JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Alteration of Chemical Composition and the Oxidative Stability of Bleached Pomace−Olive Oil on Activated Clays

W. JAHOUACH, K. ESSID, M. TRABELSI,* AND M. H. FRIKHA

Laboratoire de Chimie Appliquée, Hétérocycles Corps Gras et Polymères, Faculté des Sciences de Sfax, Route de Soukra, Km 3,5 3018 Sfax, Tunisia

This work is a contribution to the study of the bleaching process, which is a very important stage in the refining process of vegetable oils and used to reduce or convert undesired constituents to harmless ones from fats and oils. The virgin olive oil, taken as reference, and the pomace−olive oil were bleached in the optimal conditions using Tunisian bleaching earths (South of Tunisia) which were activated in our laboratory and compared with commercial clays. 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 composition (GC), the triacylglycerol composition (HPLC), and the oxidative stability (UV spectrometry) allowed us to conclude that oils, 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: Activated clays; bleaching process; pomace−olive oil; fatty acids; triacylglycerols; oxidative stability

1. INTRODUCTION

Many contaminant substances can be introduced during the refining process which must be eliminated to obtain valuable products for human consumption. Crude oils are generally processed by degumming, alkali refining, bleaching, and deodorizing to obtain an odorless, bland, and oxidatively 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. The objective of refining an edible oil is to remove unacceptable materials with the least possible effect on desirable components and with the least possible loss of oil (*1*). Indeed, a certain number of minor compounds, such as triterpenic alcohols, sterols, and particularly tocopherols and polyphenols, are responsible for the resistance to oxidation (*2*, *3*) and must be preserved in the refined product (*4*).

Bleaching of alkali-refined 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 (*5*, *6*, *7*). Some of these adsorbents involve the formation of isomers of conjugated unsaturation (*8*).

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 is dominating, and it is significant to minimize the formation of these compounds during the bleaching operation (*9*). 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, mainly tocopherols and polyphenols, which function in synergy with sterols (*10*).

The acid activation of a raw clay has permitted it to increase enormously its adsorptive capacity (an increase of specific surface) (*11*, *12*) and its catalytic activity.

The aim of this study was to prepare bleaching earths by an acid activation process according to the classic method (*11*) 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. A study, bearing on the oxidation resistance of bleached oils, was also carried out.

2. MATERIALS AND METHODS

2.1. Materials. *2.1.1. 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

* Corresponding author. E-mail: Mah.Trabelsi@fss.rnu.tn. Fax: 0021674676606

10.1021/jf0610108 CCC: $33.50
Published on Web 08/19/2006

Table 1. Chemical Composition of Raw Material

chemical composition (%)								
SiO_2	Al_2O_3	MgO	Fe_2O_3	CaO	K_2O	Na_2O	PF	total
40.78	9.2	15.9	2.06	5.51	1.98	2.02	22.92	100.37

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 (*12*) 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.

2.1.2. 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 a 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

$$\frac{[A_{500}(\text{neutral oil})][A_{500}(\text{bleached oil})]}{A_{500}(\text{neutral oil})}$$

where A_{500} is the optical density of oil at 500 nm (*11*).

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 a quantification by a spectrophotometric method (*4*). The total content of carotenoids (expressed out in β-carotene) was determined in a NOVASPEC II spectrophotometer (*4*).

2.2. Analytical Procedures. *2.2.1.* Physicochemical Analysis of Neutralized and Bleached Oils. The bleaching operation permitted mainly the elimination of the dissolved pigments in oil. Other components of the vegetable oils, especially the minor components, can undergo transformations or can be eliminated during bleaching (*9*, *13*). The study of the physicochemical stability of treated oils was released to measure the importance of these alterations.

2.2.2. Determination of Current Indexes. Determination of oil's physicochemical 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 (*14*, *15*). 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 (*16*) 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 (*17*). 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.

2.2.3. 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) (*18*, *19*). 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.

2.2.4. 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 μL of the prepared solution was injected using an isocratic HPLC system, type Shimadzu provided with a detector UV ($\lambda = 210$ 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 (*20*).

2.2.5. 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 (*20*, *21*).

