DEBITTERING OLIVE OIL BY LIQUID- LIQUID EXTRACTION: KINETICS AND THE EFFECT ON THE QUALITY OF ARBEQUINA OLIVE OIL

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Running tittle: Liquid-liquid extraction and Arbequina olive oil quality

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Keywords: Debittering, Olive oil, Phenols, Arbequina, Quality, Liquid-liquid extraction.
Abbreviations: TPC - total phenol content, TPC_o – total phenols content in olive oil, TPC_e – total phenols content in olive oil estimated, TPC_t – concentration of phenols in the oil along the time of solute extracted after a time, TPC_{oi} – concentration of phenols in the oil at the beginning of the extraction, TPC_{eq} – concentration of phenols in the oil at equilibrium, k – extraction constant, t – extraction time, Y – percentage of oil in the mix, PE – percentage of phenols to be removed from the oil.

ABSTRACT

Bitter taste is a positive sensory attribute in olive oil. Although if the bitter taste is too strong, it may be perceived as a negative attribute for the consumers. The aim of this work was to design a liquid-liquid extraction using water as a solvent to decrease the total phenol content of Arbequina olive oil and as consequence its bitter taste. Different olive oil-to-water ratios were assayed and mixing was conducted by flowing nitrogen to avoid oxidation. After 15 min, a constant total phenols concentration was reached in the olive oil independently of the olive oil-to-water ratios assayed. Lower percentage of olive oil in the mix was more efficient for phenol extraction. A mathematical equation was proposed to calculate the Arbequina olive oil-to-water ratio in the mix to remove a desired phenol percentage. After removing phenols, the obtained olive oil had a similar physico-chemical quality as untreated olive oil with a similar content of phenols.

Practical applications: By using obtained mathematical equations the phenolic content in Arbequina olive oil can be modulated by liquid- liquid extraction. It is a fast and easy method to achieve the desired bitterness in olive oil.

When in a mill oils with high intensity of bitterness are obtained, this method could be a solution to open new markets by offering more equilibrated products for consumers not used to olive oil.

1. INTRODUCTION

Olive oil is obtained directly from olives exclusively through physical processes, preserving a high nutritional value and sensory quality. This oil is the main edible fat used in the Mediterranean area, and consumption of it has increased in other countries in recent years [1]. One of the positive organoleptic attributes in olive oil is bitterness. This parameter is mostly due to the total phenol content and depends on many factors (processing, maturity degree, variety, etc.) [2]. However, the excessive intensity of the bitter taste in virgin olive oil may determine its rejection by the consumers of some markets (China, Japan, United States, Canada, etc.) who are not used to the taste of olive oil and that are more familiar with refined and tasteless oils obtained by solvent extraction. Some previous works described postharvest treatments in the olives that decrease the bitterness in the olive oil. Some of them used heat [3, 4] cold [5], or stored olives in atmosphere with CO_2 [6]. Usually the main procedure to regulate the bitterness in olive oil is to control the different stages during processing. Temperatures used in malaxation have more influence on the phenol extraction than malaxation time [7, 8]. However, results reported on temperature influence are contradictory. While some authors have reported an increase of the phenol concentration and bitterness when the malaxation temperature increased [9, 10, 11], others have observed that when the malaxation temperature increased, the phenol content decreased [7, 12]. The centrifugation system also has an influence on the olive oil's bitterness [13, 3]. A few works have studied the reduction of bitterness after the extraction of the olive oil. Pripp et al. [14] added sodium caseinate in an olive oil/ water emulsion and reduced the bitterness by 65%. Koprivnjak et al. [15] added lecithin and reduced the bitterness by

70%. However the addition of these compounds is not allowed to market virgin olive oil category [16].

The removal of phenols from olive oils is a potential strategy in order to reduce its bitterness. Some olive oil phenols are hydrophilic in nature and are more soluble in water than in the oil phase [17]. The aim of this work is to propose a system (liquid-liquid extraction using water as solvent) to decrease the total phenol content and the bitterness in Arbequina olive oil while maintaining the physico- chemical quality and without causing sensorial defects. A mathematical model describing the kinetics of the process will be proposed to establish the experimental conditions to extract different amount of phenols and in this way to modulate the excess of bitterness in Arbequina olive oil.

