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Performance of virgin and refined avocado oils during deep-frying and thermoxidation simulating frying in comparison with olive and sunflower oils

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SUMMARY: Avocado oil stability at frying temperatures has been scarcely studied. In this work, the performance of virgin (VAO), minimally refined (MRAO) and refined (RAO) avocado oils was evaluated in deep-frying and thermoxidation experiments in comparison with sunflower (SO), high-oleic sunflower (HOSO), and virgin olive (VOO) oils. Polar compounds, polymers and tocopherols were determined. For all oils, no significant differences in polymers levels were found after 10h thermoxidation and 9 discontinuous deep-frying operation. The most stable oils were HOSO, VAO, VOO and MRAO, all showing less than 20% polar compounds after 9 frying operations. Besides the stability conferred by the predominant monounsaturated fatty acids (oleic acid), the better frying performance shown by these four oils was attributed to the high content of tocopherols and DMPS in HOSO, and the presence of protective minor compounds in virgin oils (VAO and VOO) and MRAO.

***KEYWORDS**: Avocado oil; Frying; Refining; Sunflower oil; Thermoxidation; Virgin olive oil*

RESUMEN: ***Comportamiento de aceites de aguacate virgen y refinados en fritura y en termoxidación simulando fritura, en comparación con aceites de oliva y girasol***. La estabilidad de los aceites de aguacate a temperaturas de fritura ha sido escasamente estudiada. En este trabajo se evaluó el comportamiento en fritura y en termoxidación de aceites de aguacate virgen (AAV), mínimamente refinado (AAMR) y refinado (AAR) en comparación con aceites de girasol (AG), girasol alto oleico (AGAO) y oliva virgen (AOV). Se analizaron compuestos polares, polímeros y tocoferoles. No se encontraron diferencias significativas en los niveles de polímeros tras 10 h de termoxidación y tras 9 frituras. Los aceites más estables fueron AGAO, AAV, AOV y AAMR, todos ellos con niveles inferiores a 20% de compuestos polares tras 9 frituras. Además de la estabilidad conferida por los ácidos grasos monoinsaturados mayoritarios (ácido oleico), el mejor comportamiento en fritura mostrado se atribuye al alto contenido de tocoferoles y DMPS en AGAO, y a la presencia de compuestos menores protectores en los aceites vírgenes AAV y AOV, y AAMR.

***PALABRAS CLAVE**: Aceite de aguacate; aceite de girasol; aceite de oliva virgen; fritura; refinación, termoxidación*

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1. INTRODUCTION

Avocado (*Persea Americana* Mill.) oil is nowadays present in the worldwide market, being Mexico one of the main exporter countries and Hass the most abundant cultivar of avocados. As occurs with olive oils, avocado oils are obtained from fruits with high oil content. Thus, avocados contain 15-30% oil in the fresh pulp, depending on season and variety. Avocado oils also show a healthy fatty acid profile, i.e., low content of saturated fatty acids and high levels of oleic acid, presence of antioxidants as tocopherols and phenolic compounds, pigments

with antioxidant effects as carotenoids and bioactive compounds as squalene and phytosterols (Woolf *et al.*, 2009; Fernandes *et al.*, 2018).

Avocado oil has been widely used in the pharmaceutical and cosmetic industries while food applications have been scarce so far and used mostly as a gourmet oil for salads and dressings. However, the increasing production of avocado worldwide and therefore fruit rejected from the fresh-fruit trade or discarded as a subproduct in the avocado industry, together with the high consumers' demand for monounsaturated oils, make avocado oil a product of increasing exploitation interest for producers (Cervantes-Paz and Yahia, 2021). As to research interests, the number of publications on avocado oil has increased considerably during the last 10 years, especially focused on the improvement of extraction methods (Qin and Zhong, 2016; Green and Huang, 2022) physicochemical characterization (Fernandes *et al.*, 2018; Green and Wang, 2023a, 2023b) and health-promoting effects (Cervantes-Paz and Yahia, 2021).

Regarding quality of avocado oils, joint FAO and WHO Codex Alimentarius Commission has established a working group and proposed a set of standards (Codex Alimentarius Commission, 2021). For that purpose, studies on authenticity and quality of avocado oils in the marketplace are essential (Fernandes *et al.*, 2018, Green and Wang, 2023a, 2023b).

