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Original Research Article

INFLUENCE OF THE NEUTRALIZATION STEP ON THE HYDROLYSIS STABILITY OF ACID OLIVE OIL

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Abstract

The aim of this study was to investigate the effects of neutralizing process, using soda or lime, on fatty acid and triglyceridic compositions. 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 on the physicochemical properties of treated oils. A study, bearing on the hydrolysis resistance of neutralized oils, was also carried out.

The fatty acid composition (GC-MS) and the triacylglycerol composition (HPLC) allowed us to conclude that oils, neutralized with soda or lime, do not undergo considerable physicochemical alterations and remain corresponding to the international standards for refined oils for human consumption.

Keywords: Olive oil, oil stability, fatty acids, triacylglycerols, neutralization.

1. Introduction

Vegetable 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. 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 [2, 3] and chemical refining with soda (sodium hydroxide). 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.

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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 a previous work we have describe a new technique to neutralize olive oils with very high acidities (10-30%) using lime in a slightly-hydrated liquid-solid medium [5].

The objective of this study is to elucidate the modification that could undergo the olive oil during the neutralization step. 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.

Neutralization with soda or lime 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 with soda or lime.

2. Materials and methods

2.1. 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.

2.2. Analyses

2.2.1. Current 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].

2.2.2. 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 300ºC at a rate of 10°C/min. The eluted FAME were identified by MS.

2.2.3. 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 (l = 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.

3. Results and discussion

The physicochemical stability of the acidified olive oil was after neutralization with soda or lime.

3.1. Neutralization of artificially acidified olive oil

3.1.1. Physicochemical stability of neutralized oils

The physicochemical stability was evaluated by measuring the current index of the olive oils. The principal physicochemical properties of the oils neutralized with lime and soda are listed in Table 1. The yields of oils neutralized with lime were similar to those neutralized with soda. All 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 since the neutralized oils result from the same virgin olive oil. We noted that the hydrolysis was more significant in the case of the neutralization with soda than that with lime.

The reduction in iodine value (IV), which measures the degree of unsaturation, was more significant in the case of soda neutralization. This reduction could 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 [12].

In light of these analyses, it appears that the principal physicochemical characteristics of olive oil were slightly modified after artificial acidification followed by FA neutralization.

3.1.2. Determination of the Fatty Acid 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.

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 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.

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 [13, 14]. The distribution, expressed as a molar percentage, of each fatty acid in internal and external positions in the TAG is summarized in Table 4.

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 5 are in concord with the experimental results that were reported by HPLC (Table 3). 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 Tables 5, 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 [15, 16].

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 6, 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 [17].

3.2. Neutralization of olive-residue oil

3.2.1. Physicochemical stability of neutralized oils

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.

3.2.2. Determination of the Fatty Acid Composition

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.

3.2.3. Determination of the Triglyceridic Composition

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.

3.2.4. Determination of the Theoretical Triacylglycerols (ECN42)

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 olive-residue oils are in conformity with the international standards adopted for olive oils, the highest difference between them did not exceed 0.5 (*28*).

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. Neutralization with lime reduced hydrolysis.

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 olive-residue oils were completely reflected in their TAG compositions. 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 olive-residue oils are in conformity with the international standards adopted for olive oils.

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Table 1: *Chemical characteristics[a] of neutralized artificially acidified olive oils*

Analysis	Reference virgin olive oil	Oil neutralized with soda	Oil neutralized with lime
1. Neutralization yields (%)	—	91.8 (6.4)	90.5 (6.3)
2. Acidity *(mass %)*	0.50 (0.07)	0.08 (0.01)	0.08 (0.01)
Saponification value *(mg of KOH/g of fatty substance)*	191.44 (5.08)	175.31 (4.65)	185.13 (4.91)
Iodine value *(g of iodine/100g of fatty substance)*	107.86 (0.95)	98.66 (0.87)	106.59 (0.94)
Amount of unsaponifiable matter *(mass %)*	0.45 (0.01)	0.42 0.01)	0.45 (0.01)

[a] Mean of three replicates with SD in parentheses.

