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1 **Processing factors that affect the balance of alcohols and alkyl esters during**
2 **‘Arbequina’ olive oil production: separation and clarification steps**

3 ***Boudebouz, Abdelaziz*** ^(1,4); ***Romero, Agustí*** ⁽²⁾; ***Hermoso, Juan F.*** ⁽²⁾; ***Boqué, Ricard*** ⁽³⁾; ***Mestres,***
4 ***Montserrat*** ⁽⁴⁾

5 ⁽¹⁾: *IRTA-URV (Scholarship granted by the International Olive Council-COI)*

6 ⁽²⁾: *Institut de recerca i tecnologia agroalimentaria (IRTA-Mas de Bover), Ctra Reus-El Morell Km 3,8.*
7 *43120-Constantí (Spain)*

8 ⁽³⁾: *Universitat Rovira i Virgili (URV). Chemometrics, Qualimetrics and Nanosensors Group. Department*
9 *of Analytical Chemistry and Organic Chemistry. Campus Sescelades, 43007 - Tarragona (Spain)*

10 ⁽⁴⁾: *Universitat Rovira i Virgili (URV). Instrumental Sensometry Research Group (i-Sens). Department of*
11 *Analytical Chemistry and Organic Chemistry. Campus Sescelades, 43007 - Tarragona (Spain)*
12

13 **Abstract**

14 The aim of this study was to assess how different conditions used on the centrifugation
15 step during olive oil extraction affect its quality by considering the balance of fatty acid
16 alkyl esters (FAAEs) and their precursor alcohols. All the experiments were carried out
17 under real working conditions in a two-phase decanter followed by a vertical centrifuge
18 (VC) and different water injection doses and paste injection rates were tested. The fruits
19 used were from ‘Arbequina’ variety at two different maturity stages and the balances of
20 alcohols and FAAEs were measured at the outlets of both, decanter and VC, with respect
21 to the system inlet.

22 Results show that the paste injection rate affects the content of alcohols and FAAEs in
23 the final oil, which tend to increase when working closer to the maximum capacity of the
24 decanter. Different behaviors have been detected when dealing with unripe or ripe fruits.
25 Similarly, the water addition doses have different effects on the FAAEs and alcohols
26 formation depending on the maturity status. Therefore, both the decanter and the step
27 decanter to vertical centrifuge are key points that, when properly controlled, allow
28 minimizing FAAEs formation, which is essential for obtaining quality oils.

29

30 **Keywords:** Virgin olive oil; Centrifugation processing factors; Alcohols; FAAEs;
31 Quality control

32

33 **1. Introduction**

34 Virgin olive oil (VOO) is the juice of the olive fruits extracted only by physico-
35 mechanical process and when its chemical composition is good enough and it
36 organoleptic evaluation is excellent it is called extra virgin olive oil (EVOO). To
37 maximize the product quality, the olives must be harvested in their optimal health and
38 maturity state and the processing conditions must be controlled (Di Giovacchino, Sestili,
39 & Di Vincenzo, 2002; Masella, Guerrini, Angeloni, Zanoni, & Parenti, 2019). Given that
40 identifying reliable tools that preserves the quality of olive oil when maximizing
41 extraction efficiency is still a challenge, there is growing number of studies related to
42 EVOO processing (Fregapane & Salvador, 2013; Jabeur, Zribi, Abdelhedi, & Bouaziz,
43 2015; Masella et al., 2019; Parenti, Spugnoli, Masella, & Calamai, 2007). Currently, the
44 most commonly used mechanical process for extracting virgin olive oil is the so called
45 "continuous"(Uceda, Jiménez, & Beltrán, 2006), although it is not completely continuous,
46 as it consists of several steps (Fig_1): crusher, malaxer, horizontal centrifugation
47 (decanter) and oil clarification (vertical centrifuge).

48 Among the different EVOO quality parameters, one of the most studied in recent years
49 has been the content of fatty acids alkyl esters (FAAEs), which includes both ethyl esters
50 (FAEEs) and methyl esters (FAMEs) (Alcalá et al., 2017; Beltrán et al., 2016;
51 Biedermann, Bongartz, Mariani, & Grob, 2008; Di Serio et al., 2017; Gómez-Coca,
52 Fernandes, Pérez-Camino, & Moreda, 2016; Lanza, Di Serio, & Di Giacinto, 2016). Their
53 formation is due to the esterification and/or transesterification of free fatty acids with low

54 molecular weight alcohols such as methanol or ethanol (Costa et al., 2017; Guillaume,
55 Ravetti, Ruiz, & Zaparenkov, 2013; Pérez-Camino, Moreda, Mateos, & Cert, 2002). It is
56 well known that alcohols are present in olive oil (García-Vico et al., 2018; Gómez-Coca,
57 Cruz-Hidalgo, Fernandes, Pérez-Camino, & Moreda, 2014). When their origin is the
58 natural pathway of fruit metabolism (what produces the so-called endogenous alcohols)
59 their concentration depends on maturity, health status and olive cultivars (Beltrán,
60 Bejaoui, Jimenez, & Sanchez-Ortiz, 2015; Boudebouz et al., 2020; García-Vico et al.,
61 2018). However, when their presence is derived from the fermentation of olive sugars
62 during the olive processing, the content of alcohols also depends on the manufacturing
63 practices (Biedermann et al., 2008; Pérez-Camino, Cert, Romero-Segura, Cert-Trujillo,
64 & Moreda, 2008). Thus, since alcohols and, consequently, the FAAEs values will
65 increase when inappropriate practices are carried out during the processing, they have
66 been used to assess the quality of olive fruits processed during EVOO extraction, to check
67 the cleanliness of the material and to ensure a good management of the process (Pérez-
68 Camino et al., 2008).

69 Both the International Olive Council (2013) and the European Commission (2013) have
70 adopted the FAAEs standard to distinguish between EVOO and non-EVOO (Conte et al.,
71 2019). However, the latest update of the standard, limiting the amounts of FAEEs to 30
72 mg/kg (IOC, 2013), has led to a worrying situation for the sector since, in some cases, it
73 is very difficult not to exceed these limits, which would imply significant economic
74 losses.

75 To guarantee the limit values of FAEEs, the olive status control is not enough but it is
76 also necessary to control the different stages during VOO production in order to intervene
77 in those with major risk of increasing the content of alkyl esters. In the present work, we
78 focused on how the separation steps of the process (decanter and the vertical centrifuge)

79 affect the quality by evaluating the balance between FAAEs and short-chain alcohols
80 (ethanol and methanol).

81 Specifically, the main objective was to study the effect of water addition flow and paste
82 injection rate into the decanter, as these are two easy interventions that can be
83 implemented at any time or type of decanter without stopping the process. The secondary
84 goal of this work was to study the step decanter-to-vertical centrifuge and how is the oil
85 at the end of the process. The experiments were carried out under optimal conditions for
86 VOO production at a mill in operation (Cooperative La Granadella, Catalonia).

87

88 **2. Material and methods**

89 **2.1. Experiments**

90 The experiments were performed under the same extraction conditions on two different
91 days (December 08th and 15th) using healthy ‘Arbequina’ olive fruits with a maturity
92 index (MI) of 2.6 and 3.9, respectively (Table 1). The MI was assessed using the method
93 proposed by Uceda & Frias (1975).

94 Olive fruits were crushed using a hammer crusher operating at 3000 rpm, equipped with
95 a 5mm sieve and with a capacity of 4500 kg per hour. Then, the olive paste was malaxed
96 during 65 min at 27°C. The separation of the oil was carried out using a two-phase
97 decanter DC-180 (TACSA, Técnicas Andaluzas de Centrifugación S.L.), operating at
98 ~2410 RCF and with a theoretical capacity of 5000 kg/h, followed by an automatic
99 vertical centrifuge (HAUS-Centrifuge technology), operating at ~10080 RCF.

100 While the vertical centrifuge (VC) operated under the same conditions for all
101 experiments, different treatments of olive paste rates and water flow injected into the
102 decanter were experimented.

103 The first group of experiments evaluated the effect of olive paste injection rate on the
104 balance of alcohols and FAAEs between fruit, pomace and oil. This study was carried out
105 by fixing at a constant flow of 150 L/h the water injected into the decanter and testing
106 different rates of olive paste: 68% (3400 kg/h), 76% (3800 kg/h), 82% (4100 kg/h) and
107 90% (4500 kg/h) of theoretical decanter capacity. This range of working rates is within
108 the recommended levels proposed by several authors for two-phase decanters (Di
109 Giovachino, 2013). The second group of experiments focused on the effect of small
110 volumes of water injected into the decanter. Thus, the pumping of the olive paste was set
111 at 76% of the decanter capacity and the water flow injection ranged between 0 L/h (0%),
112 100 L/h (3%), 200 L/h (5%) and 300 L/h (8%) respectively. In a two-phase system, in
113 order to improve oil extraction, it is recommended to add small amounts of water into the
114 decanter when working with difficult pastes (as is the case of ‘Arbequina’) as long as a
115 limit of 10-15% water addition is not exceeded (Hermoso et al., 1996; Nieto et al., 2019).
116 Samples of pomace and oils were taken, in duplicate, at the decanter and VC outlets at
117 approximately 10 min intervals. In all treatments, chemical characteristics of both pomace
118 and oils samples were determined (Table 2 and Table 3).

119

120

121 **2.2. Samplings**

122 Sampling was carried out at different steps in order to study the balance of the compounds
123 studied between the phases (oil, pomace) in each step. To get suitable conclusions, in all
124 cases the results of the analysis were compared with those of the reference samples. In
125 this way, it was possible to determine alcohols and FAAEs coming from olives, those
126 formed during a specific production process or even alcohols lost by

127 evaporation/transesterification (Masella et al., 2019; Vidal, Alcalá, de Torres, Moya, &
128 Espínola, 2019; Alcalá et al., 2017; Pérez-Camino et al., 2008).

129 **2.2.1. Initial content in the olives**

130 To check whether the compounds studied are generated throughout the process or if they
131 enter the system coming from the fruits, initial amounts were measured when the olives
132 arrived to the mill. These olives were called reference samples and to ensure that they
133 were homogeneous and representative of the batch, small amounts were taken from the
134 hopper every 10 minutes to get a final sample of ~5 kg of olives. Then reference samples
135 were split into two parts. The first part was ground at room temperature and the
136 homogenized paste obtained was used to quantify the alcohols. The second part was
137 processed using the ABENCOR system to obtain olive oil and its content in ethanol
138 (EtOH), methanol (MeOH) and FAAEs (FAEEs, FAMES) was determined. The contents
139 in the oil obtained by this controlled system were considered as reference values of these
140 compounds at the inlet of the system (Table 4).

141 **2.2.2. Final content in pomace**

142 Olive pomace samples were taken at the decanter outlet for each one of the tested water
143 flow and paste injection rates. For each experiment two samples of 100 g pomace were
144 taken, and these were analyzed to determine their moisture, oil content (Table 2) and also
145 their alcohol amounts.

146 **2.2.3. Final content in the oil**

147 Olive oil samples were taken after the two separation steps of the process: at the decanter
148 outlet (crude oil) and at the VC outlet (clean oil). For each value of the tested parameters,
149 two samples of 250 ml each were taken. Samples from the decanter were centrifuged in
150 the laboratory at 5°C and 5000 RCF during 3 minutes. In all cases, alcohols and FAAEs

151 were quantified and moisture and impurities were measured (Table 2) to evaluate the
152 distribution of alcohols between the oil and wastes.