3. RESULTS AND DISCUSSION

3.1. 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 (*11*). 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 (*22*). The attack of a raw clay with an acid solution causes a change of its chemical composition and its physical properties. Previous studies (*11*, *23*) 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),

Table 2. 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.

and the commercial earths (actisyl and tonsil) which are taken as reference are given in **Table 2**.

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 (*12*). Indeed, the specific surfaces determined by the BET method pass from 68.25 m^2/g in the case of raw clay to 191.5 m^2/g in the case of bleaching earth T_1 and to 186.3 m^2/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 (chemisorption of chlorophylls and carotenoids). 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 (*9*, *24*, *25*, *26*, *27*) 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. A similar study was carried out on a virgin olive oil, of acidity 1.7%, which was bleached under the same conditions and was taken as reference oil, with the aim of studying the effect of the bleaching step on the physicochemical stability of oil.

3.2. Physicochemical Analysis of Bleached Oils. In the first part of the analytical study, the acid index I_A, the unsaponifiable contents, and the fatty acid and triglyceridic compositions of bleached oils were determined. 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 second part of the analytical study, carried out by the UV absorbance, concerns the modifications on the level of the polyunsaturated fatty acids.

3.2.1. FFA and Unsaponifiable Contents. The physicochemical stability was carried out on pomace−olive oil neutralized with soda and on virgin olive oils bleached with activated clays (T_1 and T_2) and with commercial bleaching earths. The amounts of unsaponifiable materials were also determined, and the obtained results are gathered in **Table 3**.

The acidity of the virgin olive oil decreased following the treatment with different bleaching earths. This reduction generally results from the elimination, by adsorption, of the free fatty acids on the activated clays in the reaction medium. 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 (*4*). 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.

Table 3. Acid Value and Unsaponifiable Matter Content[a] Of Bleached Olive Oils

samples	A^b (%)	unsaponifiable matter contents % (g/g)
VOO[c]	1.70	0.86
BOO_{act}[d]	0.55	0.83
BOO_{ton}[e]	0.66	0.84
$BOOT_1$[f]	0.46	0.75
$BOOT_2$[g]	0.69	0.73
NPOO[h]	0.07	0.88
$BPOO_{act}$[i]	0.15	0.80
$BPOO_{ton}$[j]	0.10	0.86
$BPOOT_1$[k]	0.20	0.79
$BPOOT_2$[l]	0.18	0.80

[a] Data are means of three replicates. [b] Acidity index. [c] VOO: virgin olive oil. [d] BOO_{act}: bleached olive oil by actisyl. [e] BOO_{ton} : bleached olive oil by tonsil. [f] $BOOT_1$: bleached olive oil by T_1. [g] $BOOT_2$: bleached olive oil by T_2. [h] NPOO: neutralized pomace–olive oil. [i] $BPOO_{act}$: neutralized pomace–olive oil bleached by actisyl. [j] $BPOO_{ton}$: neutralized pomace–olive oil bleached by tonsil. [k] $BPOOT_1$: neutralized pomace–olive oil bleached by T_1. [l] $BPOOT_2$: neutralized pomace–olive oil bleached by T_2.

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.

3.2.2. Determination of the Fatty Acid Composition. The determination of the acidic and triglyceridic compositions is of great importance to know the degree of deterioration of the fat content during bleaching.

The fatty acid composition of virgin olive oil and pomace−olive oil was determined, and the results are gathered in **Table 4**. These results showed that no changes of the employed adsorbents resulted in any significant changes of these acids, including the unsaturated C18:2 and C18:3. 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 (*4*, *9*).

3.2.3. 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) (*20*). The results of these analyses are summarized in **Table 5**.

