Research Article

Alkyl esters content and other quality parameters in oil mill: A response surface methodology study

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The quality of the olives oil can be altered during their processing. This paper studies the influence that the washing of olive oils has on their alkyl esters content and on other quality parameters. A Central Composite Rotatable Design (CCRD) with five center points has been used to modify the factors, rotational frequency of the motor of the vertical centrifuge (between 34 and 47 Hz) and percentage of added water (between 6 and 34%). Results show that the content in alkyl esters of olive oils increases by the flow from the decanter to the vertical centrifuge. The use of water in the vertical centrifuge decreases the content in ethyl and methyl esters 0.8 and 0.6 mg/kg, respectively, per percentage unit of added water. The acidity, peroxide index, K_{232}, chlorophyll, and carotenoid contents are slightly altered by the oil flow from the decanter to vertical centrifuge, but not by operating factors. K_{270}, total polyphenols and orthodiphenols change with the speed of the vertical centrifuge, but water does not affect them. For the normal operation frequency of the centrifuge (50 Hz), K_{270} is increased about 14%, while the total polyphenols and orthodiphenols decrease by 27 and 62%, respectively.

Practical applications: Quality control of olive oils (virgin and extra virgin).

Keywords: Ethyl esters / Methyl esters / Oil mill / Response surface methodology / Virgin olive oil

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1 Introduction

It is well-known that Extra Virgin Olive Oil (EVOO) has higher nutritional and sensory quality in comparison to other vegetable oils [1], especially due to the presence of a high ratio mono/polyunsaturated fatty acids and a high antioxidants content (lipophilic and hydrophilic phenolic compounds) [2]. Unfortunately, it is also well-known that probably a great number of olive oils, fraudulently sold as EVOO, are actually illegally mixed with other cheaper olive oils of lower quality and poorer characteristics [3]. The so-called "mildly deodorized" olive oils are nowadays the most used adulterants, because of their difficulty of being detected when added to EVOO [4]. Both the conditions used during processing and the agronomic practices affect the final contents of minor components and quality parameters of the oil, so their control allow the oil mill to obtain virgin olive oils with the required characteristics [5]. Olives contain a complex system of endogenous enzymes that, according to the operating conditions in oil mill, produces olive oils with different content in polyphenols and volatile compounds. The polyphenols and the volatile compounds are responsible of nutritional and sensory characteristics of the virgin olive oil [6].

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Abbreviations: EVOO, extra virgin olive oil; **FAAE,** fatty acids alkyl esters; **FAEE,** fatty acids ethyl esters; **FAME,** fatty acids methyl esters; **FFA,** free fatty acids

The search for analytical methods to demonstrate the quality and safety of food is currently a major challenge in the agrifood field. EVOO is a high quality product that greatly benefits human health. This type of oil, which is produced by subjecting olives to a mechanical process, shows exceptional features of odour and taste. Therefore, the characterization of EVOO by analytical instruments is crucial for the oleic sector in order to prevent fraud [7].

Historically, the regulated quality of oils was determined by the free acidity (degree of hydrolysis of the oils), the peroxide index, and K_{232} (initial level of oxidation) and the K_{270} (level of progress of oxidation) as analytical parameters; and the organoleptic characteristics by panel test (European Commission Regulation, Nos. 1989/2003 and 702/2007). All these parameters enabled the classification of oils by their quality. The European Commission Regulation, No 61/2011, added a new quality parameter for extra virgin olive oils: the content of methyl and ethyl esters. Fatty acid alkyl esters (FAAE) are a family of natural neutral lipids present in olive oils and formed by esterification of free fatty acids (FFA) with low molecular alcohols. Inappropriate practices during the olive oil extraction process and bad quality of the olive fruits promote their formation [3]. A high content of these compounds in the oils indicates that these have been obtained from deteriorated olives by excess of maturation in the case of methyl esters, or by fermentation of organic matter in the case of ethyl esters. The ethanol content of the olives increases during the ripening on the olive-tree, mainly in Hojiblanca variety cultivars [8]. Extra virgin olive oils contain low amounts of fatty acid methyl and ethyl esters, while oils obtained from altered olive or olive pomace show high concentrations of fatty acid alkyl esters, mainly ethyl esters [9].