2. MATERIAL AND METHODS

2.1. Raw material

The experiments were done using Arbequina olives harvested in the 2012 crop season in Zaragoza (Spain). These olives had a 2.27 maturity index following the method based on the pigmentation levels [18].

2.2. Oil extraction

Olive oil extraction was performed using Abencor™ laboratory equipment (MC2 Ingenierías y Sistemas, Sevilla, Spain) following the method described by Martínez et al. [19].

A total of 75 kg olives of the Arbequina variety were divided in three batch of 25 kg and then were crushed with a hammer mill. In order to obtain oils with different phenol content the paste was malaxated at three different temperatures (15, 26, and 32 °C) for

30 min and then it was centrifuged applying a force of 1370 g for 1 min to obtain the oils. The Arbequina olive oil with the highest phenol content (197.54 ±1.62 mg gallic acid/ kg oil) (oil malaxated at 26 °C) was used to study the phenol extraction kinetics.

2.3. Liquid- liquid extraction

Phenols from Arbequina olive oil were extracted in a laboratory vessel of a 500 ml capacity, (7 cm in diameter, and 13.8 cm in height) connected to a nitrogen gas source. The vessel contained 400 ml of a mixture of olive oil-to-water and the extraction was assayed with several sample-to-solvent ratios (olive oil-to-water ratio in % volume) (50/ 50, 70/ 30, 80/ 20, 90/ 10) to investigate the influence of this parameter on the extraction efficiency. The contents were mixed thoroughly in bubbling nitrogen at a pressure of 19.61 kPa at room temperature (20 ± 2 °C) for 30 min. In order to monitor the extraction, aliquots were collected at different times (0, 1, 2, 3, 5, 7.5, 10, 15, 20, and 30 min). These samples were centrifuged applying a force of 4293 g for 5 min to separate the two phases. Total phenol content (TPC) in olive oil was also determined at the beginning of the experiment and after 30 min of extraction. TPC in the aqueous phase along the time was determined to monitoring the removal of phenols from the oil. This is an economical and simpler analysis than in olive oil because a previous solid-phase extraction was not required. The extraction experiments were carried out in duplicate and the average values were reported.

Preliminary experiments were conducted to estimate if the concentration of phenols in the olive oil during the extraction could be estimated by subtracting the amount of phenols measured in the water phase along the time from the initial amount of phenol content in the olive oil. It was observed that the estimated concentration of phenols in

109 the olive oil was lower that the concentration directly measured in the oil. However, the
110 following linear relationship permitted to estimate the phenol concentration in the olive
111 oil from the concentration of phenols in the olive oil estimated from the phenols
112 measured in the aqueous phase.

113 $TPC_o = 1.187\ TPC_e - 9.1109$ (eq 1)

114 $R^2 = 0.90$

115 Where:

116 TPC_o: is the total phenols content in the olive oil

117 TPC_e: is the total phenols content in the olive oil estimated by subtracting the amount of
118 phenols measured in the water phase along the time from the initial amount of phenol
119 content in the olive oil

120 ***2.3.1. Kinetics of extraction of phenols***

121 Extraction curves for different Arbequina olive oil-to-water ratios were obtained by
122 plotting the total TPC_t in the olive oil along the time. All the extraction curves obtained
123 had similar shape and they were described by the following equation:

124 $TPC_t = (TPC_{oi} - TPC_{eq})*(e^{(-kt)}) + TPC_{eq}$ (eq 2)

125 Where:

126 TPC_t is the concentration of phenols in the oil along the time of solute extracted after a
127 time

128 TPC_{oi} is the concentration of phenols in the oil at the beginning of the extraction

129 TPC_{eq} is the concentration of phenols in the oil at equilibrium**.** These values were
130 obtained from the total phenol content in aqueous phase and for difference with the
131 initial total phenol content in the olive oil.

