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Original Research Article

EFFECTS OF NEUTRALIZATION AND BLEACHING PROCESS ON FATTY ACID AND TRIGLYCERIDE COMPOSITIONS OF POMACE-OLIVE OIL

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Abstract
This work is a contribution to the study of the refining process, especially the neutralization and the bleaching steps, which are very important stages in the refining process of vegetable oils. The results of the different analyses by chromatographic methods (CPG and HPLC) and a theoretical calculation, bearing on the distribution of the fatty acids on the internal and external positions of glycerol, enabled us to put in a prominent position the effects of neutralizing as well as bleaching on the physicochemical properties of treated oils. It was shown that activated Tunisian clays are characterized by a very important adsorptive capacity, comparable to that of commercial clays. Also, the physicochemical stability of bleached oils was studied. The fatty acid and triacylglycerol compositions allowed us to conclude that oils, neutralized with soda and bleached with the Tunisian activated clays, do not undergo considerable physicochemical alterations and remain corresponding to the international standards for refined oils for human consumption.

Keywords: Pomace-olive oil, Neutralization, Activated clays; bleaching process; fatty acids, triacylglycerols.

1. Introduction

Oils that have been degraded by hydrolysis must be refined before use as edible oils. These oils have a high percentage of free fatty acids (FFA) and partial glycerides (mono- and di-acylglycerol) resulting from hydrolysis. Crude oils have a high percentage of FFA (1) and are generally processed by degumming, alkali neutralization, bleaching, and deodorizing to obtain odorless, bland, and oxidative stable oil that is acceptable to consumers. Each processing step has specific functions for removing certain minor components, which can act as prooxidants or antioxidants.

Current methods of oil neutralization involve significant modification of glyceride and unsaponifiable components. For example, components of the unsaponifiables are degraded during distillation or during neutralization by

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soda. Bleaching of neutralized oils removes entrained soaps and reduces color bodies in the oil, and it is appropriately referred to as adsorption treatment. The used adsorbents allow also the elimination of residual phospholipids of neutralization, mucilage traces, and polar products of oxidation and destruction of peroxides (2-4). Some of these adsorbents involve the formation of isomers of conjugated unsaturation (5).

The bleaching earths, obtained by acid activation, can contribute to the formation of precursors of oxidation (free radicals). The influence of these precursors and the primary and secondary products of oxidation (aldehydes and ketones) on the stability of refined oils are dominating, and it is significant to minimize the formation of these compounds during the bleaching operation (6). However, the appearance of conjugated double bonds in the polyunsaturated fatty acids following the bleaching treatment takes part in the improvement of the oxidative resistance of the treated oils in spite of the partial elimination of natural antioxidants which function in synergy with sterols (*7*). The acid activation of a raw clay has permitted it to increase enormously its adsorptive capacity (an increase of specific surface) (8, 9) and its catalytic activity.

The objective of this study is to elucidate the modification that could undergo the olive oil during the neutralization and bleaching steps. So it is necessary to realize the reaction of neutralization on an artificially acidified olive oil but presenting the same FFA % of naturally degraded olive oils. All the detected modifications in the neutralized oil will be attributed only to the neutralization step. The other part of this study was to prepare bleaching earths by an acid activation process according to the classic method (8) and to investigate the effects of a bleaching process, using Tunisian earths activated in our laboratory in optimal conditions and commercial bleaching earths, on fatty acid and triglyceridic compositions and on oxidative stability. The results of the different analyses by chromatographic methods (CPG and HPLC) and a theoretical calculation, bearing on the distribution of the fatty acids on the internal and external positions of glycerol, enabled us to put in a prominent position the effects of bleaching earths on the physicochemical properties of treated oils.

2. Materials and methods

2.1. Materials

2.1.1. Neutralization

Neutralization with soda was carried out using artificially acidified oil having 10% FFA. This oil was prepared by adding a mixture of FA to virgin (undegraded) olive oil having 0.5% acidity (considered as the reference oil). The mixture of FA was prepared from the same virgin olive oil by saponification followed by acidification. Neutralization of this artificially acidified oil was used as a model. The artificially acidified oil was less complex than naturally degraded olive-residue oil. After treatment, oil degradation was attributed to neutralization. Oil (20 g) and the necessary amount plus 10% excess of neutralizing agent [sodium hydroxide], calculated from the acidity of the oil sample, were charged into an open reactor maintained at ambient temperature. The reaction mixture was subjected to agitation with a magnetic stirrer, then slightly hydrated (0.5 g of distilled water) to ensure ionization of the alkaline agent. After 30 min reaction, the acidity of the oils fell to 0.08%. Naturally acid oil was neutralized under the same experimental conditions. This procedure was effective in neutralizing oils with acidities ranging from 1 to 10%. For olive-residue oil having acidity >10%, the reaction mixture became very viscous, and the viscosity increased as the FFA percentage increased. To mitigate this problem, a volume of hexane, equivalent to the treated oil, was added at the beginning of the reaction. The separation of the neutralized oil was achieved by centrifuging at 1957 × *g* for 1 h.