Given the controversy among the different producer countries about the maximum content of the alkyl esters allowed in virgin olive oils, during 2013 there were some legislative changes which eliminated methyl esters as quality criterion and decreased the maximum content in ethyl esters for extra virgin olive oils (European Commission Regulation, Nos. 299/2013 and 1348/2013). Specifically, this regulation establishes a 30 mg/kg limit to olive oils produced from 2015/2016 olive-harvesting season onwards, facing to the 35 and 40 mg/kg limit established for 2014/2015 and 2013/2014, respectively. These reductions are a problem for the olive oil producers since some extra virgin olive oils could be classified as nonextra virgin [10].

As innovative aspect in the study of the olive oil production process, the main aim of this paper is to determine the influence of the vertical centrifuge operation factors (rotation speed and amount of added water) on the content of ethyl and methyl esters and on other quality parameters of the obtained oils.

Focusing on the last stage of the virgin olive oil production process, clarification/washing in vertical centrifuge, the oil is separated from any remaining water and olive residue [11].

Until now, there has been little research focused on the influence of vertical centrifuge in the olive oils quality. In this stage of the process, a strong interaction oil–air is produced in vertical centrifuge, which entails an increase in the concentration of oxygen in the oil [12, 13]. This causes a raise in peroxide index and K_{23}, and a significant decline of the content in volatile aromatic compounds, which could be minimized using inert atmosphere [14].

Considering the polyphenols content in olive oils, both Di Giovacchino et al. [15] and Salvador et al. [16] indicate their oil-soluble and water-soluble character, and that the concentration at one phase or another will depend on the partition coefficient between them and, therefore, on the operation temperature. According to the extraction system used, the oils with higher polyphenols content are those obtained by the two phases system, due to the fact that no water is used. Depending on the olives quality and their maturity index, polyphenols are more soluble in water or in oil. Thus, in the case of both olive and olive oils of high quality, polyphenols are more soluble in oil than in water; on the other hand, in olives of poor quality, polyphenols are more soluble in water [15].

To develop the study ethyl esters content in the olive oil, it is used a continuous line of extraction and an experimental design that fixed the vertical centrifuge operating factors, for each essay.

2 Materials and methods

2.1 Experimental design

Response surface methodology was employed in order to study the influence of washing on the ethyl and methyl ester content and on other quality parameters of olive oil. For this purpose, a central composite experimental design, with five center points, has been performed, using Design-Expert 8.0.7.1, Stat-Ease, Inc, Minneapolis, USA. This design is rotatable when axial points satisfying the condition that $\alpha = 1.414$. The chosen experimental factors were the percentage of added water to the centrifuge and its rotation speed. Table 1 shows the experimental design performed with real and coded factors, and the random order of execution of the trials necessary to minimize experimental and bias errors. The number of revolutions of the centrifuge has been modified, within the margins of safety, by changing the rotational frequency of the motor driving the rotor body, between 33 (coded factor -1) and 47 Hz (coded factor $+1$). The factor-added water has changed between 6 and 34% (coded factors -1 and $+1$, respectively) of oil supplied to the centrifuge with a digital flow meter. To the five central points of the design of Table 1 (coded factor $= 0$), vertical centrifuge

Table 1. Experimental design with real and coded factors, and oil temperature

	Frequency (Hz)		Water (%)		Temperature (°C)	
Run	Coded	Real	Coded	Real	Decanter	Vertical centrifuge
1	0	40	$+\alpha$	39.8	35.1	34.5
2	+1	47	+1	34	36.0	35.7
3	0	40	0	20	38.3	37.3
4	$+\alpha$	49.9	0	20	38.1	37.4
5	$-\alpha$	30.1	0	20	37.6	36.0
6	0	40	0	20	38.3	36.7
7	+1	47	−1	6	38.2	38.3
8	0	40	0	20	38.2	37.0
9	0	40	$-\alpha$	0.2	37.4	39.1
10	0	40	0	20	38.2	36.8
11	−1	33	−1	6	37.8	37.8
12	−1	33	+1	34	39.5	36.0
13	0	40	0	20	37.8	37.2

maintained a rotating speed of 6130 rpm (frequency 40 Hz) and the addition of water was 4.3 L/min (20%).

The response surface methodology was applied to study the influence of these factors in the analytical parameters of oils (ethyl and methyl esters, free acidity, peroxide index, K_{232} and K_{270}, chlorophyll and carotenoid contents, total polyphenols, and orthodiphenols contents). The quadratic model has been used to adjust the responses to the factors, Eq. (1).

$$R = a_0 + a_1 \cdot A + a_2 \cdot B + a_{12} \cdot \mathrm{AB} + a_{11} \cdot A^2 + a_{22} \cdot B^2 \pm \varepsilon \quad (1)$$

where R is the response studied, A the rotational frequency of the motor, B the percentage of added water, and ε the error made by the model in reproducing the experimental results (standard deviation).