153 **2.2.4. Sensory evaluation**

154 The sensory evaluation of the oil samples was carried out by the Official Tasting Panel
155 of Virgin Olive Oils of Catalonia (Reus, Spain), which has been recognized by the IOC
156 since 1997 and by the Spanish Government since 2004. It relies under ISO 17025 standard
157 since 2007. The final aroma evaluation represents the median from eight different trained
158 tasters. Table 3 shows the results of the positive attributes of the sensorial analysis
159 (fruitiness, bitterness and pungency). The panel was unable to test the intermediate oil
160 samples from the decanter, because the tasters are not trained for that purpose and because
161 the oil contains high levels of moisture and impurities (Table 2) that could interfere with
162 taster's perception.

163

164 **2.3. Analysis of alcohols**

165 **2.3.1. Sample preparation**

166 To determine alcohols in olive homogenates and in pomace, 2 g of the homogenized paste
167 were weighed into 20 mL vials together with 2g of saturated CaCl₂ solution. The vials
168 were tightly sealed with a septum cap and kept in the freezer (-18°C) until their analysis.
169 Regarding the quantitation of the alcohols in oil (either centrifuged crude oil or clean oil),
170 the samples were prepared by pouring 3 g of oil together with 100 µL internal standard
171 into a 10 mL vial. After hermetically sealed with a septum cap, it was kept in the freezer
172 (-18°C) until its analysis.

173 **2.3.2. Materials and Reagents**

174 All chemical reagents were of gradient HPLC grade. Ethanol and methanol were
175 purchased from Scharlab (Barcelona, Spain). Calcium chloride (CaCl₂) and 1-propanol,

176 used as internal standard, were provided by Sigma-Aldrich (St. Louis, USA). For the
177 Headspace-Solid Phase Microextraction (HS-SPME) of the analytes, 2 cm length fibers
178 50/30 μ m StableFlex divinylbenzene/carboxen/polydimethylsiloxane
179 (DVB/CAR/PDMS) (Supelco, USA) were used.

180 **2.3.3. Analytical procedure**

181 The quantification of alcohol contents in olive homogenates and pomace was carried out
182 by using an HS-SPME CTC CombiPAL autosampler (CTC Analytics, Switzerland) and
183 an HP-6890N gas chromatograph (GC) coupled to a mass detector (MSD) HP-5973
184 (Hewlett-Packard, USA). The optimal extraction conditions were: 15 min of pre-
185 equilibration at 50°C; HS-SPME during 50 min at 40°C under medium agitation; thermal
186 desorption at 270°C for 1 min in the GC injector port in splitless mode.

187 Chromatographic separations were carried out using a fused silica capillary column,
188 Chromapack CP-WAX 57CB, 50m x 0.25mm i.d. and 0.2 μ m film thickness (Varian.
189 Middelburg, Netherlands). The oven temperature program was: 40°C (5 min), 5°C.min⁻¹
190 to 100°C and 10°C.min⁻¹ to 215°C (5 min). The carrier gas was helium (He) at a constant
191 flow of 1.8 mL/min. Interface, ion source and mass quadrupole temperatures were 200°C,
192 230°C and 150°C, respectively. The mass-to-charge (m/z) ratio range used was 28-300
193 amu, and spectra matching were performed using the Wiley/NBS library. To avoid
194 quantification errors due to the matrix effect, the calibration lines were built by using
195 matrix-matched calibration technique as explained in a previous study (Boudebouz et al.,
196 2020).

197 When dealing with oil samples, alcohols were determined by using an A G1888
198 Automatic Static Headspace Sampler (Hewlett-Packard, USA) coupled to a GC-MSD
199 system. The optimal operating conditions were similar to the ones described by Gómez-

200 Coca et al., (2014) so 3 g of sample into a 10 mL vial were heated at 80°C during 50
201 minutes under medium agitation. Then, 500 µL of the headspace sample were injected
202 into the GC port through a transfer line at 110°C. The chromatographic conditions are the
203 ones described above and the quantification of alcohols was carried out by means of the
204 internal standard method by using 1-propanol for this purpose.

205 It should be noted that oil samples from the decanter were centrifuged to eliminate the
206 water, therefore, part of alcohols were also eliminated due to their distribution between
207 both phases. Since the different experiments carried out implied different oil:water ratios,
208 a previous study to determine the repartition factor in each case was necessary. Thus,
209 different oil/water mixtures were prepared ranging from 100:0 to 88:12 ratios. All the
210 mixtures were spiked with the same amount of alcohols and then were agitated to
211 facilitate partitioning of the analytes between both phases. Finally, the mixtures were
212 centrifuged to separate the phases and the amounts of alcohols in each one were
213 determined. In this way, we obtained the distribution factors that could be applied to the
214 different samples to avoid quantification errors.

215 **2.4. Analysis of fatty acids alkyl esters**

216 **2.4.1. Sample preparation**

217 To determine the amount of FAAEs (FAMEs and FAEEs) in the different oil samples
218 coming both from the decanter and from the VC, the IOC official method (COI/T.20/Doc.
219 No31. 2012) was applied. Thus, a glass column for liquid chromatography was filled with
220 3 g of silica gel suspended in a hexane:ether mixture (98:2). This column was used to
221 fractionate the sample (100±2 mg of the oil added with 25 µL of the internal standard
222 (methyl heptadecanoate in heptane 0.02%)) and to get FAAEs fraction after evaporation
223 of the solvent in a rotatory evaporator at 40°C and subsequent dissolution of the residue
224 in 1 mL of heptane. For each experiment, three extractions were performed and the

225 extracts obtained were stored in the freezer, into 1.5mL vials hermetically closed until
226 their analysis.

227 **2.4.2. Materials and Reagents**

228 The glass columns for liquid chromatography (10mm i.d, 40cm length) were provided by
229 POBEL (Madrid, Spain). The solvents used were ethyl ether for HPLC, $\geq 99.00\%$
230 (CHROMASOLV®), n-hexane for HPLC, $\geq 97.00\%$ (CHROMASOLV®) and n-heptane
231 for GC, $\geq 97.00\%$ (LICHROSOLV®). The Silica gel used was Silica 60 from Merck
232 KGaA (Darmstadt, Germany).

233 The chemical standards for FAAEs identification (methyl palmitate, methyl linoleate,
234 methyl oleate, methyl stearate, ethyl palmitate, ethyl linoleate, ethyloleate and ethyl
235 stearate) and the internal standard (methyl heptadecanoate) were supplied by Sigma-
236 Aldrich (Madrid, Spain) and their purity was $\geq 97\%$ in all cases.

237 **2.4.3. Analytical procedure**

238 The GC analyses of FAAEs were carried out with an Agilent 6890N gas chromatograph
239 equipped with an Agilent G1530 flame ionization detector (FID) (Agilent Technologies,
240 USA) coupled to an automatic injector equipped with a programmable temperature
241 vaporizing (PTV) inlet for on-column injection of the sample extracts. The
242 chromatographic separations were done using a fused silica capillary column, Zebron ZB-
243 5MS, 30m x 0.25mm i.d. and 0.25 μm d.f. from Phenomenex (Alcobendas, Spain), which
244 was protected with an empty pre-column of 30-40 cm. The oven temperature was
245 programmed at 70°C for 2 min, followed by a ramp of 10°C.min⁻¹ until 180°C, then
246 5°C.min⁻¹ until 220°C and 10°C.min⁻¹ until 320°C, and held for 16.5 min. The detector
247 temperature was 350°C. Hydrogen was used as carrier gas at a constant flow of 1.5
248 mL/min. A sample volume of 1 μL was injected in on-column mode.

249 The identification of FAAEs was performed by injecting individual standards of C16 and
250 C18 FAAEs and FAMEs. The quantification of each identified compound was performed
251 based on the area ratio between the analyte and the IS by using the following
252 mathematical relationship (COI, 2012; Pérez-Camino et al., 2008; Gómez-Coca,
253 Moreda, & Pérez-Camino, 2012):

$$254 \quad \text{FAAEs (mg/kg)} = \frac{(Ax * ms) * 1000}{(As * m)}$$

$$255 \quad \text{FAAEs (mg/kg)} = ((Ax*ms)*1000)/((As*m))$$

256 *Ax*: area corresponding to the peak for the individual C16 and C18 esters

257 *As*: area corresponding to the peak for the internal standard (methyl heptadecanoate)

258 *ms*: mass of the internal standard added (in milligrams)

259 *m*: mass of the oil sample taken for determination (in grams)

260

261 **2.5. Statistical analysis**

262 Statistical analysis of the results was performed using the SAS-Stat Software (V9.4. SAS
263 Institute Inc., Cary). The effects of water addition and paste injection rate were analyzed
264 by one-way ANOVA (Analysis of Variance) using the Generalized Linear Model (GLM)
265 procedure, exploring both linear and quadratic models. Comparison of means was
266 performed by using the Duncan's multiple range tests ($\alpha < 0.05$).

267

268

269 **3. Results and discussion**

270 As described above, each set of experiments was performed with olives at two different
271 maturity stages. For each experiment, only olives of good visual quality were used, which
272 implies that more than 94% of the fruits were healthy (data not shown).

273 To avoid any fermentation reaction from harvest to processing, the olive fruits were
274 pressed no later than two hours after receiving them at the mill. This precaution is enough
275 as the contents of alcohols in the reference samples averaged 9 mg.kg⁻¹ for ethanol and
276 120 mg.kg⁻¹ for methanol (data not shown), values very similar to those reported for
277 healthy fruits in previous studies (Beltrán et al., 2015; Boudebouz et al., 2020; García-
278 Vico et al., 2018).

279 To evaluate the effects of the decanter adjustments on the evolution of the content of
280 alcohols and FAAEs when working under different conditions, the rest of the process
281 steps (crushing, malaxing and vertical centrifugation) were kept constant. In this way the
282 different experiments focused on the effect of the paste injection rate and the water
283 injection flow into the decanter (keeping constant the spin and outlet diaphragm).

284 The values of the factors studied were chosen within the working ranges recommended
285 by the decanter manufacturer. Specifically, the paste injection rates studied ranged
286 between 3400 kg.h⁻¹ and 4500 kg.h⁻¹ and water addition between 0 and 300 L.h⁻¹, values
287 within the interval recommended for a two-phase system (70-90% of decanter capacity
288 and less than 10% water injection). It is well known that the use of water negatively
289 affects minor compounds of VOOs, mainly volatiles and polyphenols, and eventually the
290 VOOs shelf-life. However, when the contents of FAAEs are high, the concern is more
291 related to the final commercial category than to the nutritional values. Furthermore, there
292 are new injector devices that allow water to be delivered directly into the decanter without
293 mixing it with the paste and thus overcome the problem of polyphenol loss (Hermoso,
294 Boudebouz, Ninot, & Romero, 2021). In Catalonia, the prevalence of oils with high risk
295 of being downgraded due to an excess of FAAEs is 15%. It must also be stated that master
296 millers need to balance between quantity and quality of the oil extracted, based on many

297 reasons that have not been considered in this study, which aims to give them more criteria
298 to take such decision.

299 The two batches of fruits used for the experiments were different (table 1). On December-
300 8th the olives were turning from green to red color and were very rich in water, while on
301 December-15th the olives were black and with less moisture. The oil yield of both batches
302 was the expected for the 'Arbequina' cultivar in the Garrigues area in December.
303 Regarding the characteristics of the pomace at the outlet of the decanter (Table 2), the fat
304 content was higher than expected for all experiments (12-14% dry basis, while the
305 theoretical one is 8-12% db). This may be due to the fact that, to avoid the interference of
306 too many variables, coadjutants were added to the malaxing step and mechanical
307 adjustments of decanter were made (diaphragm, differential spin, distance of paste
308 download in the decanter), apart from the two conditions studied.

309 The highest pomace fat content was observed at the minimum paste injection rate without
310 water addition (table 2). This behavior may be because, under these conditions, a change
311 of the decanter diaphragms was required. Therefore, the extractability of the oil was
312 improved by increasing the paste injection rate or by adding a little water to the paste.