2.1.2. Acid activation process

Before the activation process, a characterization of the raw material was released by the determination of its chemical and mineralogical composition, and also its specific surface area. The chemical composition of raw clay (table 1)

was determined by the technique of fluorescence X. The X-ray pattern of the raw material was obtained using a Philips PW 3710 X-ray diffractometer with Cu Kα radiation. The chemical and mineralogical analysis (9) permitted the identification of the used raw clay as an interstratified smectite-illite with the predominance of smectite and containing quartz, calcite, and kaolinite as impurities. Specific surfaces of the raw and activated clays in the optimal conditions were determined using the BET (Brunauer, Emmett, and Teller) method with N_2 as an adsorbent and using an ASAP 2010 system.

An experimental study of acid activation enabled us to determine the optimum conditions of acid activation of a raw clay (63 μm) harvested from the south of Tunisia and the obtained bleached earths (T_1 and T_2) and to determine that the bleaching capacity on the pomace-olive oil is comparable to that of the commercial earths (actisyl and tonsil). It should be noted that clays T_1 and T_2 were prepared under the following conditions of activation: a quantity of raw clay 20 g was treated by 200 mL of a solution of H_2SO_4 with a concentration of 7.5 mol/L and at a temperature of 70 °C during 3 h to lead to earth T_1 and during 6 h to lead to the T_2.

2.1.3. Bleaching conditions

The effectiveness of activated clays was prepared by tests of bleaching the pomace-olive oil which was offered by the Agro-zitex refinery (Sfax). The pomace-olive oil (acidity 12.46%) was neutralized by soda (10% in excess). An atmospheric bleaching batch is practiced. The process consists of mixture of the neutralized oil with 2% (g/g) acid activated earth at 85-90 °C in presence of N_2 in the oil surface and stirring for 45 min followed by centrifugation and filtration to give clear oil.

The effectiveness of bleaching is determined by the measurement of the absorbance of neutralized and treated oils with different activated clays. The absorbance was measured for each sample at 500 nm using a NOVASPEC II spectrophotometer. The adsorptive capacity of bleached oils is given by the ratio

[*A*500(neutral oil)][*A*500(bleached oil)] / *A*500(neutral oil)

where *A*500 is the optical density of oil at 500 nm (8). The bleaching index of neutralized oil (taken as a reference) and of treated oils, the chlorophyll and carotenoid (pheophytin) contents, residual of the alkali refining, were determined. The amounts of chlorophyll were given according to the method described by Wolff, based on quantification by a spectrophotometric method (10). The total content of carotenoids (expressed out in *â*-carotene) was determined in a NOVASPEC II spectrophotometer (10).

2.2. Analytical procedures

2.2.1. Physicochemical analysis of neutralized and bleached oils

Determination of oil's hysicochemical parameters was carried out following the analytical methods described by COI standards: The FFA or free fatty acid content and the peroxide index were carried out according to COI official ethods (11, 12). The acidity index was determined by a volumetric titration of the free acidity by a 7.1 g/L aqueous solution of sodium hydroxide. It was expressed as a percentage mass of oleic acid. The conjugated diene level of neutralized and bleached oils was determined by a UV spectrophotometric method (13) with the aim of deducing their respective oxidative state. In the last part of this study, the influence of the bleaching time on treated oils was released according to their UV absorbances. The extraction and the measuring out of the unsaponifiable materials were carried out according to an IUPAC standard method (14). The amounts of unsaponifiable materials were determined by saponifying a specimen of fatty substance (2 g) for 1 h with a solution of potassium hydroxide in the ethanol. The excess of KOH was dosed with a 0.5 M solution of HCl to deduce the mass of KOH necessary to saponify 1 g of fatty substance.