As seen in the Eq. (1), with this model and experimental methodology it is possible to determine the influence that each factor has on the different responses and possible interactions that may exist between them, that is, the possible influence that one factor may have on the other's action if their real value is higher or lower.

2.2 Raw material

The study was developed with olives, variety Picual, from the municipality of Écija. By the date of realization (late December), the quality of the raw material was low, so the oil obtained was also poor in terms of quality. Throughout the process, about 72 000 kg of olives from different provenances and very heterogeneous characteristics were milled, as evidenced below. Given the heterogeneity of the raw material, the approximate average moisture, oil and total solids content was 48, 28, and 24%, respectively. Although these data were not important for the research to be performed, they were important to set the rate of milling since the oil flow supplied to the vertical centrifuge and the added water to the decanter were fixed, because the olives were very dry.

2.3 Industrial process

The trials were performed in an exclusive line of the oil mill "Molino del Genil," located at Écija, Sevilla (Spain). The line has a nominal milling capacity of 250 000 kg/day, but it adjusted so that the oil supply to the vertical centrifuge was 1300 L/h, fixed and constant by adjusting the rotational speed of the volumetric pump. The olive paste supply into the decanter was set at 6000 kg/h, to which the 10% water was added to facilitate the operation.

A horizontal mixer with five chambers was used with a capacity to maintain olive paste beating up to 120 min. The full line is brand Westfalia, the separating decanter and vertical centrifuge correspond to the OSD 50-02-007 and RCD 539-08-34 models, respectively.

It should be noted that, according to the real factors of Table 1, some trials should be performed with high water flows. Thus, initially, a flowmeter with a control range between 0 and 50 L/min and precision of 0.5 L/min was placed in the water inlet line. The flowmeter allowed control a minimum flow of 5 L/min, so it was necessary to place another with which to perform other trials with a lower flow. We used one that was similar to the other used before but with a flow control range from 0 to 4 L/min and precision of 0.05 L/min, which, for the safety margin, also enable the control of the flows of the central points. Considering the two flowmeters and programming of trials, it was decided to perform the trials 1, 2, and 12 with the flowmeter of higher range and the rest of the trials with the other. The use of two flowmeters with different control range and precision increases the experimental error of the design, which will be considered, with the rest of errors, in the standard deviation of the models considered for each response.

The trials of Table 1 were performed sequentially and following a method which assure the uniformity of treatment of the samples; so that only the factors studied were the cause of any change in analytical parameters. The operation mode was as follows: once fixed the operation factors, it was expected to centrifuge self-cleaning and after 10 min, reached steady state, oil samples were taken at the decanter way out and at the vertical centrifuge way out. For the following trial, the factors were fixed and the process continued as described previously. At the exit of both operations, samples of about 500 mL of oil were taken, which were immediately bottled in dark glass bottles and were closed hermetically. Then, they were labeled and were kept in a box for shipping; a total of 26 bottles of 500 mL. Simultaneously, the oil temperature was

taken at both locations using a double-beam laser thermometer (Table 1). In total, 12 h were needed to complete the trials because it was difficult to operate on the vertical centrifuge, due to changing operating factors. In the case of water flow change, it was necessary to wait to reach the regime of work with the new flow, but to change the rotating speed was more complex because this affected work security. Therefore, in some cases, we were forced to stop the vertical centrifuge and to start with the new frequency.

The temperatures of the oils in Table 1 are very high because the raw material (olives) was of low quality and, to increase industrial performance, the pastes were heated to 40°C in the mixer. Temperature variation between the decanter and centrifuge can be explained by the fact that the washing water was cold and it cooled the oils, depending on the percentage of added water. Temperature variation at the decanter way out is due to the oscillations in its operation.

The oils were stored in a refrigerator and the next day, in the laboratory, they were filtered with a special paper filter. Nitrogen was added to the bottles with filtered oils and kept in freezer until their analysis.