The results presented in **Table 6** 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. These results do not show any modification on the level of the triglyceridic composition of the bleached olive oils compared to the untreated olive oil. The triglyceridic compositions of the neutralized pomace−olive oil, of the bleached

Table 4. Fatty Acid Composition[a] of Virgin Olive Oil and Bleached Oils

samples	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1
VOO	17.33	2.31	0.03	0.08	2.56	58.79	17.62	0.62	0.49	0.17
	(1.05)	(0.98)	<0.01	<0.01	(0.40)	(0.51)	(1.23)	(0.19)	(0.04)	(0.01)
BOO_{act}	18.97	2.59	0.07	0.06	2.26	57.59	17.52	0.58	0.32	0.06
	(1.09)	(1.01)	<0.01	<0.01	(0.45)	(0.43)	(1.21)	(0.18)	(0.05)	(0.02)
BOO_{ton}	18.97	2.44	0.06	0.07	2.38	57.32	17.40	0.71	0.41	0.24
	(1.11)	(1.30)	<0.01	<0.01	(0.38)	(0.46)	(1.12)	(0.18)	(0.05)	(0.03)
$BOOT_1$	18.40	2.46	0.10	0.05	2.53	57.82	17.43	0.64	0.43	0.08
	(1.10)	(1.25)	<0.01	<0.01	(0.32)	(0.34)	(1.30)	(0.18)	(0.05)	(0.04)
$BOOT_2$	17.64	2.35	0.05	0.07	2.57	58.55	17.50	0.64	0.44	0.20
	(1.18)	(1.40)	<0.01	<0.01	(0.33)	(0.51)	(1.22)	(0.19)	(0.05)	(0.02)
NPOO	13.88	1.78	0.05	0.07	2.58	64.22	16.20	0.58	0.40	0.24
	(1.15)	(0.95)	<0.01	<0.01	(0.34)	(0.60)	(1.32)	(0.17)	(0.04)	(0.31)
$BPOO_{act}$	13.36	1.70	0.05	0.07	2.44	65.15	15.99	0.58	0.42	0.24
	(1.25)	(0.97)	<0.01	<0.01	(0.31)	(0.53)	(1.42)	(0.18)	(0.05)	(0.32)
$BPOO_{ton}$	13.43	1.77	0.05	0.08	2.41	65.12	16.00	0.53	0.43	0.17
	(1.02)	(0.98)	<0.01	<0.01	(0.40)	(0.54)	(1.40)	(0.20)	(0.05)	(0.33)
$BPOOT_1$	13.84	1.46	0.05	0.07	2.45	65.08	15.82	0.58	0.39	0.25
	(0.98)	(1.13)	<0.01	<0.01	(0.36)	(0.45)	(1.37)	(0.19)	(0.10)	(0.25)
$BPOOT_2$	13.99	1.79	0.06	0.08	2.55	64.83	15.54	0.57	0.36	0.21
	(0.88)	(1.22)	<0.01	<0.01	(0.34)	(0.60)	(1.40)	(0.17)	(0.06)	(0.40)

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

Table 5. Analysis of Triacylglycerols (TAG)[a] by HPLC in Treated Olive and Pomace–Olive Oils