2.2.2. Analysis of fatty acid composition

The determination of the fatty acid composition of neutralized oil (taken as reference) and bleached oils was carried out following the analytical method described by standard

methods (ISO-5509 and 5508) (15, 16). A gas chromatograph Shimadzu (GC14B) equipped with a flame ionization detector has been used. Methyl esters of fatty acids were analyzed on a Carbowax capillary column (20 m, 0.25 mm internal diameter). The oven temperature was programmed from 100 to 150 °C at 3 °C/min, then from 150 °C to 180 °C at 1 °C/min, and then held at 180 °C for 10 min. The fatty acid composition (%) was calculated by a normalization method using a Shimadzu integrator. Analyses were performed three times, and the mean values are reported.

2.2.3. Analysis of glyceridic composition by HPLC

The triacylglycerols were analyzed by HPLC on a reversed phase column. The triacylglycerols were separated from other components of the oil on column chromatography. The dissolved oil (in petroleum ether/ethylenic ether) was loaded on a chromatographic column containing a previously conditioned absorbent silica gel. An acetone solution (5%) was prepared from the vaporized eluted material. A volume of 10 *í*L of the prepared solution was injected using an isocratic HPLC system, type Shimadzu provided with a detector UV (*ì*) 210 nm). Separation of triacylglycerols was accomplished with an RP18 column (25 cm). The used eluant is a mixture of acetone-acetonitrile 50:50 (v/v). The identification of the triacylglycerols was carried out by a comparison with a reference chromatogram (17).

2.2.4. Determination of theoretic composition of triacylglycerols

The calculation of the triacylglycerol composition using the fatty acid composition permitted us to study the distribution of fatty acids between the internal position and the external positions of glycerol. In this work, the theoretical number of molecules in the internal position of a fatty acid relative to 100 molecules of this acid (relative proportion) was determined. Thus the calculation of the triacylglycerols was carried out, by taking the isomers of position into account, according to the COI international standard method (17, 18).

3. Results and discussion

3.1. Neutralization step

3.1.1. Physicochemical stability of neutralized oils

The physicochemical stability was evaluated by measuring the current index of the olive oils. Neutralization with soda was carried out using artificially acidified olive oil. This oil was prepared by adding a mixture of FA to a virgin olive oil (considered as the reference oil). Neutralization of this artificially acidified oil was used as a model because it is less complex than naturally degraded olive oils. After treatment, oil degradation was attributed to neutralization. The principal physicochemical properties of the oils neutralized with soda are listed in Table 2.

Neutralized oils had low acidities. The reduction of the saponification value (SV) of the neutralized oils, compared with the oil of reference, was attributed to the hydrolysis of TAG. The reduction in iodine value (IV), which measures the degree of unsaturation, can also be attributed to the elimination by hydrolysis of some unsaturated esters of TAG and to oxidation leading to the formation of epoxides and hydroperoxides (19). The amount of unsaponifiable matter in the neutralized pomace-olive oil was relatively higher than that of the neutralized, artificially acidified oil. In light of these initial analyses, it appears that the principal physicochemical characteristics of pomace-olive oil were slightly modified during neutralization.

3.1.2. Determination of the Fatty Acid Composition

The FA compositions of the different oils are shown in Table 3. The percentages of linoleic acid in the neutralized oils were lower than those in the reference virgin olive oil. This leads to a decrease of the iodine index Ii and the saponification index IS of the neutralized oils.

The determination of the triacylglycerol compositions of neutralized oils, in particular those who contain the linoleic acid, allows clarifying this hypothesis another time. The

composition of triacylglycerol, containing the linoleic acid, was determined, at first, with an experimental analysis of triacylglycerols by HPLC, secondly, by a theoretical calculation which takes into account the distribution of fatty acids between the internal position (2) and the external positions (1,3) of the molecule of glycerin. The results of this theoretical calculation are confronted.

3.1.3. Determination of the Triglyceridic Composition

The TAG compositions of the neutralized oils (Table 4) were consistent with those of the acid composition. Indeed, the reduction in linoleic acid content was accompanied by a small reduction in the percentages of TAG esterified with linoleic acid in the internal position (LLL, LnLO, LLO, PLP and ALO). It appears that neutralization was accompanied by hydrolysis, which attacks primarily the TAG having linoleic acid in the *sn*-2 position. This position is preferentially occupied, in natural TAG with unsaturated FA chains, in the order: linoleic acid > oleic acid > linolenic acid. The internal position corresponds to a secondary alcohol ester, which is easier to hydrolyze than a primary alcohol ester.