2.4 Analysis of fatty acids alkyl esters (FAAE)

Fatty acids alkyl esters were determined by gas chromatography (GC-FID) according to European Commission Regulation [17]. The analysis of esters and waxes was performed on a 7890A Agilent Technologies gas Chromatograph System equipped with a flame ionization detector (FID). The column used was a capillary HP-5 (length 30 m, id 0.32 mm, and film thickness 0.25 μm). The operating conditions were as follows: oven temperature, 80°C for 1 min and then increased from 20°C/min to 140°C, then increased from 5°C to 335°C and maintained for 20 min; injector was programmed from 70°C to 300°C; detector temperature was 350°C. Helium was used as the carrier gas, with a flow through the column of 1 mL/min and 1:50 split ratio.

2.5 Analysis of quality parameters

The quality parameters of the olive oils, i.e., free acidity, peroxide index, and the determination of UV spectrophotometric indices (K_{232}, K_{270}), were determined from the extracted oils according to the European Union standard methods Regulation [18]. Free acidity, given as % of oleic acid, was determined by titration of a solution of oil dissolved in ethanol–ether (1:1) with ethanolic potash. Peroxide index (mEq O_2/kg), was determined as follows: a mixture of 1.5 g of oil and 25 mL of chloroform–acetic acid (10:15 v/v), was left to react with 1 mL of saturated solution of potassium iodide in darkness. The free iodine was then titrated with a sodium thiosulfate solution (0.1 N). The UV absorbance were measured at two wavelengths (232 and 270), using a 1% solution of oil in spectrophotometric grade cyclohexane and a quartz cuvette. The equipment used was a Shimadzu double-beam UV-VIS Spectrophotometer UV-1800.

2.6 Analysis of pigments, total polyphenols, and orthodiphenols

Carotenoids and chlorophylls (mg/kg of oil) were determined at 470 and 670 nm, respectively, in cyclohexane using the specific extinction values according to the method of Mínguez et al. [19].

Polyphenols were determined according to the methodology proposed by Vázquez et al. [20]. Olive oil samples were dissolved in *n*-hexane and extracted, three times, with a water/methanol mixture (60:40 v/v). Total polyphenols were determined by adding the Folin–Ciocalteau reagent and a 20% sodium carbonate solution to a suitable aliquot of the combined extracts. After an hour in the darkness, the absorbance at 725 nm was measured. Results were given as mg/kg of caffeic acid. Orthodiphenols were determined by adding of sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$) and a suitable aliquot of the combined extracts. After 15 min in the darkness, the absorbance at 375 nm was measured.

In all cases, the spectrophotometer Shimadzu UV-1800 has been used.

3 Results and discussion

Table 2A and B shows the responses obtained by oils analysis at the decanter way out and Table 3A and B shows the responses of oils analysis at the vertical centrifuge way out. The first column on Tables 1–3, indicates the random order of execution of the tests and related the operating conditions to the analysis of oils. Given the high oil flow rate to the vertical centrifuge, the sampling is performed sequentially and it is considered that these samples correspond to oils from the same olives. As previously stated, the line has a nominal milling capacity of 250 000 kg/day. About 72 000 kg of olives were milled, from different provenance and conditions, so the variability was high, as it can be seen in the analytical values in Table 2A and B.

The REGULATION (EU) No 61/2011 permits a concentration of FAAE between 75 and 150 mg/kg if the ratio of FAEE and FAME is lower or equal to 1.50. The FAME are typically formed by the technological transformation of overripe olive fruits [21]. In good quality EVOO, FAME and FAEE are present in very small amounts, yet they are present in higher amounts in lampante olive oils [22] and in oils from the second centrifugation, also called "repaso" [23]. As it can be seen in Table 4, the results do not exceed the quantity of 150 mg/kg and the ratio is lower or equal to 1.50, but samples cannot be considered EVOO because the others quality parameters are out of the established limits.