	ECN 42			ECN 44			ECN 46			ECN 48			ECN 50		
	LLL	LnLO	LnLP	LLO	LnOO	PLL	LOO	LOP	PLP	OOO	POO	POP	ALO	SOO	SOP
VOO	0.61	0.40	0.12	5.98	4.48	0.42	16.62	15.13	2.88	19.88	22.37	6.14	0.40	3.16	1.35
	(0.02)	(0.02)	(0.01)	(0.12)	(0.12)	(0.02)	(0.80)	(0.95)	(0.54)	(1.10)	(0.90)	(0.32)	(0.08)	(0.42)	(0.90)
BOO_{act}	0.64	0.37	0.20	6.04	4.55	0.55	16.70	15.45	2.89	19.79	22.18	5.88	0.37	3.16	1.15
	(0.01)	(0.02)	(0.02)	(0.12)	(0.13)	(0.03)	(0.85)	(0.98)	(0.67)	(1.22)	(0.87)	(0.41)	(0.09)	(0.46)	(0.87)
BOO_{ton}	0.67	0.36	0.20	5.71	4.12	0.55	16.41	15.27	2.97	19.92	22.48	6.17	0.43	3.16	1.49
	(0.02)	(0.04)	(0.03)	(0.13)	(0.15)	(0.04)	(0.75)	(0.97)	(0.62)	(1.12)	(0.90)	(0.50)	(0.09)	(0.41)	(0.84)
$BOOT_1$	0.67	0.39	0.21	6.20	4.37	0.79	17.14	15.82	2.91	19.96	21.10	4.97	0.33	3.58	1.49
	(0.03)	(0.05)	(0.04)	(0.14)	(0.20)	(0.02)	(0.79)	(0.91)	(0.70)	(1.32)	(0.89)	(0.60)	(0.10)	(0.50)	(0.87)
$BOOT_2$	0.64	0.35	0.18	6.20	4.58	0.83	16.68	15.36	2.77	19.55	21.90	5.90	0.50	3.13	1.37
	(0.02)	(0.06)	(0.06)	(0.15)	(0.14)	(0.05)	(0.79)	(0.86)	(0.74)	(1.31)	(0.96)	(0.56)	(0.07)	(0.61)	(0.91)
NPOO	0.86	0.51	0.14	6.08	3.12	0.67	18.08	11.39	1.37	28.14	20.24	3.82	0.28	4.14	1.08
	(0.06)	(0.05)	(0.01)	(0.16)	(0.15)	(0.06)	(0.83)	(0.87)	(0.68)	(1.23)	(0.93)	(0.52)	(0.07)	(0.55	(0.86)
$BPOO_{act}$	0.84	0.47	0.16	5.87	3.12	0.66	17.90	11.36	1.38	27.80	20.18	3.86	0.53	4.60	1.18
	(0.02)	(0.02)	(0.03)	(0.14)	(0.13)	(0.05)	(0.91)	(0.86)	(0.72)	(1.31)	(0.87)	(0.61)	(0.09)	(0.54)	(0.89)
$BPOO_{ton}$	0.84	0.43	0.24	5.92	3.05	0.87	18.02	11.44	1.38	28.08	20.28	3.82	0.30	4.03	1.04
	(0.04)	(0.04)	(0.02)	(0.13)	(0.16)	(0.04)	(0.96)	(0.92)	(0.80)	(1.40)	(0.86)	(0.57)	(0.08)	(0.55)	(0.87)
$BPOOT_1$	0.80	0.54	0.16	6.02	3.16	0.62	18.05	11.45	1.41	28.09	20.23	3.81	0.29	3.94	1.45
	(0.05)	(0.05)	(0.04)	(0.16)	(0.12)	(0.05)	(0.98)	(0.93)	(0.75)	(1.42)	(0.85)	(0.64)	(0.10)	(0.54)	(0.89)
$BPOOT_2$	0.78	0.48	0.18	5.96	3.05	0.83	18.12	11.53	1.44	28.14	20.19	3.90	0.29	4.00	1.01
	(0.06)	(0.03)	(0.03)	(0.19)	(0.17)	(0.04)	(0.97)	(0.87)	(0.73)	(1.35)	(0.91)	(0.58)	(0.10)	(0.60)	

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

Table 6. Fatty Acid Distribution in Internal and External Positions in the TAG of Olive Oil

	VOO		BOO_{act}		BOO_{ton}		$BOOT_1$		$BOOT_2$	
fatty acids (%)	pos. (1,3)[a]	pos. (2)[b]	pos. (1,3)	pos. (2)	pos. (1,3)	pos. (2)	pos. (1,3)	pos. (2)	pos. (1,3)	pos. (2)
P	27.69	1.13	30.17	1.23	30.26	1.23	29.42	1.20	28.17	1.15
S	3.68	0.15	3.24	0.13	3.42	0.14	3.25	0.13	3.519	0.15
Po	2.21	3.17	2.43	3.60	2.29	3.41	2.33	3.42	2.238	3.24
O	50.60	72.78	48.73	72.17	48.57	72.22	49.42	72.43	50.216	72.74
L	15.28	21.98	14.93	22.12	14.85	22.08	15.01	21.99	15.123	21.90
Ln	0.54	0.78	0.49	0.73	0.61	0.91	0.59	0.73	0.557	0.80

[a] External positions (1,3). [b] Internal position (2).

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 (*28*).