3.1.4. Determination of the Theoretical Triacylglycerols (ECN42)

To confirm the experimental results concerning the stability of triacylglycerols in neutralized oils, a theoretical study by calculation of the TAG composition was investigated. The calculation of the number of molecules in the internal position of a fatty acid (relative proportion) is a convenient means to determine the preferential acylation of glycerol. In this calculation, only the fatty acids having 16 or 18 atoms of carbon were considered because they are the most abundant in olive and pomace-oilve oils (17, 18). The distribution, expressed as a molar percentage, of each fatty acid in internal and external positions in the TAG is summarized in Table 5.

This theoretical distribution was calculated using the real fatty acid composition. To determine the theoretical distribution of the fatty acids in triacylglycerols (ECN42), at first, the TAG which differ between them by nature from their fatty acids were considered. Then, for each type of TAG, the probable structures were determined by distinguishing between internal (2) and external (1,3) positions. The theoretical distributions of fatty acids in the triacylglycerols of the different samples of virgin-olive oil and neutralized oils were determined starting from the compositions of fatty acids in different positions: 2 and 1,3.

The results of this calculation reported in Table 6 are in concord with the experimental results that were reported by HPLC (Table 4). The principal TAG (ECN42) in the theoretical composition, which are the LLL, OLLn and LnLP, were detected by the chromatographic analysis. The others presented very low contents. According to the results reported in Table 6, it was noted that the distribution of fatty acids in the TAG complied with the general rules of stereospecific distribution of fatty acids in the TAG of vegetable origin. The saturated fatty acids are preferably in external positions (1 and 3). The unsaturated fatty acids esterified, mainly, the internal (2) position of the glycerol molecule (20, 21).

The difference between the real composition, determined by HPLC, and the theoretical composition of the TAG with ECN42 constitutes an international standard which permitted us to detect the presence of small quantities of seed oil which is rich in linoleic acid. The highest difference which can be tolerated between the effective and theoretical contents of the TAG in ECN42 is: 0.2 in the case of virgin olive oils, 0.3 in the case of olive oils, 0.3 in the case of refined olive oils, 0.5 in the case of pomace-olive oils.

The difference between the real and theoretical compositions of the TAG with ECN42, presented in Table 7, was in accordance with the tolerated standards. In fact, the highest difference between the real and the theoretical contents of the TAG with ECN42 for the neutralized oils did not exceed 0.3. and the difference between the theoretical composition of the TAG with ECN42 and the real composition of the pomace-olive oil are in

conformity with the international standards adopted for olive oils, the highest difference between them did not exceed 0.5 (22).

3.2. Bleaching step

3.2.1. Acid Activation and Bleaching Process

The acid activation process of clay consists in transforming silicates into colloidal silica which has an important adsorbent capacity. This transformation is carried out by the action of an acid solution (sulfuric acid or hydrochloric acid). The strong acid acts by replacing the exchangeable cations of activated clays by protons and then increasing their adsorbent surface (8). The bentonites (clays containing more than 50% of smectitic fraction) are among smectitic clays susceptible to be modified by an acid treatment. They can be activated to produce adsorbents of high effectiveness (23). The attack of raw clay with an acid solution causes a change of its chemical composition and its physical properties. Previous studies (8, 24) concerning acid activation showed that the most significant parameters are the acid concentration, the temperature, the liquid/solid ratio, and the activation time. The main purpose of this study is to prepare bleaching earths with an important adsorptive capacity and effectiveness, having less damage on the stability of treated oils, than those of commercial earths. To prepare a bleaching earth having a high adsorptive capacity, each one of these parameters was varied with the other ones maintained fixed. Activated clays, obtained in the different conditions of activation, were submitted for testing of bleaching pomace-olive oil. The adsorptive capacities of raw clay, clays T_1 and T_2 (activated in the optimal conditions), and the commercial earths (actisyl and tonsil) which are taken as reference are given in table 8. The acid activation led to a very significant increase in the adsorptive capacity of the raw clay which reaches those of the commercial bleaching earths taken as references. The improvement of the adsorptive capacity of activated clays is allotted, generally, with the activity of amorphous silica, responsible for the increase in specific surface (9). Indeed, the specific surfaces determined by the BET method pass from 68.25 m2/g in the case of raw clay to 191.5 m2/g in the case of bleaching earth T_1 and to 186.3 m2/g in the case of bleaching earth T_2. The bleaching process of vegetable oils was carried out by the adsorption or the transformation of colored pigments. The behavior of acid activated clay as an adsorbent is governed mainly by the level of its surface area and the degree of surface activity. Previous studies (6, 25-28) suggest that, during the acid activation process, amorphous silicic acid develops in the montmorillonite crystal, which in combination with the remaining intact crystalline portion is responsible for the high efficiency of the mineral and showed that the stability of treated vegetable oils depends largely on the activation conditions of the bleaching earth. Accordingly, the study of the physicochemical stability of the pomace-olive oil neutralized with soda and bleached with activated clays was considered.

