Effects of Neutralization with Lime on the Quality of Acid Olive Oil

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ABSTRACT: The technique described in this study makes it possible to neutralize olive oils having very high acidities. The neutralization is carried out in a solid-liquid biphasic medium, which is slightly hydrated, by substituting lime (calcium hydroxide, an inexpensive and locally manufactured product) for soda (sodium hydroxide) as the neutralizing agent. Neutralization by lime limits TAG hydrolysis. The use of lime as a neutralizing agent makes it possible to preserve almost 80% of the α-tocopherol. Oils neutralized with lime have higher temperatures of thermal decomposition than oils neutralized with soda. Oils neutralized with lime had better oxidative stability because natural antioxidants were preserved. Oils neutralized by lime conformed with international olive oil standards.

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KEY WORDS: Lime, neutralization, oil stability, olive oil, olive-residue oil, oxidation.

Vegetable oils that have been degraded by hydrolysis must be refined before use. Olive-residue oil has a high percentage of FFA, ranging from 5 to 60% by weight (1). The elimination of FFA is the most important refining operation. Several methods to remove FFA have been developed to enhance the value of degraded vegetable oils, for example, physical refining based on distillation and chemical refining with soda (sodium hydroxide).

Distillation is an effective method to neutralize vegetable oils with high acidity, but this operation is also energy intensive. Moreover, heating oils to high temperature under reduced pressure generates secondary reactions that alter their physicochemical characteristics and organoleptic properties (2). Thermal and catalytic reactions lead to the formation of olefin hydrocarbons, di- and triterpenes, and aromatic products as a consequence of tocotrienol degradation (3).

Sodium hydroxide not only neutralizes FA, which is the aim, but also attacks neutral oil by saponification, leading to reduced output and the formation of MAG and DAG in neutral oil. From an economic viewpoint, neutralization with soda in a liquid-liquid biphasic medium cannot be applied to oils having acidity >15% (4) because the quantity of neutral oil that is saponified becomes very significant. In addition, soda eliminates some of the principal components responsible for oxidative stability, such as tocopherols (5), and can involve losses of sterols, which constitute 30–60% of the unsaponifiables. Total sterol losses can be as much as 15%.

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 soda. All of these reactions limit the use of these oils as food. Techniques generally used to neutralize edible oils do not apply to oils having very high acidities. In the present work we describe a new technique to neutralize vegetable oils with very high acidities using lime in a slightly-hydrated liquid-solid medium.

MATERIALS AND METHODS

Neutralization. Neutralization with soda or lime 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 with soda or lime.

Oil (20 g) and the necessary amount plus 10% excess of neutralizing agent [sodium hydroxide or lime containing 69% $Ca(OH)_2$], 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 amount of oil neutralized with lime and extracted by centrifugation was >80%. The separation of the neutralized oil was achieved by centrifuging at 1957 × *g* for 1 h.

Analyses. To characterize the oils used in this work, we measured acidity (6), saponification value (7), iodine value (8), and amount of unsaponifiable matter (9).

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(i) FA composition by GC–MS. The FA compositions of oils were measured as FAME by using a Trio 1000 (Fisons, Manchester, England) gas chromatograph-mass spectrometer (10). The capillary column was CARBOWAX (Fisons), 20 m in length, and with helium as the carrier gas. The column temperature was held at 60°C for 10 min and then increased to 300°C at a rate of 10°C/min. The eluted FAME were identified by MS.

(ii) TAG composition. The oil sample (2 g) was dissolved in acetone in a 25-mL volumetric flask. Acetone was then added, with swirling, to fill to the mark, and 25 µL of the solution was analyzed. The analyses were carried out by using a JASCO PU 980 HPLC (Hachioji, Tokyo, Japan) under the following conditions (11): UV detector (λ = 210 nm), C18 column in reversed phase, 250 mm length, 4 mm i.d., 5 µm film thickness, acetone/acetonitrile (50:50 vol/vol) eluant, and 1 mL/min liquid phase flow rate.

Analysis of chlorophyll. The chlorophyll contents of the oils were determined by visible spectrophotometry (12). The oil sample was dissolved in carbon tetrachloride, and the solution was filtered through Whatman #3 filter paper before absorbance was measured.

