# MODULATING THE BITTERNESS OF EMPELTRE OLIVE OIL BY PARTITIONING POLYPHENOLS BETWEEN OIL AND WATER PHASES: EFFECT ON QUALITY AND SHELF LIFE

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**ABSTRACT**

Bitterness is a positive sensorial attribute of olive oil that mainly depends on phenol concentration. However, excessive bitterness may result unpleasant for consumers. The aim of this investigation was to evaluate if partitioning polyphenols between oil and water phases could modulate the bitterness of a Empeltre olive oil containing a phenolic concentration higher than the typical content for this olive oil variety.

The linear relationship observed between the percentage of oil in the extraction system and the percentage of phenols removed from the oil permitted estimating the olive oil-to-water ratio required to reduce the concentration of phenols for a given value in order to modulate Empeltre olive oil bitterness. Olive oils after liquid-liquid extraction did not develop any negative sensory attributes, and their physicochemical parameters were not substantially affected.

Liquid-liquid extraction using water as a solvent is a procedure capable of effectively reducing the total phenol compounds of Empeltre extra virgin olive oil and, as a consequence, of reducing its bitterness intensity without affecting the highest commercial category determined by the parameters legally established by EC regulations just after extraction and during nine months of storage.

***Keywords:*** Bitterness, Phenolic compounds, Liquid- liquid extraction, Olive oil, Empeltre.

**Abbreviations:** TPC - total phenol content, Y – percentage of oil in the mix, PE – percentage of phenols removed from the oil.

## INTRODUCTION

Empeltre is an olive variety that is mainly cultivated in the northeast regions of Spain. These olives are known to provide yellow-colored oils with a soft taste, a fruity touch and not excessively bitterness. This makes them ideal for consumers accustomed to the taste of refined oils obtained by solvent extraction.

Phenolic compounds are the main responsible agent for the pungency and bitterness of olive oil (Angerosa et al., 2004; Servili et al., 2004). Bitterness is regarded as a positive sensory attribute of olive oil. However, thoroughly bitter olive oils may be rejected by consumers, especially if they come from varieties such as Empeltre with a low or moderate level of bitterness. Therefore, producers of Empeltre olive oil have to find an equilibrium in the polyphenol content in the order to ensure the oil stability during shelf-life, but without resulting in an excessively bitter taste.

The phenolic content of olive oil depends on the olive variety, but climatic conditions, olive maturity, or processing may also affect the amount of polyphenols in the oil (Romero et al., 2003; Servili et al., 2004). Although the Empeltre olives are characterized by their low polyphenolic content (Gracia-Gómez et al., 2009), in some campaigns, as a consequence of climatic conditions, the polyphenol content of the olives increases, resulting in oils that do not have the soft taste characteristic of the oils obtained from this olive oil variety.

Several physical treatments, such as cold storage or heating of the olives, have been suggested as methods to reduce the olive oil's bitterness (García et al., 2001; García et al., 2005; Yousfi et al., 2008). It has been demonstrated that these postharvest treatments applied to the olive fruits before oil extraction can reduce the olives' polyphenolic concentration and, consequently, the bitterness of the extracted oils without significantly affecting the physicochemical parameters established to evaluate the olive oil's quality. However, this is not a widespread practice in olive oil factories, probably due to the energetic cost required to chill or to heat the olives.

Recently it was demonstrated that liquid-liquid extraction using water as a solvent was a viable method for reducing the concentration of phenols and the bitterness in Arbequina extra virgin olive oil (Abenoza et al., 2015). Regulated parameters established to measure the commercial category of quality "extra

91 virgin" were not affected just after the extraction process. However the effect of this process on the
92 quality of the obtained oil with a low phenolic concentration during its shelf-life is unknown.

93

94 The aim of this study was to assay if liquid-liquid extraction using water as a solvent was a viable method
95 for reducing the concentration of phenols and the bitterness of Empeltre olive oil containing a phenolic
96 concentration higher than the typical content for this olive oil variety and to evaluate the physicochemical
97 and nutritional characteristics of olive oils with lower phenolic content for nine months of shelf-life.

98

99 **MATERIAL AND METHODS**

100 ***Olive Oil***

101 Olive oil from olives of the Empeltre variety with a total phenolic content (TPC) of 332.52 mg gallic acid
102 $kg^{-1}$ oil was supplied by an olive oil mill from Teruel (Aragón, Spain).

103 ***Liquid-liquid extraction***

104 Liquid-liquid extraction was performed in a laboratory container of 500 ml (7 cm in diameter, and 13.8
105 cm in height) containing 400 ml of a mixture of olive oil and water at different ratios. The olive oil-to-
106 water ratios (% volume) assayed to investigate the influence of this parameter on extraction efficiency
107 were 10/90, 30/70, 50/50, 70/30, and 90/10. The contents were mixed by applying bubbling nitrogen at a
108 pressure of 19.61 kPa at room temperature (20 ± 3°C) for 15 min. Bubbling nitrogen was used to mix
109 both liquids thoroughly and to conduct the extraction in an inert atmosphere, in order to prevent oxidation
110 reactions during the extraction process. In order to eliminate variations in the mixing system employed,
111 the position of the nitrogen tube on the bottom of the mixing vessel and the nitrogen flow were fixed for
112 all extractions alike.

113 Preliminary experiments demonstrated that, after 15 min of mixing, equilibrium in both phases was
114 reached for any olive oil-to-water ratio. After equilibrium, the two phases were separated by
115 centrifugation (1370 g for 5 min). The extraction experiments were carried out in duplicate and average
116 values were reported. Total phenol content (TPC) in the oil phase was determined before and after the
117 extraction process. Total phenol content in the water was determined after the extraction process.

