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Processing factors that affect the balance of alcohols and alkyl esters during 'Arbequina' olive oil production: separation and clarification steps

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Abstract

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

Results show that the paste injection rate affects the content of alcohols and FAAEs in the final oil, which tend to increase when working closer to the maximum capacity of the decanter. Different behaviors have been detected when dealing with unripe or ripe fruits. Similarly, the water addition doses have different effects on the FAAEs and alcohols formation depending on the maturity status. Therefore, both the decanter and

the step decanter to vertical centrifuge are key points that, when properly controlled, allow minimizing FAAEs formation, which is essential for obtaining quality oils.

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

1. Introduction

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

Among the different EVOO quality parameters, one of the most studied in recent years has been the content of fatty acids alkyl esters (FAAEs), which includes both ethyl esters (FAEEs) and methyl esters (FAMEs) (Alcalá et al., 2017; Beltrán et al., 2016; Biedermann, Bongartz, Mariani, & Grob, 2008; Di Serio et al., 2017; Gómez-Coca, Fernandes, Pérez-Camino, & Moreda, 2016; Lanza, Di Serio, & Di Giacinto, 2016). Their formation is due to the esterification and/or transesterification of free fatty acids with low molecular weight alcohols such as methanol or ethanol (Costa et al., 2017; Guilaume, Ravetti, Ruiz, & Zaparenkov, 2013; Pérez-Camino, Moreda, Mateos, & Cert, 2002). It is well known that alcohols are present in olive oil (García-Vico et al., 2018; Gómez-Coca, Cruz-Hidalgo, Fernandes, Pérez-Camino, & Moreda, 2014). When their origin is the natural pathway of fruit metabolism (what produces the so-called endogenous alcohols) their concentration depends on maturity, health status and olive cultivars (Beltrán, Bejaoui, Jimenez, & Sanchez-Ortiz, 2015; Boudebouz et al., 2020; García-Vico et al., 2018). However, when their presence is derived from the fermentation of olive sugars during the olive processing, the content of alcohols also depends on the manufacturing practices (Biedermann et al., 2008; Pérez-Camino, Cert, Romero-Segura, Cert-Trujillo, & Moreda, 2008). Thus, since alcohols and, consequently, the FAAEs values will increase when inappropriate practices are carried out during the processing, they have been used to assess the quality of olive fruits processed during EVOO extraction, to check the cleanliness of the material and to ensure a good management of the process (Pérez-Camino et al., 2008).

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

To guarantee the limit values of FAEEs, the olive status control is not enough but it is also necessary to control the different stages during VOO production in order to intervene in those with major risk of increasing the content of alkyl esters. In the present work, we focused on how the separation steps of the process (decanter and the vertical centrifuge) affect the quality by evaluating the balance between FAAEs and short-chain alcohols (ethanol and methanol).

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

2. Material and methods

2.1. Experiments

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

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

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

The first group of experiments evaluated the effect of olive paste injection rate on the balance of alcohols and FAAEs between fruit, pomace and oil. This study was carried out by fixing at a constant flow of 150 L/h the water injected into the decanter and testing different rates of olive paste: 68% (3400 kg/h), 76% (3800 kg/h), 82% (4100 kg/h) and 90% (4500 kg/h) of theoretical decanter capacity. This range of working rates is within the recommended levels proposed by several authors for two-phase decanters (Di Giovachino, 2013). The second group of experiments focused on the effect of small volumes of water injected into the decanter. Thus, the pumping of the olive paste was set at 76% of the decanter capacity and the water flow injection ranged between 0 L/h (0%), 100 L/h (3%), 200 L/h (5%) and 300 L/h (8%) respectively. In a two-phase system, in order to improve oil extraction, it is recommended to add small amounts of water into the decanter when working with difficult pastes (as is the case of 'Arbequina') as long as a limit of 10-15% water addition is not exceeded (Hermoso et al., 1996; Nieto et al., 2019).

Samples of pomace and oils were taken, in duplicate, at the decanter and VC outlets at approximately 10 min intervals. In all treatments, chemical characteristics of both pomace and oils samples were determined (Table 2 and Table 3).

2.2. Samplings

Sampling was carried out at different steps in order to study the balance of the compounds studied between the phases (oil, pomace) in each step. To get suitable conclusions, in all cases the results of the analysis were compared with those of the reference samples. In this way, it was possible to determine alcohols and FAAEs coming from olives, those formed during a specific production process or even alcohols lost by evaporation/transesterification (Masella et al., 2019; Vidal, Alcalá, de Torres, Moya, & Espínola, 2019; Alcalá et al., 2017; Pérez-Camino et al., 2008).

2.2.1. Initial content in the olives

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

2.2.2. Final content in pomace

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

2.2.3. Final content in the oil

Olive oil samples were taken after the two separation steps of the process: at the decanter outlet (crude oil) and at the VC outlet (clean oil). For each value of the tested parameters, two samples of 250 ml each were taken. Samples from the decanter were centrifuged in the laboratory at 5°C and 5000 RCF during 3 minutes. In all cases, alcohols and FAAEs were quantified and moisture and impurities were measured (Table 2) to evaluate the distribution of alcohols between the oil and wastes.

2.2.4. Sensory evaluation

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

2.3. Analysis of alcohols

2.3.1. Sample preparation

To determine alcohols in olive homogenates and in pomace, 2 g of the homogenized paste were weighed into 20 mL vials together with 2g of saturated $CaCl_2$ solution. The vials were tightly sealed with a septum cap and kept in the freezer (-18°C) until their analysis. Regarding the quantitation of the alcohols in oil (either centrifuged crude oil or clean oil), the samples were prepared by pouring 3 g of oil together with 100 µL internal standard into a 10 mL vial. After hermetically sealed with a septum cap, it was kept in the freezer (-18°C) until its analysis.

