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Influence of Picual Olive Ripening on Virgin Olive Oil Alteration and Stability during Potato Frying

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ABSTRACT: Ripening modifies oil attributes and composition. However, the influence of olive ripening on virgin olive oil (VOO) thermal oxidative stability on food-frying has not been studied yet. Oils from Picual olives of low (VOO1), medium (VOO2), and high (VOO3) ripeness were obtained, and their thermal oxidative stability during 40 potato-fryings was tested. Unused VOO1 showed higher antioxidant content and oxidative stability than VOO2 and VOO3. Polar compounds (PC), oligomers, and altered fatty acid methyl esters (polar-FAME) increased, whereas linoleic acid, polyphenols, and tocopherols decreased in the three VOOs through frying. The alteration was lower in VOO1, followed by VOO2 (0.105, 0.117, and 0.042 g/100 g oil less of PC, oligomers and polar-FAME per frying, respectively, in VOO1 than in VOO3). In conclusion, VOO obtained from low-ripeness Picual olives should be preferred when frying fresh-potatoes due to its higher thermal and oxidative stability, permitting a higher number of potato-frying uses.

KEYWORDS: *olive fruit, ripening, frying, potatoes, thermal oxidation, oxidative stability, virgin olive oil*

INTRODUCTION

Frying is one of the oldest and most popular methods of cooking food.[1] Although frying seems rather a simple process of dehydration accompanied by entry of hot oil into the food and superficial browning, a complex series of various chemical reactions depending on the food, the oil, and the process takes place.[1,2] During frying, oil or fat is subjected to high temperatures in the presence of air and water from the food, thus producing a wide range of compounds resulting from thermal, oxidative, and hydrolytic reactions.[3,4] Therefore, fried foods obtained from inappropriate oil may contain high levels of thermally oxidized and polymerized products that could be undesirable from a nutritional point of view[1,3,5−7] because some of those potentially toxic compounds are to some degree absorbed.[5,8,9] Oil selection is a complex issue, influenced by factors such as price, taste, health, and efficiency.[1,10−12] In such selection, fatty acids and minor compound profiles are of main importance to get an adequate balance between nutrition and stability. Thus, oils very rich in essential fatty acids can be adequate when used at raw conditions but become very unstable at high temperatures, whereas very stable oils at high temperatures could be inadequate from a nutritional point of view as they would enrich food with saturated fatty acids (SFA).[3,4,6−9]

Among vegetable oils, olive oil and virgin olive oil (VOO), in particular, are considered premium oils from both points of view, nutritional[12−15] and thermal stability,[1,3,16,17] both amply related to their fatty acid profiles and the presence of polyphenols and other antioxidants.[11,18,19] Stability and frying oil performance are affected by many factors such as cultivar, geographical origin, olive ripening, processing, and storage that influence minor compounds (e.g., tocopherols, polyphenols, phytosterols) and fatty acid profiles and concentrations.[19−21] Thus, oils obtained from green olives normally contain higher amounts of antioxidants such as total polyphenols (e.g., oleouropein, hydroxytyrosol).[20−22]

Polar compound (PC) content and the level of different thermal oxidation and hydrolytic compounds are good quality indicators of frying fats' alteration.[23] Most European countries have set a maximum PC level of 25%. They have also indicated that oils with 10% oligomer (polymers plus dimers of triglycerides) content should be discarded. Nonetheless, in other countries the limit for oil discarding has been set at 12% oligomer level.[24] To obtain broader information on fat alteration, analysis of oxidized and polymerized fatty acids included in the triglyceride molecules has been proposed.[1,3,25] According to previous studies of our group, 25% PC corresponds to 9−11% of polar methyl esters (polar-ME).[1,25]

Although olive ripeness modifies the content and profile of all oil components, those of minor ones are also affected.[20,21] This has nutritional and technological importance and deserves research looking for the most adequate stage for olive gathering and oil uses. To the best of our knowledge, the influence of the olive ripening index on the oil stability during foodstuff frying

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has not been studied yet. This study hypothesizes that VOO obtained from Picual olives of lower ripening index shows higher stability and lower thermal oxidation than VOO from higher ripening index counterparts when used in repeated frying operations of fresh potatoes. Thus, the aim of the present study was to evaluate the effect of olive ripeness on the thermal oxidation stability during frying of fresh potatoes of VOO obtained from Picual cultivars by quantifying (a) polar compounds, (b) thermal oxidation and hydrolytic compound profiles, (c) polar and nonpolar methyl esters, (d) fatty acid and tocopherol contents and profiles, (e) oxidative stability, and (f) the theoretical number of frying operations at which the three Picual VOOs must be discarded.

MATERIALS AND METHODS

Olive Harvesting, Ripening Index, and Oil Elaboration. Olives of Picual variety were harvested during the 2011/2012 olive season in the Agricultural Experimental Station "La Chimenea", Instituto Madrileño de Investigación y Desarrollo Rural, Agrario y Alimentario (IMIDRA), located in the region of Madrid (Spain).

About 35 kg of olives was hand-picked in perfect sanitary conditions from olives trees at three ripening stages at three weekly intervals from November 19 to December 31. These three ripening stages were selected according to the normal harvesting period for the Picual olive variety. The olive ripening index was determined according to the method described by Uceda and Frias[26] based on the evaluation of the olive skin and pulp colors. Seven maturity states of the fruit (0, bright green skin; 1, green-yellowish skin; 2, green skin with reddish spots; 3, reddish-brown skin; 4, black skin with white flesh; 5, black skin with <50% purple flesh; 6, black skin with ≥50 and >100% purple flesh; and 7, black skin and purple flesh) were used.[22]

Olive oils were extracted using the Abencor system (MC2 Ingenierías y Sistemas, Sevilla, Spain). The oil was decanted, filtered, transferred into 500 mL amber glass bottles, and stored at 4 °C in darkness until analysis. Approximately 4.6 L of VOO was obtained in the first harvesting, 5.0 L in the second, and 6.1 L in the third.

Fresh Potato Frying. Domestic deep-fat fryers of 1.1 L stainless steel vessel equipped with a thermostat (SOLAC, Vitoria, Spain) were used for potato frying. A total of six fryers, two per VOO, were used. Spunta variety fresh potatoes (Valencia, Spain) were fried. The proportion of food to frying oil in the repeated frying was kept at 183 g to 1.1 L by replenishment with unused oil every five frying operations to maintain, insofar as possible, a constant food/oil ratio. Oil was heated to 180 °C for about 10 min. Potatoes were sliced to 2 mm thick using a mandolin slicer and introduced into the oil at 180 °C and fried for 8 min. Oil was allowed to cool until 30−35 °C between same-day fryings. The cooling time was approximately 4 h between frying operations. On the basis of previous studies,[27,28] a total of 40 frying operations at the rate of 4 fryings per day were performed with each VOO. The whole procedure was performed in duplicate by using two fryers for each oil. Thirty-five milliliters of oil from each of the two fryers was taken after 5, 10, 15, 20, 25, 30, 35, and 40 frying operations and kept frozen at −20 °C until analysis.