313 The oil moisture and impurity levels at the decanter outlet (table 2) were relatively high
314 for all conditions and justified the use of the vertical centrifuge in order to clarify and
315 stabilize the oil.

316 Finally, table 3 reports that all the processing conditions tested allowed to obtain oils of
317 such good quality, as all quality criteria matched the category of extra virgin (free acidity,
318 peroxide values, K_{232} , K_{270} and sensory evaluation).

319 **3.1. The balance of alcohols and FAAEs**

320 To better evaluate the effect of each processing factor studied, the balance of alcohols and
321 FAAEs was made between the input and output of every studied step. Thus, the amount

322 of each compound in every fraction (olive paste, pomace and oil) was calculated and
323 expressed in grams per hour (g/h), taking into account the total amount of each fraction
324 processed in one hour and the concentration of alcohols and FAAEs measured in aliquot
325 samples of each fraction (Table 4). Table 5 shows the balance taking into account inputs
326 and outputs in each centrifugation step (decanter and VC) and the results are expressed
327 as percentage relative to the inputs.

328 In most of the experiments, the samples showed lower contents of methanol both at the
329 decanter and VC inlet than at the decanter and VC outlet, respectively. However, when
330 looking at the ethanol contents, the values showed an opposite behavior as no generation
331 of ethanol was observed in any experiment (Table 4). This different trend seems to be
332 related to the activity of pectin methyl-esterase and its hydrolytic processes that occur
333 during the olive oil production process, which implies methanol generation but has no
334 effects on the ethanol contents. This corroborates the results found in the literature (Conte,
335 et al., 2019).

336 Alcohols exist naturally in olives, so they can pass into the oil during the extraction
337 process (Beltrán et al., 2015; Luna, Morales, & Aparicio, 2006; Boudebouz et al., 2020).
338 However, as in previous studies (Biederman et al., 2008), the results showed that large
339 amounts of ethanol and methanol are removed with water during processing although
340 each alcohol has a different behavior. As shown in table 5, while 90-95% of methanol is
341 removed from the oil in the decanter, a significant amount of ethanol (15-25%) reaches
342 the oily fraction. Therefore, special attention must be paid to ethanol and ethyl esters
343 because, if the decanter does not work in the right conditions, these compounds can reach
344 the oil.

345 According to the literature, the evaporation of a part of the alcohols can occur throughout
346 the different process steps (Masella et al., 2019). However, our data do not support such
347 fact but rather attribute some variations in the content of alcohols to their esterification
348 into alkyl esters (Pérez-Camino et al., 2008). As shown in tables 4 and 5, these
349 esterification reactions showed a different yield depending on the olive ripeness status.
350 Thus, when working with less mature fruits, the FAEE content at the decanter outlet can
351 double the value found in the olive fruits. However, these values do not reach the final oil
352 since they are drastically reduced when the oil passes through the VC. Although
353 centrifugation facilitates the elimination of a part of these compounds (Vidal et al., 2019),
354 this separation process does not explain such a marked decrease. After carefully studying
355 the results, it was concluded that at this point of the process, a certain hydrolysis of the
356 alkyl esters can happen, which should be favored by the presence of high water content.
357 Therefore, as less mature olives provided up to twice the water content in the oil obtained
358 at the decanter outlet than more mature olives, the hydrolysis process in the latter should
359 be much less. The results in table 5 show that mature samples are not only unaffected by
360 hydrolysis but even increase the value of the concentration of EE's. This behavior means
361 that there must be an intermediate step between the decanter outlet and VC where ethyl
362 esters are synthesized. This step can be related to the design of La Granadella mill, which
363 implies that liquids can remain under the vibro-filter for a while and can facilitate the
364 fermentation of sugars diluted in the vegetative water that is mixed with the oil in this
365 step.

366 Regarding the contents of FAMEs, there is a similar trend for all the experiments and, as
367 can be seen in Table 5, these values decrease in the decanter but increase again when
368 passing through the VC. This opposite behavior to that observed for the EE's may be due
369 to the high amount of methanol in the oil obtained at the decanter outlet. These great

370 concentrations can promote the esterification reaction into the vertical centrifuge, with
371 the consequent reduction of the methanol content in the final oil (due to both VC effect
372 and esterification).

373 **3.2. Effect of the paste injection rate**

374 To easily visualize whether or not there was a relationship between the different paste
375 injection rates and the concentration of the analytes studied in the final product, the plots
376 shown in Figures 2 and 3 were drawn up. These figures also show the balances between
377 the input and output of the system from the ratio “analyte contents in VC/analyte contents
378 in fruits” (VC/Fruit).

379 Concerning the relationship between the paste injection rate and EtOH content in the oil
380 at VC outlet (that is, in the final product), a very weak quadratic trend was found, with a
381 maximum between 76-82% of pumping rate and slightly higher values for the ripe olives
382 (Fig. 2a). Regarding FAEE (Fig. 2b), a significant quadratic trend was pointed out for
383 ripe olives. Maximum values of EEs were found when working at an injection rate
384 between 76-82% of the whole decanter capacity. Therefore, about 15% to 25% of the
385 EtOH that enters the system can reach the oil, either as ethanol or ethyl esters (Fig. 2c).
386 When EE's in the oil are compared to those in the olive fruits (Fig. 2d), it can be seen that
387 during the process a significant EE synthesis occurs, which ranges between 100-150% of
388 the values in the fruits. In the case of ripe olives processed at a very high rate (90%),
389 significant losses of EEs are observed (balance below 100%) that possibly are carried
390 along with the pomace due to a better separation as it happens with ethanol (Fig. 2g).

391 When only the decanter is considered, EtOH and mainly EE show a significant quadratic
392 behavior related to the paste injection rates. As expected, ethanol and ethyl esters show
393 inverse trends (Fig. 2e and 2f), that can be related to the ethanol conversion into EE since

394 the esterification of free fatty acids with these alcohols is a fast reaction (Pérez-Camino
395 et al., 2008). Thus, within the range 76% to 82% of working capacity, higher the injection
396 rate lowers the time that the oil remains in the decanter and lower the EtOH transformed
397 into EE. However, around the maximum capacity of the decanter (95-100%), the system
398 deviates from the optimal working conditions and worsens the separation yield.
399 Specifically, both the dry matter oil losses and oil moisture content increase (Table 2), so
400 this higher water content will drag more ethanol that could be esterified. This hypothesis
401 is confirmed by observing the acidity of the final oil. As shown in Table 3, when working
402 under these conditions, the acidity significantly decreases because some of the acids
403 disappear when reacting with ethanol, giving rise to ethyl esters, which are the ones that
404 increase their content.

405 In addition, and according to Guerrini, Pantani, & Parenti (2016), the effect of
406 centrifugation together with the existence of a greater amount of vegetative water caused
407 higher K_{232} . These higher values were observed at the highest injection rates in all
408 experiments, suggesting more oxidative conditions (Table 2).

409 Regarding MeOH in the oil at the VC outlet, a significant data dispersion was found
410 when it came from mature olives, probably due to its high inherent reactivity. However,
411 for green olives a quadratic effect was observed with the paste injection rate (Fig. 3a).
412 This is a trend opposite to ethanol's, as the recovery of methanol at the VC outlet increases
413 with higher injection rates, especially with more than 90% of the total decanter capacity.
414 The balance shows that between 1.5% and 2.5% of the methanol from the fruit reaches
415 the final oil (Fig. 3c). Regarding ME, no significant effect was observed (Fig. 3b).

416 Although most of the MeOH from the olive paste drags into the pomace at the decanter
417 outlet, a certain amount reaches the oil following a quadratic trend, with higher

418 concentrations of methanol in the oil at a higher injection rate (Fig. 3e), mainly when it
419 comes to green olives. Regarding MEs, these showed an opposite behavior with lower
420 concentrations at higher injection rates and, again, green fruits better fit the quadratic
421 trend (Fig. 3f).

422 **3.3. Effect of water addition**

423 The relationship between the addition of water and the compounds studied (Fig. 4 and 5)
424 shows different trends depending on the stage of the process. When looking at the
425 amounts of ethanol that reaches the oil at outlet of the VC, no statistically significant
426 effect was observed neither on green nor on ripe olives (regardless of the amount of water
427 injected). However, the concentrations of ethanol in the oil were significantly lower for
428 ripe olives in all experiments (Fig. 4a). The amounts of EE, which ranged between 10 and
429 20 mg.kg⁻¹, also did not show any significant trend. Therefore, as it can be seen in Figures
430 4c and 4d, whereas only between 12% and 25% of the total ethanol from the olives
431 reaches the oil at the VC outlet, almost all the ethyl esters from the olives reach this oil,
432 independently of the amount of water added.

433 When considering the decanter outlet, the results showed a significant quadratic
434 relationship between the ethanol content in the oil and the water injection (Fig. 4e). In
435 addition, a clear interaction with the state of maturity of the olives was identified, mainly
436 in the greater addition of water (8%). Under these conditions, the ethanol concentration
437 in the oil is reduced in green olives but increases in ripe olives.

438 At the decanter, water injection has an opposite behavior compared to the paste injection
439 rate. In fact, the addition of water tends to drag ethanol with pomace following a quadratic
440 trend with a maximum of 5% injection, which resulted in the highest oil extractability
441 (Fig. 2g and 4g). However, the paste injection rate follows the opposite trend, with a

442 minimum of ethanol dragged in the pomace when paste injection rate allows maximum
443 oil extractability (Fig. 2g). This suggests that water injection is a better regulation option
444 when processing low quality fruits (i.e, with significant amounts of ethanol).

445 Regarding MeOH contents in the oil at the VC outlet, no significant relationship with the
446 water injection flow was observed (Fig. 5a). This can be due to the fact that less than
447 2.5% of the total methanol coming from the fruits reaches the oil at the VC outlet, either
448 as MeOH or as esterified in MEs (Fig. 5c). Methyl esters showed a slight but not
449 significant trend to decrease with the addition of water (Fig. 5b). This trend is more
450 evident in terms of ME balance, referred to the initial methyl esters in the olives,
451 especially in ripe olives (Fig. 5d). Within the studied range (0 to 8% of water addition),
452 the trend is almost linear.

453 At the decanter level, the results for MeOH in oil suggest an interaction between the water
454 injection rate and the type of olives. For green olives, methanol decreases with the
455 addition of water following a quadratic trend with a minimum outside of the range studied
456 (and possibly over 10% of water addition). However, for ripe olives (with less moisture
457 content), the trend was inverse, with a maximum greater than approximately 5% (Fig.
458 5e). This interaction is equivalent, though opposite in trend, to that observed for ethanol.
459 Regarding ME, the addition of water tends to increase methyl esters in the oil at the
460 decanter outlet, following a quadratic trend that does not depend on the type of olives
461 (Fig. 5f). Finally, no significant relationship between methanol dragged into the pomace
462 and water injection rate was observed (Fig. 5g)

463 **4. Conclusions**

464 In summary, during the EVOO extraction process, there is no generation of ethanol but a
465 positive synthesis of methanol. However, decanter paste injection rate affects the content

466 of alcohols and alkyl esters in the oil, which tend to increase when working closer to the
467 maximum capacity of the decanter. Although most of alcohols are dragged within the
468 aqueous phase, significant amounts can reach the oil at the outlet of the vertical centrifuge
469 (up to 25% of alcohols present in olives), which increases the risk of FAAEs formation
470 during the decantation and storage of the oil. On the other hand, most of the alkyl esters
471 are removed through by-products and few of them hydrolyze according to the fruit
472 moisture content and the total water available in the system. In fact, unripe and ripe fruits
473 result in different FAAEs amounts depending on water injection and paste injection rate
474 used during the EVOO extraction process.

475 Thus, it can be concluded that the decanter and the passage from the decanter outlet to
476 the vertical centrifuge could be key points that must be controlled to avoid FAAEs
477 formation, and that water injection flow is a good regulation option when low quality
478 fruits are processed.