2.3.2. Materials and Reagents

All chemical reagents were of gradient HPLC grade. Ethanol and methanol were purchased from Scharlab (Barcelona, Spain). Calcium chloride ($CaCl_2$) and 1-propanol, used as internal standard, were provided by Sigma-Aldrich (St. Louis, USA). For the Headspace-Solid Phase Microextraction (HS-SPME) of the analytes, 2 cm length fibers 50/30μm StableFlex divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, USA) were used.

2.3.3. Analytical procedure

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

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

When dealing with oil samples, alcohols were determined by using an A G1888 Automatic Static Headspace Sampler (Hewlett-Packard, USA) coupled to a GC-MSD system. The optimal operating conditions were similar to the ones described by Gómez-Coca et al., (2014) so 3 g of sample into a 10 mL vial were heated at 80°C during 50 minutes under medium agitation. Then, 500 μL of the headspace sample were injected into the GC port through a transfer line at 110°C. The chromatographic conditions are the ones described above and the quantification of alcohols was carried out by means of the internal standard method by using 1-propanol for this purpose.

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

2.4. Analysis of fatty acids alkyl esters

2.4.1. Sample preparation

To determine the amount of FAAEs (FAMEs and FAEEs) in the different oil samples coming both from the decanter and from the VC, the IOC official method

(COI/T.20/Doc. No31. 2012) was applied. Thus, a glass column for liquid chromatography was filled with 3 g of silica gel suspended in a hexane:ether mixture (98:2). This column was used to fractionate the sample (100±2 mg of the oil added with 25 µL of the internal standard (methyl heptadecanoate in heptane 0.02%)) and to get FAAEs fraction after evaporation of the solvent in a rotatory evaporator at 40°C and subsequent dissolution of the residue in 1 mL of heptane. For each experiment, three extractions were performed and the extracts obtained were stored in the freezer, into 1.5mL vials hermetically closed until their analysis.

2.4.2. Materials and Reagents

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

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

2.4.3. Analytical procedure

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

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

$$\boldsymbol{FAAE}s\ (mg/kg) = \frac{(\boldsymbol{A}x * \boldsymbol{m}s) * 1000}{(\boldsymbol{A}s * \boldsymbol{m})}$$

__A__x: area corresponding to the peak for the individual C16 and C18 esters

__A__s: area corresponding to the peak for the internal standard (methyl heptadecanoate)

__m__s: mass of the internal standard added (in milligrams)

__m__: mass of the oil sample taken for determination (in grams)

2.5. Statistical analysis

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

3. Results and discussion

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

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

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

The values of the factors studied were chosen within the working ranges recommended by the decanter manufacturer. Specifically, the paste injection rates studied ranged between 3400 kg.h^{-1} and 4500 kg.h^{-1} and water addition between 0 and 300 L.h^{-1}, values within the interval recommended for a two-phase system (70-90% of decanter capacity and less than 10% water injection). It is well known that the use of water negatively affects minor compounds of VOOs, mainly volatiles and polyphenols, and eventually the VOOs shelf-life. However, when the contents of FAEEs are high, the concern is more related to the final commercial category than to the nutritional values. Furthermore, there are new injector devices that allow water to be delivered directly into the decanter without mixing it with the paste and thus overcome the problem of polyphenol loss (Hermoso, Boudebouz, Ninot, & Romero, 2021). In Catalonia, the prevalence of oils with high risk of being downgraded due to an excess of FAAEs is 15%. It must also be stated that master millers need to balance between quantity and quality of the oil extracted, based on many reasons that have not been considered in this study, which aims to give them more criteria to take such decision.

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

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

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

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

3.1. The balance of alcohols and FAAEs

To better evaluate the effect of each processing factor studied, the balance of alcohols and FAAEs was made between the input and output of every studied step. Thus, the amount of each compound in every fraction (olive paste, pomace and oil) was calculated and expressed in grams per hour (g/h), taking into account the total amount of each fraction processed in one hour and the concentration of alcohols and FAAEs measured in aliquot samples of each fraction (Table 4). Table 5 shows the balance taking into account inputs and outputs in each centrifugation step (decanter and VC) and the results are expressed as percentage relative to the inputs.

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

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

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

Regarding the contents of FAMEs, there is a similar trend for all the experiments and, as can be seen in Table 5, these values decrease in the decanter but increase again when passing through the VC. This opposite behavior to that observed for the EE's may be due to the high amount of methanol in the oil obtained at the decanter outlet. These great concentrations can promote the esterification reaction into the vertical centrifuge, with the consequent reduction of the methanol content in the final oil (due to both VC effect and esterification).

3.2. Effect of the paste injection rate

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

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

When only the decanter is considered, EtOH and mainly EE show a significant quadratic behavior related to the paste injection rates. As expected, ethanol and ethyl esters show inverse trends (Fig. 2e and 2f), that can be related to the ethanol conversion into EE since the esterification of free fatty acids with these alcohols is a fast reaction (Pérez-Camino et al., 2008). Thus, within the range 76% to 82% of working capacity, higher the injection rate lowers the time that the oil remains in the decanter and lower the EtOH transformed into EE. However, around the maximum capacity of the decanter (95-100%), the system deviates from the optimal working conditions and worsens the separation yield. Specifically, both the dry matter oil losses and oil moisture content increase (Table 2), so this higher water content will drag more ethanol that could be esterified. This hypothesis is confirmed by observing the acidity of the final oil. As shown in Table 3, when working under these conditions, the acidity significantly decreases because some of the acids disappear when reacting with ethanol, giving rise to ethyl esters, which are the ones that increase their content.