Determination of tocopherol composition by HPLC. The normal phase-HPLC (NP-HPLC) procedure was based on AOCS Official Method Ce 8-89 (13). The sample was dissolved in hexane and injected into a NP silica-based column (Alltima Silica 5U, 250 mm × 4.6 mm i.d.; particle size: 5 µm; Alltech, Lokeran, Belgium). The mobile phase consisted of hexane to which a small amount of isopropanol was added, and its flow rate was 1.2 mL/min (= 32 bar column pressure). The chromatograph was a Hewlett-Packard (series 1050; Palo Alto, CA), equipped with a UV detector set at 292 nm and a sample loop of 10 µL.

The tocopherol content was measured in all samples. The percentage loss (14) in neutralized, artificially acidified oils was calculated with respect to the virgin olive oil (reference) used to prepare the artificially acidified oil. In the case of the neutralized olive-residue oil, calculation of the real loss of tocopherols must take into account the initial amount of tocopherols in this oil, the initial acidity, and the yield after neutralization. A theoretical percentage of tocopherols was calculated assuming that the amount lost will be totally recovered in the mass amount of the olive-residue oil after neutralization.

Determination of sterol composition by GC. Oils were analyzed by using a Shimadzu 14 B gas chromatograph (Kyoto, Japan) under the following conditions (15): 25 m length capillary tube; 0.25 mm column i.d., 0.25 mm film thickness; Permabond SE 54 (Macherey-Nagel, Düren, Germany) fixed phase; 300°C injector temperature; 265°C column temperature; and helium carrier gas. The sterols were identified by comparison with the sterols of the reference oil analyzed under the same conditions.

Thermal and oxidative properties. (i) Thermal analysis by DSC. DSC was used to study the effects of heat treatment on initial degradation temperatures of oils (16). DSC profiles were obtained by using a NETZSCH-204 DSC (Waldkraiburg, Germany). The temperature program was initiated at 20°C and increased at 10°C/min to 600°C (17).

(ii) Oxidative stability. A Metrohm (Herisau, Switzerland) Rancimat model 679 was used to measure the oxidative stability of oils (18). Five grams of oil was introduced into each reaction tube, and the temperature was fixed at 100°C with an air flow regulated to 20 L/h for 48 h.

RESULTS AND DISCUSSION

Analysis of the lipid composition. (i) Physicochemical stability. The principal physicochemical properties of the oils neutralized with lime and soda are listed in Table 1. The yields of oils

TABLE 1
Chemical Characteristics of Neutralized Olive Oils[a]

Analysis	Reference virgin olive oil	Oil neutralized with soda[b]	Oil neutralized with lime[b]	Oil neutralized with soda[c]	Oil neutralized with lime[c]
Neutralization yields (%)	—	91.8	90.5	80.0	80.0
		(6.4)	(6.3)	(5.6)	(5.3)
Acidity (mass %)	0.50	0.08	0.08	0.10	0.20
	(0.07)	(0.01)	(0.01)	(0.01)	(0.03)
Saponification value (mg of KOH/g of fatty substance)	191.44	175.31	185.13	184.22	188.83
	(5.08)	(4.65)	(4.91)	(4.89)	(5.01)
Iodine value (g of iodine/100 g of fatty substance)	107.86	98.66	106.59	90.94	89.88
	(0.95)	(0.87)	(0.94)	(0.80)	(0.79)
PV (mequiv/kg of fatty substance)	11.00	13.00	19.00	17.50	18.00
	(0.39)	(0.46)	(0.67)	(0.62)	(0.64)
Amount of unsaponifiable matter (mass %)	0.45	0.42	0.45	1.20	1.30
	(0.01)	(0.01)	(0.01)	(0.03)	(0.04)

[a] Mean of three replicates with SD in parentheses.
[b] Artificially acidified oil.
[c] Olive-residue oil.