132 k: extraction constant (min^{-1})

133 t: extraction time

134 **2.4. Analytical determinations**

135 *Determinations of the physicochemical parameters* (free acidity, peroxide value, and
136 UV absorption coefficients K_{270} and K_{232}) were made following the methods described
137 in Regulation EEC/2568/91 of the Commission of the European Union [20].

138 *Determination of fatty acids.* The fatty acid profile of samples was determined by gas
139 chromatography using a modified fatty-acid methyl-esters (FAMEs) method as
140 described by Frega and Bocci [21].

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

146 *Total phenol content.* After liquid- liquid extraction the determination of the phenol
147 content in aqueous phase was performed by colorimetric determination using Folin and
148 Ciocalteau method.

149 For the extraction of total phenols from the olive oil, we used the method described by
150 Favati et al. [22]. The phenols were extracted by solid phase extraction (SPE), using

151 Isolute^{TM} C18 cartridges (5 ml/ 1 g solid phase). The determination in the extract was
152 done using the same procedure used above for the water.

153 *Individual phenols.* Phenolic compounds were extracted from olive oil following the
154 method described by Gutfinger [23]. The HPLC analysis was performed by following
155 the procedure of Montedoro et al. [24]. Phenolic compounds were identified and
156 quantified on the basis of their retention times compared to those of the standard
157 compounds.

158 *Oxidative stability.* This was expressed as the oxidation induction time (hours),
159 measured with a Rancimat^{TM} 743 instrument (Metrohm, Switzerland), using 3 g of oil,
160 warmed to 120 °C at 20 l h^{-1} air flow.

161 *Bitterness index.* Bitterness (K_{225}) was determined by SPE of bitter compounds using
162 Isolute C18 cartridges (6 ml/ 500 mg solid phase) following the method of Gutierrez-
163 Rosales et al., [25].

164 *Sensory analysis.* The sensory analysis of the samples was carried out by 10 selected
165 and trained panelists. The existence or not of negative attributes (fusty, winey, musty,
166 muddy, rancid, metallic, etc.) was evaluated.

167 **2.5. Treatment of experimental data**

168 The experiments were conducted in duplicate. Results were expressed as mean ±
169 standard deviation of two measurements of each experiment (two replicas of each olive
170 oil-to-water ratio).

171 To model the extraction curves GraphPad PRISM 3.3 software were used. The
172 goodness of the fitting was evaluated by determination coefficient (R^2) and root mean
173 square error (RMSE).

3. RESULTS AND DISCUSSION

3.1. Kinetics of extraction of phenols

The effect of the sample-to-solvent ratio (oil-to-water ratio) along the extraction time on the efficiency of partial remaining phenols was investigated. Bubbling nitrogen was used for mixing both liquids throughly and preventing oxidation reactions during the extraction process. In order to eliminate variations from the mixing system employed, the position of the nitrogen tube on the bottom of the mixing vessel and the nitrogen flow were fixed for all the extractions.

The influence of the olive oil-to-water ratio on the liquid- liquid extraction kinetics of phenols from olive oil is shown in Figure 1. TPC_o in the olive oil along the time was calculated using equation 1. Symbols correspond to the experimental values and lines to fit equation 2 to these values. Extraction curves indicated that the concentration of phenols in olive oil decreased exponentially for all the olive oil-to-water ratios investigated. The high initial rate of phenols extraction was followed by slower extraction rate and asymptotically approaching to the equilibrium concentration. At any olive oil-to-water ratio assayed concentration of phenols at equilibrium was achieved after 15 min of mixing confirming suitable performance of the mixing system used.