Avocado oils have been proposed for frying due to their high content in oleic acid, neutral flavor, and high smoke point (Woolf *et al.*, 2009) Furthermore, the possible migration of valuable bioactive compounds from the frying avocado oil to the food would enrich the fried product (Samaniego-Sánchez *et al*., 2021). However, in contrast to the myriad of studies carried out in most used vegetable oils, the frying stability of avocado oils is practically unknown. In the context of avocado oils, refined oils have special interest for use in frying due to their generally lower cost as compared to virgin oils.

Regarding studies at frying temperatures, to the best of our knowledge, the only studies published were based on thermoxidation experiments not simulating frying conditions. The first results obtained revealed that avocado (mixture of virgin and refined) and extra virgin olive oils heated at 180°C showed similar stability (Berasategi *et al.*, 2012). In the study undergone by De Alzaa *et al.* (2018), the total content of polar compounds after 10 h at 180°C indicated that avocado oil behaved similarly to olive and virgin olive oils, the three of them being more stable that sunflower, grapeseed, canola, rice bran and peanut oils and less stable than extra virgin olive and coconut oils. Likewise, in another study, olive and avocado oils heated at 180°C showed similar values in the indexes evaluated (Machado da-Costa *et al.*, 2021). Both oils have been recommended for frying practices in view of the lesser amounts of volatile aldehydes formed at 180°C (Wann *et al.*, 2021). However, neither of these studies was carried out under real food frying conditions, the thermoxidation conditions used were much different than those typical of frying and only one study applied an official method, the polar compounds determination, to evaluate frying oil quality (De Alzaa *et al.*, 2018). Since it is the only method that quantitates the cumulative degradation compounds that are non-volatile and produced during the process of frying, polar compound determination has been adopted in countries where frying used oils are regulated to establish limits for human consumption, usually set at 25% polar compounds (Firestone, 2007). Determination of polymers is also included in regulations of certain countries (Firestone, 2007) because polymerization is favored at high temperatures (Márquez-Ruiz *et al*., 2014).

In frying experiments conducted in different laboratories, the results are often not consistent because of the multitude of variables at play and their intricate interactions (Machado *et al*., 2007). In this regard, we developed a method using the Rancimat apparatus taking advantage of its technical characteristics, i.e., utilizing standard vessels, implementing temperature correction, and ensuring temperature uniformity across all vessels. The amounts of oil sample used were those providing oil surface-to-volume ratios closely resembling those in discontinuous fryers, and no air bubbling was applied (Barrera-Arellano *et al*., 1997). Good correlation of simulated frying with real frying and high repeatability have been obtained, provided that well established standard conditions were applied (Ruiz-Méndez *et al*., 2021).

The objectives of this work were i) to evaluate the performance of avocado oils during potato deep-frying and compare them with some of the most used vegetable frying oils; ii) to use the Rancimat

equipment as a tool for simulating frying procedure in avocado oils. Follow-up of oil alteration was done by polar compounds and polymer determinations. The avocado oils selected were virgin, minimally refined and refined, as stated in the labels, to be compared with virgin olive oil and two sunflower oils, i.e, conventional high-linoleic sunflower oil and high-oleic sunflower oil.

2. MATERIALS AND METHODS

2.1. Samples

Avocado oils, i.e., virgin (VAO), minimally refined (MRAO) and refined (RAO), of different brands were acquired in Mexican markets. Sunflower oil (SO), high-oleic sunflower oil (HOSO) and virgin olive oil (VOO) of the same brand were obtained from Spanish suppliers. All oils were stored at 4°C until analysis. As stated in the labels, VAO was cold-pressed extracted avocado oil while MRAO and RAO were pure avocado oils (not blends), minimally refined and refined, respectively. Minimal refining processes are intended to remove undesirable minor compounds while improving retention of healthy components. It usually involves minimal neutralization using weak alkalis or application of physical refining with alternative bleaching treatments and low deodorization temperature. Potatoes of Agria variety were purchased locally in Spanish markets.

2.2. Chemicals

Reagents and standards were purchased from MilliporeSigma (St. Louis, MO, USA). Purity of α, β, γ and δ-tocopherol standards was ≥ 95% and purity of monostearin was ≥ 99%. Solvents were purchased from Panreac SA (Barcelona, Spain).

