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Alkyl Esters of Fatty Acids a Useful Tool to Detect Soft Deodorized Olive Oils

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Fatty acid alkyl esters (FAAEs) are a family of natural neutral lipids present in olive oils and formed by esterification of free fatty acids (FFAs) with low molecular alcohols. Inappropriate practices during the olive oil extraction process and bad quality of the olive fruits promote their formation. Quantification can be done by isolation with a silica gel solid phase extraction cartridge followed by analysis on a gas chromatograph equipped with a programmed temperature vaporizer injector using a polar capillary column. The application of the method to more than 100 Spanish olive oils from different categories, varieties, and geographical origin allowed for establishing the average content of FAAEs and distinguishing the Spanish protected denomination of origin (PDO) and extra virgin olive oils from other categories of olive oils. Those other categories of oils can be subjected to a mild refining process, which leads to blending with extra virgin olive oils. Studies on low quality oils subjected to mild refining showed that FAAEs remain after that process. Thereby, blends of extra virgin olive and mildly refined low quality olive oils can be detected by their alkyl ester concentrations.

KEYWORDS: Fatty acid alkyl ester; virgin olive oil; mild refining

INTRODUCTION

Olive oils are characterized by more than 10 analytical parameters included in several official international regulations (*1, 2*). Only those oils, which fulfill all the requirements, can be classified as extra virgin olive oils. Good manufacturing practices in the olive oil extraction process and good quality of the fruits are essential conditions to produce high quality olive oils. Nevertheless, the obtained oils do not always present high quality; moreover, they could have some unpleasant organoleptic characteristics related mainly to the quality of the olive fruit, resulting in a low quality olive oil. This implies a decrease in their market value and consequently significant economic loss. On the other hand, some oils, which do not have organoleptic defects but their attributes are not balanced as occurs in the case of oils with strong bitter or pungent taste, are not accepted by the consumers. In order to overcome these drawbacks, a forbidden practice (mild refining) is used. Low quality olive oils with weak organoleptic defects, moderate free acidity, or strong bitter or pungent taste are subjected to a mild refining process consisting of neutralization and/or soft deodorization at a low temperature. The resulting oils are blended with genuine extra virgin olive oils circumventing the methods currently included in the regulations and commercialized as extra virgin category. Great effort has been made to determine if an olive oil has been subjected to the above-mentioned practice (*3*). In that way, several methods have been proposed based on the determination of new compounds, such as chlorophyllic derivatives, conjugated linoleic acid, dimers, and 1,3-DG isomers, formed in those processes, and some papers have been published (*4–7*). Nevertheless, some of those "new compounds" can also be found in aged olive oils. In the absence of a clear and specific compound produced during the processing, we have to focus our attention on those compounds that exceed the normal values found in extra virgin olive oils as is the case of fatty acid alkyl esters (FAAEs) mainly ethyl (FAEEs) and methyl esters (FAMEs) (*8*). Esterification of free fatty acids (FFAs) with low molecular alcohols (e.g., methanol or ethanol) is a typical and well-known reaction of second order that takes place in an acid medium and is catalyzed by the presence of the certain enzymes. The reaction depends strongly on both reactive, FFAs and alcohol and also on the temperature. In some cases, the esterification of FFAs to the corresponding methyl esters can be up to 99% in a few minutes (*9*). In the case of olive fruits stored for days before milling, the increase in FFAs formed by lipolysis of triglycerides and the alcohols formed by the action of micro-organisms reacts producing FAAEs (*10, 11*). Thus, molecules of triglycerides are transformed into their methyl or ethyl esters producing glycerol as a byproduct. The aim of the present paper is to determine the average content of FAAEs in olive oils of different qualities, evaluate the influence of soft deodorization