479

480

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491 **Bibliography**

- 492 Alcalá, S., Ocaña, M. T., Cárdenas, J. R., Miquel, M. Á., Vilar, J., Espínola, F., Moya, M. (2017).
493 Alkyl esters content and other quality parameters in oil mill: A response surface methodology
494 study. *European Journal of Lipid Science and Technology*. 119(1).
495 <https://doi.org/10.1002/ejlt.201600026>
- 496 Beltrán, G., Sánchez, R., Sánchez-Ortiz, A., Aguilera, M. P., Bejaoui, M. A., Jimenez, A. (2016).
497 How “ground-picked” olive fruits affect virgin olive oil ethanol content, ethyl esters and
498 quality. *Journal of the Science of Food and Agriculture*. 96(11), 3801–3806.
499 <https://doi.org/10.1002/jsfa.7573>
- 500 Beltrán, G., Bejaoui, M. A., Jimenez, A., Sanchez-Ortiz, A. (2015). Ethanol in Olive Fruit.
501 Changes during Ripening. *Journal of Agricultural and Food Chemistry*. 63(22), 5309–5312.
502 <https://doi.org/10.1021/acs.jafc.5b01453>
- 503 Biedermann, M., Bongartz, A., Mariani, C., Grob, K. (2008). Fatty acid methyl and ethyl esters
504 as well as wax esters for evaluating the quality of olive oils. *European Food Research and*
505 *Technology*. 228(1), 65–74. <https://doi.org/10.1007/s00217-008-0907-x>
- 506 Boudebouz, A., Romero, A., Boqué, R., Aceña, L., Busto, O., Mestres, M. (2020). Quantitation
507 of endogenous amount of ethanol, methanol and acetaldehyde in ripe fruits of different Spanish
508 olive varieties. *Journal of the Science of Food and Agriculture*. 100 (7), 3173-3181.
509 <https://doi.org/10.1002/jsfa.10352>.
- 510 Conte, L., Bendini, A., Valli, E., Lucci, P., Moret, S., Maquet, A., Gallina Toschi, T. (2019).
511 Olive oil quality and authenticity: A review of current EU legislation, standards, relevant
512 methods of analyses, their drawbacks and recommendations for the future. *Trends in Food*
513 *Science and Technology*. (February), 0–1. <https://doi.org/10.1016/j.tifs.2019.02.025>
- 514 Clodoveo, M. L. (2012). Malaxation: Influence on virgin olive oil quality. Past, present and *future*
515 - An overview. *Trends in Food Science and Technology*. 25(1), 13–23.
516 <https://doi.org/10.1016/j.tifs.2011.11.004>
- 517 Costa, R., Bartolomeo, G., Saija, E., Rando, R., Albergamo, A. and Dugo, G. (2017).

518 Determination of alkyl esters content in PDO extra virgin olive oils from Sicily. *Journal of*
519 *Food Quality*. Vol. 2017. <https://doi.org/10.1155/2017/3078105>

520 Di Giovacchino, L., Sestili, S., Di Vincenzo, D. (2002). Influence of olive processing on virgin
521 olive oil quality. *European Journal of Lipid Science and Technology*. 104(9–10), 587–601.
522 [https://doi.org/10.1002/1438-9312\(200210\)104:9/10<587::AID-EJLT587>3.0.CO;2-M](https://doi.org/10.1002/1438-9312(200210)104:9/10<587::AID-EJLT587>3.0.CO;2-M)

523 Di Giovacchino, L. (2013). In Aparicio, R., Harwood, J. Handbook of olive oil. Analysis and
524 properties. Second Edition. Chap. 3: Technological Aspects. Pp 57-96.
525 <https://doi.org/10.1007/978-1-4614-7777-8>

526 Di Serio, M. G., Giansante, L., Di Loreto, G., Faberi, A., Ricchetti, L., Di Giacinto, L. (2017).
527 Ethyl esters versus fermentative organoleptic defects in virgin olive oil. *Food Chemistry*. 219,
528 33–39. <https://doi.org/10.1016/j.foodchem.2016.09.109>

529 Fregapane, G., Salvador, M.D. (2013). Production of superior quality extra virgin olive oil
530 modulating the content and profile of its minor components. *Food Research International*.
531 54(2), 1907–1914. <https://doi.org/10.1016/j.foodres.2013.04.022>

532 García-Vico, L., Belaj, A., León, L., Rosa, R., De la Sanz, C., Pérez, A.G. (2018). A survey of
533 ethanol content in virgin olive oil. *Food Control*. 91, 248–253.
534 <https://doi.org/10.1016/j.foodcont.2018.04.006>

535 Gómez-Coca, R. B., Fernandes, G. D., Pérez-Camino, M. del C., Moreda, W. (2016). Fatty acid
536 ethyl esters (FAEE) in extra virgin olive oil: A case study of a quality parameter. *LWT - Food*
537 *Science and Technology*. 66, 378–383. <https://doi.org/10.1016/j.lwt.2015.10.063>

538 Gómez-Coca, R. B., Cruz-Hidalgo, R., Fernandes, G. D., Pérez-Camino, M.D.C., Moreda, W.
539 (2014). Analysis of methanol and ethanol in virgin olive oil. *MethodsX*. 1, e207–e211.
540 <https://doi.org/10.1016/j.mex.2014.09.002>

541 Gómez-Coca, R. B., Moreda, W., Pérez-Camino, M. C. (2012). Fatty acid alkyl esters presence in
542 olive oil vs. organoleptic assessment. *Food Chemistry*. 135, 1205e1209.
543 <https://doi.org/10.1016/j.foodchem.2012.05.053>

544 Guerrini, L., Pantani O.L. and Parenti, A. (2016). The impact of vertical centrifugation on olive

545 oil quality. *Journal of Food Process Engineering*. vol. 40, no.3, 2017. e12489.
546 <https://doi.org/10.1111/jfpe.12489>

547 Guillaume, C., Ravetti, L., Ruiz, N., Zaparenkov, D. (2013). Survey to determine olive oil
548 compliance with new methodologies in international standards. *Rural Industries Research and*
549 *Development Corporation (Australia)*. no. 13/094.

550 Hermoso, M.F., González, J.D., García-Ortiz, A.R., Morales, J.B., Frías, L.R., Ferenandez, A.G.
551 (1996). Manufacture of olive oil quality. Obtaining by the two-phase system. 3^a Edición.
552 Consejería de agricultura y pesca. Junta de Andalucía. Pp 83.

553 Hermoso, J.F., Boudebouz, A., Ninot, A., Romero, A. (2021). Evaluation of the effect of a
554 perimetral water injector at the decanter on the extractability and quality of olive oil. In:
555 Abstracts book from Network Congress of Oliviculture, Citrus and Fruit Culture of the Spanish
556 Society of Horticultural Sciences, on-line. 110-111.

557 International Olive Council. (2013). Trade standard applying to olive oils and olive-pomace oils.
558 COI/T. 15/Doc. No 3/Rev., 7.

559 International Olive Council. (2012). Determination of the content of waxes, fatty acid methyl
560 esters and fatty acid ethyl esters by capillary gas chromatography using 3 grams of silica.
561 COI/T. 20/Doc. No, 31.

562 Jabeur, H., Zribi, A., Abdelhedi, R., Bouaziz, M. (2015). Effect of olive storage conditions on
563 Chemlali olive oil quality and the effective role of fatty acids alkyl esters in checking olive
564 oils authenticity. *Food Chemistry*. 169, 289–296.
565 <https://doi.org/10.1016/j.foodchem.2014.07.118>

566 Lanza, B., Di Serio, M. G. & Di Giacinto, L. (2016). Fatty-acid alkyl esters in table olives in
567 relation to abnormal fermentation and poorly conducted technological treatments. *Grasas y*
568 *Aceites*. 67(2). <https://doi.org/10.3989/gya.0630152>

569 Luna, G., Morales, M.T. and Aparicio, R. (2006). Characterisation of 39 varietal virgen olive oils
570 by their volatile compositions. *Journal of Food Chemistry*. volume 98 (2), 243-252.
571 <https://doi.org/10.1016/j.foodchem.2005.05.069>

572 Masella, P., Guerrini, L., Angeloni, G., Zanoni, B., Parenti, A. (2019). Ethanol from Olive paste
573 during Malaxation, Exploratory Experiments. *European Journal of Lipid Science and*
574 *Technology*. 121(1). <https://doi.org/10.1002/ejlt.201800238>

575 Nieto, J., Montaña, A.M., Caravaca, M.J., Cuberos, J.D., Cuberos F.J., Fernandez, P.M., Abad, M.,
576 Molero, C., Pérez, D., Cardenal, P., Amezcua, C., Miquel, M.A., Peña, M.D., Ramón, S.,
577 Méndez, T., Moreda, W., López, J.E. (2019). Elaboration of quality virgin olive oil.
578 Considerations from experience and knowledge. 1st Edition (Spanish).

579 Parenti, A., Spugnoli, P., Masella, P., Calamai, L. (2007). Influence of the extraction process on
580 dissolved oxygen in olive oil. *Eur. J. of Lipid Science and Technology*. 109(12), 1180–1185.
581 <https://doi.org/10.1002/ejlt.200700088>

582 Pérez-Camino, M.D.C., Cert, A., Romero-Segura, A., Cert-Trujillo, R., Moreda, W. (2008). Alkyl
583 esters of fatty acids a useful tool to detect soft deodorized olive oils. *Journal of Agricultural*
584 *and Food Chemistry*. 56(15), 6740–6744. <https://doi.org/10.1021/jf801131b>

585 Pérez-Camino, M. C., Moreda, W., Mateos, R., Cert, A. (2002). Determination of esters of fatty
586 acids with low molecular weight alcohols in olive oils. *Journal of Agricultural and Food*
587 *Chemistry*. 50(16), 4721–4725. <https://doi.org/10.1021/jf025542>

588 Uceda, M., Frías, L. (1975). Harvest dates. Evolution of the fruit of content, oil composition and
589 oil quality. In Proceedings of the II Seminario Oleícola Internacional, International Olive
590 Council, Córdoba, Spain, 6–17 October 1975, pp. 125–130

591 Uceda, M., Jiménez, A., Beltrán, G. (2006). Olive oil extraction and quality. *Grasas y Aceites*.
592 57(1), 25–31. <https://doi.org/10.3989/gya.2006.v57.i1.19>

593 Vidal, M.A., Alcalá, S., de Torres, A., Moya, M., Espínola, F. (2019) Centrifugation, Storage,
594 and Filtration of Olive Oil in an Oil Mill: Effect on the Quality and Content of Minority
595 compounds. *Journal of Food Quality*. 7381761-7. <https://doi.org/10.1155/2019/7381761>

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597

598 **FIGURE CAPTIONS**

599

600 **Figure 1:** Olive oil processing scheme (Two-phase system)

601

602 **Figure 2.** Paste injection rates effect on ethanol and ethyl esters in the decanter and the
603 vertical centrifuge outlets.