2.3. Frying experiments

Frying operations were performed under discontinuous conditions, using three one-liter fryers (Moulinex AF2200, France) for each oil. Experiments were carried out following a previous procedure (Holgado *et al*., 2021) with some slight modifications. Homogeneous sticks (1 cm × 1 cm × 6 cm) of peeled potatoes were washed with water. Nine batches of 200 g potatoes were fried in one L oil at 180°C for 10 min with intervals of 20 min between frying operations, while the fryers remained open. The endpoint of frying operations was established when, for at least one of the oils, polar compound content, as monitored by Testo 270, was close to 25%, the alteration limit established for frying fats and oils in countries where regulations are established (Firestone, 2007). Surface-to-oil volume ratio changed from initial 0.3 to final 0.4 cm^{-1}. Fryers were kept closed at room temperature for 48h and the tenth, last frying operation was done with another batch of 200 g potatoes. Frying oil samples collected were kept under nitrogen atmosphere and stored at -20°C until analysis.

2.4. Thermoxidation experiments

The thermoxidation experiments were carried out using a Rancimat apparatus (Rancimat 743 equipment, Metrohm, Switzerland) and following a procedure described in detail, including reproducibility data, in a previous publication (Barrera-Arellano *et al*., 1997). Three Rancimat tubes were used for each oil, with 8.00 ± 0.01g of oil each. The tubes were inserted in the heating block, previously heated at 180 ± 1°C, for 10 h. Surface-to-oil volume ratio was 0.4 cm^{-1}, close to that in the domestic fryers used in the frying experiments. During heating, the tubes were left open, and no bubbling of air was applied. Samples collected were kept under nitrogen atmosphere and stored at -20 °C until analysis.

2.5. Analytical determinations

2.5.1. Fatty acid composition

Fatty acid composition was determined by GC after derivatization to fatty acid methyl esters (FAME) with 2M KOH in methanol, according to the IUPAC Standard Method 2.301 and 2.302 (IUPAC, 1992). FAMEs were analyzed using a Hewlett-Packard 6890 Series gas chromatograph equipped with a DB-23-fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness) and a flame ionization detector. The detector and injector temperatures were held at 240°C while the column temperature remained isothermal at oven temperature of 185°C. The FAMEs were identified by comparison of their retention times with those obtained with a FAME reference standard mix and quantified based on peak area percentages.

2.5.2. Tocopherols

Tocopherols were analyzed by HPLC with fluorescence detection following IUPAC Standard Method 2.432 (IUPAC, 1992). The oil samples were dissolved in n-heptane at concentration of 50 mg/mL and analyzed in an Agilent 1260 Infinity HPLC chromatograph (Agilent Technologies, Santa Clara, CA, USA). The chromatograph was equipped with a quaternary pump VL (G1311C), a standard autosampler (G1329B), a thermostated column compartment (G1316A), and a fluorescence detector (G1321A). A silica HPLC column (LiChrospher® Si 60, 250 mm × 4 mm i.d., 5 μm particle size) (Merck, Darmstadt, Germany) was used. The volume of the sample analyzed was 20 μL. The temperature of the thermostated column compartment was set at 25°C. The separation of tocopherols was performed using n-heptane:isopropanol (99:1, v/v) with a flow rate of 1 mL/min. The excitation and emission wavelengths in the detector were 290 nm and 330 nm, respectively. Quantification was made by external calibration using α, β, γ and δ-tocopherol standards.

2.5.3. Acidity

Acidity (expressed as % oleic acid) was determined by titration according to method ISO 660:2020 (ISO, 2020).

2.5.4. Peroxide value

Peroxide value (meq O_2 kg^{-1}oil) was determined by the iodometric assay according to method ISO 3960:2017 (ISO, 2017).

2.5.5. Oil stability index

Oil stability index (h) was determined using a Rancimat apparatus (Rancimat 743 equipment, Metrohm, Switzerland) at 100 °C following AOCS Official Method Cd-12b-92 (AOCS, 2003).

2.5.6. Smoke point

Smoke point (°C) was determined following AOCS Official Method Cc-9a-48 (AOCS, 2003).

2.5.7. Rapid measurement of polar compounds

Testo 270 (Testo AG, Germany), a dielectric constant sensor, was used to follow-up formation of polar compounds during frying and thermoxidation. The probe was immersed into the hot oil and data were collected after 3 seconds while gently stirring the oil for uniform measurement.