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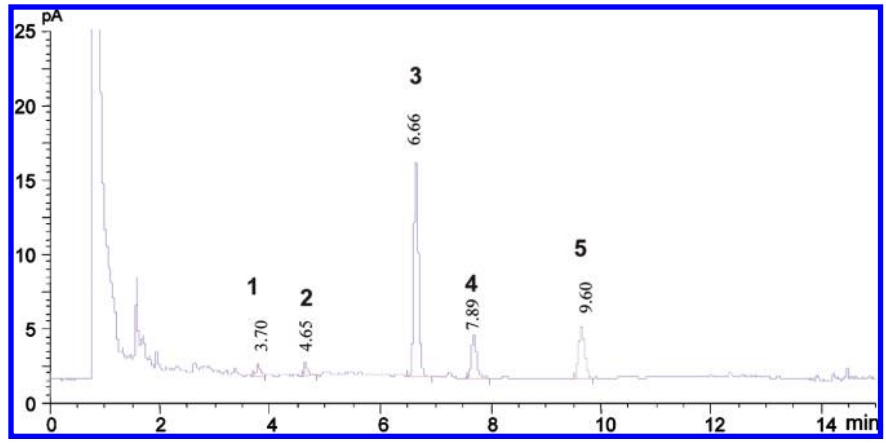

Figure 1. GC-FID chromatogram profile of FAAEs from an extra virgin olive oil (total FAAEs = 35 mg/kg): (1) C16:0 ME; (2) C16:0 EE; (3) Internal standard, C17:0 EE; (4) C18:1 ME; (5) C18:1 EE. Chromatographic conditions as stated in the Materials and Methods section.

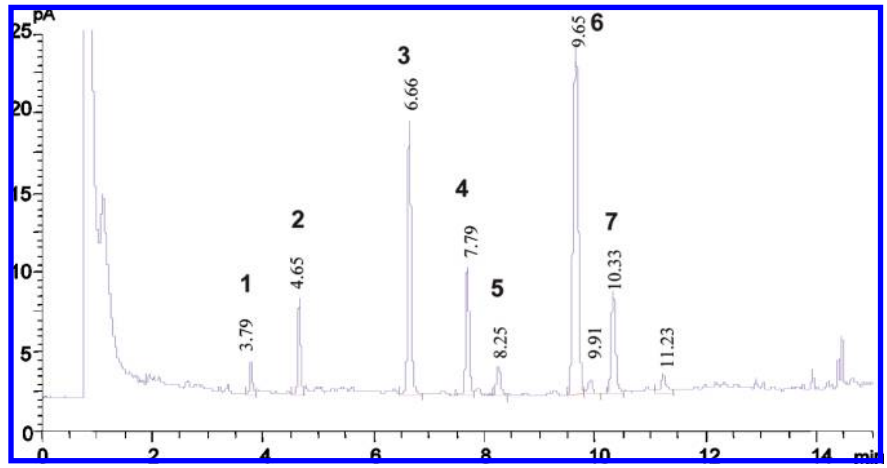

Figure 2. GC-FID chromatogram profile of FAAEs from an olive oil (total FAAEs = 100 mg/kg): (1) C16:0 ME; (2) C16:0 EE; (3) Internal standard, C17:0 EE; (4) C18:1 ME; (5) C18:2 ME; (6) C18:1 EE; (7) C18:2 EE. Chromatographic conditions as stated in the Materials and Methods section.

on the concentration of these compounds and the use of the method to detect blends of mildly refined olive oils with extra virgin olive oils.

MATERIALS AND METHODS

Samples. A set of 15 Spanish protected denomination of origin (PDOs) extra virgin olive oils were bought in local markets. A second set formed by 45 Spanish olive oils of several varieties, origin, and qualities was bought as virgin olive oils (samples 16−46) and as lampante olive oils (samples 47−60) also from local markets and directly purchased from producers. A third set was prepared in a laboratory-scale mild refining plant consisting of two sets of six samples each. They were obtained by submitting two originally low quality oils to a mild refining process at 98 °C using nitrogen as stripping gas for up to 2 h. Finally, a set of 36 mildly deodorized olive oils was obtained after four different and controlled processes. The first was obtained subjecting a Lampante olive oil to a mild refining process at 98 °C for 4 h under nitrogen stripping; the second was made from the same lampante oil but under steam stripping; the third process was carried out increasing the temperature to 150 °C, with stripping nitrogen, for 4 h, and finally, the oil was previously neutralized, washed and filtered through trysil, and then deodorized at 150 °C using steam as stripping gas.