Fatty Acid Profile. Analysis of the fatty acid composition of oils was performed by GC after derivatization to fatty acid methyl esters (FAME) with 2 N KOH in methanol.[29] FAME were analyzed by GC using a chromatograph (Agilent Technologies 6850 series II network GC system) equipped with one capillary column Supelco 24111 of 60 m × 0.25 mm i.d. × 0.20 μm (Agilent Technologies). Samples were introduced into the column at 170 °C during 30 min; after this time, the temperature was increased at 5 °C/min to 200 °C and maintained for 12 min. The flow rate of He, used as carrier gas, was 0.6 mL/min. Injector and flame ionization detector temperatures were 230 and 250 °C, respectively.

The absolute amount of each individual FAME was calculated by multiplying the percentage of each FAME by the amount of nonoxidized fatty acid monomers (FAM) giving results equivalent to those obtained using internal standard.[30]

Polar Compound Assessment. Total PC contents in the unused oil and used frying oils were determined by gravimetric measurement using silica column chromatography.[23] One gram of sample was separated by column chromatography using 150 mL of hexane/diethyl ether (90:10, v/v) and 150 mL of diethyl ether to elute the nonpolar and polar fractions, respectively.

Quantitation of Thermal Oxidation and Hydrolytic Compounds. To obtain further information about changes due to thermal oxidation and hydrolysis during frying, high-performance size exclusion liquid chromatography (HPSEC) analysis of the PC fraction present in the unused and used frying oils was performed following a slight modification of the AOCS method.[23] Briefly, a sample concentration of the previously isolated PC (10−15 mg/mL tetrahydrofuran) was applied in a high-performance liquid chromatograph (HPLC) (Agilent 1100 series, Madrid, Spain) with a 20 μL sample loop. A refractive index detector (Agilent Technologies 1260 infinity, Madrid, Spain) and two 300 mm × 7.5 mm i.d. (5 μm particle size), 0.01 and 0.05 μm, PL gel columns (Agilent, Bellefonte, PA, USA), connected in series, were operated at 40 °C. HPLC grade tetrahydrofuran was used as the mobile phase with a flow of 1 mL/min. Polymers of triglycerides (PTG), dimers of triglycerides (DTG), oxidized triglycerides (OTG), diglycerides (DG), monoglycerides (MG), and free fatty acids (FFA) were quantified in the PC fraction. Hydrolytic compounds (HC) were calculated as the sum of DG, MG, and FFA, whereas thermally oxidized compounds (TC) were calculated as the sum of PTG, DTG, and OTG. Oligomers (OLIG) were calculated as the sum of DTG and PTG.

Isolation and Quantitation of Altered and Unaltered Fatty Acid Methyl Esters. Samples of the unused and used frying oils were saponified during 10 min with 0.5 N NaOH in methanol. Methylation was performed according to an AOAC method,[31] using 20% BF_3 in methanol, and required 15 min. After methylation, 1 g of sample was separated by column chromatography using 150 mL of hexane/diethyl ether (88:12, v/v) and 150 mL of diethyl ether to elute the nonpolar and polar fractions of methyl esters, respectively.[4,25] Thermal fatty acid dimers (thermal-FAD) and nonoxidized FAM were quantified in the nonpolar fraction, whereas fatty acid polymers (FAP), oxidized fatty acid dimers (oxFAD), and oxidized fatty acid monomers (oxFAM) were quantified in the polar fraction. Aliquot samples of both fractions (10−15 mg/mL tetrahydrofuran) were injected in a HPLC (Agilent 1100 series) with a 20 μL sample loop. The same equipment (refractive index detector and columns) and conditions (temperature and mobile phase flow) as for thermal oxidation and hydrolytic compound quantitations were used.

Oxidative Stability. Oxidative stability was analyzed in unused and used frying oils using the Rancimat equipment at 120 °C (Metrohm Ltd., Herisau, Swiss) with a continuous air flow of 20 L/h passing and 2.5 g oil samples. The inflection point of the curve was assigned as the induction time, measured in hours.[32,33]

Determination of Total Polyphenolic Compounds. Total phenolic compounds in unused and used frying oils were determined after methanol extraction and subsequent reaction with Folin−Ciocalteu reagent and measured in a Lambda 25 UV−vis spectrophotomer (PerkinElmer, Waltham, MA, USA) at a wavelength of 725 nm.[34]

Determination of Tocopherols. The determination of α- and γ-tocopherols present in unused and used frying oils was carried out by HPLC according to IUPAC methods.[35]

Statistical Analyses. All determinations were done in duplicate. The Pearson product−moment correlation test was used to find correlations between parameter data. Linear adjustments between the concentration of polar compounds, different thermal oxidation and hydrolytic compounds, various methyl esters compounds, and frying number were assessed. A stepwise multiple regression procedure was used to identify which of the following parameters, total phenolic content, tocopherol content, oleic, linoleic, and linolenic acids, explained better the extent of VOO thermal oxidation during frying. Regression collinearity diagnostics and regression case wise diagnostics were performed to prevent multicollinearity and influential cases. The SPSS 15.0 statistical packet was employed. Comparisons between linear adjustments for the different compounds in the three VOOs were checked by ANCOVA test using SAS 9.2 statistical package. Statistical significance was set at $p < 0.05$.

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Table 1. Changes (Grams per 100 g of Oil) in the Fatty Acid Profile after 40 Fryings of Fresh Potatoes with Virgin Olive Oils Obtained from Picual Olives of Different Ripeness Indices[a]

acid	VOO1-0	VOO1-40	VOO2-0	VOO2-40	VOO3-0	VOO3-40
myristic (14:0)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
palmitic (16:0)	12.81 ± 0.14	11.55 ± 0.02	10.69 ± 0.07	10.11 ± 0.03	10.88 ± 0.14	10.38 ± 0.20
palmitelaidic (*t* 16:1 n-7)	0.09 ± 0.00	0.09 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
palmitoleic (16:1 n-7)	0.45 ± 0.01	0.98 ± 0.02	0.42 ± 0.01	0.86 ± 0.01	0.40 ± 0.00	0.39 ± 0.01
margaric (17:0)	0.20 ± 0.00	0.04 ± 0.00	0.18 ± 0.00	0.04 ± 0.00	0.19 ± 0.01	0.18 ± 0.00
margaroleic (17:1 n-7)	0.28 ± 0.00	0.08 ± 0.00	0.28 ± 0.00	0.07 ± 0.01	0.27 ± 0.00	0.26 ± 0.01
stearic (18:0)	3.20 ± 0.04	2.56 ± 0.04	3.21 ± 0.01	2.55 ± 0.07	3.51 ± 0.05	3.34 ± 0.08
oleic (18:1 n-9)	70.35 ± 0.68	71.60 ± 0.51	72.16 ± 0.44	70.66 ± 0.18	72.24 ± 0.84	69.60 ± 0.70
α-linoleic (18:2 n-6)	9.39 ± 0.11	2.26 ± 0.02	9.41 ± 0.04	2.68 ± 0.04	9.30 ± 0.11	2.55 ± 0.04
arachidic (20:0)	0.34 ± 0.00	0.45 ± 0.00	0.33 ± 0.00	0.43 ± 0.01	0.35 ± 0.01	0.34 ± 0.01
α-linolenic (18:3 n-3)	0.76 ± 0.02	0.37 ± 0.01	0.77 ± 0.01	0.43 ± 0.02	0.75 ± 0.01	0.50 ± 0.01
gadoleic (20:1 n-9)	0.26 ± 0.00	0.24 ± 0.00	0.29 ± 0.01	0.29 ± 0.02	0.29 ± 0.00	0.27 ± 0.01
behenic (22:0)	0.08 ± 0.00	0.13 ± 0.00	0.08 ± 0.00	0.13 ± 0.00	0.08 ± 0.00	0.09 ± 0.00
lignoceric (24:0)	0.03 ± 0.00	0.07 ± 0.00	0.03 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
SFA	17.07 ± 0.01	16.49 ± 0.15	14.95 ± 0.00	15.20 ± 0.05	15.42 ± 0.03	16.46 ± 0.15
MUFA	72.70 ± 0.03	80.08 ± 0.15	74.66 ± 0.02	81.28 ± 0.12	74.37 ± 0.03	80.08 ± 0.15
PUFA	10.33 ± 0.02	3.47 ± 0.01	10.39 ± 0.02	3.52 ± 0.07	10.21 ± 0.00	3.47 ± 0.01