2.5.8. Total content and analysis of polar compounds

Polar compounds were determined by combination of adsorption chromatography and high-performance size-exclusion chromatography as previously described (Márquez-Ruiz *et al*., 1996). Polar compounds were separated from the non-oxidized triacylglycerols by solid-phase extraction. Fifty milligrams of oil were dissolved in 2 mL of hexane containing 1 mg of monostearin added as internal standard. The sample solution was poured into a 1 g silica Sep-Pak cartridge (Waters, Darmstadt, Germany) and the non-oxidized triacylglycerols were eluted with 15 mL hexane/diethyl ether (90:10, v/v). After that, the polar fraction was eluted with 25 mL diethyl ether. The solvent of the polar fraction was evaporated in a rotary evaporator and redissolved in 1 mL diethyl ether. The polar fraction was analyzed by high performance size exclusion chromatography in a liquid chromatograph equipped with a Rheodyne injector with a 20 μL sample loop, a Waters 510 pump (Waters, Milford, MA, USA), and a Waters refractive index detector. The separation was performed on two PLgel columns (Agilent Technologies, Palo Alto, CA, USA) packed with 5 mm particles of 100 and 500 Å pore size, respectively, and placed into an oven set at 35°C. High performance liquid chromatography grade tetrahydrofuran was the mobile phase with a flow of 1 mL/min. Resolved peaks of triacylglycerol oligomers, triacylglycerol dimers, oxidized triacylglycerol monomers, diacylglycerols, monostearin (used as internal standard), and a peak corresponding to free fatty acids and polar unsaponifiable matter, were obtained. Total polar compounds were calculated as the sum of all the groups of compounds quantitated.

2.5.9. Polymers

Aliquots of 50 mg of oil were dissolved in 1 mL tetrahydrofuran for direct analysis by high-performance size-exclusion chromatography according to IUPAC Standard Method 2.508 (IUPAC, 1992) under the same chromatographic conditions described above. The sum of triacylglycerol dimers and higher oligomers is referred as total polymers.

2.6. Statistical analysis

Data were expressed as mean ± standard deviations of three analytical determinations performed on independent samples. For the comparison of two means, Student's t-test was applied, while for three or more, one-factor analysis of variance (ANOVA) and Tukey's test were used to establish differences between means. A P value of 5% was considered. Statistical analysis was performed with the SPSS 27 statistical program (IBM Corp., Chicago, IL, USA).

3. RESULTS

3.1. Characterization and quality parameters

Table 1 shows characterization and quality parameters of the oils used. The fatty acid compositions of all oils were within the values normally found (García-González *et al*., 2013, Grompone 2020; Green and Huang, 2023a). Specifically, the three avocado oils tested showed values within the proposed CODEX standards as of 2021 (Codex Alimentarius Commission, 2021). Fatty acid composition reflected, as expected, the high proportion of oleic acid in VOO, avocado oils,

Table 1. Characterization and quality parameters of unused oils.

	VAO	MRAO	RAO	SO	HOSO	VOO
FA (%)						
C16:0	14.96 ± 0.02	16.32 ± 0.02	11.61 ± 0.02	6.62 ± 0.01	4.62 ± 0.01	11.59 ± 0.01
C16:1	7.82 ± 0.01	7.71 ± 0.01	9.02 ± 0.02	0.12 ± 0.00	ND	0.97 ± 0.01
C18:0	0.38 ± 0.00	0.59 ± 0.00	0.29 ± 0.00	4.27 ± 0.01	4.28 ± 0.01	3.05 ± 0.01
C18:1	63.75 ± 0.04	62.73 ± 0.02	68.07 ± 0.03	24.83 ± 0.01	81.11 ± 0.03	77.66 ± 0.02
C18:2	11.80 ± 0.02	11.44 ± 0.01	10.07 ± 0.01	62.62 ± 0.02	8.75 ± 0.01	5.09 ± 0.01
C18:3	0.90 ± 0.01	0.82 ± 0.01	0.53 ± 0.01	0.17 ± 0.01	< 0.10	0.69 ± 0.01
C20:0	0.11 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.27 ± 0.01	< 0.10	0.35 ± 0.01
C20:1	0.19 ± 0.00	0.21 ± 0.01	0.30 ± 0.01	0.22 ± 0.01	< 0.10	0.29 ± 0.01
C22:0	< 0.10	< 0.10	ND	0.58 ± 0.01	0.12 ± 0.01	< 0.10
Toc ($mg \cdot kg^{-1}$)						
α	82	59	351	486	487	191
β	9	64	9	16	8	0
γ	210	126	21	0	246	13
δ	33	42	18	0	31	0
Total	334 ± 10	291 ± 8	399 ± 11	502 ± 14	782 ± 20	204 ± 4
Acidity (% oleic acid)	0.10 ± 0.03	0.10 ± 0.02	0.09 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.14 ± 0.06
PV (meq $O_2 \cdot kg^{-1}$)	2.0 ± 0.6	1.9 ± 0.5	4.0 ± 0.5	5.8 ± 1.1	3.8 ± 0.8	3.6 ± 0.6
OSI (h)	25.3 ± 0.6	11.4 ± 0.2	9.7 ± 0.1	10.9 ± 0.3	32.7 ± 0.2	30.9 ± 0.5
Smoke point (°C)	249 ± 2	212 ± 2	220 ± 3	234 ± 1	233 ± 3	188 ± 2
PC (%)						
OxTAG	0.9	1.0	0.8	1.2	0.9	1.2
TAGD	0.5	0.7	1.5	1.6	0.4	0.5
DAG	2.2	2.1	2.2	1.7	1.9	2.6
FFA[a]	0.2	0.2	0.2	0.5	0.3	0.3
Total	3.8 ± 0.1	4.0 ± 0.1	4.8 ± 0.1	5.0 ± 0.1	3.5 ± 0.1	4.7 ± 0.1