TABLE 2
FA Compositions of Neutralized Olive Oils[a] (mass %)

OIL	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1
Reference virgin olive oil	21.33	3.33	0.03	0.07	2.66	51.37	17.20	0.55	0.35	0.16
	(1.25)	(1.93)	(< 0.01)	(<0.01)	(0.11)	(0.56)	(1.54)	(0.19)	(0.04)	(0.01)
Oil neutralized with soda[b]	24.11	3.10	0.03	0.07	2.79	51.58	12.94	0.53	0.40	0.16
	1.42)	(1.80)	(< 0.01)	(<0.01)	(0.11)	(0.56)	(1.15)	(0.18)	(0.05)	(0.01)
Oil neutralized with lime[b]	20.82	2.94	0.03	0.07	2.69	51.69	15.32	0.52	0.39	0.16
	(1.22)	(1.71)	(< 0.01)	(<0.01)	(0.11)	(0.56)	(1.37)	(0.18)	(0.05)	(0.01)
Crude olive-residue oil	17.35	2.01	0.04	0.07	2.64	60.57	16.46	0.53	0.40	0.26
	(1.02)	(1.16)	(< 0.01)	(<0.01)	(0.11)	(0.66)	(1.47)	(0.18)	(0.05)	(0.02)
Oil neutralized with soda[c]	13.88	1.64	0.05	0.07	2.58	64.22	16.20	0.52	0.40	0.24
	(0.81)	(0.95)	(< 0.01)	(<0.01)	(0.11)	(0.70)	(1.45)	(0.18)	(0.05)	(0.02)
Oil neutralized with lime[c]	14.19	1.78	0.06	0.08	2.56	63.83	16.43	0.58	0.44	0.19
	(0.83)	(1.03)	(< 0.01)	(<0.01)	(0.10)	(0.70)	(1.47)	(0.20)	(0.05)	(0.02)

[a] Mean of three replicates with SD in parentheses.
[b] Artificially acidified oil.
[c] Olive-residue oil.

neutralized with lime were similar to those neutralized with soda. We noted, however, reduced yields of neutralized olive-residue oils, which we attributed to more neutral oil being entrained with the soaps because of the high acidities (30%) of these oils. All neutralized oils had low acidities.

The reduction of the saponification value (SV) of the neutralized oils, compared with the reference, was attributed to the hydrolysis of TAG. Comparison between the SV results proved that hydrolysis was more significant in the case of soda neutralization than of lime neutralization. The reduction in iodine value (IV), which measures the degree of unsaturation, was 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 PV confirmed these conclusions and showed quantities of active oxygen in the two neutralized samples compared with the reference oil. This increase was more significant in the case of the neutralization with lime; however, these values remained low and did not affect the quality of neutralized oil because they conformed with international standards adopted for vegetable oils (20).

The neutralized olive-residue oils had low acidities. The olive-residue oil neutralized with soda had lower SV than oils neutralized with lime. This indicated again that the hydrolysis of TAG was more significant in the case of soda neutralization. The amount of unsaponifiable matter in the neutralized olive-residue oils was relatively higher than that of the neutralized, artificially acidified oils.

In light of these initial analyses, it appears that the principal physicochemical characteristics of olive-residue oils were slightly modified during neutralization. These modifications were less in samples neutralized with lime than in those neutralized with soda.

(ii) FA composition. The FA compositions of the different oils, before and after neutralization, are shown in Table 2. The percentages of linoleic acid in the neutralized, artificially acidified oils were lower than those in the reference virgin olive oil. This reduction was more important with soda neutralization.

Contrary to what was observed with neutralized, artificially acidified oils, the percentage of linoleic acid (C18:2n-6) remained practically unchanged in the neutralized olive-residue oils compared with that of the crude oil. A reduction in the percentage of palmitic acid (C16:0) was noted in the neutralized oils, as well as a very clear increase in the percentage of oleic acid (C18:1n-9). Most of the free acidity of crude olive-residue oil was attributable to palmitic acid.

(iii) Analysis of TAG. The TAG compositions of the neutralized oils (Table 3) were consistent with those of the acid composition. Indeed, the reduction in linoleic acid content of the neutralized, artificially acidified oils 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, where L = linoleic acid, Ln = linolenic acid, O = oleic acid, P = palmitic acid, and A = arachidic acid). 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. Neutralization with lime reduced hydrolysis.