[a]VOO1, VOO2, and VOO3 correspond to harvesting period; for more details see Material and Methods. Values (mg/100 mg oil) are the mean ± SD of two determinations. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Sample and frying effects were not statistically tested as only duplicates of oil samples when unused (−0) and after 40 fryings (−40) were tested.

Table 2. Changes (Grams per 100 g of Oil) of Polar Compounds and Thermal Oxidation Compounds during 40 Fryings of Fresh Potatoes with Virgin Olive Oils Obtained from Picual Olives of Different Ripeness Indices

frying no.	ripeness[a]	polar content	PTG	DTG	OTG	TC
0	VOO1	1.85 ± 0.08	nd	0.03 ± 0.00	0.37 ± 0.00	0.39 ± 0.00
	VOO2	2.39 ± 0.00	nd	0.03 ± 0.00	0.49 ± 0.01	0.52 ± 0.00
	VOO3	2.54 ± 0.19	nd	0.05 ± 0.02	0.61 ± 0.05	0.67 ± 0.08
5	VOO1	3.03 ± 0.01	0.03 ± 0.01	0.42 ± 0.04	1.38 ± 0.01	1.83 ± 0.04
	VOO2	3.86 ± 0.04	0.04 ± 0.00	0.70 ± 0.07	1.80 ± 0.09	2.54 ± 0.16
	VOO3	4.78 ± 0.33	0.04 ± 0.01	0.72 ± 0.04	1.57 ± 0.11	2.34 ± 0.15
10	VOO1	5.10 ± 0.14	0.10 ± 0.00	1.34 ± 0.03	1.86 ± 0.06	3.30 ± 0.09
	VOO2	6.18 ± 0.25	0.14 ± 0.07	1.56 ± 0.65	2.80 ± 0.67	4.50 ± 1.39
	VOO3	7.27 ± 0.04	0.25 ± 0.02	2.30 ± 0.02	2.88 ± 0.02	5.43 ± 0.02
15	VOO1	7.18 ± 0.11	0.22 ± 0.01	2.16 ± 0.06	3.00 ± 0.07	5.38 ± 0.11
	VOO2	9.94 ± 0.35	0.48 ± 0.11	3.22 ± 0.11	4.07 ± 0.26	7.78 ± 0.48
	VOO3	9.83 ± 0.24	0.38 ± 0.08	3.03 ± 0.25	4.16 ± 0.01	7.57 ± 0.32
20	VOO1	9.00 ± 0.04	0.45 ± 0.04	3.15 ± 0.07	3.58 ± 0.02	7.18 ± 0.05
	VOO2	10.50 ± 0.37	0.53 ± 0.02	3.59 ± 0.13	4.15 ± 0.10	8.28 ± 0.21
	VOO3	11.38 ± 0.30	0.63 ± 0.02	3.89 ± 0.08	4.73 ± 0.31	9.25 ± 0.41
25	VOO1	10.34 ± 0.08	0.61 ± 0.02	3.60 ± 0.05	4.31 ± 0.07	8.53 ± 0.09
	VOO2	12.23 ± 0.11	0.77 ± 0.07	4.06 ± 0.08	5.20 ± 0.08	10.04 ± 0.08
	VOO3	13.93 ± 0.04	1.02 ± 0.07	5.04 ± 0.03	5.79 ± 0.06	11.84 ± 0.16
30	VOO1	11.74 ± 0.08	0.72 ± 0.01	4.06 ± 0.02	5.09 ± 0.05	9.87 ± 0.08
	VOO2	13.11 ± 0.13	0.91 ± 0.04	4.52 ± 0.02	5.36 ± 0.03	10.78 ± 0.09
	VOO3	15.80 ± 0.20	1.35 ± 0.15	5.75 ± 0.10	6.48 ± 0.00	13.59 ± 0.25
35	VOO1	12.99 ± 0.04	0.76 ± 0.25	4.73 ± 0.08	5.44 ± 0.15	10.92 ± 0.02
	VOO2	14.46 ± 0.14	1.24 ± 0.17	5.09 ± 0.11	5.90 ± 0.13	12.23 ± 0.15
	VOO3	17.56 ± 0.26	1.41 ± 0.16	6.19 ± 0.02	7.31 ± 0.04	14.91 ± 0.13
40	VOO1	13.95 ± 0.10	1.17 ± 0.04	5.06 ± 0.11	5.78 ± 0.02	12.00 ± 0.17
	VOO2	14.85 ± 0.21	1.22 ± 0.05	5.13 ± 0.03	6.13 ± 0.04	12.48 ± 0.12
	VOO3	18.59 ± 0.27	1.96 ± 0.04	6.71 ± 0.13	7.58 ± 0.03	16.25 ± 0.20

[a]VOO1, VOO2, and VOO3 correspond to harvesting periods; for more details see text. Values are the mean ± SD of two determinations. PTG, triglyceride polymers; DTG, triglyceride dimers; OTG, oxidized triglycerides; TC, thermal oxidized compounds (PTG, DTG plus OTG); nd, nondetected.