The estimated parameters of equation 2 (TPC_{eq} and k), correlation coefficients, and RMSE of the fits are shown in Table 1. The correlation coefficient for all the fits was higher than 0.9 and RMSE lower than 7.93, which implied good concordance between experimental and calculated data. The increase of the k value and the decrease of total phenolic concentration in olive oil with the decreased of the olive oil-to-water ratio shown in Table 1 are consistent with mass transfer principles. The driving force during

mass transfer is considered to be the concentration gradient, which was higher when a lower olive oil-to-water ratio was used, resulting in an increase of the diffusion rate. On the other hand, the TPC equilibrium was also significantly affected by the olive oil-to-water ratio. The phenols concentration in olive oil at equilibrium was the lowest (97.7 mg/ kg) when the extraction was conducted with the highest proportion of water in the mixed (50/ 50) (olive oil-to-water ratio) and the highest (152.1 mg/ kg) in the case of the lower proportion of water in the mixed (90/ 10) (olive oil-to- water ratio).

3.2. Estimation of olive oil-to-water ratio to obtaining Arbequina oils with different amount of phenols

From a practical point of view, it should be very useful to be able to estimate the proportion of Arbequina olive oil and water (olive oil-to water ratio) that should be mixed in a liquid- liquid extraction for reducing the phenols concentration to a given level. Figure 2 show the relationship between the percentage of oil in the mix and the percentage of phenols partly removed from Arbequina olive oil. A linear relationship was observed between both parameters. Therefore the following equation permits estimating the olive oil-to-water ratio required to reduce a given percentage the concentration of phenols in the oil:

$$Y = -1.23^{*} PE + 116.07 \quad R^2 = 0.99 \qquad \text{(eq 3)}$$

Where Y is the % of oil in the mix and PE is the % of phenols to be removed from the oil.

3.3. Comparison of physico-chemical parameters of olive oils in which phenols was partly removed with untreated olive oils with the same amount of phenols

219 The effect of the liquid-liquid extraction on the physico-chemical parameters of olive oil
220 was studied. For this purpose, two olive oils with similar phenol concentration as those
221 obtaining by malaxation at 15 ºC and 32 ºC were obtained by removing phenols from
222 the olive oil with the highest phenol concentration (olive oil obtaining by malaxation at
223 26 ºC). The equation 3 was used to calculate the Arbequina olive oil-to-water ratio
224 needed to get the desired removal of phenol by liquid-liquid extraction. In order to
225 obtain an olive oil with around 139 mg gallic acid/ kg oil (the content in the 15 °C
226 malaxated olive oil) and with around 158 mg gallic acid/ kg oil (the content in the 32 °C
227 malaxated olive oil), an olive oil-to-water ratio of 80/ 20 and 92/ 8 was used,
228 respectively. The time of contact between the water and the oil was 15 minutes since in
229 preliminary experiments the total phenol content reached the equilibrium at this time.

230 Physicochemical parameters of the control oils malaxated at 15 ºC and 32 ºC and the
231 removed phenol oils with similar phenol content are compared in Table 2. The values
232 obtained were under the limits established by the European regulations for the category
233 of extra virgin olive oils in all cases. When control olive oils were compared with
234 phenol removed oils with similar phenol content we observed differences that did not
235 have practical implications for acidity, peroxide value, K_{232}, K_{270} and oxidative stability.
236 A similar behaviour was observed for SFA, MUFAS, PUFAS and MUFAS/ PUFAS
237 ratio. Differences were not observed for α-tocopherol and total phenol content when
238 comparing control olive oils with the phenol removed oils. The bitterness index was
239 reduced from 0.20 (value in the olive oil obtained with a malaxation at 26 ºC) to 0.15
240 (the same value of the olive oil obtained with a malaxation at 32ºC) when the 92/8 olive
241 oil-to-water ratio was used. A higher reduction was obtained when the liquid-liquid
242 extraction was done with an olive oil-to-water ratio of 80/20. Concerning to the

243 individual phenols the biggest differences between control olive oils and removed
244 phenol oils were observed for the most hydrophilic phenols (hydroxytyrosol and
245 tyrosol). Concentration of 3, 4-DHPEA-AC and 3, 4- DHPEA-EDA was higher for the
246 removed phenol oil when the oil-to-water 80/20 ratio in the liquid-liquid extraction was
247 used. When less water was added in the mix (oil-to-water 92/8) the differences
248 observed for these two phenols were smaller. Parenti et al. [9] reported that 3,4-
249 DHPEA-EDA content increased linearity with olive oil extraction temperatures until 30
250 °C and then decreased. This may be the reason for the higher concentration of this
251 compound in the removed phenol olive oils obtained from the 26 °C malaxated olive oil
252 even after liquid-liquid extraction. For the other individual phenols the concentration
253 was quite similar between control olive oils and removed phenol oils.