Table 2. Olive oil responses at decanter way out

			A		
Run	Ethyl esters (mg/kg)	Methyl esters (mg/kg)	Acidity (%)	Peroxide index (mEq O_2/kg)	K_{232}
1	85	55	2.48	5.60	1.34
2	77	64	2.44	5.73	1.34
3	37	31	1.70	8.43	1.42
4	39	30	1.64	7.58	1.46
5	34	31	1.58	6.66	1.42
6	42	33	1.56	7.58	1.37
7	31	27	1.54	6.51	1.42
8	38	31	1.53	6.73	1.40
9	48	38	1.55	6.87	1.46
10	45	35	1.53	6.83	1.42
11	32	22	1.56	6.92	1.38
12	78	62	2.62	6.28	1.43
13	24	19	1.57	5.85	1.42
			B		
Run	K_{270}	Chlorophylls (mg/kg)	Carotenoids (mg/kg)	T. polyphenols (mg/kg)	Orthodiphenols (mg/kg)
1	0.147	37.4	15.1	186.3	133.7
2	0.137	30.7	13.2	327.8	181.5
3	0.171	18.7	10.3	297.5	197.0
4	0.177	20.1	9.1	346.1	144.8
5	0.165	20.1	9.0	372.2	164.1
6	0.159	18.7	8.3	313.0	131.4
7	0.161	21.8	9.6	338.9	135.8
8	0.154	21.7	9.8	359.3	216.4
9	0.173	22.2	9.9	349.8	118.6
10	0.168	20.5	9.2	325.0	170.7
11	0.161	20.5	9.2	267.9	94.5
12	0.194	21.1	12.9	167.6	55.1
13	0.167	20.5	9.1	282.4	155.4

Figure 1 shows the content of methyl and ethyl esters of oils at the decanter way out, and it can be seen that there is a great variability of milled olives. The average value of all samples is showed in dashed line.

After analyzing the oils and given the variability of milled olives, it was necessary to find a way to minimize the influence of raw material on the analyses of oils samples, so only the action of vertical centrifuge was considered on the variations between Tables 2A and 3A and between 2B and 3B. The differences between Tables 3A and 2A, and 3B and 2B were calculated and adjusted to Eq. (1), as the value of the vertical centrifuge response minus the value of the decanter response.

The response data were processed into the Design-Expert 8.0.7.1 data analysis software and the models shown in Table 5A and B was obtained, for real factors. The models for the differences of ethyl and methyl esters, between oils taken from the vertical centrifuge and oils taken from the decanter, were significant and the coefficient of determination (R^2) was acceptable given the high variability of the samples. For the rest of the responses studied, only the models of total polyphenols, orthodiphenols and K_{270} were significant. For acidity, peroxide index, K_{232}, chlorophylls and carotenoids, the models have not been significant (Table 5A and B). The standard deviations of the differences are relatively large, but as the absolute values of responses are (in some cases) high, its value is not modified significantly.

The statistical parameters R^2, *P*-value, Lack of Fit, and Coefficient of Variation, have been included in Table 5A and B to evaluate the goodness of the models. R^2, multiplied by a hundred, represents the percentage of the response variability explained by the model. In this case, the obtained values of R^2 (from 0.633 to 0.789) indicate that all the models could be considered good, as [24] indicate, which is confirmed by the other statistics included in Table 5A and B.

By definition, *P*-value model indicates the probability that the model provides an erroneous value with respect to the actual responses (a *P*-value less than 0.05 indicates that model is significant, so there is a 5% chance of getting an erroneous value). Regarding the Lack of Fit, tabulated values indicate that there is no lack of fit, so that the models

Table 3. Olive oil responses at vertical centrifuge way out

			A		
Run	Ethyl esters (mg/kg)	Methyl esters (mg/kg)	Acidity (%)	Peroxide index (mEq O_2/kg)	K_{232}
1	64	45	2.38	6.76	1.38
2	70	51	2.41	6.04	1.37
3	47	32	1.70	7.22	1.40
4	40	28	1.63	6.29	1.38
5	26	25	1.57	6.36	1.40
6	35	31	1.54	6.07	1.46
7	43	33	1.53	5.66	1.45
8	37	30	1.50	5.34	1.41
9	38	39	1.53	5.19	1.44
10	24	23	1.53	6.26	1.50
11	42	34	1.57	6.70	1.38
12	71	58	2.60	5.56	1.37
13	27	22	1.54	5.51	1.35
			B		
Run	K_{270}	Chlorophylls (mg/kg)	Carotenoids (mg/kg)	T. polyphenols (mg/kg)	Orthodiphenols (mg/kg)
1	0.161	35.2	16.3	206.2	110.0
2	0.166	24.9	13.1	219.9	144.8
3	0.162	19.6	10.2	260.6	144.2
4	0.154	19.9	10.3	284.0	155.2
5	0.157	19.5	10.2	299.7	220.6
6	0.169	19.1	10.0	339.6	208.1
7	0.170	18.5	9.8	296.3	268.7
8	0.165	19.4	10.0	363.7	171.0
9	0.173	20.0	10.5	301.9	111.8
10	0.159	19.1	10.0	269.5	260.0
11	0.157	19.0	10.1	306.8	142.0
12	0.184	20.9	10.3	147.1	107.3
13	0.162	20.5	10.5	322.9	133.9