604

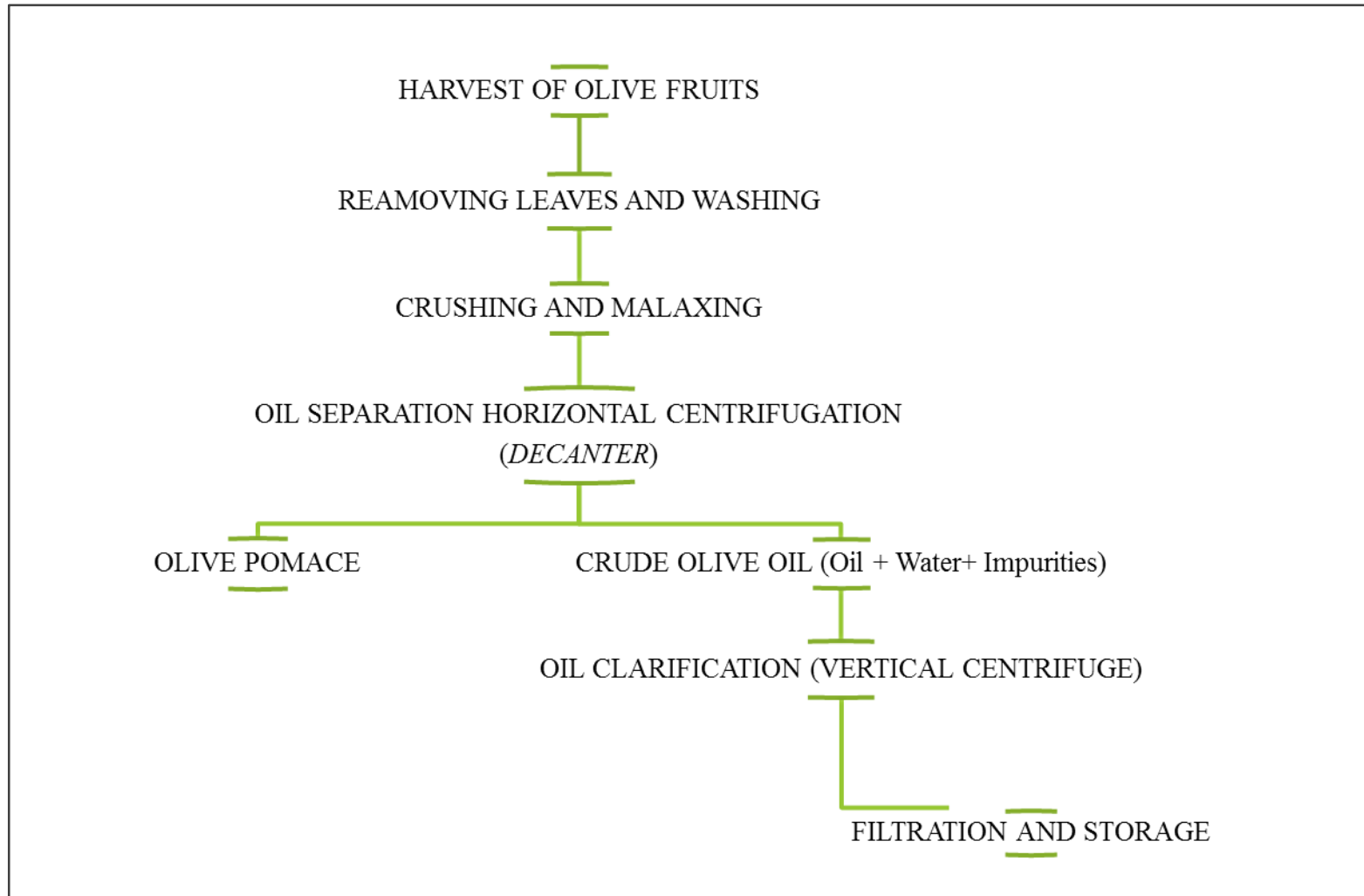
605 **Figure 3.** Paste injection rates effect on methanol and methyl esters in the decanter and
606 the vertical centrifuge outlets.

607

608 **Figure 4.** Water addition effect on ethanol (EtOH) and ethyl esters (EE) in the decanter
609 and the vertical centrifuge outlets.

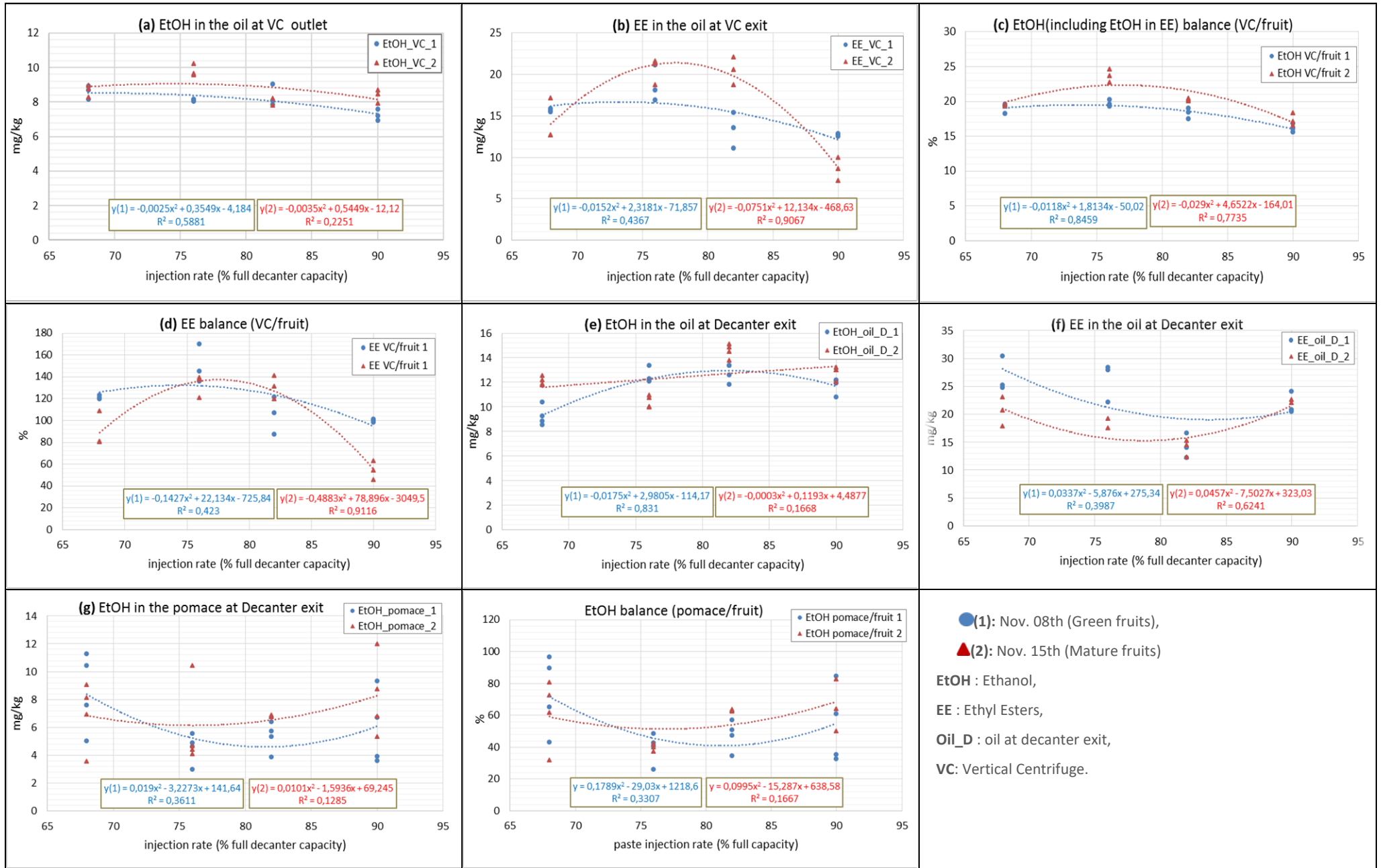
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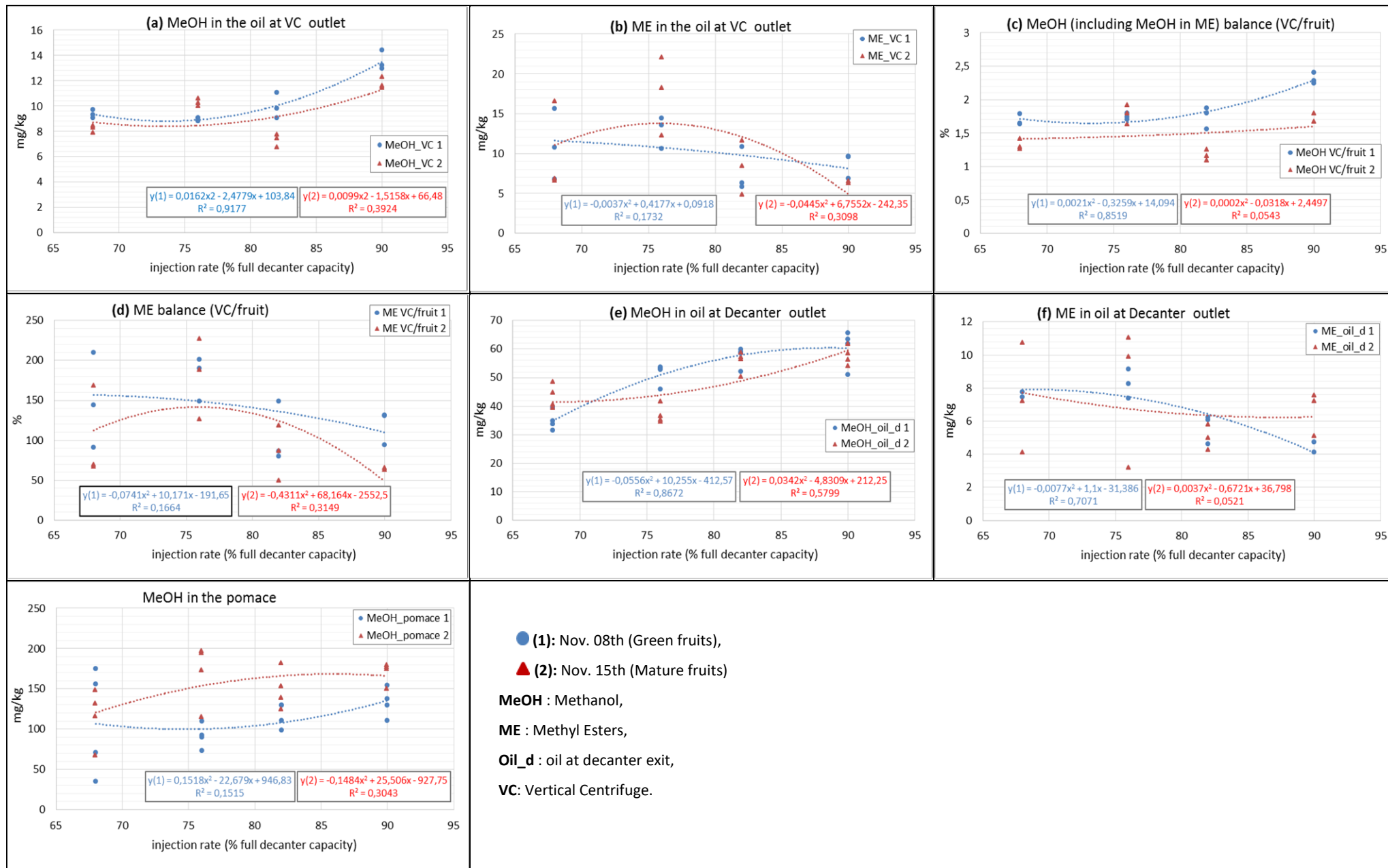
611 **Figure 5.** Water addition effect on methanol (MeOH) and methyl esters (ME) in the
612 decanter and the vertical centrifuge outlets.