254 After sensory analysis of the treated olive oils, no negative attributes or defects were
255 observed (results not shown).

256 **4. CONCLUSION**

257 Phenol diffusion by liquid- liquid extraction from the olive oil to the water depended on
258 the olive oil-to-water ratio. Lower olive oil-to-water ratios in the mixture were more
259 efficient for phenol extraction.

260 A mathematical equation was used to estimate the Arbequina olive oil-to-water ratio in
261 the mix needed to remove a given phenol content by liquid- liquid extraction.

262 With the liquid- liquid extraction system used, the phenols in olive oil were reduced
263 without significant changes in the physico- chemical parameters and no negative
264 attributes were detected in the sensorial analysis. This could be a solution to open new
265 markets demanding less bitter Arbequina olive oil. However more research is needed

using other olive varieties and to determine if the stability of the Arbequina olive oils in which phenols were partly removed is sufficient for commercialization.

5. ACKNOWLEDGEMENTS

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6. CONFLICT OF INTEREST

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

7. REFERENCES

[1] Luchetti, F. Importance and future of olive oil in the world market -an introduction to olive oil. *Eur.J. Lipid Sci. Techn.* 2002, 104, 559-563.

[2] Fregapane, G., Salvador, M.D. Production of superior extra virgin olive oil modulating the content and profile of its minor components. *Food Res. Intern.* 2013, 54 (2) 1907-1914.

[3] García, J.M., Yousfi, K., Mateos, R., Olmo, M., Cert, A. Reduction of oil bitterness by heating of olive fruits. *J. Agric. Food Chem.* 2001, 49, 4231- 4235.

[4] Yousfi, K., Moyano, M.J., Martínez, F., Cayuela, J.A., García, J.M. Postharvest heat treatment for olive oil debittering at the industrial scale. *J. Am. Oil Chem. Soc.* 2010, 87, 1053-1061.

[5] Yousfi, K., Cayuela, J.A., García, J.M. Reduction of virgin olive oil bitterness by fruit cold storage. J. Agric. Food Chem. 2008, 56, 10085- 10091.

287 [6] Dourtoglou, V.G., Mamalos, A., Makris, D.P. Storage of olives (*Olea europaea*)
288 under CO2 atmosphere: Effect of anthocyanins, phenolics, sensory attributes and in
289 vitro antioxidant properties. *Food Chem.* 2006, 99, 342- 349.

290 [7] Angerosa, F., Mostalllino, R., Basti, C., Vito, R. Influence of malaxation
291 temperature and time on the quality of virgin olive oils. *Food Chem.* 2001, 72, 19-28.

292 [8] Clodoveo, M.L. Malaxation: influence on virgin olive oil quality. Past, present and
293 future- an overview. *Trends Food Sci. Techn.*. 2012, 25(1), 13-23.

294 [9] Parenti, A., Spugnoli, P., Masella, P., Calamai, L. The effect of malaxation
295 temperature on the virgin olive oil phenolic profile under laboratory-scale conditions.
296 *Eur.J. Lipid Sci.Techn.* 2008, 110, 735-741.

297 [10] Boselli, E., Di Lecce, G., Strabbioli, R., Pieralisi, G., Frega, N.G. Are virgin olive
298 oils obtained below 27 °C better than those produced at higher temperatures? *LWT-*
299 *Food Sci. Techn.* 2009, 42(3), 748-757.

300 [11] Gómez-Rico, A., Inarejos-García, A., Salvador, M.D., Fregapane, G. Effect of
301 malaxation conditions on phenol and volatile profiles in olive paste and the
302 corresponding virgin olive oils (Olea europaea L. cv. Cornicabra). *J. Agric. Food Chem.*
303 2009, 57, 3587-3595.