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

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

Although most of the MeOH from the olive paste drags into the pomace at the decanter outlet, a certain amount reaches the oil following a quadratic trend, with higher concentrations of methanol in the oil at a higher injection rate (Fig. 3e), mainly when it comes to green olives. Regarding MEs, these showed an opposite behavior with lower concentrations at higher injection rates and, again, green fruits better fit the quadratic trend (Fig. 3f).

3.3. Effect of water addition

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

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

At the decanter, water injection has an opposite behavior compared to the paste injection rate. In fact, the addition of water tends to drag ethanol with pomace following a quadratic trend with a maximum of 5% injection, which resulted in the highest oil extractability (Fig. 2g and 4g). However, the paste injection rate follows the opposite trend, with a minimum of ethanol dragged in the pomace when paste injection rate allows maximum oil extractability (Fig. 2g). This suggests that water injection is a better regulation option when processing low quality fruits (i.e, with significant amounts of ethanol).

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

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

4. Conclusions

In summary, during the EVOO extraction process, there is no generation of ethanol but positive synthesis of methanol. However, decanter paste injection rate affects the of alcohols and alkyl esters in the oil, which tend to increase when working closer to the maximum capacity of the decanter. Although most of alcohols are dragged within the aqueous phase, significant amounts of ethanol can reach the oil at the outlet of the vertical centrifuge (up to 25% of alcohols present in olives), which increases the risk of FAEEs formation during the decantation and storage of the oil. On the other hand, most of the alkyl esters are removed through by-products and few of them could be hydrolyzed according to the fruit moisture content and the total water available in the system. In fact, unripe and ripe fruits result in different FAAEs amounts depending on water injection and paste injection rate used during the EVOO extraction process.

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

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FIGURE CAPTIONS

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

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

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

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

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

TABLES

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 8th	2.65	2.52	Arbequina	52.31	22.37	46.91
December 15th	3.90	2.61	Arbequina	48.69	22.64	44.13

Table 2. Olive pomace and oil characteristics at the outlet of horizontal centrifuge (decanter) (mean ± 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-8th	3400	63.39 ^{a} ± 0.32	5.32 ^{a} ± 0.01	14.55 ^{a} ± 0.10	5.73 ^{a} ± 1.15	0.50 ^{a} ± 0.25
		3800	63.10 ^{a} ± 0.58	4.44 ^{b} ± 0.01	12.04 ^{a} ± 0.18	3.90 ^{a} ± 1.15	0.39 ^{a} ± 0.20
		4100	61.13 ^{a} ± 0.99	4.75 ^{ab} ± 0.19	12.26 ^{a} ± 0.80	3.97 ^{a} ± 0.79	0.28 ^{a} ± 0.14
		4500	61.97 ^{a} ± 0.30	4.69 ^{ab} ± 0.12	12.35 ^{a} ± 0.41	4.30 ^{a} ± 0.86	0.50 ^{a} ± 0.25
	Dec-15th	3400	62.43 ^{b} ± 0.09	5.13 ^{a} ± 0.07	13.67 ^{a} ± 0.15	2.20 ^{a} ± 0.44	0.25 ^{a} ± 0.13
		3800	62.68 ^{b} ± 0.08	4.72 ^{a} ± 0.20	12.65 ^{a} ± 0.57	2.58 ^{a} ± 0.52	0.21 ^{a} ± 0.11
		4100	63.28 ^{a} ± 0.02	4.80 ^{a} ± 0.05	13.07 ^{a} ± 0.12	4.08 ^{ab} ± 0.82	0.23 ^{a} ± 0.11
		4500	63.54 ^{a} ± 0.02	4.94 ^{a} ± 0.10	13.56 ^{a} ± 0.26	5.75 ^{b} ± 1.15	0.18 ^{a} ± 0.09
Water	Dec-8th	0	62.83 ^{b} ± 0.23	5.41 ^{a} ± 0.13	14.56 ^{a} ± 0.25	6.31 ^{a} ± 1.15	0.66 ^{a} ± 0.33
		100	63.66 ^{ab} ± 0.24	4.91 ^{b} ± 0.05	13.52 ^{b} ± 0.23	6.53 ^{a} ± 1.31	0.62 ^{a} ± 0.31
		200	64.44 ^{a} ± 0.09	4.79 ^{b} ± 0.01	13.46 ^{b} ± 0.06	6.66 ^{a} ± 1.33	0.23 ^{b} ± 0.11
		300	63.84 ^{a} ± 0.08	4.36 ^{c} ± 0.06	12.06 ^{c} ± 0.13	6.52 ^{a} ± 1.30	0.16 ^{b} ± 0.08
	Dec-15th	0	60.29 ^{a} ± 0.15	5.85 ^{a} ± 0.07	14.74 ^{a} ± 0.12	3.22 ^{a} ± 0.64	0.27 ^{a} ± 0.13
		100	61.15 ^{a} ± 0.24	5.02 ^{b} ± 0.05	12.91 ^{b} ± 0.04	2.32 ^{a} ± 0.46	0.35 ^{a} ± 0.18
		200	60.85 ^{a} ± 0.70	4.85 ^{b} ± 0.16	12.41 ^{b} ± 0.62	3.77 ^{a} ± 0.75	0.21 ^{a} ± 0.11
		300	62.73 ^{a} ± 0.41	4.78 ^{b} ± 0.03	12.84 ^{b} ± 0.07	5.01 ^{a} ± 1.00	0.42 ^{a} ± 0.21

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

Table 3. Olive oil characteristics at the vertical centrifuge outlet (mean ± 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-8th	**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-15th	**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-8th	**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-15th	**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	$5.0^{a} \pm 0.1$	$4.3^{a} \pm 0.4$	$4.6^{a} \pm 0.2$