3.2.2. FFA and Unsaponifiable Contents of Bleached Oils

The physicochemical stability was carried out on pomace-olive oils neutralized with soda and bleached with activated clays (T_1 and T_2) and with commercial bleaching earths and the obtained results are gathered in table 9. The comparison between the results of these analyses and the results of those carried out on a virgin olive oil taken as reference permitted us to visualize the principal physicochemical modifications on the level of the general composition of treated oils. The low increase of the acidity of pomace-olive oils was associated with the decomposition of the trained soaps persisting in these oils after the step of alkali-refining (10). The decomposition of the soaps is due to the presence of the protons fixed on the activated clays. The lowest variation of acidity was noted in the case of the oil bleached by the tonsil, which is the least acid bleaching earth among all the earths used in our tests of bleaching. The content in unsaponifiable materials, expressed as a percentage mass, was not affected during the bleaching step. However, the major part of pigments was eliminated. Certain components of the

unsaponifiable fraction can be eliminated by adsorption on the activated earths. Others can be transformed in the reaction medium such as sterols and tocopherols responsible for the oxidative stability of vegetable oils.

3.2.3. Determination of the Fatty Acid Composition

The determination of the acidic composition is of great importance to know the degree of deterioration of the oil content during bleaching. The fatty acid composition of pomace-olive oil was determined, and the results are gathered in table 10. These results showed that no changes of the employed adsorbents resulted in any significant changes of these acids, including the unsaturated $C_{18:2}$ and $C_{18:3}$. This is a desirable feature considering their nutritional significance. Previous studies, however, mentioned transformations on the level of the polyunsaturated acids (linoleic and linolenic) due to the migrations of the double bonds leading to conjugated dienic or trienic systems (6, 10).

3.2.4. Determination of the Triglyceridic Composition

Triacylglycerols (TAG) in different samples of oil were analyzed, before and after the bleaching step, by high performance liquid chromatography on reversed phase. This has permitted us to obtain the triglyceridic composition directly and to determine the TAG difference between them by the nature of fatty acids. The experimental peaks were identified by the use of some literature data and the calculation of the equivalent carbon number (ECN) (17). The results of these analyses are summarized in table 11. The results showed the prevalence of six TAG (OOO, POO, LOO, LOP, LLO, and POP). The other TAG are present only in small quantities in all the considered samples. The triglyceridic compositions of the bleached pomace-olive oils by the commercial bleaching earths (actisyl and tonsil), and of the bleached pomace-olive oils by the activated clays (T_1 and T_2) were similar. They are in conformity with the international standards concerning olive oils (22).

3.2.5. Determination of the Theoretical Triacylglycerols (ECN42)

The theoretical distributions of fatty acids in the triacylglycerols of the different samples of pomace-olive oil were determined starting from the compositions of fatty acids in different positions: 2 and 1,3 (table 12). The results of this calculation reported in table 13 are in concord with the experimental results that were reported by HPLC (table 11). The principal TAG (ECN42) in the theoretical composition, which are the LLL, OLLn and LnLP, were detected by the chromatographic analysis. The others presented very low contents and did not appear on the chromatogram.

The difference between the real and theoretical compositions of the TAG with ECN42, presented in table 14, was in accordance with the tolerated standards. In fact, the highest difference between the real and the theoretical contents of the TAG with ECN42 for the pomace-olive oil did not exceed 0.5 (22). The physicochemical characteristics, the acidic and triglyceridic compositions, and the difference between the theoretical composition of the TAG with ECN42 and the real composition of the pomace-olive oils, which were bleached using the commercial earths or the activated clays, are in conformity with the international standards adopted for olive oils (22). At this step of the study, and according to the obtained results, we can consider that the pomace-olive oil bleaching using the activated earths prepared from Tunisian clay is effective. The adsorptive capacities of activated clays T_1 and T_2 were important and comparable to those of the commercial earths. The chromatographic techniques used for the determination of the acidic and triglyceridic compositions of bleached oils did not reveal notable modifications compared to the compositions of the virgin olive oil taken as reference.