Table 4. Sum of FAME and FAEE and ratio of FAEE/FAME

Run	$\sum$ FAME + FAEE (decanter)	FAEE/FAME (decanter)	$\sum$ FAME + FAEE (vertical centrifuge)	FAEE/FAME (vertical centrifuge)
1	140	1.5	109	1.4
2	141	1.2	121	1.4
3	68	1.2	79	1.5
4	69	1.3	68	1.4
5	65	1.1	51	1.0
6	75	1.3	66	1.1
7	58	1.1	76	1.3
8	69	1.2	67	1.2
9	86	1.3	77	1.0
10	80	1.3	47	1.0
11	54	1.5	76	1.2
12	140	1.3	129	1.2
13	43	1.3	49	1.2

adequately describe the relationship between factors and responses. Coefficient of Variation for a response is the error expressed as a percentage of the mean value for all oils of the decanter. Terms a_{12}, a_{11}, and a_{22} (Eq. 1) are not shown in Table 5 because they are not significant in models (P-value > 0.05).

In Table 5A it can be seen that the only factor that influences on the content of ethyl and methyl esters in oils is the percentage of added water to the vertical centrifuge; while the rotation speed is not significantly influenced. In Fig. 2, it can be appreciated there is a decreasing linear variation of the difference of ethyl esters between oils taken from the vertical centrifuge and oils taken from the decanter, with the increase in added water to the vertical centrifuge. Besides, the rotation speed does not affect the content of esters in the oils. For methyl esters content, a similar figure is obtained, according to the data in Table 5A. The variation in the content in ethyl esters of the oils at the vertical centrifuge way out can be calculated considering the model which response surface is shown in Fig. 2 and assuming that all the oils have the same content in ethyl esters at the decanter way out (46.9 mg/kg,

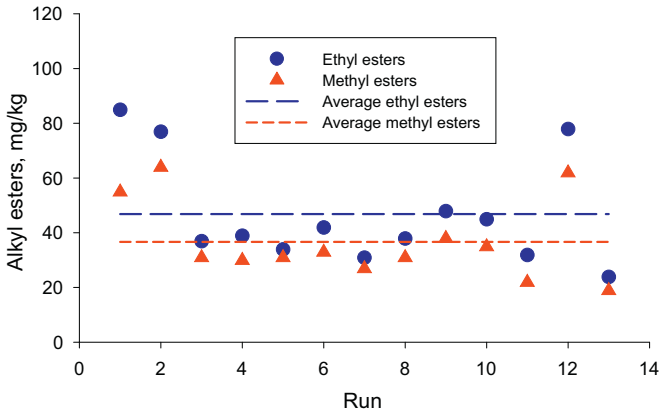

Figure 1. Methyl and ethyl esters content in oils at the decanter way out.

dashed line in Fig. 1). Thus, for values of 6, 20, and 34% of water added, the oils contain 56.7, 45.9, and 35.2 mg/kg of ethyl esters, respectively; values subject to the standard deviation of the model (4.72 mg/kg, Table 5A).

According to the models of Table 5A, the values of the independent terms indicated how responses change when oils flow from one machine to the other. If no water is used, the concentration of ethyl esters would increase around 14 mg/kg by the flow from the decanter way out to vertical centrifuge way out, points where sample gathering is performed. Similarly, the increase of the methyl esters was 11 mg/kg. This variation, in principle, should not occur because oils only flow through the sieve cleaning of the exit of decanter and by the vertical centrifuge.

Given the high flow rates used, the alteration should occur very quickly. The fermentation of organic matter is slow, so oils must be altered by being in contact with fermented organic matter and it must be in the path from the exit of decanter to the exit of vertical centrifuge. Inside the vertical centrifuge, depending of volume and oil-water flow, oils remain between 20 and 30 s, but it should not have organic matter because the vertical centrifuge is self-cleaning and this is performed periodically. The time it takes to pass the oil by the pump and the hose is insignificant, although there could be organic matter if there are dead spaces. The place with a higher alteration possibility is the cleaning sieve and the oil reception tank which is under that. According to his capacity, oils remain there between 120 and 150 s. If the tank and cleaning sieve are not cleaned regularly, filth (organic matter) can be fermented and alter the oils, as predicted by the models in Table 5A. Therefore, all process equipment should be cleaned regularly to get quality virgin oils.