3.2.4. Determination of the Theoretical Triacylglycerols (ECN42). To confirm the experimental results concerning the stability of triacylglycerols in bleached 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

Table 7. Fatty Acid Distribution in Internal and External Positions in the TAG of Pomace–Olive Oil

	NPOO		$BPOO_{act}$		$BPOO_{ton}$		$BPOOT_1$		$BPOOT_2$	
fatty acid (%)	pos. (1,3)	pos. (2)	pos. (1,3)	pos. (2)	pos. (1,3)	pos. (2)	pos. (1,3)	pos. (2)	pos. (1,3)	pos. (2)
P	22.26	0.91	21.45	0.87	21.55	0.89	22.21	0.91	22.43	0.91
S	2.53	0.15	3.53	0.14	3.48	0.14	3.54	0.14	3.68	0.15
Po	1.76	2.35	1.69	2.23	1.76	2.32	1.44	1.93	2.17	2.37
O	57.18	76.46	58.38	76.98	58.29	76.97	58.05	77.37	57.67	77.22
L	14.53	19.43	14.43	19.03	14.43	19.05	14.22	18.95	17.07	18.65
Ln	0.52	0.70	0.53	0.69	0.48	0.63	0.52	0.69	0.51	0.69

Table 8. Theoretical Distribution of Fatty Acids in TAG of Olive Oil

triacylglycerols	VOO	BOO_{act}	BOO_{ton}	$BOOT_1$	$BOOT_2$
LLL	0.513	0.493	0.487	0.495	0.501
PoPoPo	0.001	0.002	0.002	0.002	0.002
PoLL	0.222	0.241	0.226	0.231	0.222
PoPoL	0.000	0.052	0.035	0.036	0.033
OLLn	0.361	0.321	0.392	0.215	0.367
PLLn	0.133	0.134	0.165	0.082	0.139
PoOLn	0.052	0.052	0.070	0.057	0.054
SLnLn	0.000	0.000	0.000	0.000	0.000
PPoLn	0.019	0.052	0.013	0.022	0.021
ECN 42	1.335	1.309	1.392	1.141	1.340

Table 9. Theoretical Distribution of Fatty Acids in TAG of Pomace–Olive Oil

triacylglycerols	NPOO	$BPOO_{act}$	$BPOO_{ton}$	$BPOOT_1$	$BPOOT_2$
LLL	0.410	0.396	0.396	0.383	0.543
PoPoPo	0.001	0.000	0.001	0.004	0.001
PoLL	0.149	0.154	0.145	0.129	0.207
PoPoL	0.018	0.016	0.018	0.012	0.028
OLLn	0.347	0.351	0.320	0.346	0.405
PLLn	0.092	0.077	0.080	0.090	0.097
PoOLn	0.042	0.045	0.032	0.035	0.048
SLnLn	0.000	0.040	0.033	0.000	0.039
PPoLn	0.011	0.019	0.005	0.009	0.014
ECN 42	1.070	1.093	1.031	1.009	1.383

carbon were considered because they are the most abundant in olive and pomace-oilve oils (*20*, *21*). The distribution, expressed as a molar percentage, of each fatty acid in internal and external positions in the TAG is summarized in **Tables 6** and **7**. This theoretical distribution was calculated using the real fatty acid composition (**Table 4**).

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 pomace−olive oil and olive oil were determined starting from the compositions of fatty acids in different positions: 2 and 1,3. The results of this calculation reported in **Tables 8** and **9** are in concord with the experimental results that were reported by HPLC (**Table 5**). 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.

According to the results reported in **Tables 8** and **9**, 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 (*29*, *30*, *31*).

Table 10. 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
theor composition	1.09	1.03	1.01	1.38
Δ ECN 42	0.37	0.48	0.49	0.05

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 10**, 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 (*28*).

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 (*28*). At this step of the study, and according to the obtained results, we can consider that the olive and pomace-olive oils bleaching using the activated earths prepared from a 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. The decomposition of peroxides and the isomerization of polyunsaturated fatty acids following the migrations of double bonds were investigated.