4. Conclusion

It appears that neutralization was accompanied by hydrolysis, which attacks primarily the TAG having linoleic acid in the *sn*-2 position. This position is preferentially occupied, in natural TAG with unsaturated FA chains, in the order:

linoleic acid, oleic acid and linolenic acid. The internal position corresponds to a secondary alcohol ester, which is easier to hydrolyze than a primary alcohol ester. The saturated fatty acids are preferably in external positions (1 and 3). The unsaturated fatty acids esterified, mainly, the internal (2) position of the glycerol molecule. The acidic compositions of the neutralized pomace-olive oils were completely reflected in their TAG compositions. To conclude, The physicochemical characteristics, the acidic and triglyceridic compositions, and the difference between the theoretical composition of the TAG with ECN42 and the real composition of the pomace-olive oils are in conformity with the international standards adopted for olive oils.

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Tables:

Table 1: *Chemical composition of raw material*

Chemical composition (%)								
SiO_2	Al_2O3	MgO	Fe_2O_3	CaO	K_2O	Na_2O	PF	total
40.78	9.20	15.90	2.06	5.51	1.98	2.02	22.92	100.37

Table 2: *Chemical characteristics[a] of neutralized olive oils*

Analysis		[b]VOO	[c]NAOO	[d]NPOO
-8018990. yields (%)	Neutralization	—	91.8 (6.4)	80.0 (5.6)
-8018989.	Acidity *(mass %)*	0.50 (0.07)	0.08 (0.01)	0.10 (0.01)
Saponification value *(mg of KOH/g of fatty substance)*		191.44 (5.08)	175.31 (4.65)	184.22 (4.89)
Iodine value *(g of iodine/100g of fatty substance)*		107.86 (0.95)	98.66 (0.87)	90.94 (0.80)
Amount of unsaponifiable matter *(mass %)*		0.45 (0.01)	0.42 0.01)	1.20 (0.03)

[a]Mean of three replicates with SD in parentheses. [b]VOO: virgin olive oil. [c] NAOO: neutralized artificially acidified olive oil. [d]NPOO: neutralized pomace-olive oil.

Tableau 3: *Fatty acid (FA) compositions of neutralized olive oils* [a] *(mass %)*

	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$
VOO	21.33 (1.25)	3.33 (1.93)	0.03 (<0.01)	0.07 (<0.01)	2.66 (0.11)	51.37 (0.56)	17.20 (1.54)	0.55 (0.19)	0.35 (0.04)	0.16 (0.01)
NAOO	24.11 (1.42)	3.10 (1.80)	0.03 (<0.01)	0.07 (<0.01)	2.79 (0.11)	51.58 (0.56)	12.94 (1.15)	0.53 (0.18)	0.40 (0.05)	0.16 (0.01)
NPOO	13.88 (0.81)	1.64 (0.95)	0.05 (<0.01)	0.07 (<0.01)	2.58 (0.11)	64.22 (0.70)	16.2 (1.45)	0.52 (0.18)	0.40 (0.05)	0.24 (0.02)

[a]Mean of three replicates with SD (standard deviation) in parentheses.

Tableau 4: ***Analysis of Triacylglycerols (TAG) by HPLC in neutralized olive oils*** [a] ***(mass %)***

E.C.N.	**TAG**	VOO	NAOO	NPOO
42	**LLL**	**0.63**	**0.35**	**0,80**
42	**LnLO**	**0.30**	**0.20**	**0,45**
42	**LnLP**	**0.17**	**0.17**	**0,16**
44	**LLO**	**5.49**	**5.06**	**6,02**
44	**LnOO**	**4.28**	**4.24**	**3,16**
44	**PLL**	**0.46**	**0.57**	**0,62**
46	**LOO**	**15.79**	**15.99**	**18,05**
46	**OLP**	**15.74**	**15.69**	**11,45**
46	**PLP**	**2.63**	**2.49**	**1,41**
48	**OOO**	**19.35**	**19.65**	**28,09**
48	**POO**	**24.19**	**24.13**	**20,23**
48	**POP**	**5.84**	**5.65**	**3,81**
50	**ALO**	**0.26**	**0.14**	**0,29**
50	**SOO**	**3.45**	**3.37**	**3,94**
50	**SOP**	**1.36**	**1.33**	**1,45**

[a]Mean of three replicates.

L, linoleic acid; Ln, linolenic acid; O, oleic acid; P, palmitic acid; A, arachidic acid; S, stearic acid.