Materials and Reagents. All reagents were of analytical grade unless otherwise stated. A standard heptadecanoic acid ethyl ester (C17:0 EE) was purchased from Sigma (St. Louis, MO). The SPE cartridges (6 mL), packed with silica gel phase (1000 mg), were from Varian (EA Middleburg, the Netherlands).

Standard Solutions. Two solutions of 0.05 and 0.5 mg/mL, respectively, of C17:0 EE in hexane were used as the internal standard for determining esters.

Solid Phase Extraction (SPE). The procedure for isolation has been previously published by Pérez-Camino et al. (*8*), but several modifications have been introduced, mainly concerning the eluents. Olive oils (0.2 g ±0.001 g) were weighed into a graduated tube, 250 μL of standard solution was added and filled up to 2 mL with hexane. Silica SPE cartridges (1000 mg) were placed in an automatic vacuum elution apparatus, and previously conditioned by passing 12 mL of hexane, then 1 mL of the oil solution in hexane containing the standards was introduced onto the column, and the solvent was pulled through at 0.5 mL/min, leaving the sample and the standards on the top of the column. The column was eluted with 7 mL of the solvent mixture hexane/toluene 85:15 and the fraction rejected. Next, a fraction was collected passing 10 mL of the same admixture at a flow rate of 1 mL/min. The eluate was evaporated in a rotary evaporator at room temperature under vacuum until dry. The residue was redissolved with 200 μL of heptane, and a volume (1 μL) of the solution was injected into the GC.

GC Analysis of FAAEs. A chromatographic analysis of the esters was performed using a HP 6890N GC (Palo Alto, CA) fitted with a flame ionization detector and equipped with a programmable temperature-vaporizing inlet (PTV). Separations were carried out on an Rtx-65TG capillary column (30 m, 0.25 mm id) coated with 35% dimethyl−65% diphenylpolysiloxane (Restek Corporation, Bellefonte, PA). The operating conditions were as follows: oven temperature, 160 °C for 10 min and then increased at 10 °C/min up to 290 °C and maintained for 10 min; injector was programmed from 60 to 260 °C; detector temperature was 320 °C. Hydrogen was used as the carrier gas at a column head pressure of 110 psi.

GC Analysis of Alcohols. Chromatographic analysis of volatile alcohols was performed by static headspace analysis, heating the sample

Table 1. Fatty Acid Alkyl Esters of Olive Oils[a]