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

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			Decanter inlet		Decanter outlet				Vertical centrifuge outlet		
			Olive paste		Pomace	Oil			Oil		
Factor		**dose**	**EtOH (g/h)**	**EE^{z} (g/h)**	**EtOH (g/h)**	**EtOH (g/h)**	**EE (g/h)**	**EtOH_in_EE (g/h)^{x}**	**EtOH (g/h)**	**EE (g/h)**	**EtOH_in_EE (g/h)**
Rhythm (kg/h)	**Dec-8th**	**3400**	33.29	7.72	24.49^{a} ± 2.72	5.85^{c} ± 0.17	16.92^{a} ± 0.66	2.63^{a} ± 0.10	5.13^{b} ± 0.08	9.33^{b} ± 0.04	1.45^{b} ± 0.01
		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	12.96^{a} ± 0.51	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
		4500	44.06	10.22	23.48^{a} ± 3.57	9.85^{a} ± 0.19	18.28^{a} ± 0.56	2.84^{a} ± 0.09	5.80^{a} ± 0.09	10.19^{b} ± 0.04	1.58^{b} ± 0.01
	Dec-15th	**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-8th**	**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-15th	**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-8th**	**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-15th	**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-8th**	**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} ± 0.04
		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^{yh}	**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} ± 0.01
		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} ± 0.05
		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

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

[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 equation:

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

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

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 decanter inlet (mean ± standard deviation)

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

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

[(Z)] *: 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.*

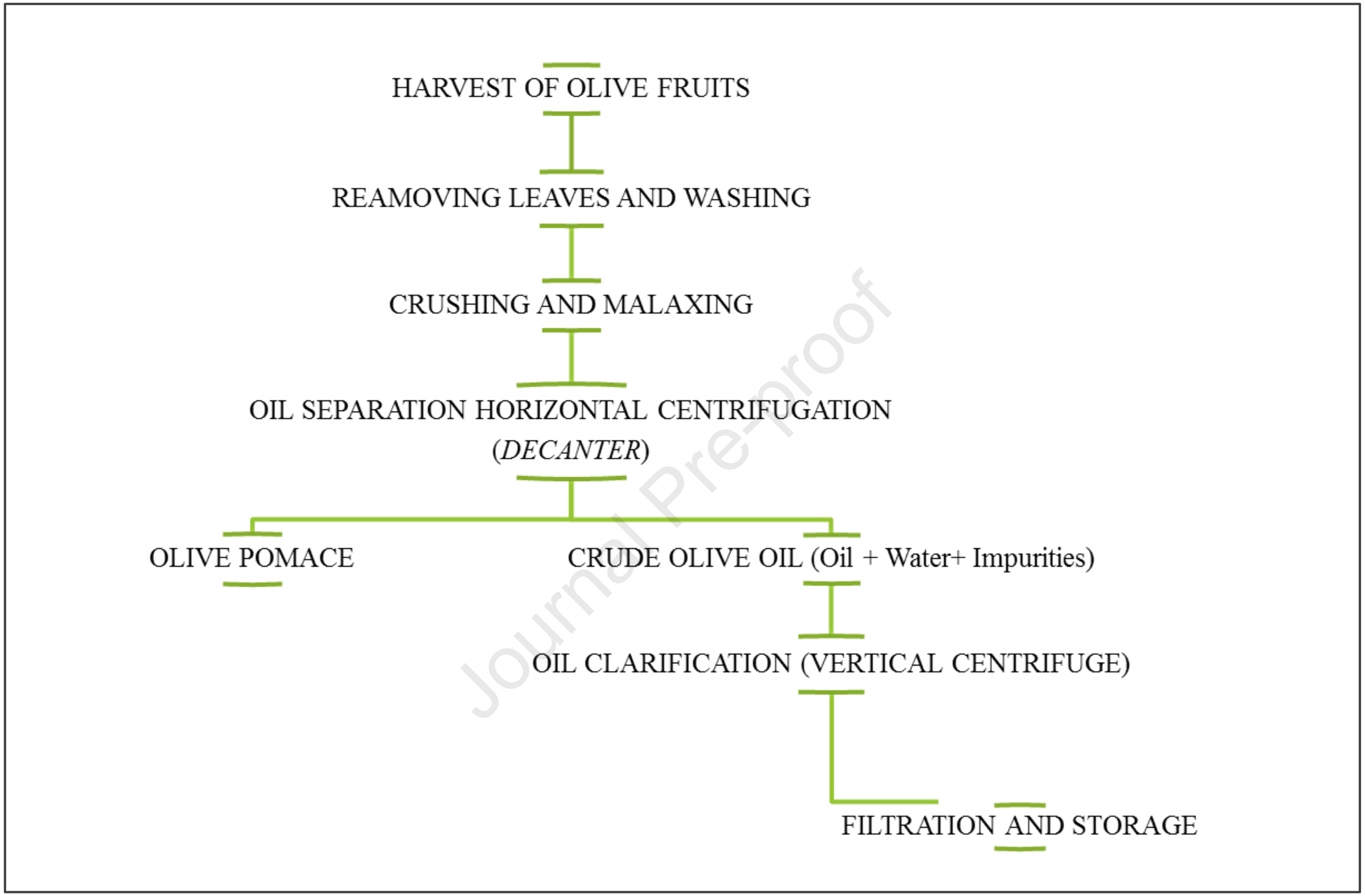

HARVEST OF OLIVE FRUITS
REAMOVING LEAVES AND WASHING
CRUSHING AND MALAXING
OIL SEPARATION HORIZONTAL CENTRIFUGATION (DECANTER)
OLIVE POMACE
CRUDE OLIVE OIL (Oil + Water+ Impurities)
OIL CLARIFICATION (VERTICAL CENTRIFUGE)
FILTRATION AND STORAGE

Figure 1.