On the other hand, according to the coefficients of the obtained models for percentage of added water, it can be stated that for each percentage unit of added water, the content in ethyl and methyl esters was reduced 0.8 and 0.6 mg/kg, respectively.

Table 5. Model fit of Eq. (1)

			A		
Coefficient	Ethyl esters	Methyl esters	Acidity	Peroxide index	K_{232}
a_0	14.33	11.15	−0.015	−0.91	0.007
a_1	–	–	–	–	–
a_2	−0.77	−0.58	–	–	–
ε	4.72	3.59	0.010	0.52	0.049
R^2	0.789	0.767	–	–	–
P-value model	0.0006	0.0004	–	–	–
Lack of Fit	0.633	0.170	0.896	0.505	0.937
Coefficient of Variation	10.06	9.75	0.55	7.75	3.46
			B		
Coefficient	K_{270}	Chlorophylls	Carotenoids	Total polyphenols	Orthodiphenols
a_0	−0.063	−1.06	0.76	302.6	277.6
a_1	0.002	–	–	−7.7	−7.4
a_2	–	–	–	–	–
ε	0.008	1.28	0.60	31.2	23.2
R^2	0.633	–	–	0.678	0.756
P-value model	0.006	–	–	0.012	0.002
Lack of Fit	0.710	0.575	0.818	0.585	0.319
Coefficient of Variation	4.75	5.66	5.80	10.31	15.85

Real factors of Eq. (1).
Adjustment of values in Table 3 minus values in Table 2 (vertical centrifuge oils minus decanter oils).
–, term statistically non-significant (*P*-value > 0.05).

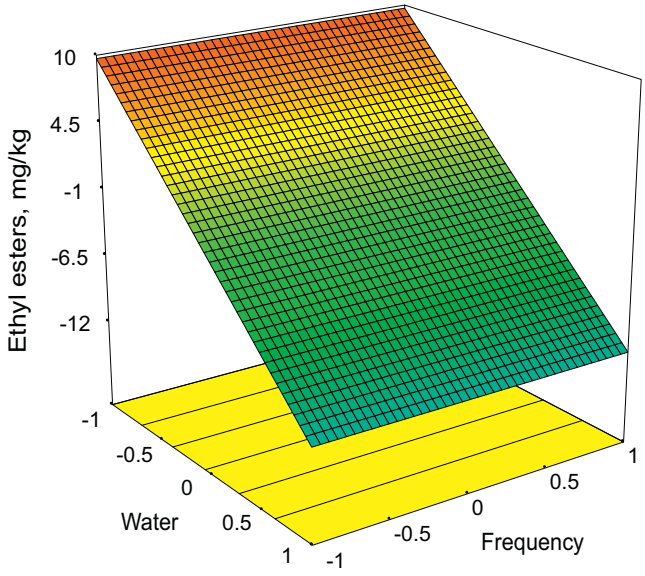

Figure 2. Variation of the difference of ethyl esters, between oils from the vertical centrifuge and from the decanter with the coded factors.

The content in ethyl esters can only be increased by the presence of FFA and ethanol in oils. The former are generated by the hydrolysis reaction between triglycerides and water, and the latter by fermentation of organic matter. Therefore, it is advisable to remove the water and organic matter present in the oils by filtering them in the shortest time from the moment they are obtained and their subsequent decanting. Gómez-Coca et al. [25] show a protocol for the determination of methanol and ethanol in oils, although, as fermentation products and reagents for forming FAAE, its content can be quite variable over time. This implies that it is necessary to implement good working practices that minimize their formation [10], as filter the oils and control the temperature.

For some parameters studied, for example, acidity, peroxide index, K_{232}, chlorophylls, and carotenoids, non-significant models have been obtained. This means that these parameters have not been influenced by the rotational speed of the vertical centrifuge and the added water. By contrast, mean value and its standard deviation indicate a slight variation by the flow from one machine to the other, as well, acidity, peroxides index, and chlorophylls content decrease slightly, while K_{232} is not altered and carotenoids content increase. It must be consider the significant decrease of the peroxides index, 14% of the average value of all oil samples of the decanter, which can be related to the increase of K_{270}.