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Table 3. Changes (Grams per 100 g of Oil) in Hydrolytic Compounds and the Thermal Oxidized/Hydrolytic Compounds Ratio during 40 Fryings of Fresh Potatoes with Virgin Olive Oils Obtained from Picual Olives of Different Ripeness Indices

frying no.	ripeness[a]	DG	MG	FFA	HC	TC/HC
0	VOO1	1.07 ± 0.06	0.01 ± 0.01	0.38 ± 0.04	1.46 ± 0.09	0.27 ± 0.02
	VOO2	1.33 ± 0.00	0.06 ± 0.00	0.48 ± 0.01	1.87 ± 0.00	0.28 ± 0.00
	VOO3	1.20 ± 0.05	0.09 ± 0.04	0.58 ± 0.02	1.87 ± 0.11	0.36 ± 0.02
5	VOO1	1.36 ± 0.05	0.05 ± 0.08	0.62 ± 0.05	2.03 ± 0.08	0.91 ± 0.05
	VOO2	1.47 ± 0.09	nd	0.76 ± 0.08	2.24 ± 0.17	1.14 ± 0.01
	VOO3	1.75 ± 0.16	nd	0.72 ± 0.06	2.46 ± 0.10	0.95 ± 0.02
10	VOO1	1.32 ± 0.04	0.07 ± 0.00	0.41 ± 0.00	1.80 ± 0.05	1.83 ± 0.00
	VOO2	1.30 ± 0.76	0.09 ± 0.03	0.47 ± 0.34	1.86 ± 1.13	2.86 ± 1.20
	VOO3	1.34 ± 0.05	0.10 ± 0.01	0.40 ± 0.01	1.84 ± 0.02	2.96 ± 0.02
15	VOO1	1.31 ± 0.01	0.08 ± 0.00	0.40 ± 0.00	1.80 ± 0.01	2.99 ± 0.07
	VOO2	1.57 ± 0.12	0.10 ± 0.01	0.48 ± 0.02	2.16 ± 0.13	3.62 ± 0.45
	VOO3	1.62 ± 0.02	0.10 ± 0.04	0.54 ± 0.02	2.26 ± 0.08	3.36 ± 0.25
20	VOO1	1.31 ± 0.05	0.09 ± 0.03	0.41 ± 0.03	1.82 ± 0.01	3.95 ± 0.04
	VOO2	1.62 ± 0.13	0.07 ± 0.03	0.52 ± 0.01	2.22 ± 0.16	3.74 ± 0.18
	VOO3	1.55 ± 0.08	0.08 ± 0.02	0.50 ± 0.01	2.13 ± 0.11	4.35 ± 0.42
25	VOO1	1.30 ± 0.03	0.10 ± 0.02	0.41 ± 0.02	1.81 ± 0.01	4.72 ± 0.46
	VOO2	1.58 ± 0.07	0.10 ± 0.02	0.52 ± 0.03	2.19 ± 0.02	4.59 ± 0.01
	VOO3	1.56 ± 0.10	0.07 ± 0.01	0.46 ± 0.01	2.09 ± 0.12	5.68 ± 0.41
30	VOO1	1.35 ± 0.01	0.08 ± 0.01	0.43 ± 0.01	1.87 ± 0.01	5.29 ± 0.02
	VOO2	1.68 ± 0.02	0.09 ± 0.02	0.55 ± 0.01	2.32 ± 0.04	4.65 ± 0.05
	VOO3	1.59 ± 0.10	0.12 ± 0.03	0.51 ± 0.02	2.21 ± 0.05	6.18 ± 0.24
35	VOO1	1.46 ± 0.07	0.14 ± 0.01	0.47 ± 0.00	2.06 ± 0.06	5.30 ± 0.16
	VOO2	1.61 ± 0.22	0.12 ± 0.01	0.50 ± 0.08	2.23 ± 0.30	5.55 ± 0.81
	VOO3	1.93 ± 0.29	0.11 ± 0.02	0.61 ± 0.08	2.65 ± 0.40	5.70 ± 0.90
40	VOO1	1.43 ± 0.00	0.11 ± 0.00	0.42 ± 0.07	1.95 ± 0.07	6.15 ± 0.30
	VOO2	1.75 ± 0.05	0.14 ± 0.02	0.48 ± 0.01	2.37 ± 0.09	5.28 ± 0.15
	VOO3	1.72 ± 0.07	0.12 ± 0.00	0.51 ± 0.01	2.37 ± 0.07	6.94 ± 0.13

[a]VOO1, VOO2, and VOO3 correspond to harvesting periods; for more details see text. Values are the mean ± SD of two determinations. DG, diglycerides; MG, monoglycerides; FFA, free fatty acids; HC, hydrolytic compounds (DG, MG plus FFA); TC, thermal oxidized compounds (PTG, DTG plus OTG); nd, nondetected.

RESULTS AND DISCUSSION

Unused Picual Virgin Olive Oil Characteristics. Picual olives showed ripening indices of 4.9, 5.3, and 6.0 at the three ripening stages (VOO1, VOO2, and VOO3, respectively), which are normal values for this olive fruit variety.[22]

Initial fatty acid composition of the three VOOs obtained is shown in Table 1. VOO1 showed lower oleic acid and higher palmitic acid than the other two VOOs. Baccouri et al.[36] reported in virgin olive oils extracted from diverse fruits of selected oleasters that "at the beginning of fruit development, palmitic acid content was high, followed by an extreme decrease, then it remained unchanged until the end of fruit development" (sic), suggesting that very green olives are richer in this saturated fatty acid. Ontogenesis of the enzymes participating in the biosynthesis of oleic acid in olives seems to be important in determining the oil fatty acid profile and phenol content.[20] Stearoyl-acyl carrier protein (stearoyl-ACP) is obtained from acetyl-CoA by the action of an enzymatic complex called fatty acid synthase I and III (FAS III/I) followed by FAS II. Stearoyl-ACP is desaturated to oleoyl-ACP (C18:1-ACP) by the stearoyl-ACP Δ9-desaturase, which is highly active in the plastids.[20] As growth proceeds, desaturase transcripts accumulate at higher levels, and the high transcription rate remains up to 28 weeks after flowering. This transcription pattern observed during fruit development parallels well with the synthesis of oleic acid in olives.[37] Thus, Δ9-desaturase converting stearoyl in oleoyl moieties could be less active in less ripened olives.

Unused VOOs presented total PC levels ranging from 1.85 to 2.54 g per 100 g of oil (Table 2). These results suggest the good quality of the oils tested as the PC of fresh oils normally ranges between 0.4 and 6.4 g per 100 g of oil[38] and agree with previous findings of our group in olive oil[28] and extra VOO.[39] Similarly to other quality olive oils[28,39] DG were the major compounds in the polar fraction of the unused oils, whereas PTG and MG were rather low or not detected at all (Tables 2 and 3). Nevertheless, the VOO1 oil exhibited the lowest PC and OTG levels followed by the VOO2 oil. In addition, the TC/HC ratio also increased from VOO1 to VOO3 (Table 3).

As expected, unused VOOs showed low amounts of polar-ME, suggesting that the Picual monovarietal tested oils were