Table 11. Peroxide Index and UV Spectrophotometric Analysis of Treated and Untreated Oils[a]

	samples	Ip[b] (mmol/kg)	K_{232}[c]	K_{270}[c]	K_{320}[c]
1	VOO	4.83	4.94	0.34	0.13
2	BOO_{ton}	4.61	2.64	0.37	0.09
3	BOO_{act}	2.32	2.83	1.41	0.19
4	$BOOT_1$	1.70	2.63	1.30	0.12
5	$BOOT_2$	1.21	2.39	1.40	0.08
6	NPOO	6.93	5.12	2.15	0.16
7	$BPOO_{ton}$	6.80	4.30	2.30	0.17
8	$BPOO_{act}$	6.50	5.60	3.03	0.16
9	$BPOOT_1$	5.54	4.43	3.52	0.20
10	$BPOOT_2$	6.60	6.08	3.60	0.25

[a] Data are means of three replicates. [b] Ip is the peroxide index. [c] The UV specific extinction at the wavelength indicated in the subscript.

3.2.5. Stability of Peroxides and Polyunsaturated Fatty Acids during the Bleaching Process. The results illustrated in **Table 11** showed the peroxide indexes of virgin olive oil and bleached oils and their UV absorbance at 232, 270, and 320 nm.

The decrease of the peroxide index of the virgin olive oils (samples 2, 3, 4, and 5) after the bleaching treatment by the commercial earths and the activated clays (T_1 and T_2) was attributed to the decomposition of peroxides on the bleaching earths, which gradually increases from one to another as following: tonsil, actisyl, T_1, and T_2. In addition, neutralized pomace−olive oils (samples 7, 8, 9, and 10) preserved, after bleaching, high peroxide indexes compared to that of virgin olive oil (oil of reference) while remaining in conformity with the international standards. The peroxide index of bleached pomace−olive oil by activated clay T_1 (sample 9) was relatively the lowest.

In addition to the decomposition of peroxides after the bleaching, other reactions such as the decomposition of the minor components and the conjugation of the double bonds in the polyunsaturated fatty acids were identified. These components (dienes and trienes) absorb in ultraviolet between 225 and 280 nm (*9*).

The conjugated dienes and primary products of oxidation of fatty acids, when they have a conjugated dienic structure such as linoleic hydroperoxide, absorb at 232 nm. The conjugated trienes and the secondary products of oxidation, α-unsaturated aldehydes and ketones, absorb at 270 nm. The determination of the absorbances at 232 nm and at 270 nm allowed the detection and the evaluation of the oxidation products. In certain cases, the measurements of the absorbance at 232 nm also permitted us to detect the presence of the conjugated dienic system of fatty acids.

In the case of the bleached virgin olive oils, the decrease in the peroxide indexes was accompanied by a decrease in the absorbance at 232 nm and by an increase in the absorbance at 270 nm which can be explained by transformation of a part of the hydroperoxides into secondary products of oxidation (aldehydes and α-unsaturated ketones). The contribution of conjugated trienes, suitable to be formed during the bleaching step, whose absorbance, at 270 nm, was not probable because the content of the trienic acid (linolenic) was very low in the virgin olive oil sample (approximately 0.6%).

The decrease in the absorbance at 232 nm of bleached virgin olive oil samples compared to that of the virgin olive oil (reference) cannot be associated exclusively with the decomposition of the hydroperoxides (decrease of the peroxide index). Indeed, the bleached oil using the tonsil (sample 2), which has not lost its peroxides, had an absorbance at 232 nm equivalent to those of sample 3 (bleached by actisyl) having lost approximately half of its peroxides and of samples 4 and 5 (bleached respectively by the earths T_1 and T_2) having lost 75% of their peroxides. Thus, it appears that other components, essentially conjugated dienes, were identified by their absorbance at 232 nm. The latter was formed during the bleaching steps but with a low average. The elucidation of this assumption was released by the increase of the bleaching time. The study was carried out on bleached virgin olive oil under the same experimental conditions during 90 and 120 min instead of 45 min. The obtained results are presented in **Table 12**.