The acidic compositions of the neutralized olive-residue oils were completely reflected in their TAG compositions. We also observed that the percentages of TAG having linoleic acid in the internal position were slightly higher in the olive-residue oil neutralized with lime.

The neutralization of olive-residue oils having very high acidity with lime or soda was not accompanied by saponification of TAG. The absence of saponification can be explained by the trapping of the excess alkaline agent in the soapy phase, which was very abundant when oils with very high acidity were neutralized. Diluting oil with hexane at the beginning of neutralization also contributed to limiting saponification reactions of TAG.

Analysis of the composition of minor constituents. *(i) Quantitative proportioning of chlorophyll.* The colors and flavors of

TABLE 3
TAG Compositions of Neutralized Olive Oils (mass %)

ECN	TAG	Reference virgin olive oil	Oil neutralized with soda[b]	Oil neutralized with lime[b]	Oil neutralized with soda[c]	Oil neutralized with lime[c]
42	LLL	0.63	0.35	0.60	0.80	0.83
42	LnLO	0.30	0.20	0.28	0.45	0.49
42	LnLP	0.17	0.17	0.17	0.16	0.15
44	LLO	5.49	5.06	5.44	6.02	5.88
44	LnOO	4.28	4.24	4.21	3.16	3.14
44	PLL	0.46	0.57	0.49	0.62	0.64
46	LOO	15.79	15.99	15.85	18.05	17.89
46	LOP	15.74	15.69	15.87	11.45	11.35
46	PLP	2.63	2.49	2.52	1.41	1.38
48	OOO	19.35	19.65	19.58	28.09	27.76
48	POO	24.19	24.13	24.21	20.23	20.17
48	POP	5.84	5.65	5.75	3.81	3.87
50	ALO	0.26	0.14	0.14	0.29	0.40
50	SOO	3.45	3.37	3.38	3.94	4.64
50	SOP	1.36	1.33	1.35	1.45	1.20

[a] Mean of three replicates.
[b] Artificially acidified oil.
[c] Olive-residue oil.; L, linoleic acid; Ln, linolenic acid; O, oleic acid; P, palmitic acid; A, arachidic acid; S, stearic acid.

olive oils are partly affected by their chlorophyll concentrations (21). Chlorophyll has antioxidant activity in darkness and prooxidant activity in light. Chlorophyll contents were reduced during neutralization of the artificially acidified olive oils (Table 4). This reduction was much more important when neutralizing with soda (80%) than with lime (8%).

The presence of chlorophylls in olive oils is desirable, however, their concentration should not exceed 20 ppm because at that level chromophoric pigments, particularly chlorophylls, promote photo-oxidation, which can cause oil deterioration. The contents of chlorophyll pigments were lower than the limits (<20 ppm) required by international standards (20).

The chlorophyll content of neutralized olive-residue oils was relatively higher than that of neutralized artificially acidified oils. These neutral oils were yellow when treated with soda and yellow-green when treated with lime. We also noted that most of the pigments and solid particles were eliminated from the oily phase during neutralization and were recovered in the soapy phase. The contents of chlorophyll pigments in the neutralized olive-residue oils were also lower than the limits (<20 ppm) of international standards (20).

(ii) Tocopherols. Tocopherols possess vitamin and natural antioxidant activities. The amounts of tocopherol in oils before and after neutralization are shown in Table 4. Neutralizing the artificially acidified oil and the olive-residue oil reduced the tocopherol contents. This reduction, determined as percentage of loss (14), was more important during neutralization of olive-residue oil. Indeed, this oil was more acid and neutralization generated more soaps. Thus, the quantity of tocopherols removed by the soaps was important. Tocopherol losses were lower when neutralizing with lime. Similar results were reported by Jung *et al.* (22), who showed that removing FFA decreases oil stability by decreasing antioxidant contents, such as tocopherols, during the neutralization of different vegetable oils with soda.