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Table 4. Changes (Grams per 100 g of Oil) of Polar Methyl Esters, Nonoxidized Fatty Acid Monomers, Fatty Acid Dimers (Oxidized and Thermal), Fatty Acid Polymers, and Oxidized Fatty Acid Monomers during 40 Fryings of Fresh Potatoes with Virgin Olive Oils Obtained from Picual Olives of Different Ripeness Indices

frying no.	ripeness[a]	polar-ME	thermal-FAD	nonoxidized FAM	FAP	oxFAD	oxFAM
0	VOO1	1.75 ± 0.99	nd	98.25 ± 0.99	nd	0.04 ± 0.02	1.71 ± 0.97
	VOO2	2.03 ± 0.56	nd	97.98 ± 0.56	nd	0.05 ± 0.03	1.97 ± 0.53
	VOO3	1.58 ± 0.62	nd	98.43 ± 1.18	nd	0.05 ± 0.03	1.52 ± 1.15
10	VOO1	2.32 ± 0.01	3.22 ± 0.40	94.47 ± 0.42	0.15 ± 0.02	0.56 ± 0.04	1.61 ± 0.05
	VOO2	2.68 ± 0.40	3.02 ± 0.16	94.30 ± 0.57	0.18 ± 0.05	0.57 ± 0.09	1.93 ± 0.27
	VOO3	3.49 ± 0.22	2.93 ± 0.24	93.58 ± 0.02	0.17 ± 0.01	0.69 ± 0.10	2.63 ± 0.11
20	VOO1	3.85 ± 0.36	3.66 ± 0.41	92.49 ± 0.77	0.28 ± 0.04	1.10 ± 0.09	2.46 ± 0.46
	VOO2	4.48 ± 0.52	3.23 ± 0.28	92.29 ± 0.80	0.26 ± 0.00	1.07 ± 0.02	3.16 ± 0.54
	VOO3	4.69 ± 1.27	3.57 ± 0.85	91.74 ± 0.12	0.27 ± 0.03	0.98 ± 0.11	3.44 ± 1.35
30	VOO1	4.41 ± 0.04	4.14 ± 0.50	91.46 ± 0.55	0.34 ± 0.04	1.04 ± 0.00	3.03 ± 0.09
	VOO2	5.96 ± 0.06	4.10 ± 0.54	89.94 ± 0.59	0.28 ± 0.04	1.47 ± 0.03	4.21 ± 0.04
	VOO3	6.38 ± 0.01	4.53 ± 0.00	89.09 ± 0.01	0.32 ± 0.03	1.60 ± 0.05	4.46 ± 0.02
40	VOO1	5.62 ± 0.22	3.94 ± 0.29	90.44 ± 0.51	0.46 ± 0.06	1.46 ± 0.05	3.69 ± 0.11
	VOO2	6.31 ± 0.21	5.25 ± 0.12	88.44 ± 0.09	0.33 ± 0.06	1.58 ± 0.13	4.40 ± 0.02
	VOO3	7.14 ± 0.73	4.79 ± 0.31	88.08 ± 1.04	0.41 ± 0.05	1.77 ± 0.07	4.95 ± 0.71

[a]VOO1, VOO2, and VOO3 correspond to harvesting periods; for more details see Materials and Methods. Values are the mean ± SD of two determinations. Polar-ME, polar methyl esters; thermal-FAD, thermal fatty acid dimers; nonoxidized FAM, nonoxidized fatty acid monomers; FAP, fatty acid polymers; oxFAD, oxidized fatty acid dimers; oxFAM, oxidized fatty acid monomers; nd, nondetected.

good-quality oils (Table 4). oxFAM where the main compounds followed by far from oxFAD as thermal-FAD and FAP were not detected in the polar-ME of the three unused VOOs tested. These results agree with those reported in previous studies.[40]

The determination of the oxidative stability of VOOs by the Rancimat test is a widely used method, as it is an easy, standardized, and well-controlled method. The oxidative stability, given by the Rancimat method, also decreases with olive fruit ripening, with values of 12.9, 7.6, and 4.0 h in VOO1, VOO2, and VOO3, respectively (Figure 1A). Total tocopherol content exhibited values of 236, 207, and 189 ppm in VOO1, VOO2, and VOO3, respectively (Figure 1B). α-Tocopherol was the most abundant isoform of vitamin E in the three VOOs (about 92−93% of the total tocopherol content). Picual VOO1, VOO2, and VOO3 showed average total polyphenol contents of 394, 289, and 78 ppm, respectively (Figure 1C). Differences in the polyphenol content between VOOs were high, suggesting a dramatic effect of ripening in these antioxidant compounds.[20] Gómez-Rico et al.,[21] Baccouri et al.,[36] Beltrán et al.,[41] and Salvador et al.,[42] reported decreases in phenolic compounds and tocopherol content during olive ripening. Yousfi et al.[22] studied the effect of fruit ripening on the quality of the oil extracted and on the amount of phenolic compounds in the Arbequina and Picual varieties and observed that the oil quality was not affected by fruit ripening or by the increase in rainfall of the season. However, Yousfi et al.[22] reported that the changes in oil stability and phenolic compounds strongly differ depending on olive variety and ripeness of the olives from which oils were obtained.

Oils Changes during Repeated Potato Frying. *Changes in the Fatty Acid Composition.* As commented, VOO1 presented a higher amount of SFA. The differences in the fatty acid profile of the three tested VOOs have probably determined, at least partially, that VOO1 showed higher stability during frying (Table 1). It is well-known that oils rich in saturated fatty acids are highly thermostable.[1,43] Nonetheless, VOO1 also presents higher amounts of tocopherols and phenolic compounds, contributing to the higher thermal stability of VOO1.

After 40 frying operations, linoleic and linolenic acids markedly decreased (72−74 and 33.3−51.3%, respectively), whereas little difference was observed for oleic acid. These results are in line with general frying bibliography, reflecting that oleic acid decreases relevantly only when reduced linoleic acid content remains in the oil.[3,39,44] Results also confirm that the degradation rate of unsaturated fatty acids is higher than that of monounsaturated and saturated ones.

Polar Content and Thermal Oxidation and Hydrolytic Changes during 40 Fryings. As reported in previous studies with olive oils,[3,16,28,39,45] thermal oxidation compounds increased with the number of frying operations, whereas hydrolytic alteration products remained quite stable (Table 2). Casal et al.,[46] frying potatoes with commercial monovarietal extra VOO from the same cultivar region, a refined sunflower oil, and a refined oil and VOO blend, also observed PC increases. These authors reported that PC increased with the number of frying operations in all oils but that extra VOO showed the highest stability, whereas sunflower oil reached the 25% PC cutoff point at least 9 h before the other oils. However, in the present study, after 40 frying operations, the PC and OLIG contents (Table 2) were far from legal limits.[24] Table 2 also shows that after 40 frying operations, a large proportion of the polar fraction (~75%) was constituted by OTG and DTG and to a lesser degree by PTG. Changes in PC and thermal oxidation compounds but not those of most hydrolytic compounds were significantly fitted to linear adjustments (Table 5). These linear adjustments explain >92% (r^2 > 0.92) of the data variability of all the thermal oxidation compounds tested.