Partition coefficients (PC) for different olive oil-to-water ratios were calculated from the equation:

$$PC = TPC_o / TPC_w \quad \text{(Eq. 1)}$$

where $TPC_o$ and $TPC_w$ are the total phenol content in oil and water, respectively, at equilibrium (mg $kg^{-1}$) after 15 min of mixing.

***Evaluation of effect of liquid-liquid extraction on the shelf-life of the obtained olive oils with lower phenolic content***

Once the olive oil-to-water ratio to obtain an olive oil with a given concentration of phenols was stablished, Empeltre olive oils with a concentration of phenols of around 150, 200, 250 mg gallic acid/ kg were obtained. These oils were stored in the dark at 20ºC for 9 months and every 3 months physicochemical and nutritional characteristics were analyzed.

***Analytical Measurements***

*Total phenol content (TPC):* TPC in water and oil was performed using the Folin-Ciocalteu method. An extraction step was required before determining TPC in the olive oil. The phenols were extracted by solid phase extraction (SPE), using Isolute™ C18 cartridges (6 ml, 1000 mg solid phase) (Favati et al., 1994). The results were expressed as mg gallic acid $kg^{-1}$oil.

*Measurement of physicochemical parameters:* Free acidity, peroxide value, and UV absorption coefficients ($K_{270}$ and $K_{232}$) were determined following the methods described in Regulation EEC/2568/91 of the Commission of the European Union. (EEC, 1991).

*Bitterness index*. Bitterness ($K_{225}$) was determined by SPE of bitter compounds following the method of Gutiérrez-Rosales et al., (1992) using Isolute C18 cartridges (6 ml, 500 mg solid phase). The results were expressed as the absorbance of 1 g oil per 100 ml.

*α-Tocopherol measurement*. A sample of oil in hexane was analyzed by high-pressure liquid chromatography (HPLC) with a Zorbax SB-C18 phase-reverse column (Agilent) eluted with acetonitrile/ water (99/1 v/v), using a flow rate of 1 ml/min. A photodiode matrix detector was used. Chromatograms were registered at 295 nm. The results were expressed as mg of α-tocopherol $kg^{-1}$ oil.

144 *Oxidative stability*. This was expressed as the oxidation induction time (hours), measured with a
145 Rancimat$^{TM}$ 743 instrument (Metrohm, Switzerland), using 3 g of oil warmed to 120 °C with 20 l $h^{-1}$ air
146 flow. Induction time is the time required to reach the breaking point of the curve.

147 *Individual phenols*. Phenolic compounds were extracted from olive oil following the method described by
148 Gutfinger, (1981). An HPLC analysis was performed according to the procedure of Montedoro et al.
149 (1992). Phenolic compounds were tentatively identified on the basis of their retention times, compared to
150 those of the standard compounds. Quantitative determination was performed using standards. The results
151 were expressed as mg $kg^{-1}$ oil.

152 *Sensory analysis.* The sensory analysis of the samples was carried out by 10 selected and trained panelists
153 from the accredited panel of Aragón and the Zaragoza Faculty of Veterinary Science, following the
154 method described in Regulation EEC/640/2008 (EC, 2008). The intensities of positive (fruity, bitter and,
155 pungent) and negative attributes (fusty, winey, musty, muddy, rancid, metallic, and other) were evaluated
156 for each oil sample on a non-structured scale of 10 cm anchored by its origin.

157 ***Statistical analysis***

158 Statistical analysis was performed using Statgraphics Plus 5.1. Extraction experiments were conducted in
159 duplicate. Physicochemical and nutritional analysis during storage was carried out in triplicate. The
160 results of olive oil analysis during storage were expressed as a mean ± standard deviation.

161 Significant differences between the samples were determined by the One-way ANOVA and Multiple
162 Range Test. Different letters indicate statistically significant differences ($p \leq 0.05$).

163

164 **RESULTS AND DISCUSSION**

165 *Influence of the olive oil-to-water ratio on the extraction of phenols from Empeltre olive oil*

166 The TPC of the Empeltre olive oil before extraction was 332.52 mg gallic acid $kg^{-1}$ oil. It has been
167 reported that the concentration of phenols in such olive oil ranges from 50 to 1000 mg $kg^{-1}$ of oil,
168 depending on the variety (Montedoro et al., 1992). Although phenol content of the olive oil used in this
169 investigation was in this range, TPC of Empeltre olive oil is generally lower than 300 mg kg (Angerosa et
170 al., 2004).

171 Concentration of phenols in the Empeltre olive oil after liquid-liquid extraction for 15 min at different
172 oliveoil-to-water ratios are shown in Table 1. A gradual decrease in TPC of the oils was observed when
173 the amount of water in the olive oil-to-water ratio increased. The lowest TPC in the oil when extraction
174 was conducted with the highest proportion of water in the mixture (10/90 olive oil-to-water ratio) was
175 113.9 mg $kg^{-1}$ oil, and the highest TPC was 245.9 mg $kg^{-1}$ oil when the olive-oil-to-water ratio was 90/10.

176 Liquid-liquid extraction has shown to be more effective in reducing TPC in olive oil than other suggested
177 procedures such as the heating or cold storage of olive fruits before extracting the olive oil. For example,
178 in order to decrease the TPC by around 65 % in Verdial olive oil, it was necessary to heat the olive fruits
179 at 40ºC for 72 hours (García et al., 2001) alternatively, 6 weeks of storage at 5ºC decreased the TPC of
180 the obtained oil by 55%. (Yousfi et al., 2010). Furthermore, energetic requirements for liquid-liquid
181 extraction are lower than for heating or cooling the olive fruits.