Tableau 5: ***Fatty acid distribution in internal and external positions of the TAG in neutralized artificially acidified olive oils***

Fatty acid (%)	VOO		NAOO		NPOO	
	Positions (1,3)	**Position (2)**	**Positions (1,3)**	**Position (2)**	**Positions (1,3)**	**Position (2)**
P	**34.861**	**1.423**	**39.866**	**1.627**	**22.423**	**0.915**
S	**3.917**	**0.160**	**4.155**	**0.169**	**3.737**	**0.152**
Po	**3.295**	**5.296**	**2.808**	**4.926**	**1.522**	**2.037**
O	**43.108**	**69.298**	**42.095**	**73.84**	**57.615**	**77.141**
L	**14.544**	**23.375**	**10.641**	**18.660**	**14.467**	**19.369**
Ln	**0.466**	**0.750**	**0.438**	**0.768**	**0.253**	**0.339**

Tableau 6: ***Theoretical distribution of fatty acids in TAG of neutralized artificially acidified olive oils***

TAG	VOO	NAOO	**NPOO**
LLL	**0.494**	**0.214**	**0,400**
PoLL	**0.036**	**0.166**	**0,135**
PoPoL	**0.075**	**0.043**	**0,015**
OLLn	**0.282**	**0.206**	**0,299**
PLLn	**0.154**	**0.131**	**0,043**
PoOLn	**0.044**	**0.054**	**0,033**
SLnLn	**0.000**	**0.000**	**0,000**
PPoLn	**0.034**	**0.034**	**0,008**
ECN 42	**1.119**	**0.848**	**0.933**

Table 7: ***The difference between the real and the theoretical composition of TAG with ECN 42 in neutralized artificially acidified olive oils***

TAG	VOO	NAOO	**NPOO**
LLL	**0.494**	**0.214**	**0,800**
LnLO	**0.282**	**0.206**	**0,450**
LnLP	**0.154**	**0.131**	**0,160**
∑ % ECN 42 real composition	**0.930**	**0.551**	**1.410**
∑ % ECN 42 theoretical composition	**1.119**	**0.848**	**0.933**
Δ ECN 42	**0.189**	**0.297**	**0,477**

Table 8: Adsorptive capacity and chlorophyll content of raw and activated clays and commercial bleaching earths via pomace-olive oil

Bleaching earths	Adsorptive capacity[a] (%)	Chlorophyll content[a] (ppm)
raw clay	18.18	21.68
T_1	81.11	1.00
T_2	80.07	1.33
actisyl	80.89	1.33
tonsil	75.72	2.66

[a] Data are means of three replicates.

Table 9: *Acid value and unsaponifiable matter content[a] of bleached olive oils*

Samples	Acidity *(mass %)*	Unsaponifiable matter contents % (g/g)
[e]$BPOO_{act}$	0.15	0.80
[f]$BPOO_{ton}$	0.10	0.86
[g]$BPOOT_1$	0.20	0.79
[h]$BPOOT_2$	0.18	0.80

[a] Data are means of three replicates. [e]BPOOact: neutralized pomace-olive oil bleached by actisyl. [f]BPOOton: neutralized pomace-olive oil bleached by tonsil. [g]BPOOT1: neutralized pomace-olive oil bleached by T1. [h]BPOOT2 : neutralized pomace-olive oil bleached by T2.

Table 10: *Fatty acid composition of olive oils*

	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$
$BPOO_{act}$	13.36 (1.25)	1.70 (0.97)	0.05 (<0.01)	0.07 (<0.01)	2.44 (0.31)	65.15 (0.53)	15.99 (1.42)	0.58 (0.18)	0.42 (0.05)	0.24 (0.32)
$BPOO_{ton}$	13.43 (1.02)	1.77 (0.98)	0.05 (<0.01)	0.08 (<0.01)	2.41 (0.40)	65.12 (0.54)	16.00 (1.40)	0.53 (0.20)	0.43 (0.05)	0.17 (0.33)
$BPOOT_1$	13.84 (0.98)	1.46 (1.13)	0.05 (<0.01)	0.07 (<0.01)	2.45 (0.36)	65.08 (0.45)	15.82 (1.37)	0.58 (0.19)	0.39 (0.10)	0.25 (0.25)
$BPOOT_2$	13.99 (0.88)	1.79 (1.22)	0.06 (<0.01)	0.08 (<0.01)	2.55 (0.34)	64.83 (0.60)	15.54 (1.40)	0.57 (0.17)	0.36 (0.06)	0.21 (0.40)