Previous studies by our group indicate that oil thermal oxidation changes fit linear adjustments when frying was

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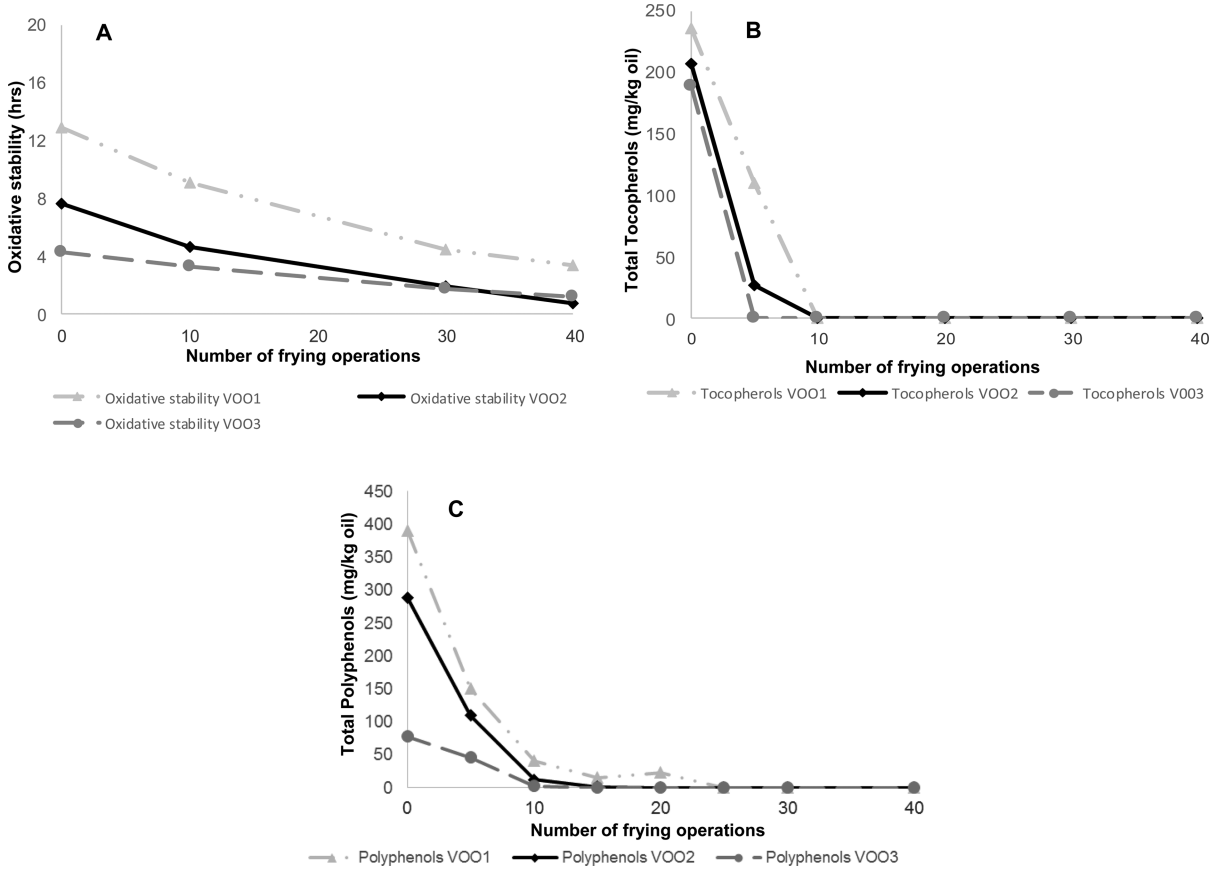

Figure 1. Changes in the oxidative stability, total tocopherols, and total polyphenols during 40 discontinuous fryings with virgin olive oils (VOO). (A) Oxidative stability changes (h) in the three Picual VOOs. VOO1, VOO2, and VOO3 correspond to virgin olive oils obtained from Picual olives at different ripening periods; for more details see text. Linear adjustment: Y = intercept (with their values 95% CI) + slope (with their values 95% CI) × X, where Y is the oxidative stability and X the number of frying operations: (▲) VOO1, Y = 11.571 (9.900; 13.242) − 0.236 (−0.309; −0.163) × X; r^2 = 0.839; (p < 0.01); (◆) VOO2, Y = 6.621 (5.567; 7.676) − 0.167 (−0.213; −0.121) × X; r^2 = 0.867; (p < 0.01); (●) VOO3, Y = 3.941 (3.484; 4.397) − 0.087 (−0.098; −0.058) × X; r^2 = 0.884; (p < 0.01). Mean values of oxidative stability were significantly different for VOO1 versus VOO3 (p = 0.01) and marginally significant for VOO2 versus VOO3 (p = 0.054) although not significantly different for VOO1 versus VOO2 (p = 0.099). (B) Total tocopherol changes (mg/kg oil) and (C) total polyphenol changes (mg/kg oil) in the three Picual VOOs. VOO1, VOO2, and VOO3 correspond to virgin olive oils obtained from Picual olives at different ripening periods; for more details see text. Nonlinear adjustments were found; thus, comparisons between oils were not performed.

performed with low or null oil turnover but to a power, logarithmic, or quadratic adjustment when frequent turnover was done.[47] The equations presented in Table 5 suggest that during frying of potatoes each frying operation induces increases of 0.307 and 0.317 g/100 g oil of PC and 0.161 and 0.172 mg/100 g oil OLIG in VOO1 and VOO2 oils, respectively, whereas these increases were of 0.412 and 0.222 g/100 g oil of PC and OLIG, respectively, in VOO3 oil. According to OLIG adjustments (Table 5), and in the case of keeping the same experimental frying conditions and trends, the VOO3 oils should be discarded at the 53rd frying, whereas the VOO1 oil should be discarded at the 73rd frying. The increased stability of the oils prepared from olive fruits of minor ripeness index seems to be related to their fatty acid profile and higher (4 and 5 times) polyphenol and higher (14 and 25%) tocopherol contents with respect to their VOO2 and VOO3 oil counterparts.

No linear relationships were found between the frying number and any hydrolytic compounds in any of the Picual VOO tested (data not shown).

Changes in the Composition of FAME and Formation of Oxidative and Thermal Dimers and Polymers of FAME during 40 Fryings. Evaluation of specific changes occurring in the fatty acyl chains of triglycerides, by far the most abundant compounds in oils, is an important tool to study in-depth oil degradation.[1] During frying, the formation of dimers (thermal-FAD and oxFAD) and polymers (oxFAD) derived of FAME increases the analysis complexity because of the presence of oxygenated (C−O−C) and nonoxygenated (C−C) bindings between triglyceride fatty acyl groups.[1] Following derivatization, results showed that thermal fatty acid monomers (thermal-FAM) and oxidized oxFAM significantly increased with the number of frying study (Table 4). These increases were fitted to linear adjustments (at least p = 0.05) (Table 6). On the contrary, when the number of frying operations increased, nonoxidized FAM decreased (0.133−0.173 g/100 g oil per frying) (Table 6). After 40 frying operations, VOO3 oil showed less nonoxidized FAM and more oxFAM compared with the other two VOOs (Table 4). Linear adjustments for the different FAME related compounds tended to be higher, although in most cases nonsignificant, in VOO3 oil with respect to VOO1 and VOO2 counterparts. VOO1 showed lower polar-ME increase than VOO3 (0.098 vs 0.140 g/100 g oil increase each frying, p = 0.029) (Table 6). Márquez-Ruiz et al.[25] observed a major increase in the altered fatty acid methyl esters content