182 The behavior of the liquid-liquid extraction process for Empeltre olive oil was similar to behavior
183 previously observed for Arbequina olive oil (Abenoza et al., 2015). For an olive oil-to-water ratio of
184 70/30, the effectiveness of the reduction in TPC was similar for both oils (36.4% and 39% of reduction in
185 Arbequina and in Empeltre olive oil, respectively). However, when the olive oil-to-water ratio of the mix
186 decreased, phenolic extraction was more efficient for Arbequina that for Empeltre olive oil. For example,
187 a 50/50 olive oil-to-water ratio reduces the TPC of Arbequina olive oil by around 53.5% but only by
188 47.1% in Empeltre olive oil. Although the decrease of TPC in olive oil is consistent with mass transfer
189 principles, the different composition and polarity of individual phenols contained in both varieties of olive
190 oil could explain the differences observed in the efficacy of liquid-liquid extraction for different oils when
191 the proportion of water is increased in the mixture. For example hydroxytyrosol, one of the most
192 hydrophilic phenols in olive oil (Rodis et al., 2002), represented approximately 14% of the TPC of
193 Arbequina olive oil, but only the 4.5% of the TPC of Empeltre olive oil.

194 The partition coefficient (PC) for different olive oil-to-water ratios calculated from the ratio of TPC in the
195 oil and in the water at equilibrium is also shown in Table 1. In liquid-liquid extraction, the universal rule
196 is that at a given temperature the ratio of concentration of a solute in each solvent is always a constant; in
197 this study, however, it was observed that the partition coefficient depended on the olive oil-to-water ratio
198 used in the mix due to the different solubility of the individual phenols in water (Rodis et al., 2002). It
199 was previously observed that, with Arbequina olive oil, the PC was always lower than 1 regardless of the

olive oil-to-water ratio used in the extraction process (Abenoza et al., 2015).. However, in the case of Empeltre olive oil, the PC was lower or higher than 1 depending on the olive oil-to-water ratio. As can be seen in table 1, partition coefficients higher than 1 (indicating that phenols preferentially partitioned to the oil phase) were obtained when the proportion of water in the liquid-liquid system was the same or lower than the oil proportion. This behavior confirms the lower proportion of hydrophilic phenols in Empeltre olive oil as compared to Arbequina olive oil.

*Obtaining olive oils with different phenol content*

The following linear relationship was observed between the percentage of oil in the mix and the percentage of phenols removed from Empeltre olive oil.

$$Y = - 2.09\ PP + 147.19 \qquad R^2 = 0.99 \qquad \text{(Eq. 2)}$$

where Y is the percentage of oil in the mix and PP is the percentage of phenols to be removed from the oil.

Eq. 2 may result useful for estimating the olive oil-to-water ratio required to reduce the concentration of phenols in Empeltre olive oil by a given value in order to modulate the bitterness intensity. In order to validate the equation, the olive oil-to water ratio required to obtain Empeltre olive oil with 150, 200 and 250 mg gallic acid $kg^{-1}$ oil was estimated. Table 2 shows the olive oil-to water ratio estimated from Eq 2 to obtain oils with different TPC, and the TPC obtained after liquid-liquid extraction with the olive-oil-to-water ratios calculated using equation 2. In addition to the TPC, Table 2 also shows other parameters that are associated with TPC such as $K_{225}$, sensorial bitterness, and oxidative stability. Results displayed in Table 2 confirm the usefulness of Eq 2 to estimate the olive oil-to-water ratio required to obtain Empeltre olive oil with a given TPC. It is observed that the difference between the estimated TPC and the TPC obtained after liquid-liquid extraction was lower than 3% independently of the olive oil-to-water ratio used for decreasing TPC. On the other hand, a strong correlation was observed between the TPC of the oil and the oxidative stability ($R^2$=0.99) for olive oils in the range of 154.3 to 332.5 mg gallic acid $kg^{-1}$ oil. However, for the bitterness index ($K_{225}$) and sensorial bitterness, the TPC was very highly correlated in the range from 154.26 to 255.91 mg gallic acid $kg^{-1}$ oil, indicating the existence of a TPC threshold above which the bitterness of Empeltre olive oil does not grow stronger by increasing polyphenol concentration.

227 Sensory characteristics of the control olive oil and the olive oil with reduced content TPC after extraction
228 are shown in Figure 1. It is remarkable to note that, after liquid-liquid extraction, the olive oils did not
229 develop any negative sensory attributes and thus were able to maintain the commercial category of "extra
230 virgin" quality. However, the reduction of polyphenol content affected the oils' sensorial profile
231 depending on the olive-oil-to-water ratio used in the extraction. Testers found the same fruity, sweetness
232 and pungency as control for the oil with the highest TPC (255.9 mg gallic acid $kg^{-1}$ oil). On the other
233 hand, oil with the intermediate TPC (198.7 mg gallic acid $kg^{-1}$ oil) was significantly less bitter than the
234 control olive oil. Although testers described this olive oil as less fruity, pungent and sweet than the
235 control, differences were not statistically significant. Finally, the olive oil with the lowest TPC (154.3 mg
236 gallic acid $kg^{-1}$ oil) was the sweetest one, due to its significantly lower bitterness and pungency.

237 *Evolution of physico-chemical and nutritional parameters of Empeltre olive oils during storage*

238 Table 3 shows the physico-chemical and nutritional parameters for the control olive oil and the olive oils
239 obtained after liquid-liquid extraction with different TPC at zero, three, six, and nine months of storage.