Table 11: Analysis of triacylglycerols (TAG)[a] by HPLC in treated olive oils

		$BPOO_{act}$	$BPOO_{ton}$	$BPOOT_1$	$BPOOT_2$
ECN 42	LLL	0.84 (0.02)	0.84 (0.04)	0.80 (0.05)	0.78 (0.06)
	LnLO	0.47 (0.02)	0.43 (0.04)	0.54 (0.05)	0.48 (0.03)
	LnLP	0.16 (0.03)	0.24 (0.02)	0.16 (0.04)	0.18 (0.03)
ECN 44	LLO	5.87 (0.14)	5.92 (0.13)	6.02 (0.16)	5.96 (0.19)
	LnOO	3.12 (0.13)	3.05 (0.16)	3.16 (0.12)	3.05 (0.17)
	PLL	0.66 (0.05)	0.87 (0.04)	0.62 (0.05)	0.83 (0.04)
ECN 46	LOO	17.90 (0.91)	18.02 (0.96)	18.05 (0.98)	18.12 (0.97)
	LOP	11.36 (0.86)	11.44 (0.92)	11.45 (0.93)	11.53 (0.87)
	PLP	1.38 (0.72)	1.38 (0.80)	1.41 (0.75)	1.44 (0.73)
ECN 48	OOO	27.80 (1.31)	28.08 (1.40)	28.09 (1.42)	28.14 (1.35)
	POO	20.18 (0.87)	20.28 (0.86)	20.23 (0.85)	20.19 (0.91)
	POP	3.86 (0.61)	3.82 (0.57)	3.81 (0.64)	3.90 (0.58)
ECN 50	ALO	0.53 (0.09)	0.30 (0.08)	0.29 (0.10)	0.29 (0.10)
	SOO	4.60 (0.54)	4.03 (0.55)	3.94 (0.54)	4.00 (0.60)
	SOP	1.08 (0.89)	1.04 (0.87)	1.45 (0.89)	1.01 (0.87)

[a] Data are means of three replicates and SD (standard deviation) is given in parentheses.

Table 12: ***Fatty acid distribution in internal and external positions of the TAG of pomace-olive oil***

		P	*S*	*Po*	*O*	*L*	*Ln*
$BPOO_{act.}$	*Pos. (1,3)*	*21.45*	*3.53*	*1.69*	*58.38*	*14.43*	*0.53*
	Pos. (2)	*0.87*	*0.14*	*2.23*	*76.98*	*19.03*	*0.69*
$BPOO_{ton}$	*Pos. (1,3)*	*21.55*	*3.48*	*1.76*	*58.29*	*14.43*	*0.48*
	Pos. (2)	*0.89*	*0.14*	*2.32*	*76.97*	*19.05*	*0.63*
$BPOOT_1$	*Pos. (1,3)*	*22.21*	*3.54*	*1.44*	*58.05*	*14.22*	*0.52*
	Pos. (2)	*0.91*	*0.14*	*1.93*	*77.37*	*18.95*	*0.69*
$BPOOT_2$	*Pos. (1,3)*	*22.43*	*3.68*	*2.17*	*57.67*	*17.07*	*0.51*
	Pos. (2)	*0.91*	*0.15*	*2.37*	*77.22*	*18.65*	*0.69*

Table 13: *Theoretical distribution of fatty acids in TAG of pomace-olive oil*

TAG	$BPOO_{act.}$	$BPOO_{ton}$	$BPOOT_1$	$BPOOT_2$
LLL	**0.396**	**0.396**	**0.383**	**0.543**
PoPoPo	**0.000**	**0.001**	**0.004**	**0.001**
PoLL	**0.154**	**0.145**	**0.129**	**0.207**
PoPoL	**0.016**	**0.018**	**0.012**	**0.028**
LnLO	**0.351**	**0.320**	**0.346**	**0.405**
LnLP	**0.077**	**0.080**	**0.090**	**0.097**
PoOLn	**0.045**	**0.032**	**0.035**	**0.048**
SLnLn	**0.040**	**0.033**	**0.000**	**0.039**
PoLnP	**0.019**	**0.005**	**0.009**	**0.014**
ECN 42	**1.093**	**1.031**	**1.009**	**1.383**

Table 14**: *The difference between the real and the theoretical composition of TAG with ECN 42***

	$BPOO_{act.}$	$BPOO_{ton}$	$BPOOT_1$	$BPOOT_2$
LLL	**0.84**	**0.84**	**0.80**	**0.78**
LnLO	**0.47**	**0.43**	**0.54**	**0.48**
LnLP	**0.16**	**0.24**	**0.16**	**0.18**
∑ % ECN 42 real composition	**1.47**	**1.51**	**1.50**	**1.44**
∑ % ECN 42 theoretical composition	**1.09**	**1.03**	**1.01**	**1.38**
Δ ECN 42	**0.37**	**0.48**	**0.49**	**0.05**