Table 2: *Fatty acid (FA) compositions of neutralized artificially acidified olive oils* [a] *(mass %)*

	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$
Reference virgin olive oil	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)
Oil neutralized with soda	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)
Oil neutralized with lime	20.82 (1.22)	2.94 (1.71)	0.03 (<0.01)	0.07 (<0.01)	2.69 (0.11)	51.69 (0.56)	15.32 (1.37)	0.52 (0.18)	0.39 (0.05)	0.16 (0.01)

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

Table 3: ***Analysis of Triacylglycerols (TAG) by HPLC in neutralized artificially acidified olive oils*** [a] ***(mass %)***

E.C.N.	**TAG**	Reference virgin olive oil	Oil neutralized with soda	Oil neutralized with lime
42	**LLL**	**0.63**	**0.35**	**0.60**
42	**LnLO**	**0.30**	**0.20**	**0.28**
42	**LnLP**	**0.17**	**0.17**	**0.17**
44	**LLO**	**5.49**	**5.06**	**5.44**
44	**LnOO**	**4.28**	**4.24**	**4.21**
44	**PLL**	**0.46**	**0.57**	**0.49**
46	**LOO**	**15.79**	**15.99**	**15.85**
46	**OLP**	**15.74**	**15.69**	**15.87**
46	**PLP**	**2.63**	**2.49**	**2.52**
48	**OOO**	**19.35**	**19.65**	**19.58**
48	**POO**	**24.19**	**24.13**	**24.21**
48	**POP**	**5.84**	**5.65**	**5.75**
50	**ALO**	**0.26**	**0.14**	**0.14**
50	**SOO**	**3.45**	**3.37**	**3.38**
50	**SOP**	**1.36**	**1.33**	**1.35**

[a] Mean of three replicates.

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

Table 4: ***Fatty acid distribution in internal and external positions of the TAG in neutralized artificially acidified olive oils***

Fatty acid (%)	**Reference virgin olive oil**		Oil **neutralized with soda**		**Oil neutralized with lime**	
	Positions (1,3)	**Position (2)**	**Positions (1,3)**	**Position (2)**	**Positions (1,3)**	**Position (2)**
P	**34.861**	**1.423**	**39.866**	**1.627**	**34.939**	**1.426**
S	**3.917**	**0.160**	**4.155**	**0.169**	**4.066**	**0.166**
Po	**3.295**	**5.296**	**2.808**	**4.926**	**2.806**	**4.527**
O	**43.108**	**69.298**	**42.095**	**73.84**	**44.469**	**71.733**
L	**14.544**	**23.375**	**10.641**	**18.660**	**13.270**	**21.406**
Ln	**0.466**	**0.750**	**0.438**	**0.768**	**0.459**	**0.713**

Table 5: ***Theoretical distribution of fatty acids in TAG of neutralized artificially acidified olive oils***

TAG	Reference virgin olive oil	Oil neutralized with soda	Oil neutralized with lime
LLL	**0.494**	**0.214**	**0.380**
PoLL	**0.036**	**0.166**	**0.139**
PoPoL	**0.075**	**0.043**	**0.050**
OLLn	**0.282**	**0.206**	**0.258**
PLLn	**0.154**	**0.131**	**0.136**
PoOLn	**0.044**	**0.054**	**0.054**
SLnLn	**0.000**	**0.000**	**0.000**
PPoLn	**0.034**	**0.034**	**0.029**
ECN 42	**1.119**	**0.848**	**1.046**

Table 6: ***The difference between the real and the theoretical composition of TAG with ECN 42 in neutralized artificially acidified olive oils***

TAG	Reference virgin olive oil	Oil neutralized with soda	Oil neutralized with lime
LLL	**0.494**	**0.214**	**0.380**
LnLO	**0.282**	**0.206**	**0.258**
LnLP	**0.154**	**0.131**	**0.136**
∑ % ECN 42 real composition	**0.930**	**0.551**	**0.774**
∑ % ECN 42 theoretical composition	**1.119**	**0.848**	**1.046**
∆ ECN 42	**0.189**	**0.297**	**0.272**