oil sample	FAME (ppm)	FAEE (ppm)	total FAAE (ppm)	FAEE/FAME
PDO-1	7.1	10.0	17.1	1.4
PDO-2	5.0	5.3	10.3	1.1
PDO-3	11.6	20.1	31.7	1.7
PDO-4	5.4	9.4	14.8	1.7
PDO-5	16.5	22.5	38.9	1.4
PDO-6	9.6	9.6	19.2	1.0
PDO-7	15.1	4.1	19.2	0.3
PDO-8	13.5	17.5	31.0	1.3
PDO-9	6.7	9.0	15.7	1.3
PDO-10	1.9	2.6	4.5	1.4
PDO-11	0.0	0.0	0.0	
PDO-12	13.9	11.6	25.5	0.8
PDO-13	4.3	4.8	9.1	1.1
PDO-14	0.0	0.0	0.0	
PDO-15	8.7	10.1	18.9	1.2
16	3.5	3.2	6.7	0.9
17	3.8	5.4	9.2	1.4
18	7.9	5.4	13.3	0.7
19	5.0	9.0	14.0	1.8
20	6.3	10.6	16.9	1.7
21	8.7	10.7	19.4	1.2
22	10.1	9.4	19.5	0.9
23	10.0	9.5	19.5	1.0
24	5.5	15.1	20.6	2.8
25	8.9	13.8	22.7	1.6
26	12.9	10.6	23.5	0.8
27	10.0	14.6	24.6	1.5
28	10.5	14.4	24.9	1.4
29	12.6	12.4	25.0	1.0
30	7.7	17.6	25.3	2.3
31	10.9	15.5	26.4	1.4
32	10.2	18.9	29.1	1.9
33	13.9	15.2	29.1	1.1
34	9.1	20.1	29.2	2.2
35	11.6	18.3	29.9	1.6
36	10.6	19.8	30.4	1.9
37	13.1	19.2	32.3	2.3
38	12.5	19.8	32.3	1.6
39	16.7	15.8	32.5	0.9
40	10.3	22.4	32.7	2.2
41	11.2	22.6	33.8	2.0
42	10.8	25.1	35.9	2.3
43	17.6	21.8	39.4	1.2
44	20.2	19.5	39.7	1.0
45	19.7	43.2	62.9	2.2
46	23.4	40.0	63.4	1.7
LOO-47	28.7	41.5	70.2	1.5
LOO-48	28.7	56.9	85.6	2.0
LOO-49	38.0	61.0	99.0	1.6
LOO-50	23.9	80.4	104.3	3.4
LOO-51	53.3	68.1	121.4	1.3
LOO-52	42.0	123.0	165.0	2.9
LOO-53	49.0	177.6	226.6	3.6
LOO-54	65.4	175.3	240.7	2.7
LOO-55	97.8	203.9	301.7	2.1
LOO-56	135.0	343.0	478.0	2.5
LOO-57	130.5	1399.5	1530.0	10.7
LOO-58	160.8	1450.0	1610.8	9.0
LOO-59	150.7	1766.0	1916.7	11.7
LOO-60	192.5	3636.2	3828.7	18.9

[a] Data are the average of two replicates. PDO: protected denomination of origin. LOO: lampante olive oil.

(3 g) at 50 °C set in a closed vial for 30 min, and the volatile was injected (500 μL) into a polar capillary column. Maintaining the oven temperature at 40 °C isocratic, both methyl and ethyl alcohols were separated after 5 min.

Statistical Analysis. Statistical analyses of the data were performed using SPSS v12. Statistical differences between means were determined using a one-way ANOVA, p values of <0.05 were considered significant.

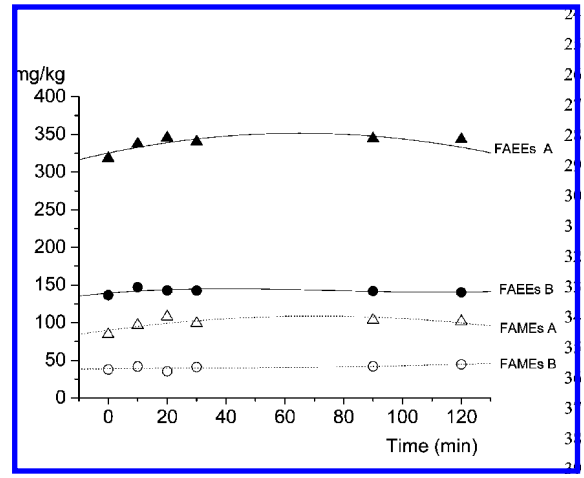

Figure 3. Evolution of FAAEs when two low quality olive oils (Sample A ≈ 400 mg/kg FAAEs and sample B ≈ 170 mg/kg FAAEs) are submitted to a soft deodorization process (98 °C under nitrogen) for 2 h.