240 Official physico-chemical parameters (acidity, peroxide index, UV absorption) established to evaluate the
241 quality of virgin olive oils (EEC, 1991) were not significantly affected immediately after liquid-liquid
242 extraction (time 0) conducted to obtain olive oils with different TPC. On the other hand, the evolution of
243 these parameters was similar for the control olive oil and for the olive oils with reduced content of
244 polyphenols in the course of storage time. In all cases, a slight increase of acidity and peroxide value
245 along storage time was observed, and the increment of the $K_{232}$ and $K_{270}$ values indicated the progress of
246 oil oxidation. These increments, however, although statistically significant in some cases, do not imply a
247 loss of quality level: the values remained considerably under the established limit for the highest
248 commercial category of extra virgin olive oils.

249 The α-tocopherol content of the extracted olive oils during storage is also shown in Table 3. Due to this
250 compound's lipophilic nature, α-tocopherol remained in the oily phase after liquid-liquid extraction and,
251 therefore, significant differences in α-tocopherol content between the control olive oil and the olive oil
252 obtained after liquid-liquid extraction were not observed. After nine months of storage, a decrease in α-
253 tocopherol content lower than 10% was observed in all cases. This lower decrease in α-tocopherol content
254 after nine months of storage as compared with other reported studies (Gómez- Alonso et al., 2007) could

255 be due to the fact that the samples were stored in the dark, and that nitrogen was added in the headspace
256 of the bottles after storing.

257 Table 3 also shows the evolution of the TPC and of oxidative stability for the different olive oils during
258 nine months of storage. Both parameters decreased along the storage time in both control olive oil and
259 olive oils with reduced content of polyphenols. The liquid-liquid extraction process did not significantly
260 affect the decrease in polyphenols that takes place in oil with the passage of time. Whereas, in the control
261 olive oil, TPC decreased by 32% after nine months of storage, in the oils obtained after liquid-liquid
262 extraction the TPC decreased between 25 and 35% after the same storage interval. Similarly to TPC,
263 oxidative stability was not significantly affected by the liquid-liquid extraction process. At the end of
264 storage time, this parameter decreased about 7.5% for the control and around 10% for the three olive oils
265 obtained after liquid-liquid extraction. Oxidative stability decreased in a lower proportion than TPC
266 throughout a storage time of nine months due to the α-tocopherol concentration.

267 Individual phenol contents of the different olive oils are shown in Table 4. There is not a general
268 consensus concerning which individual polyphenols are the main ones responsible for bitterness, probably
269 because of the existence of saturation values for the human senses (Yousfi et al., 2008). Some authors
270 consider that hydroxytyrosol, tyrosol, and their derivatives are the main polyphenols responsible for the
271 bitterness and pungency of olive oil (García et al., 2001). As these polyphenols are hydrophilic, a
272 reduction of 91% and a 71% for hydroxytyrosol and tyrosol, respectively, was obtained using the lowest
273 olive oil-to-water ratio (32.5/ 67.5) assayed in this study. Other authors have attributed olive oil
274 bitterness to 3.4-DHPEA-EA (Mateos et al., 2004; Siliani et al., 2006; Yousfi et al., 2010) and p-HPEA-
275 EDA.(Gutiérrez- Rosales et al., 2003). These compounds that were present in the control Empeltre olive
276 oil used in this investigation at a concentration lower than 10 mg $kg^{-1}$ have more affinity for the oil phase
277 than hydroxytyrosol and tyrosol. A maximum decrease of around 30% was observed for 3.4-DHPEA-EA
278 and of around 19% for p-HPEA-EDA. Affinity for the oil phase of 3.4-DHPEA-EDA, the main
279 polyphenol in the Empeltre oil (195.54 mg gallic acid $kg^{-1}$ oil) used in this investigation, was intermediate
280 between hydrophilic and hydrophobic phenols. Concentration of 3.4-DHPEA-EDA in the oils ranged
281 between 33% and 55%, depending on the olive-oil-to-water ratio used in the extraction.

282 Regarding individual polyphenols whose concentration was lower than 1 mg $kg^{-1}$, differences for luteolin,
283 apigenin and vanillin between the control oils and the olive oils obtained after extraction were not

observed. On the other hand, the concentration of vanillic and cumaric acids decreased by a maximum of 50 and 35 %, respectively, indicating a higher hydrophilicity for those phenols.

As a consequence of the number of individual polyphenols in Empeltre olive oil and their different concentration in the oils obtained after liquid-liquid extraction, it is quite complicated to associate changes in the bitterness intensity of oil with changes in the content of different phenolic compounds. However, as reported above, a gradual decrease in the bitterness of the olive oils coincided with a decrease in total concentration of phenols (García et al., 2001; Yousfi et al., 2010).

Over the nine months of storage, hydroxytyrosol and tyrosol progressively increased in the control and olive oils obtained by liquid-liquid extraction. This behavior has been previously observed by other authors (Brenes et al., 2001; Gómez- Alonso et al., 2007; Morelló et al., 2004; Mulinacci, et al., 2013) and it is attributed to the hydrolysis of the secoiridoid derivatives during storage that cause a decrease in 3.4-DHPEA-EDA concentration. A significant increase for p-HPEA-EDA during storage was also observed in all cases: this trend is different to those reported in previous investigations featuring olive oils of other varieties (Gómez Alonso et al., 2007; Morelló et al., 2004). Finally, as has been reported by other authors (Lozano- Sánchez et al., 2009) 3.4-DHPEA-EA showed an irregular trend. The compound 3.4-DHPEA-EA increased up to sixth months of storage, and then decreased for those oils possessing a higher polyphenol content; however, it remained constant in the case of other olive oils.