TABLE 4
Amounts of Chlorophyll and Tocopherols in Neutralized Olive Oils[a]

Minor component	Reference virgin olive oil	Oil neutralized with soda[b]	Oil neutralized with lime[b]	Crude olive-residue oil	Recovered olive-residue oil	Oil neutralized with soda[b]	Oil neutralized with lime[c]
Chlorophyll (ppm)	1.32	0.18	1.22	17.70	—	9.82	15.91
	(0.07)	(0.01)	(0.09)	(1.25)	(0.73)	(1.13)	
α-Tocopherol (ppm)	220.00	97.57	208.18	218.18	272.72[d]	94.93	217.60
	(9.66)	(4.28)	(9.14)	(9.58)		(4.16)	(9.55)
γ-Tocopherol (ppm)	61.70	47.80	52.50	52.6	65.75[d]	26.29	40.90
	(1.35)	(1.04)	(1.14)	(1.15)		(0.57)	(0.89)
Total tocopherols (ppm)	281.70	145.37	260.68	270.78	338.47[d]	121.22	258.50
α-Tocopherol losses (%)	—	55.65	5.37	—	—	65.19	20.21
Total tocopherols losses (%)	—	48.39	7.46	—	—	64.18	23.62

[a] Mean of three replicates and SD is given in parentheses.
[b] Artificially acidified oil.
[c] Olive-residue oil.
[d] Calculated percentage in recovered olive-residue oil.

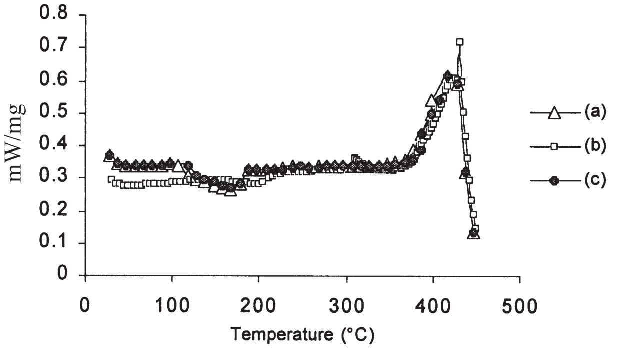

FIG. 1. DSC curves of (a) acid olive oil neutralized with soda, (b) acid olive oil neutralized with lime, and (c) virgin olive oil.

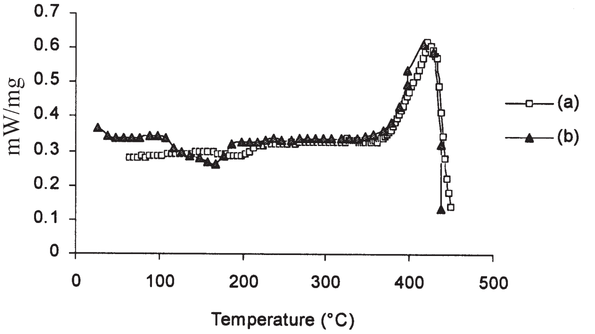

FIG. 2. DSC curves of (a) olive-residue oil neutralized with soda and (b) olive-residue oil neutralized with lime.

α-Tocopherol represents nearly 80% of the total content of tocopherols and, as an antioxidant, has a significant protective effect. Considerable loss in α-tocopherol content was observed when neutralizing with soda. The use of lime as a neutralizing agent for the olive-residue oil preserved 80% of the α-tocopherol. Amounts of tocopherol in oils neutralized with lime conform with the international standards (>200 mg/kg of α-tocopherol) adopted for the edible olive oils (20).

Thermal and oxidative stability of neutralized oils. (i) Thermal analysis by DSC. The thermal stabilities of the oils are shown in Figure 1. The DSC thermograms exhibited three distinct phases: (i) an initial loss in mass as temperature approached 100°C, which was related to the loss of absorbed water; (ii) a second loss in mass between 250 and 350°C owing to the vaporization of volatile products; and (iii) a third loss in mass corresponding to the decomposition of oil, which appeared in all thermograms starting at 350°C.

We noticed a slight difference in the values of thermal decomposition temperatures of the oils. The oil neutralized with lime had a temperature of decomposition of 365°C, which was slightly higher than that of the oils neutralized with soda (350°C). The two temperatures of decomposition were lower than that of the reference oil (380°C). The neutralized olive-residue oils (Fig. 2) had a decomposition temperature of nearly 360°C.