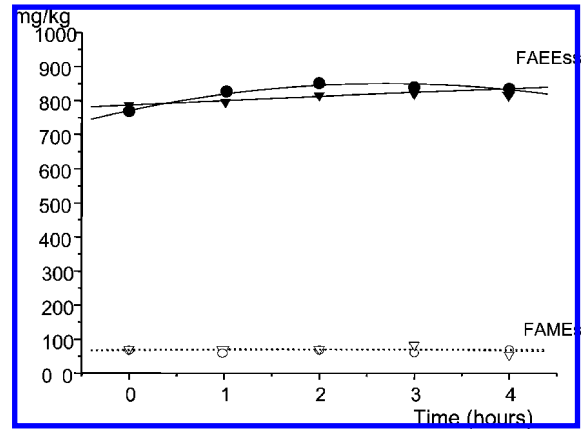

Figure 4. Evolution of FAAEs when an olive oil containing >800 mg/kg FAAEs is submitted to a soft deodorization process for 4 h at 98 °C under nitrogen (▼,▽) and steam water (●,○).

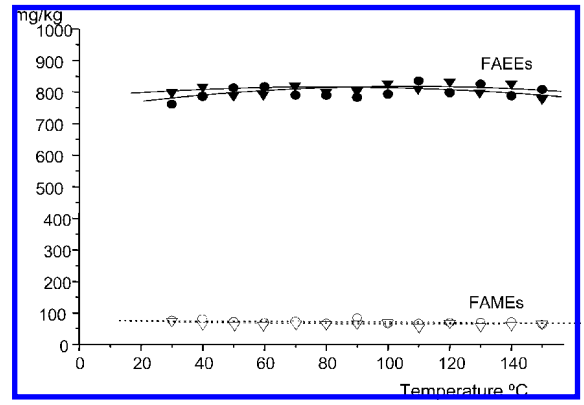

Figure 5. Evolution of FAAEs when an olive oil containing >800 mg/kg FAAEs is submitted to a deodorization process up to 150 °C for 4 h under nitrogen (▼,▽) and steam water (●,○).

RESULTS AND DISCUSSION

The main fatty acid alkyl esters (FAAEs) found in olive oils are those corresponding to palmitic, oleic, and linoleic acids. In the genuine high quality extra virgin oils, the methyl esters are similar to the corresponding ethyl esters although only palmitic and oleic are clearly observed and quantified. **Figure 1** shows a profile of FAAEs in an extra virgin olive oil (∑esters

= 35 mg/kg of oil). On the other hand, **Figure 2** shows the profile of an olive oil in which the total quantity = 100 mg/kg, and also, esters of linoleic acid can be detected. In **Table 1**, data 1−15 belong to samples of extra virgin olive oils from Spanish PDOs. The total amount of FAAEs found in this set of 15 samples does not exceed the quantity of 40 mg/kg (mean value = 17.1). The average in the FAEEs/FAMEs ratio arising from those extra virgin oils was 1.0. As the total amount of FAAEs increases, the amount of FAEEs are much higher than FAMEs, reaching in some cases, a ratio value higher than 10. In those oils, propyl and butyl esters are also encountered.They are low quality lampante oils (samples 47−60) and are refined by normal procedures. The results of the analysis of samples 16−46 included in **Table 1** show that these olive oils contain a mean value of total alkyl esters of 27.9 mg/kg with an upper value of 63.4 mg/kg and present an average ratio FAEEs/FAMEs of 1.5. No differences between means have been found among samples 1−15 (PDO) and samples 16−46. Taking into account this group of 46 samples, 90% of them showed a FAEEs/FAMEs ratio of <2.

In the lampante olive oil group (samples 47−60), the total amount of FAAEs is higher than 70 mg/kg (mean value = 770 mg/kg) and presents an average ratio FAEEs/FAMEs over 5. Their difference with the above-discussed groups is highly significant ($p > 0.001$).