304 [12] Servili, M., Selvaggini, R., Taticchi, A., Esposto, S., Montedoro, G.F. Volatile
305 compounds and phenolic composition of virgin olive oil: optimization of temperature
306 and time exposure of olive pastes to air contact during the mechanical extraction
307 process. *J.Agric. Food Chem.* 2003, 51, 7980-7988.

308 [13] Di Giovacchino, L., Costantini, N., Serraiocco, A., Surricchio, G., Basti, C. Natural
309 antioxidants and volatile compounds of virgin olive oils obtained by two or three-phases
310 centrifugal decanters. *European J. Lipid Sci. Techn.* 2001, 103, 279-285.

311 [14] Pripp, A.H., Busch, J., Vreeker, R. Effect of viscosity, sodium caseinate and oil on
312 bitterness perception of olive oil phenolics. *Food Qual. Pref.* 2004, 15, 375-382.

313 [15] Koprivnjak, A., Skevin, D., Petricevic, S., Brkic Bubola, K., Mokrovcak, Z.
314 Bitterness, odor properties and volatile compounds of virgin olive oil with
315 phospholipids addition. *LWT-Food Sci.Techn*. 2009, 42, 50-55.

316 [16] IOC (International Olive Council) Trade Standard applying to olive oils and olive-
317 pomace oils. *COI/T.15/NC N° 3/Rev. 7*, 2013.

318 [17] Rodis, P.S., Karathanos, V.T., Mantzavinou, A. Partitioning of olive oil
319 antioxidants between oil and water phases. *J.Agric. Food Chem*. 2002, 50, 596- 601.

320 [18] Hermoso, M., Uceda, M., García, A., Morales, B., Frías, M.L., Fernández, A.
321 Elaboración de aceite de oliva de calidad. Sevilla, (Spain). Consejería de Agricultura y
322 Pesca, *Serie Apuntes:* 5/92. 1991.

323 [19] Martínez, J.M., Muñoz, E., Alba, J., Lanzón, A. Informe sobre la utilización del
324 analizador de rendimientos "Abencor*". Grasas Aceites*. 1975, 26: 379- 385.

325 [20] Commission regulation (EEC) Nº 2568/91 of 1 July of 1991 on the characteristics
326 of olive oil and olive-residue oil and on the relevant methods of analysis. *Off. J. Europ.*
327 *Comm.* 1991, L248/ 1-114.

328 [21] Frega, N., Bocci, F. L'analisi rapida dell'olio di oliva. *Laborat. 2000*, 2001. Italy
329 328.

330 [22] Favati, F., Caporale, G., Bertuccioli, M. Rapid determination of phenol content in
331 extra virgin olive oil. *Grasas Aceites.* 1994, 45, 68-70.

332 [23] Gutfinger, T. Phenols in olive oils. *J. Am. Oil Chem. Soc.* 1981, 58, 966–968.

333

334 [24] Montedoro, G., Servilli, M., Baldioli, M., Miniati, E. Simple and hydrolysable
335 phenolic compounds in virgin olive oil. 1. Their extraction, separation and quantitative
336 and semiquantitative evaluation by HPLC. *J. Agric. Food Chem.* 1992, 40, 1571-1576.

337 [25] Gutiérrez-Rosales, F., Perdiguero, S., Gutiérrez, R., Ollas, J.M. Evaluation of the
338 bitter taste in virgin olive oil. *J. Am. Oil Chem. Soc.* 1992, 69(4), 394-395.

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344 **Table and Figure captions**

345 **Table 1-** TPC_{eq} and k values from the fitting of (eq 2) to the liquid- liquid extraction of
346 phenols at different oil-to-water ratios.

347 **Table 2-** Physicochemical parameters of the control oils malaxated at 15 ºC and 32 ºC
348 and the removed phenol oils with similar phenol content (at the end of the liquid- liquid
349 extractions, after 15 minutes).