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Table 5. Linear Adjustments between Polar Compounds, the Different Thermal Oxidation Compounds, and the Number of Fryings of Fresh Potatoes with Virgin Olive Oils Obtained from Picual Olives of Different Ripeness Indices

	ripeness[a]	r^2	β	intercept[b]	slope[b]	P[c]	VOO1 vs VOO2[d]	VOO1 vs VOO3[d]	VOO2 vs VOO3[d]
PC	VOO1	0.991	0.996	2.296 (1.938, 2.665)	0.307 (0.292, 0.323)	<0.001	ns	<0.001	<0.001
	VOO2	0.952	0.976	3.489 (2.595, 2.665)	0.317 (0.279, 0.354)	<0.001			
	VOO3	0.991	0.995	3.056 (2.558, 3.553)	0.412 (0.391, 0.433)	<0.001			
PTG	VOO1	0.927	0.963	−0.114 (−0.214, −0.014)	0.028 (0.240, 0.032)	<0.001	ns	<0.001	<0.001
	VOO2	0.952	0.975	−0.108 (−0.207, −0.009)	0.350 (0.031, 0.039)	<0.001			
	VOO3	0.955	0.977	−0.204 (−0.339, −0.068)	0.049 (0.044, 0.055)	<0.001			
DTG	VOO1	0.983	0.991	0.063 (−0.157, 0.284)	0.133 (0.124, 0.142)	<0.001	ns	<0.001	<0.001
	VOO2	0.924	0.961	0.301 (−0.195, 0.798)	0.137 (0.117, 0.158)	<0.001			
	VOO3	0.980	0.990	0.280 (−0.035, 0.594)	0.173 (0.160, 0.186)	<0.001			
PTG + DTG (OLIG)	VOO1	0.989	0.995	−0.050 (−0.261, 0.160)	0.161 (0.153, 0.170)	<0.001	ns	<0.001	<0.001
	VOO2	0.942	0.970	0.193 (−0.347, 0.734)	0.172 (0.149, 0.195)	<0.001			
	VOO3	0.989	0.994	0.076 (−0.220, 0.373)	0.222 (0.210, 0.235)	<0.001			
OTG	VOO1	0.982	0.991	0.651 (0.414, 0.888)	0.139 (0.129, 0.149)	<0.001	ns	<0.001	<0.001
	VOO2	0.931	0.965	1.135 (0.654, 1.616)	0.140 (0.120, 0.160)	<0.001			
	VOO3	0.980	0.990	0.975 (0.648, 1.303)	0.180 (0.166, 0.193)	<0.001			
TC	VOO1	0.990	0.995	0.601 (0.228, 0.973)	0.300 (0.284, 0.316)	<0.001	ns	<0.001	<0.001
	VOO2	0.942	0.971	1.328 (0.351, 2.305)	0.312 (0.271, 0.353)	<0.001			
	VOO3	0.988	0.994	1.051 (0.498, 1.605)	0.402 (0.379, 0.425)	<0.001			

[a]VOO1, VOO2, and VOO3 correspond to different harvesting periods; for more details see text. [b]Values (95% CI). [c]p, lineal regression adjustment. [d]p, significant differences between lineal adjustments. PC, polar compounds; PTG, triglyceride polymers; DTG, triglyceride dimers; OLIG, triglyceride oligomers (PTG plus DTG); OTG, oxidized triglycerides; TC, thermal oxidized compounds (PTG, DTG plus OTG); ns, nonsignificant.

when the oils presented higher PC content in sunflower oil, high-oleic sunflower oil, and palm olein submitted to frying or thermal oxidation. Taking into account the correlation between PC and polar-ME found in the present study [polar-ME = PC × 0.345) + 0.855] it can be calculated that 25% PC corresponds to 9.5% polar-ME. Márquez-Ruiz et al.[25] and Sanchez-Muniz et al.[1] reported that PC values between 21 and 27% corresponded to 8−11% total altered fatty acid values. In the case of discarding oils at this 9.5% cutoff point, the VOO1 oil shelf life could be extended up to the 80th frying in contrast with VOO2 and VOO3 oils, which would be used for only 64 and 55 fryings, respectively. These results clearly indicate the highest stability of the oil obtained from olive fruits of low ripening index.

Pokorný and Dostálová[2] and Romero et al.[47,48] have suggested that slightly higher concentrations of these altered compounds are present in food than in the oil media. These results show the benefits of frying potatoes with the most stable oil as fewer alteration compounds are formed in the oil and absorbed by the food during frying.

Changes in the Oxidative Stability and Tocopherol and Polyphenol Contents. The oxidative stability of the three VOOs decreased with the number of frying operations. Andrikopoulos et al.[49] observed while obtaining chip potato that the increase in PC with the number of frying operations in VOO and a commercial vegetable oil was related to a decrease in the oxidative stability of both oils. After 40 fryings, VOO1 appears as the most stable (Figure 1A). However, with respect to its respective basal polyphenol content, VOO2 suffered the highest polyphenol losses followed by VOO1. The oxidative stability of the oils was significantly and negatively correlated ($p < 0.001$) with the contents of PC, OLIG, OTG, and TC and the TC/HC ratio and positively correlated (at least $p < 0.05$) with the TC/OLIG ratio (Table 7). Similarly, the oxidative stability was significantly and negatively correlated (at least $p < 0.01$) with polar-ME and oligomers of methyl esters (oligomers-ME) and positively correlated ($p < 0.001$) with nonpolar-ME (Table 7). The highest stability found for VOO1 with respect to VOO2 and VOO3 was closely related with the loss of tocopherols because approximately 50% of tocopherols remained after five frying operations in VOO1, and only 10% of basal levels remained in VOO2, whereas tocopherols were not detected in VOO3. Nevertheless, tocopherols were exhausted in all tested oils beyond the 10th frying operation (Figure 1B). Frying potatoes with olive oil, Chatzilazarou et al.[3] found a decrease of 41.2% after 10 h of frying, whereas Andrikopoulos et al.[49] described a retention of 50% of tocopherols after only three successive deep-fryings. Márquez-Ruiz et al.[40] suggested that oxidized triglyceride monomers and oligomers significantly increased only when oils were highly depleted in tocopherols.

In the three studied VOOs, the initial total polyphenol content decreased by >50% after six frying operations (Figure 1C). After 10 fryings, the polyphenol contents in VOO1 and VOO2 with respect to their respective basal values were 10 and 5%, respectively, whereas those compounds were not detected in VOO3. These amounts were further reduced as 3.5−5.5% of the initial polyphenol content was found in VOO1 between the 16th and 25th fryings, whereas those compounds were not detected in VOO2 and VOO3 (Figure 1C). Andrikopoulos et al.[49] reported that the major phenols found in virgin olive oils have abruptly diminished (by 50%) after five successive deep-fryings of potatoes. The higher polyphenol content of