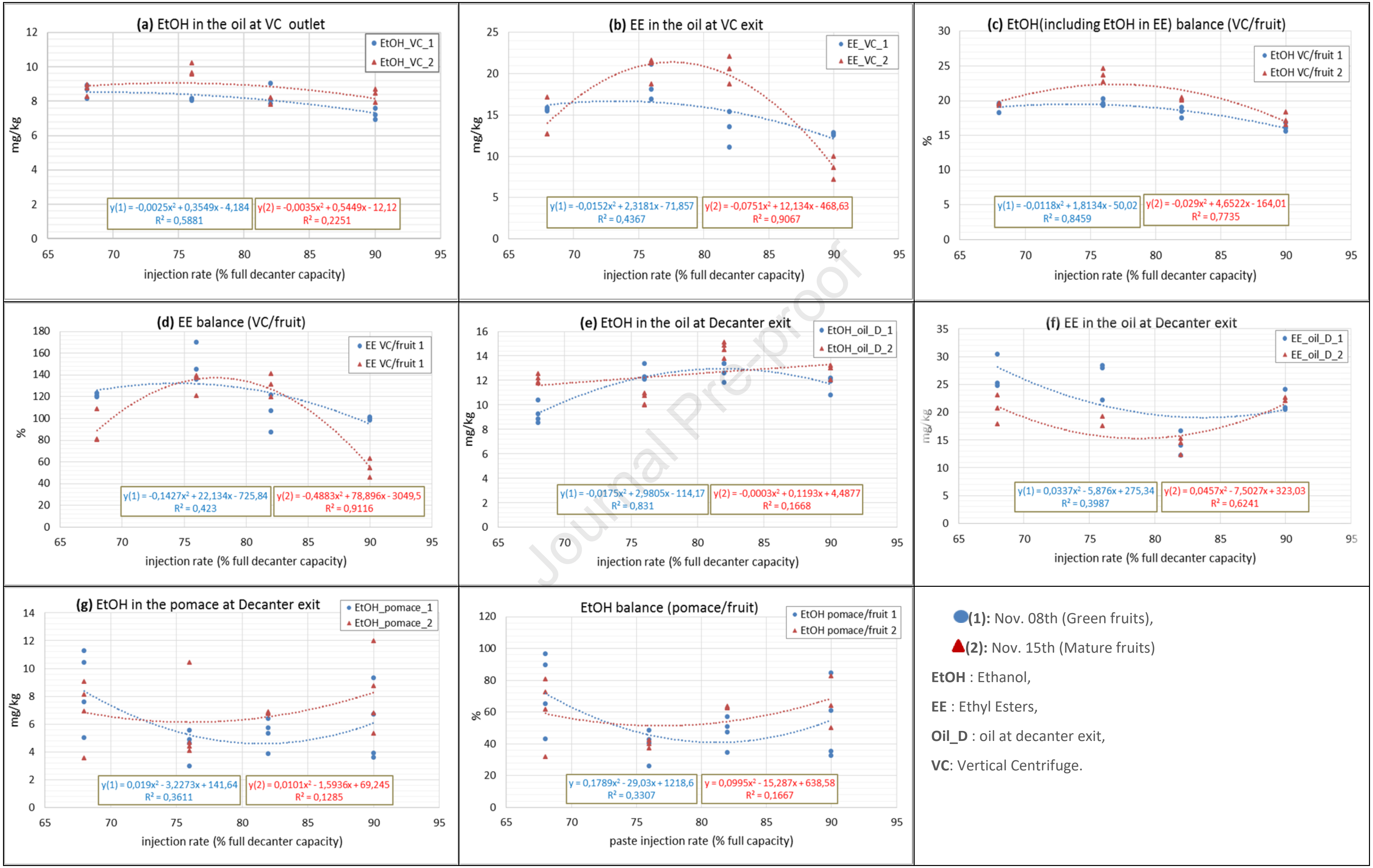
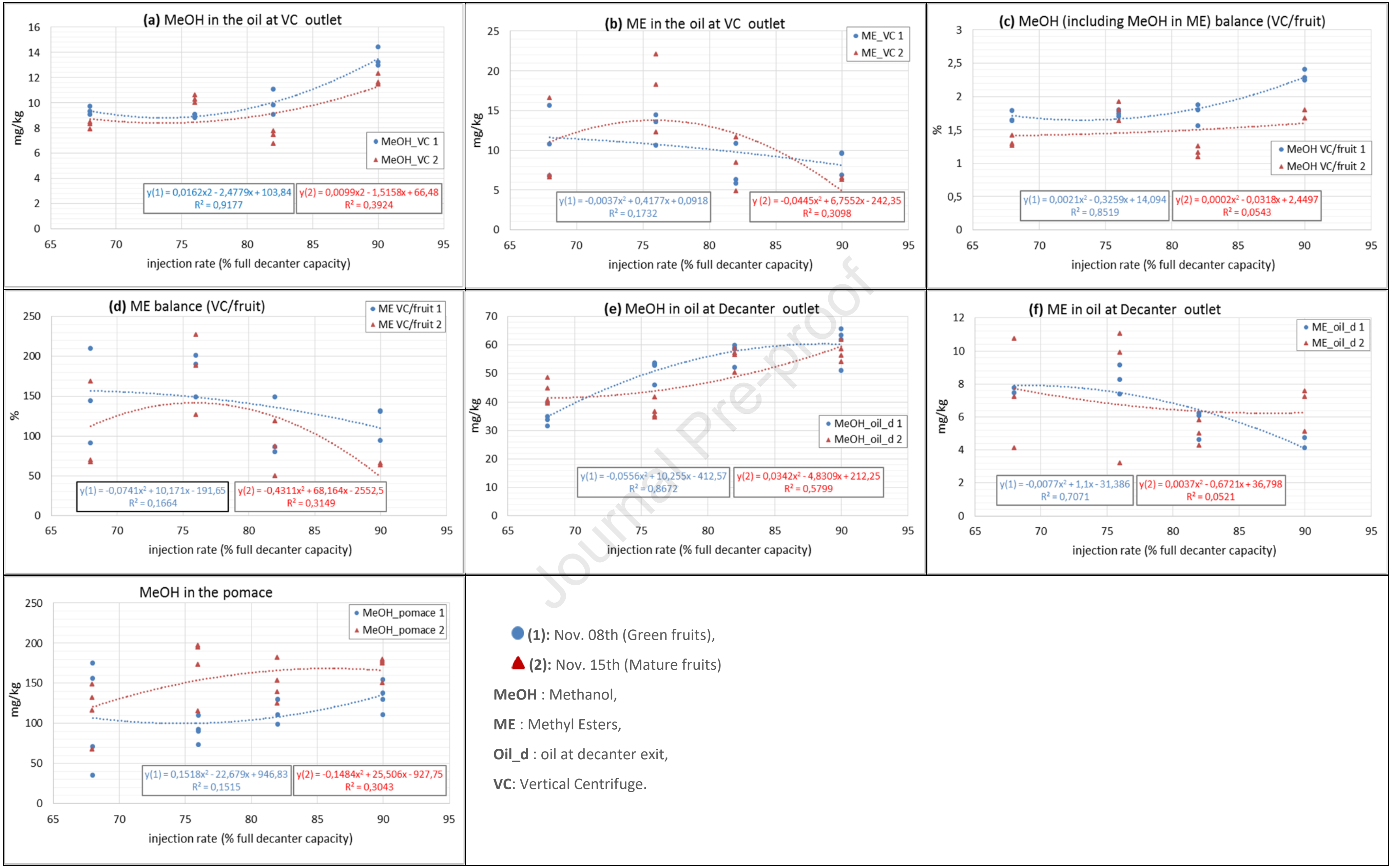