350 **Figure 1-** Total phenols content in the oil (TPC_o) during the liquid-liquid extraction at
351 different olive oil-to-water ratios 50/ 50 (▲), 70/ 30 (•), 80/ 20 (♦) and 90/ 10 (■)
352 along the time.

353 **Figure 2-** Correlation between the percentages of Arbequina oil in the mix for liquid-
354 liquid extraction and the percentage of phenols removed.

355

356 **Table 1-**

Oil-to-water ratio	TPC_{eq}*	k	RMSE	R^2
50/ 50	97.7 (86.7- 108.7)	0.55 (0.28- 0.81)	5.62	0.98
70/ 30	115.2 (99.9- 130.5)	0.29 (0.10- 0.48)	4.26	0.98
80/ 20	133.7 (107.8- 159.6)	0.20 (-0.04- 0.44)	7.93	0.90
90/ 10	152.1 (129.9- 174.3)	0.17 (-0.07- 0.41)	3.43	0.96

357

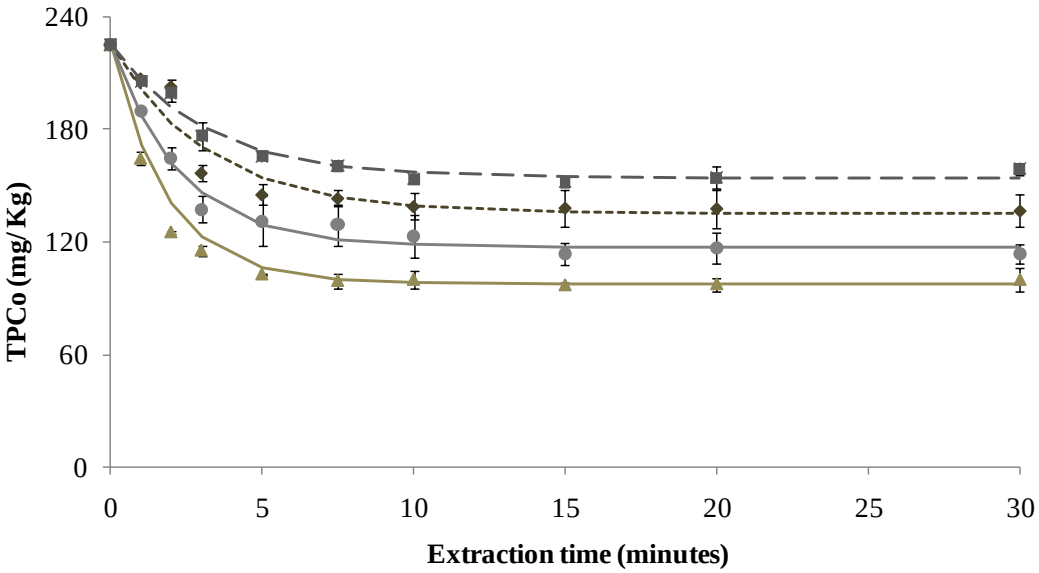
358 Values reported are mean values of two experiments.
359 * TPC_{eq}= concentration of phenols in the oil at equilibrium (mg/ kg). These values were obtained from
360 the total phenol content in aqueous phase and by difference with the initial total phenol content in the
361 olive oil.
362 RMSE was the root mean square error Results of TPC_{omax} and K value are expressed with a confidence
363 interval of 95%.
364