Table 12. The Variation of the Peroxide Index and the UV Absorbance of Bleached Olive Oils versus the Bleaching Time

samples	bleaching time (min)	K_{232}	K_{270}	K_{320}	Ip (mmol of O_2/kg)
VOO	4.92	0.36	0.12	4.83	
BOO_{ton}	45	2.64	0.37	0.09	4.61
	90	2.62	2.00	0.15	5.97
	120	3.39	1.51	0.18	4.21
BOO_{act}	45	2.83	1.41	0.19	2.32
	90	5.76	2.15	0.24	3.74
	120	8.71	1.69	0.28	5.57
$BOOT_1$	45	2.63	1.30	0.12	1.70
	90	2.25	2.34	0.31	6.17
	120	2.94	2.76	0.33	3.19
$BOOT_2$	45	2.39	1.40	0.08	1.21
	90	2.06	2.40	0.20	5.73
	120	2.73	2.83	0.28	6.04

The assumption of the formation of conjugated dienes was checked perfectly in the case of bleaching with the tonsil. Although the peroxide index decreased (disappearance of hydroperoxides), a significant increase in the absorbance at 232 nm was noted, which passed from 2.64 in 45 min to 3.39 in 120 min. The increase of the bleaching time, in the case of the acid activated earths (actisyl, T_1, and T_2), led to the increase in the peroxide indexes enhancing the oxidation of bleached oils. These primary products of oxidation (hydroperoxides) absorb at 232 nm and mask the absorbance of the conjugated dienes whose formation was rather favorable in such acid medium. In the case of the bleaching earths prepared at our laboratory (T_1 and T_2), many hydroperoxides, which are formed during the bleaching step, were transformed then into secondary products of oxidation (unsaturated ketones and aldehydes) shown by the increase of the absorbance at 270 nm.

To conclude, the fatty acid and triglyceridic compositions of the pomace−olive oils, bleached by activated clays or by commercial earths, are in conformity with the international standards concerning olive oils. However, pomace−olive oils preserved after the bleaching step a relatively high peroxide index compared to that of the virgin olive oil while remaining within the limits of the required standards.

All these factors were combined to confer on treated oils a better oxidative stability, and so a more significant durability for consumption. Further studies of the bleaching behavior of Tunisian activated clays, using ultrasonic techniques, on the glyceridic fraction and on the minor components (sterols, tocopherols, polyphenols, ..., and stigmastadienes) are in progress.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of the National Office of Oil (Tunisia) in the performance of the chromatographic analysis.

LITERATURE CITED

(1) Ferrari, R. A.; Schulte, W.; Esteves, W.; Brühl, L.; Mukherjee, K. D. Minor constituents of oils during industrial processing. *J. Am. Oil Chem. Soc.* **1996**, *73*, 587−592.

(2) Baldioli, M.; Servili, M.; Perretti, G.; Montedero, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589−1593.

(3) Chimi, H.; Cillard, J.; Cillard, P.; Rahmani, M. Peroxyl and hydroxyl radical scavenging activity of natural phenolic antioxydant. *J. Am. Oil Chem. Soc.* **1991**, *68*, 307−311.

(4) Wolff, J. P. *Manuel des corps gras*; Karleskind, A., Ed.; Lavoisier: Paris, 1992; p 790.

(5) Norris, F. A. *Bailey's Industrial Oil and Fat Products*, 4th ed.; Swern, D. Ed.; Interscience Publishers: New York, 1982; pp 294−314.

(6) Topallar, H. Bleaching Kinetics of Sunflowerseed. *J. Am. Oil Chem. Soc.* **1998**, *75*, 531−533.

(7) Kheok, S. C.; Lim, E. E. *Mechanism of Palm Oil Bleaching by Montmorillonite Clay Activated at Various Acid Concentrations. J. Am. Oil Chem. Soc.* **1982**, *59*, 129−131.

(8) Vasvazova, P. I.; Tontchev, D. T.; Totova, P. F.; Nenkova, T. P. Traitement thermiques des acides gras du type linoléique avec de la bentonite. *Ol., Corps Gras, Lipides* **1998**, *5*, 233−236.

(9) Emangeard, J. P.; Marchand, D. Utilisation de Trisyl dans le raffinage des huiles alimentaires. *Rev. Fr. Corps Gras* **1991**, *11*/*12*, 398−400.