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614 **Figure 1.**

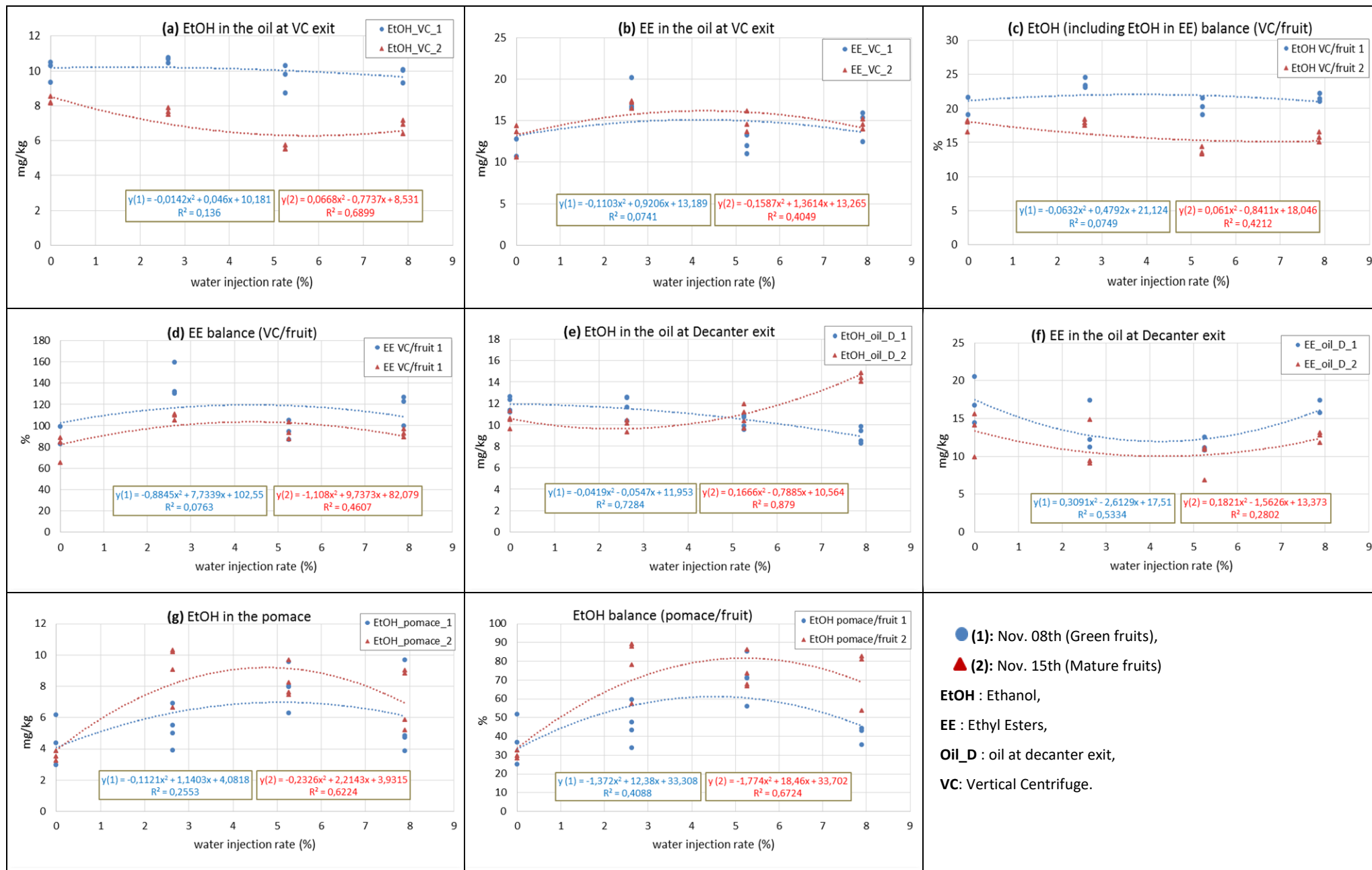




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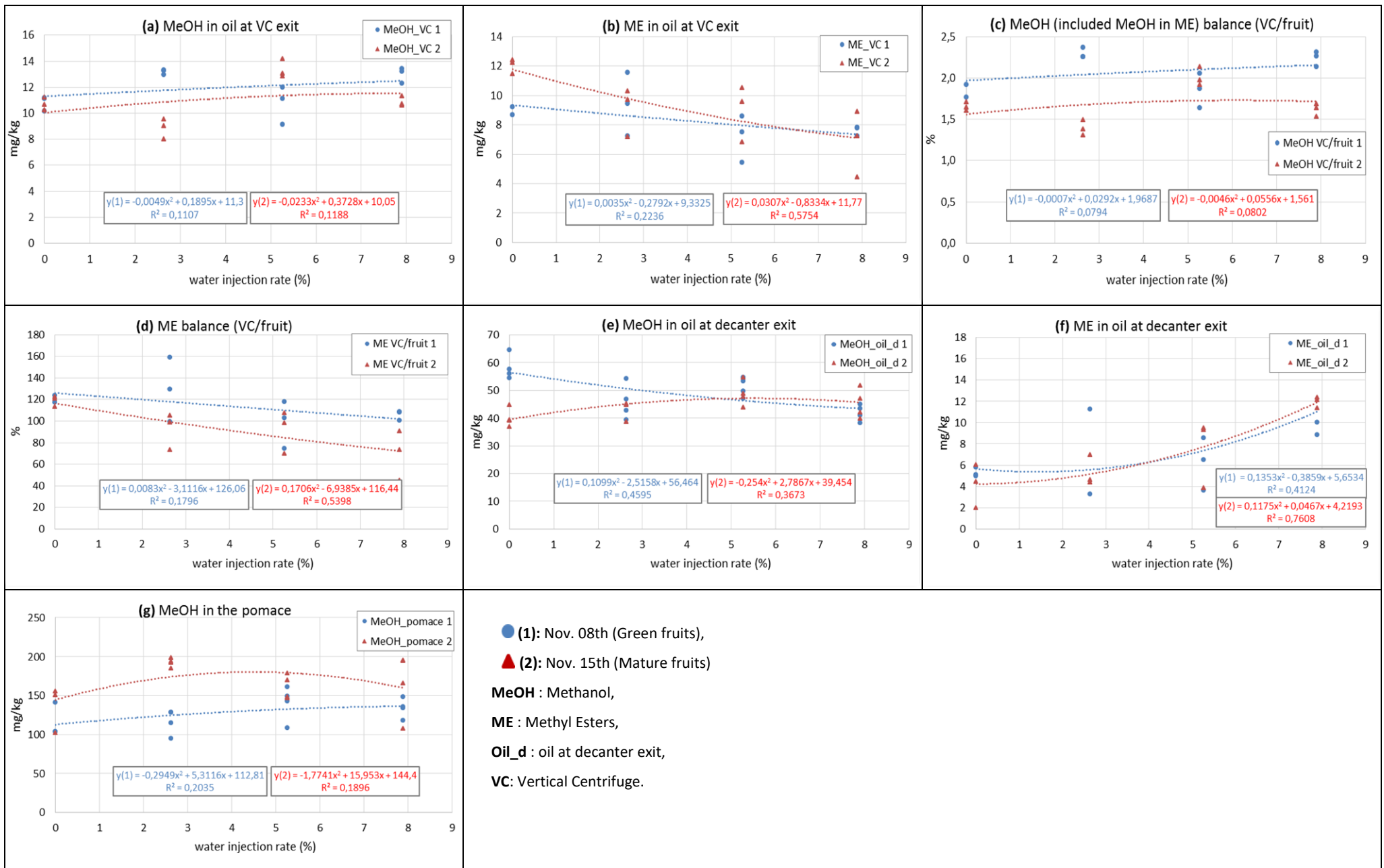
Figure 3.



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Figure 4.



620 **Figure 5.**

621 TABLES

622 Table 1. Olive characteristics according to the harvest date

date	Maturity Index (MI)	Flesh/pit Ratio	Variety	Moisture (%)	Fat in wet basis (%)	Fat in dry basis (%)
December 8 th	2.65	2.52	Arbequina	52.31	22.37	46.91
December 15 th	3.90	2.61	Arbequina	48.69	22.64	44.13

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625 Table 2. Olive pomace and oil characteristics at the outlet of horizontal centrifuge (decanter) (mean \pm
626 standard deviation)

Factor	date	Dose (kg/h)	olive pomace (decanter exit)			Oil (decanter exit)	
			Moisture %	Fat in wet basis (%)	Fat in dry basis (%)	Moisture and volatiles (%)	Impurities (%)
Rhythm	Dec-8 th	3400	63.39 ^a \pm 0.32	5.32 ^a \pm 0.01	14.55 ^a \pm 0.10	5.73 ^a \pm 1.15	0.50 ^a \pm 0.25
		3800	63.10 ^a \pm 0.58	4.44 ^b \pm 0.01	12.04 ^a \pm 0.18	3.90 ^a \pm 1.15	0.39 ^a \pm 0.20
		4100	61.13 ^a \pm 0.99	4.75 ^{ab} \pm 0.19	12.26 ^a \pm 0.80	3.97 ^a \pm 0.79	0.28 ^a \pm 0.14
		4500	61.97 ^a \pm 0.30	4.69 ^{ab} \pm 0.12	12.35 ^a \pm 0.41	4.30 ^a \pm 0.86	0.50 ^a \pm 0.25
	Dec-15 th	3400	62.43 ^b \pm 0.09	5.13 ^a \pm 0.07	13.67 ^a \pm 0.15	2.20 ^a \pm 0.44	0.25 ^a \pm 0.13
		3800	62.68 ^b \pm 0.08	4.72 ^a \pm 0.20	12.65 ^a \pm 0.57	2.58 ^a \pm 0.52	0.21 ^a \pm 0.11
		4100	63.28 ^a \pm 0.02	4.80 ^a \pm 0.05	13.07 ^a \pm 0.12	4.08 ^{ab} \pm 0.82	0.23 ^a \pm 0.11
		4500	63.54 ^a \pm 0.02	4.94 ^a \pm 0.10	13.56 ^a \pm 0.26	5.75 ^b \pm 1.15	0.18 ^a \pm 0.09
Water	Dec-8 th	0	62.83 ^b \pm 0.23	5.41 ^a \pm 0.13	14.56 ^a \pm 0.25	6.31 ^a \pm 1.15	0.66 ^a \pm 0.33
		100	63.66 ^{ab} \pm 0.24	4.91 ^b \pm 0.05	13.52 ^b \pm 0.23	6.53 ^a \pm 1.31	0.62 ^a \pm 0.31
		200	64.44 ^a \pm 0.09	4.79 ^b \pm 0.01	13.46 ^b \pm 0.06	6.66 ^a \pm 1.33	0.23 ^b \pm 0.11
		300	63.84 ^a \pm 0.08	4.36 ^c \pm 0.06	12.06 ^c \pm 0.13	6.52 ^a \pm 1.30	0.16 ^b \pm 0.08
	Dec-15 th	0	60.29 ^a \pm 0.15	5.85 ^a \pm 0.07	14.74 ^a \pm 0.12	3.22 ^a \pm 0.64	0.27 ^a \pm 0.13
		100	61.15 ^a \pm 0.24	5.02 ^b \pm 0.05	12.91 ^b \pm 0.04	2.32 ^a \pm 0.46	0.35 ^a \pm 0.18
		200	60.85 ^a \pm 0.70	4.85 ^b \pm 0.16	12.41 ^b \pm 0.62	3.77 ^a \pm 0.75	0.21 ^a \pm 0.11
		300	62.73 ^a \pm 0.41	4.78 ^b \pm 0.03	12.84 ^b \pm 0.07	5.01 ^a \pm 1.00	0.42 ^a \pm 0.21

627 By column and by group, means with the same letter are not significantly different according to Duncan's multiple range tests ($P < 0.05$).

