mean significant differences for the same X value according to a Duncan’s multiple-range test ($p < 0.05$).

Figure 1
190x254mm (96 x 96 DPI)

Figure 2
190x254mm (96 x 96 DPI)

Figure 3
190x254mm (96 x 96 DPI)