(a) EtOH in the oil at VC outlet
EtOH_VC_1
EtOH_VC_2
mg/kg
0
2
4
6
8
10
12
y(1) = -0,0025x² + 0,3549x - 4,184
R² = 0,5881
y(2) = -0,0035x² + 0,5449x - 12,12
R² = 0,2251
65
70
75
80
85
90
95
injection rate (% full decanter capacity)
(b) EE in the oil at VC exit
EE_VC_1
EE_VC_2
mg/kg
0
5
10
15
20
25
y(1) = -0,0152x² + 2,3181x - 71,857
R² = 0,4367
y(2) = -0,0751x² + 12,134x - 468,63
R² = 0,9067
injection rate (% full decanter capacity)
(c) EtOH(including EtOH in EE) balance (VC/fruit)
EtOH VC/fruit 1
EtOH VC/fruit 2
%
30
y(1) = -0,0118x² + 1,8134x - 50,02
R² = 0,8459
y(2) = -0,029x² + 4,6522x - 164,01
R² = 0,7735
injection rate (% full decanter capacity)
(d) EE balance (VC/fruit)
EE VC/fruit 1
EE VC/fruit 1
%
0
20
40
60
80
100
120
140
160
180
y(1) = -0,1427x² + 22,134x - 725,84
R² = 0,423
y(2) = -0,4883x² + 78,896x - 3049,5
R² = 0,9116
injection rate (% full decanter capacity)
(e) EtOH in the oil at Decanter exit
EtOH_oil_D_1
EtOH_oil_D_2
mg/kg
0
2
4
6
8
10
12
14
16
y(1) = -0,0175x² + 2,9805x - 114,17
R² = 0,831
y(2) = -0,0003x² + 0,1193x + 4,4877
R² = 0,1668
injection rate (% full decanter capacity)
(f) EE in the oil at Decanter exit
EE_oil_D_1
EE_oil_D_2
mg/kg
0
5
10
15
20
25
30
35
y(1) = 0,0337x² - 5,876x + 275,34
R² = 0,3987
y(2) = 0,0457x² - 7,5027x + 323,03
R² = 0,6241
injection rate (% full decanter capacity)
(g) EtOH in the pomace at Decanter exit
EtOH_pomace_1
EtOH_pomace_2
mg/kg
0
2
4
6
8
10
12
14
y(1) = 0,019x² - 3,2273x + 141,64
R² = 0,3611
y(2) = 0,0101x² - 1,5936x + 69,245
R² = 0,1285
injection rate (% full decanter capacity)
EtOH balance (pomace/fruit)
EtOH pomace/fruit 1
EtOH pomace/fruit 2
%
0
20
40
60
80
100
120
y = 0,1789x² - 29,03x + 1218,6
R² = 0,3307
y = 0,0995x² - 15,287x + 638,58
R² = 0,1667
paste injection rate (% full capacity)
●(1): Nov. 08th (Green fruits),
▲(2): Nov. 15th (Mature fruits)
EtOH : Ethanol,
EE : Ethyl Esters,
Oil_D : oil at decanter exit,
VC: Vertical Centrifuge.

Figure 2.



● **(1):** Nov. 08th (Green fruits),

▲ **(2):** Nov. 15th (Mature fruits)

MeOH : Methanol,

ME : Methyl Esters,

Oil_d : oil at decanter exit,

VC: Vertical Centrifuge.