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639 **Table 3.** Olive oil characteristics at the vertical centrifuge outlet (mean \pm standard deviation)

Factor	Date	Dose (kg/h)	Moisture and volatiles content (%)	Acidity (% oleic acid)	Peroxide value (meq O ₂ /kg)	K ₂₃₂	K ₂₇₀	Panel test			
								category	fruitiness	bitterness	pungent
Rhythm	Dec-8 th	3400	0.21 ^a \pm 0.04	0.14 ^a \pm 0.01	7 ^a \pm 1	1.60 ^a \pm 0.16	0.14 ^a \pm 0.04	Extra	4.7 ^a \pm 0.4	3.8 ^a \pm 0.5	4.3 ^a \pm 0.2
		3800	0.26 ^a \pm 0.05	0.15 ^a \pm 0.02	9 ^a \pm 1	1.52 ^a \pm 0.15	0.14 ^a \pm 0.03	Extra	4.3 ^a \pm 0.2	3.1 ^a \pm 0.3	4.3 ^a \pm 0.4
		4100	0.24 ^a \pm 0.05	0.15 ^a \pm 0.02	7 ^a \pm 1	1.54 ^a \pm 0.15	0.17 ^a \pm 0.04	Extra	4.8 ^a \pm 0.2	3.6 ^a \pm 0.2	4.4 ^a \pm 0.3
		4500	0.33 ^b \pm 0.07	0.07 ^a \pm 0.03	8 ^a \pm 1	1.68 ^a \pm 0.17	0.15 ^a \pm 0.04	Extra	4.5 ^a \pm 0.3	4.4 ^a \pm 0.5	4.4 ^a \pm 0.4
	Dec-15 th	3400	0.24 ^b \pm 0.05	0.15 ^a \pm 0.02	9 ^a \pm 1	1.51 ^a \pm 0.15	0.11 ^a \pm 0.03	Extra	4.7 ^a \pm 0.2	3.9 ^a \pm 0.1	4.5 ^a \pm 0.2
		3800	0.27 ^a \pm 0.05	0.14 ^a \pm 0.01	9 ^a \pm 1	1.64 ^a \pm 0.16	0.13 ^a \pm 0.03	Extra	4.7 ^a \pm 0.2	3.5 ^a \pm 0.2	4.7 ^a \pm 0.2
		4100	0.28 ^a \pm 0.06	0.14 ^a \pm 0.01	8 ^a \pm 1	1.58 ^a \pm 0.16	0.12 ^a \pm 0.03	Extra	4.9 ^a \pm 0.2	3.6 ^a \pm 0.2	4.6 ^a \pm 0.2
		4500	0.29 ^a \pm 0.06	0.12 ^a \pm 0.01	8 ^a \pm 1	1.76 ^a \pm 0.18	0.12 ^a \pm 0.03	Extra	4.6 ^a \pm 0.2	3.4 ^a \pm 0.2	4.4 ^a \pm 0.3
Water	Dec-8 th	0	0.28 ^a \pm 0.06	0.11 ^a \pm 0.01	6 ^a \pm 1	1.50 ^a \pm 0.15	0.09 ^a \pm 0.02	Extra	5.1 ^a \pm 0.2	3.8 ^a \pm 0.2	4.7 ^a \pm 0.4
		100	0.27 ^a \pm 0.05	0.11 ^a \pm 0.01	6 ^a \pm 1	1.49 ^a \pm 0.15	0.09 ^a \pm 0.02	Extra	5.0 ^a \pm 0.5	3.7 ^a \pm 0.1	4.4 ^a \pm 0.4
		200	0.27 ^a \pm 0.05	0.11 ^a \pm 0.01	6 ^a \pm 1	1.49 ^a \pm 0.15	0.11 ^a \pm 0.03	Extra	4.7 ^a \pm 0.4	3.5 ^a \pm 0.2	4.4 ^a \pm 0.2
		300	0.30 ^a \pm 0.06	0.12 ^a \pm 0.01	7 ^a \pm 1	1.51 ^a \pm 0.15	0.11 ^a \pm 0.03	Extra	5.0 ^a \pm 0.2	3.8 ^a \pm 0.2	4.6 ^a \pm 0.1
	Dec-15 th	0	0.18 ^a \pm 0.04	0.16 ^a \pm 0.02	7 ^a \pm 1	1.48 ^a \pm 0.15	0.12 ^a \pm 0.03	Extra	4.8 ^a \pm 0.3	4.4 ^a \pm 0.2	4.9 ^a \pm 0.4
		100	0.18 ^a \pm 0.04	0.16 ^a \pm 0.02	7 ^a \pm 1	1.52 ^a \pm 0.15	0.12 ^a \pm 0.03	Extra	5.0 ^a \pm 0.5	4.1 ^a \pm 0.3	4.8 ^a \pm 0.3
		200	0.18 ^a \pm 0.04	0.17 ^a \pm 0.02	8 ^a \pm 1	1.59 ^a \pm 0.16	0.16 ^a \pm 0.04	Extra	4.9 ^a \pm 0.3	4.0 ^a \pm 0.3	4.8 ^a \pm 0.4
		300	0.27 ^b \pm 0.05	0.17 ^a \pm 0.02	6 ^a \pm 1	1.61 ^a \pm 0.16	0.17 ^a \pm 0.04	Extra	5.0 ^a \pm 0.1	4.3 ^a \pm 0.4	4.6 ^a \pm 0.2

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641 *By column and by group, means with the same letter are not significantly different according to Duncan's multiple range tests (P<0.05).*

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R F D e	3400	33.29	7.72	24.49 ^a ± 1.72	5.85 ^c ± 0.17	16.92 ^a ± 0.66	2.63 ^a ± 1.10	5.13 ^b ± 1.08	9.33 ^b ± 1.04	1.45 ^b ± 1.01
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Table 4.

Alcohols and FAAEs contents (mean ± standard deviation) in each step (loaded paste, pomace and oil), expressed in grams per hour (g/h) of processing.

Experiment	3800	37.20	8.63	14.63 ^a ± 1.19	8.99 ^b ± 0.14	18.85 ^a ± 0.83	2.93 ^a ± 0.13	5.62 ^{ab} ± 0.01	Vertical centrifuge	10.54	2.01 ^a ± 0.08
	4100	40.14	9.31	19.05 ^a ± 1.27	9.76 ^{ab} ± 0.19	10.87 ^b ± 0.56	1.69 ^b ± 0.09	6.12 ^a ± 0.16	9.79 ^b ± 0.52	1.52 ^b ± 0.08	
Factor	dose	EtOH (g/h)	EE (g/h)	MeOH (g/h)	EtOH (g/h)	EE (g/h)	EtOH_in_EE (%) ^x	MeOH (g/h)	EE (g/h)	EtOH_in_EE (%) ^x	
Water (L/h)	Dec-15 th	3400	32.06	9.48	19.73 ^{bc} ± 2.29	7.45 ^b ± 0.07	12.65 ^b ± 0.53	1.97 ^b ± 0.08	5.23 ^c ± 0.07	8.54 ^b ± 0.52	1.33 ^b ± 0.08
		3800	35.83	10.60	19.18 ^c ± 3.29	7.30 ^b ± 0.12	12.86 ^b ± 0.28	2.00 ^b ± 0.04	6.71 ^a ± 0.08	14.04 ^a ± 0.36	2.18 ^a ± 0.06
		4100	38.66	11.44	24.30 ^b ± 0.06	11.06 ^a ± 0.15	10.65 ^b ± 0.38	1.66 ^b ± 0.06	5.89 ^b ± 0.06	14.94 ^a ± 0.4	2.32 ^a ± 0.06
		4500	42.44	12.55	32.82 ^a ± 3.83	10.78 ^a ± 0.15	18.76 ^a ± 0.11	2.92 ^a ± 0.02	6.65 ^a ± 0.10	6.82 ^b ± 0.37	1.06 ^b ± 0.06
Water (L/h)	Dec-8 th	0	37.20	8.63	10.58 ^b ± 2.71	8.46 ^a ± 0.17	12.28 ^a ± 0.73	1.91 ^a ± 0.11	6.70 ^a ± 0.14	7.83 ^b ± 0.33	1.22 ^b ± 0.05
		100	37.20	8.63	17.13 ^{ab} ± 1.33	8.57 ^a ± 0.25	9.91 ^a ± 0.81	1.54 ^a ± 0.13	7.25 ^a ± 0.04	12.11 ^a ± 0.48	1.88 ^a ± 0.07
		200	37.20	8.63	26.34 ^a ± 1.48	7.48 ^b ± 0.16	8.38 ^a ± 0.22	1.30 ^a ± 0.03	6.54 ^a ± 0.18	8.21 ^b ± 0.26	1.28 ^b ± 0.04
		300	37.20	8.63	19.66 ^{ab} ± 2.99	6.63 ^b ± 0.19	12.21 ^a ± 0.29	1.90 ^a ± 0.04	6.76 ^a ± 0.10	10.04 ^{ab} ± 0.42	1.56 ^{ab} ± 0.06
	Dec-15 th	0	35.83	10.60	11.06 ^b ± 0.32	7.09 ^b ± 0.16	8.91 ^a ± 0.67	1.38 ^a ± 0.10	5.43 ^a ± 0.05	8.41 ^b ± 0.44	1.31 ^b ± 0.07
		100	35.83	10.60	29.09 ^a ± 1.82	6.89 ^b ± 0.13	7.68 ^a ± 0.74	1.19 ^a ± 0.12	5.19 ^a ± 0.04	11.48 ^a ± 0.11	1.78 ^a ± 0.02
		200	35.83	10.60	27.35 ^a ± 1.11	7.58 ^b ± 0.23	6.75 ^a ± 0.57	0.79 ^a ± 0.19	3.78 ^c ± 0.03	10.00 ^{ab} ± 0.29	1.55 ^{ab} ± 0.04
		300	35.83	10.60	24.65 ^a ± 2.26	10.53 ^a ± 0.20	8.95 ^a ± 0.17	1.39 ^a ± 0.03	4.61 ^b ± 0.09	9.82 ^b ± 0.14	1.53 ^b ± 0.02
Factor	dose	MeOH (g/h)	ME ^y (g/h)	MeOH (g/h)	MeOH (g/h)	ME (g/h)	MeOH_in_ME(g/h) ^t	MeOH (g/h)	ME (g/h)	MeOH_in_ME (g/h)	
Rhythm (kg/h)	Dec-8 th	3400	388.55	4.44	312.10 ^b ± 13.04	22.03 ^d ± 0.70	4.80 ^{ab} ± 0.04	0.55 ^{ab} ± 0.01	5.58 ^c ± 0.07	6.58 ^a ± 0.88	0.75 ^a ± 0.10
		3800	434.26	4.96	296.00 ^b ± 12.02	37.06 ^c ± 0.88	5.96 ^a ± 0.21	0.68 ^a ± 0.02	6.22 ^c ± 0.03	8.92 ^a ± 0.46	1.01 ^a ± 0.05
		4100	468.55	5.35	418.92 ^{ab} ± 13.90	43.83 ^b ± 0.91	4.30 ^b ± 0.23	0.49 ^b ± 0.03	7.32 ^b ± 0.24	5.62 ^a ± 0.68	0.64 ^a ± 0.08
		4500	514.26	5.87	531.30 ^b ± 18.18	50.68 ^a ± 1.81	3.71 ^b ± 0.12	0.42 ^b ± 0.01	10.85 ^a ± 0.21	6.97 ^a ± 0.42	0.79 ^a ± 0.05
	Dec-15 th	3400	443.43	5.92	331.35 ^b ± 16.96	26.69 ^c ± 0.86	4.54 ^{ab} ± 0.68	0.52 ^{ab} ± 0.08	4.96 ^c ± 0.06	6.04 ^{ab} ± 1.14	0.69 ^{ab} ± 0.13
		3800	495.60	6.62	554.39 ^a ± 12.87	25.96 ^c ± 0.75	7.36 ^a ± 0.19	0.84 ^a ± 0.02	7.03 ^b ± 0.07	11.98 ^a ± 1.12	1.36 ^a ± 0.13
		4100	534.72	7.14	536.06 ^a ± 18.79	42.35 ^b ± 0.93	3.82 ^b ± 0.19	0.43 ^b ± 0.02	5.34 ^c ± 0.13	6.08 ^{ab} ± 0.82	0.69 ^{ab} ± 0.09
		4500	586.89	7.84	682.01 ^a ± 10.47	48.58 ^a ± 0.95	5.18 ^{ab} ± 0.42	0.59 ^{ab} ± 0.05	9.36 ^a ± 0.12	3.38 ^b ± 0.98	0.58 ^b ± 0.00
Water (L/h)	Dec-8 th	0	434.26	4.96	363.48 ^a ± 24.76	41.36 ^a ± 1.05	3.76 ^b ± 0.11	0.43 ^b ± 0.01	7.22 ^b ± 0.13	5.97 ^a ± 0.08	0.68 ^a ± 0.01
		100	434.26	4.96	374.99 ^a ± 10.59	33.37 ^{bc} ± 1.57	5.29 ^{ab} ± 1.37	0.60 ^{ab} ± 0.16	8.99 ^a ± 0.05	6.41 ^a ± 0.49	0.73 ^a ± 0.06
		200	434,26	4.96	465.64 ^a ± 21.93	37.23 ^{ab} ± 0.83	4.55 ^{ab} ± 0.60	0.52 ^{ab} ± 0.07	7.31 ^b ± 0.33	4.89 ^a ± 0.36	0.55 ^a ± 0.06
		300	434,26	4.96	455.18 ^a ± 13.80	30.86 ^c ± 0.72	9.36 ^a ± 0.93	1.06 ^a ± 0.11	8.94 ^a ± 0.14	5.24 ^a ± 0.08	0.60 ^a ± 0.01
	Dec-15 th	0	495,60	6.62	425.96 ^b ± 23.09	27.05 ^b ± 0.75	2.82 ^b ± 0.47	0.32 ^b ± 0.05	7.02 ^b ± 0.11	7.86 ^a ± 0.11	0.89 ^a ± 0.03
		100	495,60	6.62	619.36 ^a ± 4.44	29.6 ^{ab} ± 0.68	3.68 ^b ± 0.33	0.42 ^b ± 0.04	5.98 ^c ± 0.17	6.11 ^{ab} ± 0.37	0.69 ^{ab} ± 0.04
		200	495,60	6.62	533.23 ^{ab} ± 13.14	34.23 ^a ± 1.04	5.31 ^b ± 0.75	0.60 ^b ± 0.08	9.05 ^a ± 0.16	6.07 ^{ab} ± 0.43	0.69 ^{ab} ± 0.04
		300	495,60	6.62	567.00 ^{ab} ± 14.33	32.05 ^{ab} ± 1.25	8.51 ^a ± 0.12	0.97 ^a ± 0.01	7.34 ^b ± 0.09	4.63 ^b ± 0.51	0.53 ^b ± 0.06