## CONCLUSION

It has been demonstrated herein that liquid-liquid extraction using water as solvent is an effective procedure to reduce the TPC of Empeltre extra virgin olive oil and, as a consequence, its bitterness intensity, without affecting the best commercial category measured by the parameters legally established by EC regulations of olive oils obtained immediately after extraction and over nine months of storage. The possibility of estimating the proportion of olive-oil-to-water ratio in the mix in order to obtain an oil with a given TPC and the low energetic requirements as compared with other proposed procedures such as the heating or cooling of olive fruits support this technique's feasibility for facilitating the commercialization of olive oils with excessive bitterness. Data presented in this investigation could be of

311 interest in order to design and construct an industrial-scale reactor designed to perform liquid-liquid
312 extraction in olive oil factories.

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375 **Table 1**. Total phenols content in Empeltre olive oil after the liquid-liquid extractions at the different
376 olive oil-to-water ratio.

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| Olive oil-to-water ratio (v/ v) | Total phenols (mg $kg^{-1}$) | PC |
|---|---|---|
| 100/0 | 332.5±1.7 | - |
| 90/10 | 245.8±5.6 | 2.84 |
| 70/ 30 | 202.7±3.9 | 1.56 |
| 50/ 50 | 175.8±4.7 | 1.12 |
| 30/ 70 | 152.7±0.7 | 0.85 |
| 10/ 90 | 113.9±0.5 | 0.52 |

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380 Extraction experiments were conducted in duplicate. Total phenols analysis was carried out in triplicate. Values
381 reported are mean values and standard deviations of each olive oil-to-water ratio.
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**Table 2**. Estimated olive oil-to-water ratio required to obtain olive oils with different total polyphenol content according to equation 2 and total polyphenol content, oxidative stability, $K_{222}$ and sensorial bitterness of the oils obtained after performing extraction according to the estimated olive oil-to-water ratio.

| Olive oil-to-water ratio | TPC estimated (mg $kg^{-1}$) | TPC obtained (mg $kg^{-1}$) | Oxidative stability (hours) | $K_{225}$ (Abs 225 nm) | Sensorial bitterness (median) |
|---|---|---|---|---|---|
| 100/0 | | 332.5±1.7 | 10.0±0.1 | 0.22±0.03 | 3.7 |
| 95.3/ 4.7 | 250 | 255.9±1.4 | 9.1±0.2 | 0.21±0.01 | 3.6 |
| 63.9/ 36.1 | 200 | 198.7±1.0 | 8.0±0.1 | 0.17±0.02 | 2.6 |
| 32.5/ 67.5 | 150 | 154.3±1.4 | 7.5±0.2 | 0.12±0.01 | 2.2 |

Extraction experiments were conducted in duplicate. Analysis was carried out in triplicate. Values reported are mean values and standard deviations of each olive oil-to-water ratio.

**Table 3**. Physicochemical and nutritional parameters of extracted olive oils at different olive oil to water ratio and its evolution during storage time.