Figure 3.

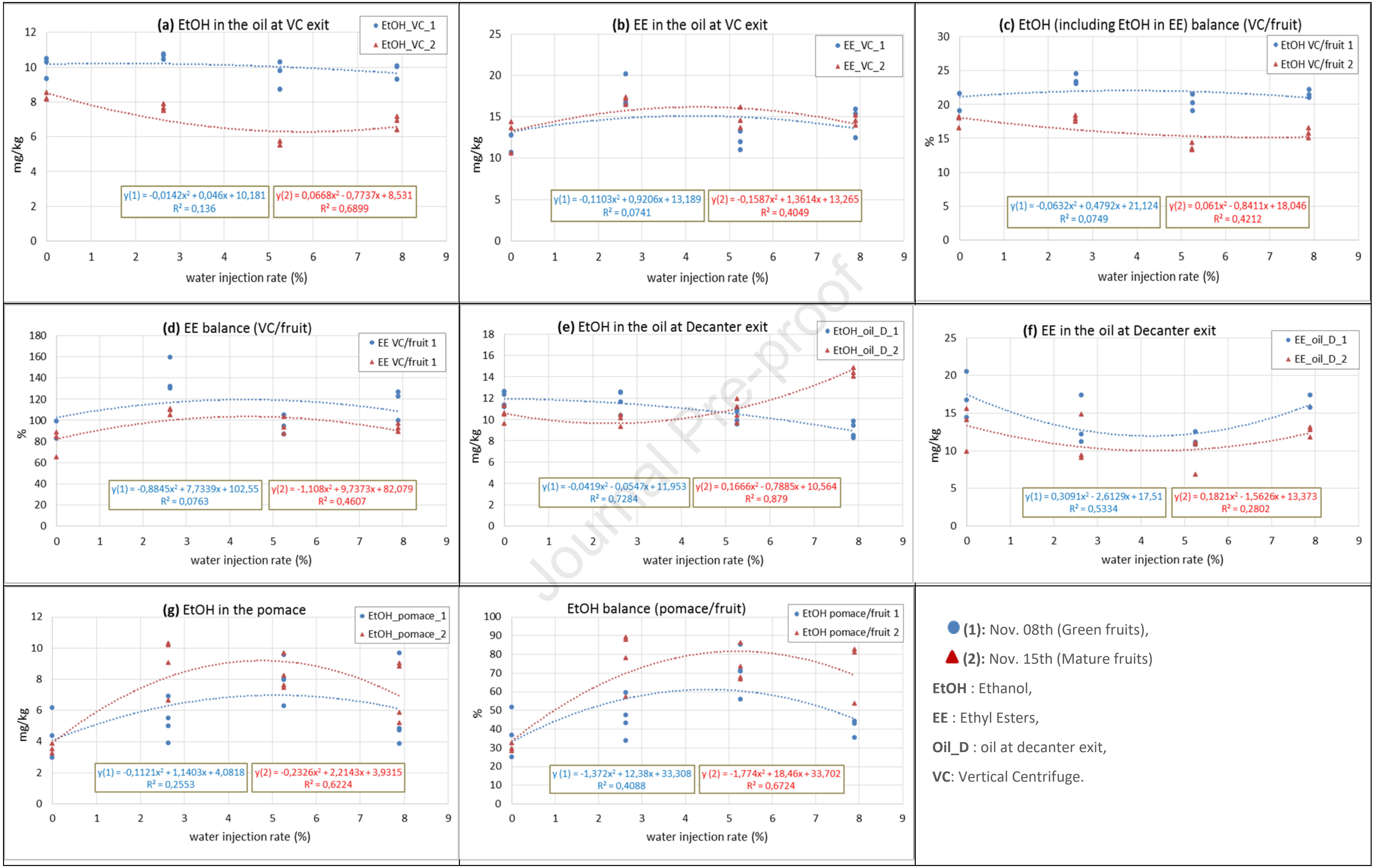
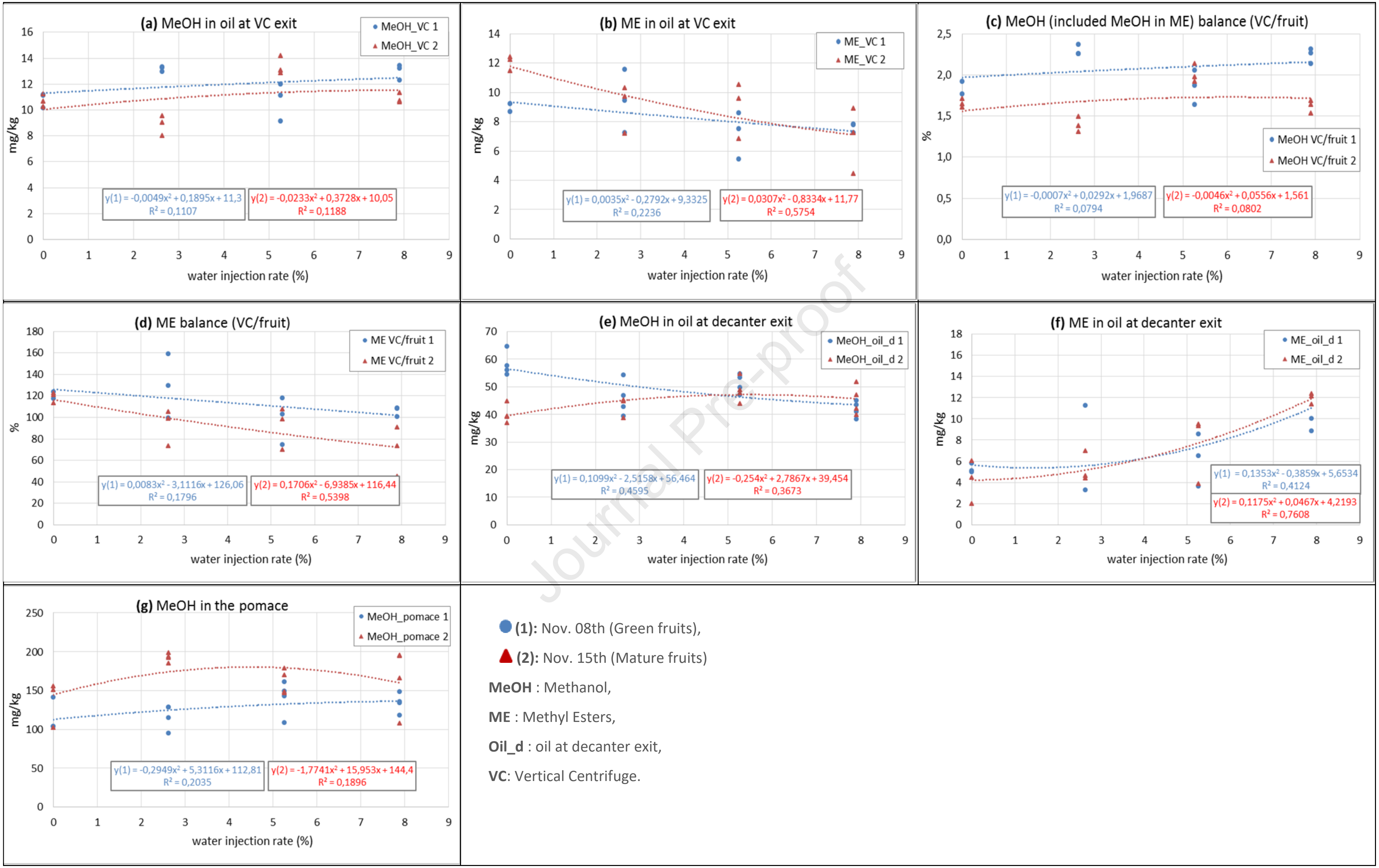