Table 2-

Parameters	Olive oil-to-water ratio 80/ 20	Control oil malaxated at 15 °C	Olive oil-to-water ratio 92/ 8	Control oil malaxated at 32 °C
Total phenols (mg/ kg)	132.52±2.41	139.26±4.29	148.70±4.49	158.31±4.40
Acidity (% oleic acid)	0.17±0.00	0.23±0.00	0.17±0.00	0.27±0.02
Peroxide value (mEq O_2 active/ kg oil)	6.33±0.03	6.00±0.00	6.17±0.03	6.04±0.00
K_{232} (Abs 232 nm)	1.60±0.01	1.64±0.02	1.55±0.01	1.50±0.01
K_{270} (Abs 270 nm)	0.06±0.00	0.05±0.00	0.08±0.01	0.05±0.00
Oxidative stability (hours)	7.88±0.13	6.13±0.14	8.37±0.16	8.77±0.14
α-tocopherol (mg/ kg)	230.05±1.58	228.66±1.85	228.51±1.43	228.02±2.11
K_{225} (Abs 225 nm)	0.11±0.00	0.15±0.00	0.15±0.00	0.15±0.00
Individual phenols (mg/ kg):				
Hydroxytyrosol	1.06±0.04	12.95±0.12	1.15±0.02	20.86±0.04
Tyrosol	1.68±0.04	9.80±0.03	2.39±0.06	8.37±0.07
Vanillic acid	0.28±0.01	0.35±0.01	0.33±0.00	0.40±0.01
Vanillin	1.20±0.01	1.14±0.01	1.20±0.02	1.33±0.01
Coumaric acid	0.54±0.01	0.67±0.00	0.64±0.01	0.67±0.00
3.4-DHPEA-AC	23.78±0.16	15.84±0.25	28.38±0.32	31.96±0.18
3.4-DHPEA-EDA	53.54±0.17	31.16±0.08	62.16±0.12	56.86±0.27
p-HPEA-EDA	10.42±0.06	9.38±0.03	10.73±0.11	11.70±0.07
Lignans	45.18±0.15	39.47±0.01	45.20±0.16	52.45±0.01
3.4-DHPEA-EA	18.57±0.29	21.00±0.30	20.01±0.25	21.58±0.28
Luteolin	1.64±0.02	1.98±0.02	1.66±0.01	1.71±0.02
Apigenin	1.14±0.00	1.19±0.00	1.14±0.01	1.15±0.01
Fatty acids (%):				
Oleic/ Linoleic	5.62±0.01	5.52±0.01	5.67±0.03	5.68±0.00
SFA	18.31±0.03	18.51±0.06	18.25±0.08	18.50±0.07
MUFAS	69.25±0.04	68.88±0.06	69.31±0.08	69.16±0.33
PUFAS	12.45±0.02	12.61±0.00	12.36±0.05	12.30±0.04
MUFAS/ PUFAS	5.56±0.01	5.46±0.01	5.61±0.02	5.62±0.01

Values reported are mean values and standard deviations of two measurements of each experiment (two replicas of the different olive oil-to-water ratios). In the control the values reported are mean values and standard deviations of two measurements. 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. SFA, saturated fatty acids; MUFAS, monounsaturated fatty acids; PUFAS, polyunsaturated fatty acids.

388 **Figure 1-**



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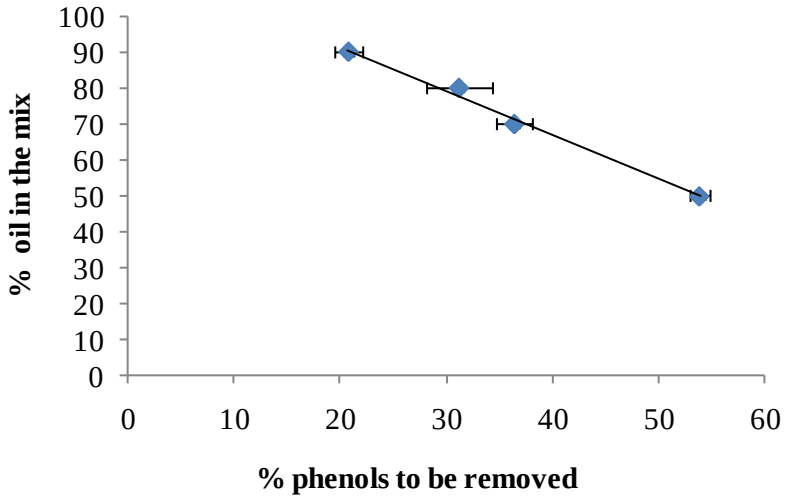
390 * Phenols in oil calculated from water have been previously corrected by equation 1

391 (Materials and Methods).

392

393 **Figure 2-**



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