| **Parameters** | **Storage time** (months) | **Control oil (100/0)** | **Olive oil-to-water ratio 95.3/ 4.7** | **Olive oil-to-water ratio 63.9/ 36.1** | **Olive oil-to-water ratio 32.5/ 67.5** |
|---|---|---|---|---|---|
| Acidity (% oleic acid) | 0 | $0.17\pm0.01^{A;F}$ | $0.17\pm0.01^{A;G}$ | $0.17\pm0.01^{A;F}$ | $0.17\pm0.02^{A;F}$ |
| | 3 | $0.17\pm0.01^{B;F}$ | $0.17\pm0.01^{A;F}$ | $0.17\pm0.02^{B;F}$ | $0.17\pm0.01^{B;F}$ |
| | 6 | $0.20\pm0.02^{A;G}$ | $0.20\pm0.02^{A;H}$ | $0.20\pm0.02^{A;G}$ | $0.20\pm0.02^{A;G}$ |
| | 9 | $0.23\pm0.02^{A;H}$ | $0.23\pm0.03^{B;I}$ | $0.23\pm0.03^{AB;H}$ | $0.23\pm0.02^{AB;H}$ |
| Peroxide value (meq $O_2$ active/ kg oil) | 0 | $13.30\pm0.05^{A;F}$ | $14.00\pm0.01^{B;F}$ | $14.06\pm0.05^{B;F}$ | $14.00\pm0.01^{B;F}$ |
| | 3 | $13.91\pm0.01^{A;G}$ | $14.70\pm0.11^{B;G}$ | $14.70\pm0.11^{B;G}$ | $14.67\pm0.10^{B;G}$ |
| | 6 | $15.40\pm0.06^{A;H}$ | $16.04\pm0.12^{B;H}$ | $15.90\pm0.18^{B;H}$ | $16.00\pm0.01^{B;H}$ |
| | 9 | $16.67\pm0.01^{A;I}$ | $17.37\pm0.07^{B;I}$ | $17.45\pm\pm0.00^{C;I}$ | $18.12\pm0.01^{D;I}$ |
| $K_{232}$ (Abs 232 nm) | 0 | $1.97\pm0.01^{A;F}$ | $1.97\pm0.02^{A;F}$ | $1.97\pm0.01^{A;F}$ | $1.97\pm0.01^{A;F}$ |
| | 3 | $2.00\pm0.01^{A;FG}$ | $2.01\pm0.01^{A;G}$ | $2.03\pm0.02^{B;G}$ | $2.04\pm0.01^{B;G}$ |
| | 6 | $2.01\pm0.02^{A;GH}$ | $2.03\pm0.02^{A;GH}$ | $2.06\pm0.01^{B;H}$ | $2.06\pm0.01^{B;GH}$ |
| | 9 | $2.03\pm0.02^{A;H}$ | $2.05\pm0.02^{AB;H}$ | $2.08\pm0.02^{C;H}$ | $2.07\pm0.01^{BC;H}$ |
| $K_{270}$ (Abs 270 nm) | 0 | $0.12\pm0.01^{A;FG}$ | $0.12\pm0.01^{A;F}$ | $0.12\pm0.01^{A;F}$ | $0.12\pm0.01^{A;F}$ |
| | 3 | $0.12\pm0.01^{A;F}$ | $0.12\pm0.01^{A;F}$ | $0.12\pm0.01^{A;F}$ | $0.12\pm0.01^{A;F}$ |
| | 6 | $0.12\pm0.00^{A;F}$ | $0.13\pm0.01^{A;F}$ | $0.13\pm0.01^{A;F}$ | $0.13\pm0.01^{A;F}$ |
| | 9 | $0.13\pm0.01^{A;G}$ | $0.13\pm0.01^{A;F}$ | $0.13\pm0.01^{A;F}$ | $0.13\pm0.00^{A;F}$ |
| α-tocopherol (mg $kg^{-1}$ oil) | 0 | $304.30\pm1.24^{A;I}$ | $301.29\pm2.97^{A;G}$ | $303.88\pm0.53^{A;H}$ | $302.17\pm2.09^{A;H}$ |
| | 3 | $296.26\pm.29^{A;H}$ | $299.02\pm0.53^{B;G}$ | $299.57\pm0.65^{B;G}$ | $297.71\pm1.38^{AB;G}$ |
| | 6 | $286.11\pm1.34^{B;G}$ | $285.81\pm2.07^{A;F}$ | $282.57\pm1.27^{A;F}$ | $284.86\pm1.61^{AB;F}$ |
| | 9 | $280.19\pm2.39^{A;F}$ | $282.12\pm2.23^{A;F}$ | $282.12\pm2.23^{A;F}$ | $281.44\pm2.85^{A;F}$ |
| Total phenols (mg gallic acid $kg^{-1}$ oil) | 0 | $332.52\pm1.74^{D;I}$ | $255.91\pm1.36^{C;I}$ | $198.74\pm1.03^{B;I}$ | $154.26\pm1.36^{A;I}$ |
| | 3 | $312.47\pm2.17^{D;H}$ | $229.16\pm2.58^{C;H}$ | $178.69\pm1.81^{B;H}$ | $142.45\pm.36^{A;H}$ |
| | 6 | $304.93\pm2.65^{D;G}$ | $212.31\pm1.78^{C;G}$ | $165.64\pm1.02^{B;G}$ | $130.59\pm1.77^{A;G}$ |
| | 9 | $228.41\pm1.48^{D;F}$ | $177.26\pm1.41^{C;F}$ | $129.26\pm2.17^{B;F}$ | $116.93\pm2.11^{A;F}$ |
| Oxidative stability (hours) | 0 | $10.03\pm0.13^{D;H}$ | $9.10\pm0.16^{C;G}$ | $8.00\pm0.08^{B;G}$ | $7.52\pm0.17^{A;H}$ |
| | 3 | $9.59\pm0.01^{C;G}$ | $8.57\pm0.13^{B;F}$ | $7.55\pm0.16^{A;F}$ | $7.32\pm0.08^{A;GH}$ |
| | 6 | $9.45\pm0.07^{C;FG}$ | $8.31\pm0.16^{B;F}$ | $7.47\pm0.08^{A;F}$ | $7.15\pm0.17^{A;G}$ |
| | 9 | $9.28\pm0.07^{D;F}$ | $8.25\pm0.16^{C;F}$ | $7.24\pm0.13^{B;F}$ | $6.78\pm0.01^{A;F}$ |

Analysis during storage was carried out in triplicate. Values reported are mean values and standard deviations. Significant differences between the samples were determined by the one-way ANOVA and Multiple Range Test.

$^{A-D}$ For each parameter, different letters for the same storage month statistically significant differences ($p\leq0.05$) among liquid-liquid extractions. $^{F-I}$ For each parameter, different letters for the same liquid-liquid extraction indicate statistically significant differences ($p\leq0.05$) among storage month.

**Table 4.** Individual phenols of extracted olive oils at different olive oil to water ratio and its evolution during storage time.