(ii) Oxidative stability. The resistance to oxidation, in hours, of the neutralized olive oils was as follows (mean ± SD, $n = 3$): reference olive oil 14.20 ± 0.95; artificially acidified oil neutralized with soda: 7.78 ± 0.52; artificially acidified oil neutralized

TABLE 5
Sterol Compositions of Neutralized Olive Oils[a] (mass %)

Sterol	Reference virgin olive oil	Oil neutralized with soda[b]	Oil neutralized with lime[b]	Oil neutralized with soda[c]	Oil neutralized with lime[c]
β-Sitosterol	82.16	78.85	82.15	80.59	82.78
	(5.55)	(5.33)	(5.55)	(5.45)	(5.60)
Δ-5-Avenasterol	10.68	13.14	11.15	9.01	9.78
	(0.78)	(0.96)	(0.81)	(0.66)	(0.71)
Campesterol	2.60	1.03	2.80	3.87	3.77
	(0.77)	(0.30)	(0.83)	(1.14)	(1.11)
Stigmasterol	1.00	0.23	0.88	0.50	1.09
	(0.15)	(0.03)	(0.13)	(0.07)	(0.16)
Chlerosterol	0.91	—	1.03	1.17	1.13
	(0.43)		(0.49)	(0.56)	(0.54)
Sitostanol	0.85	—	0.66	0.12	0.15
	(0.11)		(0.08)	(0.01)	(0.02)
Cholesterol	0.11	0.26	0.15	0.32	0.23
	(0.01)	(0.03)	(0.02)	(0.04)	(0.03)
24-Methylene-cholesterol	0.13	0.76	0.15	0.13	0.12
	(0.01)	(0.10)	(0.02)	(0.01)	(0.01)
Δ-5,24-Stigmastadienol	0.30	0.58	0.30	0.21	0.19
	(0.04)	(0.07)	(0.04)	(0.02)	(0.02)
Δ-7-Stigmastenol	0.42	0.52	—	0.40	0.50
	(0.08)	(0.08)		(0.08)	(0.08)
Δ-7-Avenasterol	0.78	1.56	0.70	0.54	0.71
	(0.16)	(0.32)	(0.14)	(0.11)	(0.14)

[a] Mean of three replicates and SD in parentheses.
[b] Artificially acidified oil.
[c] Olive-residue oil.

with lime, 2.05 ± 0.87; oil-residue oil neutralized with soda, 6.08 ± 0.40; olive-residue oil neutralized with lime, 8.20 ± 0.55. Thus reduction was much greater when oils were neutralized with soda. The artificially acidified oil neutralized with soda (having lost more tocopherols) was less resistant to oxidation than was the oil neutralized with lime. The two neutralized oils were less resistant to oxidation than the untreated reference oil.

The neutralized olive-residue oils were less resistant to oxidation than the artificially acidified neutralized oils. The olive-residue oil sample neutralized with lime, however, was more resistant to oxidation than the oil neutralized with soda.

Lime is better suited to the neutralization of vegetable oils than soda because it preserves the FA composition of TAG and the composition of minor constituents, particularly the antioxidants. The biological activity of vegetable oil is related mainly to its minor components and, more particularly, to the sterol fraction.

Sterols. The sterol compositions of the oils are shown Table 5. β-Sitosterol was the most abundant sterol in all samples, followed by Δ-5-avenasterol and campesterol. The sterol contents of the oils neutralized by lime were not affected by neutralization (Table 5). Neutralization with soda reduces the β-sitosterol content and affects the biological value of the oil. β-Sitosterol, stigmasterol, and campesterol are phytosterols that possess hypocholesterolemic activity. When neutralizing with soda, β-sitosterol, the main sterol, remained virtually the same, but stigmasterol and campesterol fell. The sterol content did not change with lime neutralization. Jung *et al.* (22) and Lanzon *et al.* (23) studied the sterol composition of soybean and corn oils during their neutralization with soda and showed that a large portion of sterols, mainly phytosterols, was lost with the soaps. Cholesterol was also present in very small quantities (Table 5). Previous studies also identified the presence of cholesterol in virgin olive oils (24,25); however, the presence of cholesterol in very small quantity does not affect the food quality.

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