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Table 6. Linear Adjustments between Polar Methyl Esters, Thermal Fatty Acid Dimers, Nonoxidized Fatty Acid Monomers, Fatty Acid Polymers, Oxidized Fatty Acid Dimers, and the Number of Fryings of Fresh Potatoes with Virgin Olive Oils Obtained from Picual Olives of Different Ripeness Indices

	ripeness[a]	r^2	β	intercept[b]	slope[b]	P[c]	VOO1 vs VOO2[d]	VOO1 vs VOO3[d]	VOO2 vs VOO3[d]
polar-ME	VOO1	0.926	0.962	1.622 (1.066, 2.178)	0.098 (0.076, 0.121)	<0.001	ns	0.029	ns
	VOO2	0.934	0.967	1.919 (1.291, 2.547)	0.119 (0.097, 0.144)	<0.001			
	VOO3	0.902	0.950	1.850 (0.928, 2.772)	0.140 (0.102, 0.178)	<0.001			
thermal-FAD	VOO1	0.647	0.804	1.229 (−0.071, 2.529)	0.088 (0.035, 0.141)	0.050	ns	ns	ns
	VOO2	0.868	0.932	0.804 (−0.099, 1.707)	0.116 (0.079, 0.153)	<0.001			
	VOO3	0.824	0.908	0.926 (−0.106, 1.958)	0.112 (0.070, 0.154)	<0.001			
nonoxidized FAM	VOO1	0.983	0.991	0.063 (−0.157, 0.284)	0.133 (0.124, 0.142)	<0.001	ns	0.067	ns
	VOO2	0.924	0.961	0.301 (−0.195, 0.798)	0.137 (0.117, 0.158)	<0.001			
	VOO3	0.980	0.990	0.280 (−0.035, 0.594)	0.173 (0.160, 0.186)	<0.001			
FAP	VOO1	0.989	0.995	−0.050 (−0.261, 0.160)	0.161 (0.153, 0.170)	<0.001	ns	ns	ns
	VOO2	0.942	0.970	0.193 (−0.347, 0.734)	0.172 (0.149, 0.195)	<0.001			
	VOO3	0.989	0.994	0.076 (−0.220, 0.373)	0.222 (0.210, 0.235)	<0.001			
oxFAD	VOO1	0.907	0.952	0.178 (−0.034, 0.390)	0.033 (0.024, 0.042)	<0.001	ns	0.033	ns
	VOO2	0.951	0.975	0.160 (−0.018, 0.338)	0.039 (0.032, 0.047)	<0.001			
	VOO3	0.965	0.982	0.145 (−0.020, 0.310)	0.044 (0.037, 0.050)	<0.001			
oxFAM	VOO1	0.982	0.991	0.651 (0.414, 0.888)	0.139 (0.129, 0.149)	<0.001	ns	0.067	ns
	VOO2	0.931	0.965	1.135 (0.654, 1.616)	0.140 (0.120, 0.160)	<0.001			
	VOO3	0.980	0.990	0.975 (0.648, 1.303)	0.180 (0.166, 0.193)	<0.001			

[a]VOO1, VOO2, and VOO3 correspond to harvesting periods; for more details see text. [b]Values (95% CI). [c]p, lineal regression significance. [d]p, significant differences between lineal adjustments. Polar-ME, polar methyl esters; thermal-FAD, thermal fatty acid dimers; nonoxidized FAM, nonoxidized fatty acid monomers; FAP, fatty acid polymers; oxFAD, oxidized fatty acid dimers; oxFAM, oxidized fatty acid monomers; NS, nonsignificant.

Table 7. Product−Moment Correlations between the Oxidative Stability Determined by the Rancimat Method and Different Thermal Oxidative and Hydrolytic Alteration Compounds Present in Virgin Olive Oils Obtained from Picual Olives of Different Ripeness Indices

ripeness[a]	PC	OLIG	OTG	TC	DG	MG	FFA	HC	TC/HC	TC/OLIG	polar-ME	non polar-ME	total OLIG-ME
VOO1	−0.947 ***	−0.940 ***	−0.948 ***	−0.944 ***	−0.864 **	−0.919 ***	−0.405	−0.876 **	−0.958 ***	0.843 **	−0.897 **	0.970 ***	−0.955 ***
VOO2	−0.978 ***	−0.951 ***	−0.974 ***	−0.964 ***	−0.396	−0.579	0.070	−0.286	−0.953 ***	0.885 **	−0.922 ***	0.971 ***	−0.968 ***
VOO3	−0.960 ***	−0.950 ***	−0.968 ***	−0.959 ***	−0.965 ***	−0.204	0.141	−0.885 **	−0.960 ***	0.731 *	−0.894 **	0.917 ***	−0.933 ***

[a]VOO1, VOO2, and VOO3 correspond to harvesting period; for more details see text. ∗, $p < 0.05$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$. PC, polar compounds; OLIG, triglyceride polymers plus of triglyceride dimers; OTG, oxidized triglycerides; TC, thermal oxidized compounds (PTG plus DTG plus OTG); DG, diglycerides; MG, monoglycerides; FFA, free fatty acids; HC, hydrolytic compounds (DG plus MG plus FFA); polar-ME, polar methyl esters (fatty acid polymers plus oxidized fatty acid dimers plus oxidized fatty acid monomers); nonpolar-ME, nonpolar methyl esters; total OLIG-ME, total oligomers of methyl esters (fatty acid polymers plus thermal fatty acid dimers plus oxidized fatty acid dimers).

VOO1 throughout repeated fryings is probably due to its initial higher value and to the partial turnover of oil performed after each five fryings to keep the food/oil ratio constant. None of the three VOOs showed polyphenol detectable amounts after the 25th frying.

To identify which parameters explained better the extent of VOO thermal oxidation during frying, the basal contents of total polyphenols, total tocopherols, and oleic, linoleic, and linolenic acids were correlated with the polar content change. Polyphenols ($r = -0.928$; $p = 0.008$) and tocopherols ($r = -0,870$; $p = 0.024$) were negatively and significantly correlated, whereas the other parameters did not show significant relationships. According to the stepwise multiple-regression procedure, a total polyphenol content of unused VOOs explains about 86% ($p = 0.008$) of data variability for the extent of oil thermal oxidation (as polar content change) during 40 fryings in our experimental conditions. Other parameters of the unused VOOs tested were excluded from the stepwise multiple-regression model. This confirms the hypothesis that olive oil phenols play an important antioxidant role during frying.[2,10,49]

In conclusion, Picual olive ripeness affects the VOO composition and stability. Although repeated fryings increased the concentration of all thermal oxidation compounds and decreased the oxidative stability in the three VOOs studied,

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oil stability was higher and degradation lower during frying in the VOOs obtained from olives of lower ripeness index. As the potential toxicity of oils used in repeated fryings is ascribed to the amount and profile of alteration compounds, the repeated domestic potato frying with VOO obtained from low ripeness index olives should be recommended to obtain high-quality fried potatoes.

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Author Contributions

F.J.S.-M. is the corresponding author and guarantor of the paper and contributed to the study design, data discussion, and writing of the paper. R.O.-D., C.M., and B.S. contributed to the data acquisition and analysis, whereas G.M.R. contributed to the oxidative stability analysis. A.P.-J. and S.B. contributed to the study design.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

DG, diglycerides; DTG, dimers of triglycerides; FAME, fatty acid methyl esters; FAP, fatty acid polymers; HC, hydrolytic compounds; HPSEC, high-performance size exclusion chromatography; MG, monoglycerides; nonoxidized FAM, nonoxidized fatty acid monomers; OLIG, oligomers; OTG, oxidized triglycerides; oxFAD, oxidized fatty acid dimers; oxFAM, oxidized fatty acid methyl esters; PC, polar compounds; polar-ME; polar methyl esters; PTG, polymers of triglycerides; TC, thermal oxidized compounds; thermal-FAM, thermal fatty acid methyl esters; thermal-FAD, thermal fatty acid dimers; VOO, virgin olive oil

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