| **Parameters** | **Storage time** (months) | **Control oil (100/0)** | **Olive oil-to-water ratio 95.3/ 4.7** | **Olive oil-to-water ratio 63.9/ 36.1** | **Olive oil-to-water ratio 32.5/ 67.5** |
|---|---|---|---|---|---|
| Hydroxytyrosol | 0 | 14.93±0.08$^{C;F}$ | 3.05±0.03$^{B;G}$ | 1.41±0.01$^{A;F}$ | 1.37±0.02$^{A;F}$ |
| | 3 | 16.65±0.06$^{C;H}$ | 2.83±0.01$^{B;F}$ | 1.43±0.06$^{A;F}$ | 1.52±0.08$^{A;G}$ |
| | 6 | 16.49±0.18$^{C;H}$ | 4.16±0.04$^{B;H}$ | 2.29±0.06$^{A;G}$ | 2.27±0.08$^{A;H}$ |
| | 9 | 16.07±0.09$^{C;G}$ | 5.15±0.08$^{B;I}$ | 3.12±0.11$^{A;H}$ | 2.99±0.09$^{A;I}$ |
| Tyrosol | 0 | 1.61±0.05$^{C;F}$ | 0.98±0.04$^{B;F}$ | 0.48±0.03$^{A;F}$ | 0.47±0.00$^{A;F}$ |
| | 3 | 1.97±0.01$^{D;G}$ | 1.16±0.03$^{C;G}$ | 0.59±0.02$^{B;G}$ | 0.47±0.00$^{A;F}$ |
| | 6 | 1.98±0.01$^{D;G}$ | 1.24±0.05$^{C;H}$ | 0.60±0.02$^{B;G}$ | 0.50±0.01$^{A;G}$ |
| | 9 | 2.06±0.02$^{D;H}$ | 1.29±0.01$^{C;H}$ | 0.65±0.01$^{B;H}$ | 0.57±0.02$^{A;H}$ |
| Vanillic acid | 0 | 0.12±0.01$^{B;G}$ | 0.12±0.01$^{B;H}$ | 0.07±0.01$^{A;F}$ | 0.06±0.01$^{A;F}$ |
| | 3 | 0.10±0.01$^{A;F}$ | 0.09±0.01$^{A;F}$ | 0.09±0.01$^{A;H}$ | 0.09±0.01$^{A;H}$ |
| | 6 | 0.09±0.01$^{A;F}$ | 0.09±0.01$^{A;F}$ | 0.09±0.01$^{C;H}$ | 0.09±0.01$^{A;H}$ |
| | 9 | 0.10±0.01$^{A;F}$ | 0.10±0.01$^{A;F}$ | 0.09±0.01$^{A;H}$ | 0.09±0.01$^{A;H}$ |
| Vanillin | 0 | 1.09±0.01$^{A;G}$ | 1.09±0.01$^{A;G}$ | 1.08±0.02$^{A;G}$ | 1.05±0.03$^{A;F}$ |
| | 3 | 1.07±0.02$^{A;FG}$ | 1.07±0.02$^{A;FG}$ | 1.05±0.01$^{A;F}$ | 1.03±0.01$^{A;F}$ |
| | 6 | 1.05±0.02$^{A;F}$ | 1.05±0.01$^{A;F}$ | 1.05±0.01$^{A;F}$ | 1.05±0.02$^{A;F}$ |
| | 9 | 1.04±0.01$^{A;F}$ | 1.04±0.02$^{A;F}$ | 1.04±0.02$^{A;F}$ | 1.04±0.01$^{A;F}$ |
| Coumaric acid | 0 | 0.49±0.01$^{D;F}$ | 0.40±0.01$^{C;H}$ | 0.36±0.01$^{B;H}$ | 0.32±0.01$^{A;F}$ |
| | 3 | 0.47±0.01$^{C;F}$ | 0.38±0.02$^{B;FH}$ | 0.34±0.01$^{A;G}$ | 0.32±0.01$^{A;F}$ |
| | 6 | 0.48±0.01$^{C;F}$ | 0.40±0.03$^{B;H}$ | 0.33±0.01$^{A;G}$ | 0.31±0.01$^{A;F}$ |
| | 9 | 0.45±0.03$^{C;F}$ | 0.36±0.01$^{B;F}$ | 0.31±0.01$^{A;F}$ | 0.31±0.01$^{A;F}$ |
| 3.4-DHPEA-AC | 0 | 18.71±0.25$^{D;H}$ | 18.30±0.26$^{C;H}$ | 12.38±0.13$^{B;I}$ | 7.43±0.09$^{A;G}$ |
| | 3 | 18.91±0.22$^{D;H}$ | 17.43±0.18$^{C;G}$ | 11.30±0.17$^{B;H}$ | 10.47±0.16$^{A;I}$ |
| | 6 | 16.68±0.36$^{C;G}$ | 17.02±0.34$^{C;G}$ | 10.47±0.31$^{B;G}$ | 8.46±0.07$^{A;H}$ |
| | 9 | 15.02±0.15$^{C;F}$ | 15.19±0.31$^{C;F}$ | 8.32±0.05$^{B;F}$ | 4.68±0.04$^{A;F}$ |
| 3.4-DHPEA-EDA | 0 | 195.54±0.76$^{D;I}$ | 131.81±0.36$^{C;I}$ | 92.04±0.17$^{B;I}$ | 87.55±0.26$^{A;I}$ |
| | 3 | 157.47±0.28$^{D;H}$ | 124.65±0.20$^{C;H}$ | 87.60±0.23$^{B;H}$ | 83.62±0.10$^{A;H}$ |
| | 6 | 156.11±0.51$^{D;G}$ | 120.65±0.29$^{C;G}$ | 86.64±0.24$^{B;G}$ | 76.67±0.07$^{A;G}$ |
| | 9 | 151.30±0.77$^{D;F}$ | 108.29±0.41$^{C;F}$ | 76.28±0.57$^{B;F}$ | 74.81±0.14$^{A;F}$ |
| p-HPEA-EDA | 0 | 9.70±0.07$^{C;F}$ | 9.57±0.16$^{C;F}$ | 8.65±0.06$^{B;F}$ | 7.85±0.08$^{A;F}$ |
| | 3 | 19.72±0.13$^{C;H}$ | 16.09±0.17$^{B;G}$ | 14.30±0.06$^{A;G}$ | 14.30±0.19$^{A;G}$ |
| | 6 | 18.15±0.08$^{D;G}$ | 16.02±0.07$^{C;G}$ | 14.87±0.13$^{B;H}$ | 14.29±0.03$^{A;G}$ |
| | 9 | 18.18±0.19$^{D;G}$ | 17.41±0.36$^{C;H}$ | 16.23±0.15$^{B;I}$ | 14.44±0.12$^{A;G}$ |
| Lignans | 0 | 13.63±0.14$^{B;I}$ | 13.68±0.14$^{B;I}$ | 13.56±0.04$^{B;H}$ | 11.67±0.09$^{A;I}$ |
| | 3 | 12.58±0.12$^{C;H}$ | 12.53±0.07$^{C;H}$ | 10.34±0.10$^{B;G}$ | 7.69±0.14$^{A;H}$ |
| | 6 | 9.63±0.06$^{C;G}$ | 9.93±0.07$^{D;G}$ | 7.75±0.13$^{B;F}$ | 6.92±0.09$^{A;G}$ |
| | 9 | 7.02±0.12$^{B;F}$ | 9.46±0.04$^{D;F}$ | 7.56±0.12$^{C;F}$ | 6.19±0.04$^{A;F}$ |
| 3.4-DHPEA-EA | 0 | 9.41±0.22$^{D;F}$ | 8.43±0.07$^{C;F}$ | 7.33±0.09$^{B;F}$ | 6.52±0.06$^{A;F}$ |
| | 3 | 21.55±0.04$^{D;I}$ | 15.45±0.17$^{C;H}$ | 12.79±0.35$^{B;G}$ | 12.12±0.07$^{A;G}$ |
| | 6 | 19.04±0.33$^{C;H}$ | 18.93±0.27$^{C;I}$ | 13.94±0.48$^{B;H}$ | 12.35±0.33$^{A;G}$ |
| | 9 | 13.48±0.11$^{B;G}$ | 13.74±0.21$^{BC;G}$ | 14.00±0.30$^{C;H}$ | 12.47±0.26$^{A;G}$ |
| Luteolin | 0 | 0.72±0.01$^{A;G}$ | 0.72±0.01$^{A;H}$ | 0.72±0.01$^{A;H}$ | 0.72±0.01$^{A;H}$ |
| | 3 | 0.71±0.02$^{B;G}$ | 0.66±0.01$^{A;G}$ | 0.66±0.01$^{A;G}$ | 0.65±0.01$^{A;G}$ |
| | 6 | 0.65±0.02$^{A;F}$ | 0.64±0.01$^{A;F}$ | 0.63±0.02$^{A;G}$ | 0.65±0.01$^{A;G}$ |
| | 9 | 0.63±0.01$^{B;F}$ | 0.64±0.01$^{B;F}$ | 0.57±0.01$^{A;F}$ | 0.58±0.01$^{A;F}$ |
| Apigenin | 0 | 0.88±0.02$^{A;F}$ | 0.88±0.03$^{A;F}$ | 0.88±0.01$^{A;F}$ | 0.87±0.02$^{A;F}$ |
| | 3 | 0.87±0.02$^{A;F}$ | 0.87±0.02$^{A;F}$ | 0.88±0.02$^{A;F}$ | 0.87±0.01$^{A;F}$ |
| | 6 | 0.87±0.03$^{A;F}$ | 0.87±0.02$^{A;F}$ | 0.87±0.02$^{A;F}$ | 0.87±0.01$^{A;F}$ |
| | 9 | 0.87±0.02$^{A;F}$ | 0.87±0.02$^{A;F}$ | 0.87±0.02$^{A;F}$ | 0.87±0.02$^{A;F}$ |