(10) Aparicio, R.; Roda, L.; Albi, M. A.; Guitierrez, F. Effect of various compounds on virgin olive oil stability measured by rancimat. *J. Agric. Food Chem.* **1999**, *47*, 4150−4155.

(11) Gannouni, A.; Bellagi, A.; Bagane, M. Préparation d'une argile activée pour la décoloration de l'huile d'olive. *Ann. Chim. Sci. Mat. Fr.* **1999**, *24*, 407−416.

(12) Perrin, R.; Scharff, J.-P. *Chimie industrielle*, 2nd ed.; Masson: Paris, 1993; p 864.

(13) Castillo, M. L.; Caja, M. M.; Herraiz, M.; Blanch, G. P. Rapid Recognition of olive oil adulterated with hazelnut oil by direct analysis of the enantiomeric composition of Filbertone, *J. Agric. Food Chem.* **1998**, *46*, 5128−5131.

(14) International standard. Animal and vegetable fats and oils. Determination of acid value and acidity. ISO 660; 1996, 2nd ed.

(15) International standard. Animal and vegetable fats and oils. Determination of peroxide index. ISO 3960; 1998, 3rd ed.

(16) International standard. COI/T-20; 1996, Doc. No. 19.

(17) International standard. Animal and vegetable fats and oils. Determination of the amount of the unsaponifiable matter. ISO 3596; 1988, 1st ed.

(18) International standard. ISO 5509; 1978, 1st ed.

(19) International standard. ISO 5508; 1990, 2nd ed.

(20) International standard. Détermination de la différence entre la composition réelle et la composition théorique des triglycérides à ECN42. COI/T.20; 1998, Doc. NO. 20.

(21) Christopoulou, E.; Lazarki, M.; Komaitis, M.; Kaselimis, K. Effectiveness of determinations of fatty acids and triglycerides for the detection of adulteration of olive oils with vegetable oils. *Food Chem.* **2004**, *84*, 463−474.

(22) Srasra, E.; Bergaya, F.; Van Damme, H.; Ariguib, N. K. Surface properties of an activated bentonite- decolorisation of rape-seed oils. *Appl. Clay Sc.* **1989**, *4*, 411−421.

(23) Kafarov, V. Méthodes cybernétiques et technologie chimiques; Mir: Moscow, 1992.

(24) Sarier, N.; Guler, C. β-Carotene Adsorption on Acid-Activated Montmorillonite. *J. Am. Oil Chem. Soc.* **1988**, *65*, 776−779.

(25) Brimberg, U. I. Kinetics of Bleaching of Vegetable Oils. *J. Am. Oil Chem. Soc*. **1982**, *59*, 74−78.

(26) Taylor, D. R.; Ungermann, C. B.; Jenkins, D. B. Bleaching with Alternative Layered Minerals—A Comparison with Acid-Activated Montmorillonite for Bleaching Soybean Oil. *J. Am. Oil Chem. Soc*. **1989**, *66*, 334−341.

(27) Habile, M.; Barlow, P. J.; Hole, M. Adsorptive bleaching of soybean oil with non-montmorillonite zambian clays. *J. Am. Oil Chem. Soc.* **1992**, *69*, 379−383.

(28) International Oleicol Council. Commercial standard applied to olive and pomace-olive oil; 1995, 2nd ed.

(29) Christie, W. W. The positions distribution of fatty acids in natural glycerides of vegetable origin. *Chem. Ind.* **1986**, 121−123.

(30) Guanstone, F. D.; Hamilton, R. J.; Padley, F. B.; Qureshi, I. M. Glycerids studies V. The distribution of unsaturated acyl groups in vegetable triglycerides. *J. Am. Oil Chem. Soc.* **1965**, *42*, 965−970.

(31) Essid, K.; Chtourou, M.; Jahouach, W.; Trabelsi, M.; Frikha, M. H. Analyse de la composition lipidique de l'huile acide de grignon d'olive neutralisée par la chaux. *J. Soc. Chem. Tunis*. **2003** (June), 41−53.

Received for review April 11, 2006. Revised manuscript received July 4, 2006. Accepted July 20, 2006.

JF0610108