Table 7: *Chemical characteristics*[a] *of neutralized olive-residue oils*

Analysis	Reference virgin olive oil	Oil neutralized with soda	Oil neutralized with lime
3. Neutralization yields (%)	—	80.0 (5.6)	80.0 (5.3)
4. Acidity *(mass %)*	0.50 (0.07)	0.10 (0.01)	0.20 (0.03)
Saponification value *(mg of KOH/g of fatty substance)*	191.44 (5.08)	184.22 (4.89)	188.83 (5.01)
Iodine value *(g of iodine/100g of fatty substance)*	107.86 (0.95)	90.94 (0.80)	89.88 (0.79)
Amount of unsaponifiable matter *(mass %)*	0.45 (0.01)	1.20 (0.03)	1.30 (0.04)

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

Table 8**: *Fatty acid (FA) compositions of neutralized olive-residue oils*** [a] ***(mass %)***

	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$
Reference virgin olive oil	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)
Crude olive-residue oil	17.35 (1.02)	2.01 (1.16)	0.04 (<0.01)	0.07 (<0.01)	2.64 (0.11)	60.57 (0.66)	16.46 (1.47)	0.53 (0.18)	0.40 (0.05)	0.26 (0.02)
Oil neutralized with soda	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)
Oil neutralized with lime	14.19 (0.83)	1.78 (1.03)	0.06 (<0.01)	0.08 (<0.01)	2.56 (0.10)	63.83 (0.70)	16.43 (1.47)	0.58 (0.20)	0.44 (0.05)	0.19 (0.02)

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

Table 9: ***Analysis of Triacylglycerols (TAG) by HPLC in neutralized olive-residue oils***[a] ***(mass %)***

E.C.N.	**TAG**	Reference virgin olive oil	Oil neutralized with soda	Oil neutralized with lime
42	**LLL**	**0,63**	**0,80**	**0,83**
42	**LnLO**	**0,30**	**0,45**	**0,49**
42	**LnLP**	**0,17**	**0,16**	**0,15**
44	**LLO**	**5,49**	**6,02**	**5,88**
44	**LnOO**	**4,28**	**3,16**	**3,14**
44	**PLL**	**0,46**	**0,62**	**0,64**
46	**LOO**	**15,79**	**18,05**	**17,89**
46	**OLP**	**15,74**	**11,45**	**11,35**
46	**PLP**	**2,63**	**1,41**	**1,38**
48	**OOO**	**19,35**	**28,09**	**27,76**
48	**POO**	**24,19**	**20,23**	**20,17**
48	**POP**	**5,84**	**3,81**	**3,87**
50	**ALO**	**0,26**	**0,29**	**0,40**
50	**SOO**	**3,45**	**3,94**	**4,64**
50	**SOP**	**1,36**	**1,45**	**1,20**

[a] Mean of three replicates.

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

Table 10**: *Theoretical distribution of fatty acids in TAG of neutralized olive-residue oils***

TAG	Oil **neutralized**	Oil **neutralized**
LLL	**0,400**	**0,440**
PoLL	**0,135**	**0,150**
PoPoL	**0,015**	**0,019**
LnLO	**0,299**	**0,340**
LnLP	**0,043**	**0,090**
PoOLn	**0,033**	**0,040**
SLnLn	**0,000**	**0,000**
PoLnP	**0,008**	**0,007**
ECN 42	**0.933**	**1.086**

Tableau 11: ***The difference between the real and the theoretical composition of TAG with ECN 42 in neutralized olive-residue oils***

	Oil **neutralized with soda**	Oil **neutralized with lime**
LLL	**0,800**	**0,830**
LnLO	**0,450**	**0,490**
LnLP	**0,160**	**0,150**
∑ % ECN 42 real composition	**1.410**	**1.470**
∑ % ECN 42 theoretical composition	**0.933**	**1.086**
∆ ECN 42	**0,477**	**0,384**