Analysis during storage was carried out in triplicate. Values reported are mean values and standard deviations. The results were expressed as mg $kg^{-1}$. Significant differences between the samples were determined by the one-way ANOVA and Multiple Range Test.

$^{A-D}$ For each parameter, different letters for the same storage month statistically significant differences ($p \leq 0.05$) among liquid-liquid extractions. $^{F-I}$ For each parameter, different letters for the same liquid-liquid extraction indicate statistically significant differences ($p \leq 0.05$) among storage month.

3.4-DHPEA-AC, 4-(acetoxyethyl)-1.2-dihydroxybenzene; 3.4-DHPEA-EDA, dialdehydric form of elenolic acid linked to hydroxytyrosol; p-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; 3.4-DHPEA-EA, oleuropein aglycone.

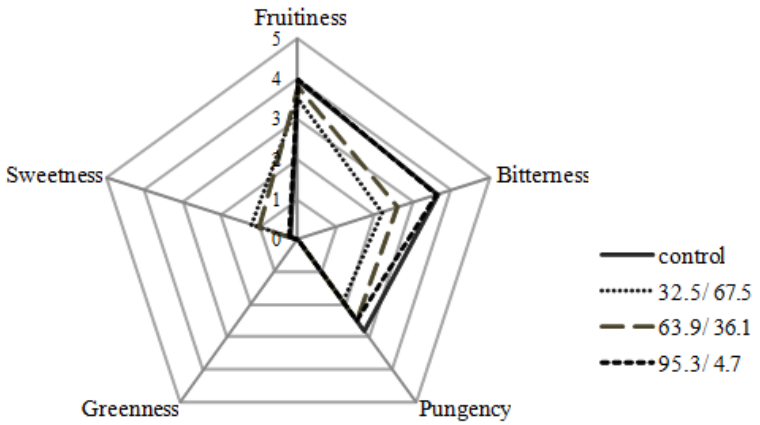


**Figure 1**. Sensory analysis of the olive oils after liquid- liquid extraction.