(a) EtOH in the oil at VC exit
EtOH_VC_1
EtOH_VC_2
mg/kg
0
2
4
6
8
10
12
0 1 2 3 4 5 6 7 8 9
water injection rate (%)
y(1) = -0,0142x² + 0,046x + 10,181
R² = 0,136
y(2) = 0,0668x² - 0,7737x + 8,531
R² = 0,6899
(b) EE in the oil at VC exit
EE_VC_1
EE_VC_2
mg/kg
0
5
10
15
20
25
y(1) = -0,1103x² + 0,9206x + 13,189
R² = 0,0741
y(2) = -0,1587x² + 1,3614x + 13,265
R² = 0,4049
(c) EtOH (including EtOH in EE) balance (VC/fruit)
EtOH VC/fruit 1
EtOH VC/fruit 2
%
0
5
10
15
20
25
30
y(1) = -0,0632x² + 0,4792x + 21,124
R² = 0,0749
y(2) = 0,061x² - 0,8411x + 18,046
R² = 0,4212
(d) EE balance (VC/fruit)
EE VC/fruit 1
EE VC/fruit 1
%
0
20
40
60
80
100
120
140
160
180
y(1) = -0,8845x² + 7,7339x + 102,55
R² = 0,0763
y(2) = -1,108x² + 9,7373x + 82,079
R² = 0,4607
(e) EtOH in the oil at Decanter exit
EtOH_oil_D_1
EtOH_oil_D_2
mg/kg
0
2
4
6
8
10
12
14
16
18
y(1) = -0,0419x² - 0,0547x + 11,953
R² = 0,7284
y(2) = 0,1666x² - 0,7885x + 10,564
R² = 0,879
(f) EE in the oil at Decanter exit
EE_oil_D_1
EE_oil_D_2
mg/kg
0
5
10
15
20
25
y(1) = 0,3091x² - 2,6129x + 17,51
R² = 0,5334
y(2) = 0,1821x² - 1,5626x + 13,373
R² = 0,2802
(g) EtOH in the pomace
EtOH_pomace_1
EtOH_pomace_2
mg/kg
0
2
4
6
8
10
12
y(1) = -0,1121x² + 1,1403x + 4,0818
R² = 0,2553
y(2) = -0,2326x² + 2,2143x + 3,9315
R² = 0,6224
EtOH balance (pomace/fruit)
EtOH pomace/fruit 1
EtOH pomace/fruit 2
%
0
10
20
30
40
50
60
70
80
90
100
y (1) = -1,372x² + 12,38x + 33,308
R² = 0,4088
y (2) = -1,774x² + 18,46x + 33,702
R² = 0,6724
● (1): Nov. 08th (Green fruits),
▲ (2): Nov. 15th (Mature fruits)
EtOH : Ethanol,
EE : Ethyl Esters,
Oil_D : oil at decanter exit,
VC: Vertical Centrifuge.

Figure 4.



● **(1):** Nov. 08th (Green fruits),

▲ **(2):** Nov. 15th (Mature fruits)

MeOH : Methanol,

ME : Methyl Esters,

Oil_d : oil at decanter exit,

VC: Vertical Centrifuge.

Figure 5.

HIGHLIGHTS

- Most content of alcohols is removed within the by-products during oil elaboration.
- The olive paste injection rhythm affects the content of FAAEs and of their precursor alcohols.
- The suitable addition of water reduces the risk of FAAEs formation in olive oil.
- The centrifugation steps are key points to controls the formation of FAAEs.

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We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.

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