In Table 5B, it can be seen that K_{270} slightly increases with frequency and the added water does not affect it. The variation of K_{270} can be justified by increased agitation experienced by oils inside the vertical centrifuge, due to the spin speed, that promotes the degradation of peroxides and formation of degradation compounds. In the same vein, [12] and [13] determined the oils oxidation by the increase in the peroxide index and K_{232}.

Considering the model and that the frequency of light in Spain is 50 Hz, K_{270} increases 0.023 units by effect of vertical centrifuge, approximately 14% of the mean value of K_{270} in oils of the decanter.

In Fig. 3 and Table 5B, it can be seen that the content of total polyphenols decreases with the rotational frequency because matter transfer controls the step, of these compounds, of the oil phase to the water. According to the data in Table 5B, the behavior for orthodiphenols content is similar to this. It follows from the above that the contact water-oil favor the mass-transfer between phases, until solubility equilibrium or partition coefficient is reached at the operating temperature, as [15] and [16] indicate. Achieving this balance depends on the mass-transfer rate and on the contact time between phases. When the agitation is greater, the contact between phases is better and the mass-transfer rate is greater. The equilibrium will not reach if the contact time between phases is insufficient and, in this case, the amount of water added will not be a significant factor. Table 5B shows that, for the performed essays, the amount of added water does not influence the process because the solubility limit is not reached, since the time contact between water-oil in the vertical centrifuge is very short.

Considering, as in K_{270}, the frequency of light, it can be indicated that the total polyphenol content decreases about 80 mg/kg and to orthodiphenols 91 mg/kg, 27 and 62% of phenols present in oils of the decanter, respectively. It is due to washing of oils in the vertical centrifuge.

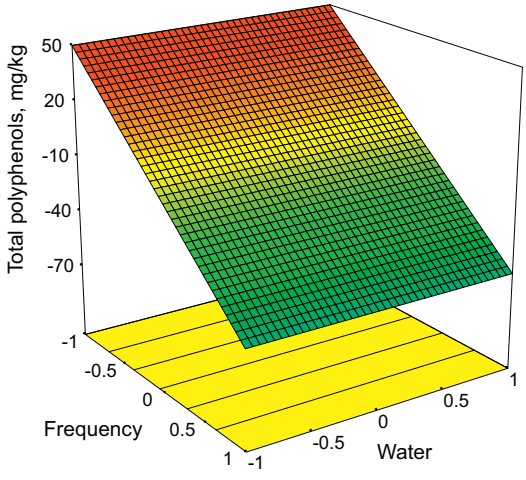

Figure 3. Variation of the difference of total polyphenols, between oils from the vertical centrifuge and from the decanter with the coded factors.

In order to obtain olive oil of higher quality, it is advisable to clean the process line periodically and to filter the oils in the shortest possible time from the moment when they are obtained.

Further works should be focused on the study the variation in the quality of olive oil during the storage, basically, by determining the variation in the content of ethanol, obtained by fermenting organic matter, ethyl esters, and other quality parameters.

4 Conclusions

According to the developed study, it can be noted that:

(1) All analyzed oils are of low quality, due to their high esters content and high acidity.
(2) The content of ethyl esters in olive oils increases by the flow from the decanter to the vertical centrifuge.
(3) The rise of the content of ethyl esters is mainly due to the lack of cleanliness of both the exit sieve of the decanter and the oil reception tank.
(4) The use of water in the vertical centrifuge decreases the content in ethyl esters at a rate of 0.8 mg/kg per each percentage point of water added.
(5) The use of water in the vertical centrifuge decreases the content in methyl esters at a rate of 0.6 mg/kg per each percentage point of water added.
(6) Acidity, peroxide index, K_{232}, chlorophylls, and carotenoids have slight variations by the flow from the decanter to vertical centrifuge. It has been determined that there is not significant influence by the rotational speed of the vertical centrifuge and the added water.
(7) K_{270} increases slightly with the speed of the vertical centrifuge, but the added water does not affect it. For normal operation of the centrifuge to the frequency of 50 Hz, K_{270} is increased 0.023 units, about 14% of the mean value of this parameter in oils of the decanter.
(8) In regard to total polyphenols and orthodiphenols, the content of these compounds decreases when the speed of the vertical centrifuge increases. For normal operation of the centrifuge to the frequency of 50 Hz, the total polyphenols and orthodiphenols decrease by 27 and 62%, respectively.

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The authors have declared no conflicts of interest.

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