Normal deodorization is a process done through pressurized steam-distillation at temperatures of 180−250 °C for 30−180 min (*12*), which removes undesirable compounds, and the oil obtained is odorless and tasteless. A previous neutralization is also sometimes required. The oils subjected to deodorization are easily detected by several analytical parameters such as *trans* isomers (*13*) (*6*), stigmastadienes (*14*) (*15*), or polar dimers (*16*). Soft deodorization uses low temperatures, which invalidate the above-mentioned methods to detect those oils. There are some characteristics of low quality oils that are not related to volatile compounds, eliminated after soft deodorization, which remain after deodorization. Among these compounds are those formed by the esterification of fatty acids with low molecular alcohol (FAAEs). In order to determine the influence of a soft refining process on the alkyl esters of fatty acids, two oil samples containing 400 and 170 mg/kg of FAAEs were submitted to a laboratory-scale soft deodorization process with similar conditions to those used at industrial scale. Depending on the quality of the oils, the volatile compounds are eliminated at different processing times. After 45 min of treatment, the sample did not contain unpleasant volatile compounds; however, the objective of this work was not to optimize soft deodorization conditions but to study the influence of the soft deodorization process on the FAAEs content, and deodorization was maintained up to 2 h. **Figure 3** shows the evolution of the total methyl and also total ethyl esters when the two above-mentioned oils were submitted to a soft deodorization process at 98 °C for up to 2 h under nitrogen stripping and a vacuum of −76 kPa. During the process, there is no loss in FAAE quantities; even more, there is a light increase (15%) in their quantities after the first sampling corresponding to FAEEs, as can be observed in the figure. The combined effect of bubbling nitrogen through the sample and the temperature may have caused the formation of FAAEs. This esterification takes place easily when the reactives (alcohols and FFA) are present in acidic medium (*9*). To confirm this hypothesis, free acidity was determined by titration, resulting in 0.82% expressed in oleic acid; next, we determined the content of alcohols by static headspace GC analysis. The results obtained show considerable amounts of alcohol, with ethyl alcohol in twice the concentration as the corresponding methyl, which explains the new formation of FAEEs. Statistical analysis of the data shows that there is no loss in FAAEs during the soft deodorization time of the samples in the laboratory, maintaining their concentrations after the process. Therefore, although the oils have been subjected to soft refining and circumvent the parameters included in the regulations, the determination of FAAEs demonstrated that oils were of uncertain quality to begin with.

In relation to oils with higher quantities of FAAEs (≈ 800 mg/kg), four different soft deodorization processes were carried out, and the data is depicted in **Figures 4** and **5**. **Figure 4** represents the evolution of FAEEs and FAMEs when the oil is submitted for 4 h at 98 °C under vacuum and nitrogen stripping (▼: FAEEs; ▽: FAMEs) and under vacuum and steam stripping (●: FAEES; ○: FAMEs). **Figure 5** represents the evolution of FAAEs when the oil is subjected to increasing temperatures up to 150 °C for 4 h. The experiment was also carried out under nitrogen and steam stripping. As can be observed, there are negligible differences either among samples after the initial step or among treatments, although in all cases, an initial increase, mainly in the ethyl esters content, is observed, which is already explained by an initial esterification process that takes place due to the remaining quantities of alcohol in the lampante oils.

In conclusion, the influence of soft deodorization on the composition of fatty acid methyl and ethyl esters in olive oils was investigated although not significantly affected by the experimental conditions. Therefore, FAAEs can be considered good markers of low quality olive oil subjected to soft deodorization. From all data analyses on the oils studied here, it can be concluded that those considered virgin olive oil presented a total FAAEs lower than 70 mg/kg (mean value = 24.3) and a FAEEs/FAMEs ratio lower than 2. Other oils that comply with the analytical requirements actually established for extra virgin olive oils and having higher FAAEs and FAEEs/FAMEs relationships are suspected of being subjected to soft deodorization.

ABBREVIATIONS USED

FFAs, free fatty acids; SPE, solid phase extraction; GLC, gas liquid chromatography; FAAEs, fatty acid alkyl esters; FAEEs, fatty acid ethyl esters; FAMEs, fatty acid methyl esters.

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