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By column and by group, means with the same letter are not significantly different according to Duncan's multiple range tests (P<0.05).

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^z EE for ethyl esters; ^y ME for methyl esters ^{t, x} The conversion (EtOH in EE and MeOH in ME) explains the quantity of ethanol transformed into ethyl ester and methanol into methyl ester. It was calculated applying the

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equation:

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$$EtOH \text{ in } EE = (ethyl \text{ ester mass} * ethanol \text{ molar mass}) / (oleic \text{ acid molar mass}) \text{ and the equivalent for MeOH in ME}$$

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The oleic acid mass was used in the equation, as it presents the dominating fatty acid in olive oil.

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674 **Table 5.** Relative content (%) of alcohols and FAAEs at the decanter and vertical centrifuge outlets as a function of their initial content in the olive paste at the
675 decanter inlet (mean \pm standard deviation)

676	Rhythm (kg/h)	Dec-8th	3400	73.57^a \pm 8.18	219.24^a \pm 8.55	17.57^b \pm 0.50	15.41^a \pm 0.28	120.90^b \pm 0.59	77.86^a \pm 1.15		
677			3800	39.32 ^b \pm 3.21	218.49 ^a \pm 9.67	24.15 ^a \pm 0.39	15.09 ^a \pm 0.04	150.18 ^a \pm 5.88	63.69 ^{bc} \pm 1.76		
			4100	47.46 ^{ab} \pm 3.17	116.72 ^b \pm 6.05	24.31 ^a \pm 0.47	15.23 ^a \pm 0.40	105.19 ^b \pm 5.63	67.51 ^b \pm 0.90		
			4500	53.29 ^{ab} \pm 8.11	178.88 ^a \pm 5.52	22.36 ^a \pm 0.42	13.17 ^b \pm 0.20	99.78 ^b \pm 0.42	58.49 ^c \pm 0.95		
678	Rhythm (kg/h)	Dec-15th	3400	61.54 ^{ab} \pm 7.14	133.33 ^{ab} \pm 5.62	23.22 ^c \pm 0.22	16.30 ^b \pm 0.22	90.03 ^b \pm 5.44	69.33 ^b \pm 0.40		
679			3800	39.80 ^b \pm 9.17	121.27 ^b \pm 2.68	20.37 ^d \pm 0.32	18.73 ^a \pm 0.22	132.45 ^a \pm 3.36	96.80 ^a \pm 1.51		
			4100	62.84 ^{ab} \pm 0.15	93.15 ^c \pm 3.37	28.61 ^a \pm 0.38	15.23 ^b \pm 0.14	130.60 ^a \pm 3.53	64.82 ^b \pm 0.59		
			4500	65.52 ^a \pm 9.03	149.43 ^a \pm 0.87	25.40 ^b \pm 0.34	15.66 ^b \pm 0.24	54.34 ^c \pm 2.92	55.68 ^c \pm 0.00		
680	Water (L/h)	Dec-8th	0	37.90 ^b \pm 4.47	142.29 ^a \pm 8.52	22.74 ^a \pm 0.46	18.01 ^a \pm 0.36	90.75 ^b \pm 3.77	74.46 ^b \pm 4.37		
681			100	46.05 ^{ab} \pm 3.58	114.89 ^a \pm 9.37	23.03 ^a \pm 0.67	19.47 ^a \pm 0.10	140.36 ^a \pm 5.52	91.93 ^{ab} \pm 0.88		
			200	70.80 ^a \pm 3.99	97.12 ^a \pm 2.50	20.12 ^b \pm 0.42	17.56 ^a \pm 0.49	95.16 ^b \pm 3.03	90.84 ^{ab} \pm 3.32		
			300	52.85 ^{ab} \pm 8.05	141.56 ^b \pm 3.35	17.82 ^b \pm 0.50	18.16 ^a \pm 0.26	116.34 ^{ab} \pm 4.83	98.25 ^a \pm 2.62		
682		Dec-15th	0	30.06 ^b \pm 0.90	84.06 ^a \pm 6.30	19.23 ^b \pm 0.43	14.76 ^a \pm 0.14	79.32 ^b \pm 4.12	81.20 ^a \pm 0.88		
			100	78.18 ^a \pm 5.09	72.43 ^a \pm 7.00	18.52 ^b \pm 0.37	13.93 ^a \pm 0.11	108.30 ^a \pm 1.02	81.89 ^a \pm 1.43		
683			200	73.53 ^a \pm 3.10	63.68 ^a \pm 5.34	20.36 ^b \pm 0.64	10.16 ^c \pm 0.08	94.36 ^{ab} \pm 2.72	62.88 ^b \pm 2.53		
			300	66.26 ^a \pm 6.32	84.40 ^a \pm 1.56	28.29 ^a \pm 0.56	12.39 ^b \pm 0.24	92.65 ^b \pm 1.33	51.65 ^c \pm 1.76		
684	Factor	date	MeOH (%)		ME (%)		MeOH (%)		ME (%)		Balance Decanter/VC
685	Rhythm (kg/h)	Dec-8th	3400	80.32 ^a \pm 2.38	108.30 ^a \pm 1.01	5.67 ^c \pm 0.18	1.43 ^b \pm 0.02	148.33 ^a \pm 19.89	29.06 ^a \pm 1.08		
			3800	68.16 ^a \pm 3.69	120.17 ^a \pm 4.27	8.53 ^b \pm 0.20	1.43 ^b \pm 0.01	179.94 ^a \pm 9.21	19.03 ^b \pm 0.19		
686			4100	89.41 ^a \pm 3.96	80.40 ^b \pm 4.22	9.35 ^{ab} \pm 0.19	1.56 ^b \pm 0.05	105.05 ^a \pm 12.76	18.66 ^b \pm 0.82		
			4500	103.31 ^a \pm 4.71	63.24 ^b \pm 2.06	9.85 ^a \pm 0.35	2.11 ^a \pm 0.04	118.72 ^a \pm 7.13	24.75 ^b \pm 1.08		
687		Dec-15th	3400	74.72 ^b \pm 7.45	76.63 ^{ab} \pm 11.51	6.02 ^b \pm 0.19	1.12 ^c \pm 0.01	102.0 ^{ab} \pm 19.25	21.079 ^b \pm 0.94		
			3800	111.86 ^{ab} \pm 8.38	111.14 ^a \pm 2.86	5.24 ^b \pm 0.15	1.42 ^b \pm 0.01	180.98 ^a \pm 16.91	34.50 ^a \pm 0.98		
688			4100	100.25 ^a \pm 5.47	53.51 ^b \pm 2.66	7.92 ^a \pm 0.17	1.00 ^d \pm 0.02	85.15 ^b \pm 11.48	14.80 ^b \pm 0.55		
			4500	116.21 ^a \pm 3.14	66.08 ^{ab} \pm 5.33	8.28 ^a \pm 0.16	1.59 ^a \pm 0.02	43.11 ^b \pm 12.46	21.07 ^b \pm 0.00		
689	Water (L/h)	Dec-8th	0	83.70 ^a \pm 5.21	75.83 ^b \pm 2.16	9.52 ^a \pm 0.24	1.66 ^b \pm 0.03	120.41 ^a \pm 23.20	19.04 ^b \pm 0.51		
			100	86.35 ^a \pm 3.95	106.80 ^{ab} \pm 27.66	7.68 ^{ab} \pm 0.36	2.07 ^a \pm 0.01	129.24 ^a \pm 9.94	28.25 ^{ab} \pm 2.00		
690			200	107.23 ^a \pm 5.77	91.79 ^{ab} \pm 12.08	8.57 ^{bc} \pm 0.19	1.68 ^b \pm 0.07	98.61 ^a \pm 7.29	21.43 ^b \pm 1.17		
			300	104.82 ^a \pm 3.22	188.84 ^a \pm 18.71	7.11 ^c \pm 0.17	2.06 ^a \pm 0.03	105.78 ^a \pm 1.53	31.06 ^a \pm 0.79		
691		Dec-15th	0	85.95 ^b \pm 6.21	42.53 ^b \pm 7.02	5.46 ^b \pm 0.15	1.42 ^b \pm 0.02	118.73 ^a \pm 1.65	29.93 ^a \pm 1.10		
			100	124.97 ^a \pm 1.20	55.56 ^b \pm 4.91	5.97 ^{ab} \pm 0.14	1.21 ^c \pm 0.03	92.30 ^{ab} \pm 5.64	21.04 ^b \pm 0.73		
692			200	107.59 ^{ab} \pm 3.54	80.17 ^b \pm 11.27	6.91 ^a \pm 0.21	1.83 ^a \pm 0.03	91.69 ^{ab} \pm 6.54	28.58 ^{ab} \pm 1.48		
			300	114.41 ^{ab} \pm 9.44	128.5 ^a \pm 1.83	6.47 ^{ab} \pm 0.25	1.48 ^b \pm 0.02	69.94 ^b \pm 7.68	23.95 ^{ab} \pm 1.20		

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696 By column and by group, means with the same letter are not significantly different according to Duncan's multiple range tests ($P < 0.05$).

697 ⁽²⁾: The balance Decanter/CV explains the proportion of alcohol (either as alcohol or alkyl ester) between the vertical centrifuge outlet and the horizontal centrifuge outlet.