Abbreviations: VAO, virgin avocado oil; MRAO, minimally refined avocado oil; RAO, refined avocado oil; SO, sunflower oil; HOSO, high-oleic sunflower oil; VOO, virgin olive oil; FA, fatty acids; Toc, tocopherols, PV, peroxide value; OSI, oil stability index; PC, polar compounds; oxTAG, oxidized triacylglycerols; TAGD, triacylglycerol dimers; DAG, diacylglycerols; FFA, free fatty acids.

Results are expressed as means ± SD (n = 3).

[a]It also includes polar unsaponifiable matter.

and especially HOSO, quite in contrast with that in SO, otherwise with high content in linoleic acid.

Regarding quality parameters, HOSO, VOO and VAO showed comparatively higher oxidative stability values. HOSO contained the highest amounts of tocopherols, including high γ-tocopherol levels. One possible reason for that is that a tocopherol mix rich in γ-tocopherol had been added. HOSO also contained dimethylpolysiloxane, as indicated in the label, which is an antifoaming additive with inhibitory action in thermoxidative reactions (Márquez-Ruiz *et al.*, 2004). With respect to VAO, the γ-tocopherol levels found were higher than expected in view of the previous data published (Fernandes *et al.*, 2018; Cervantes-Paz and Yahia, 2021) although it has been recently reported that high levels can be found in VAOs when whole fruits instead of fruit mesocarps are extracted; and harvest time and quality grade have also an influence (Green and Selina, 2023a). Data of acidity, peroxide value, and polar compounds, including total levels and specific values for the oxidation and hydrolysis groups of compounds determined, were within the ranges characteristic of unused, good quality oils. As to smoke points, it is important to note that all oils showed values above the temperature used for frying in this study (180 ± 3°C).

3.2. Polymer formation

Table 2 shows the evolution of polymers during the frying experiment and at 10 hours of thermoxidation. Polymers were analyzed directly by high-performance size-exclusion chromatography.

As commented in the Introduction, direct determination of polymers is a rapid and useful tool to follow-up degradation of used frying oils since they are the compounds predominantly formed during frying. Determinations of polar compounds and polymers show a very high correlation hence the limit for human consumption is either 25-27% of polar compounds or 10-16% polymers in countries where regulations are established. Results showed that, except for HOSO, all oils reached 10-16% polymers after the tenth frying operation, which took place after a period of 48 hours keeping the oils at room temperature. There were no significant differences between values after the ninth frying operation and 10 h-thermoxidation, showing that the order of frying stability was HOSO > VAO > VOO > MRAO > RAO > SO. This order of stability differed than that obtained when measuring oxidative stability (Table 1) which was HOSO > VOO > VAO > MRAO > SO > RAO, and this was expected since such an index does not predict frying performance but oxidative behavior al low and moderate temperatures.

Formation of polymers followed a zero-order kinetic for all oils under the conditions applied, in

Table 2. Polymers (%) in oils during frying and thermoxidation

	VAO	MRAO	RAO	SO	HOSO	VOO
Initial	0.5 ± 0.1a	0.7 ± 0.1a	1.5 ± 0.1b	1.6 ± 0.1b	0.4 ± 0.1a	0.5 ± 0.1a
Frying operation:						
First	1.2 ± 0.1a	1.6 ± 0.1b	2.3 ± 0.1c	2.6 ± 0.1c	1.3 ± 0.1ab	1.5 ± 0.1ab
Third	2.5 ± 0.1b	3.4 ± 0.1c	3.9 ± 0.1d	4.9 ± 0.2e	1.1 ± 0.0a	2.7 ± 0.2b
Sixth	4.3 ± 0.1b	5.7 ± 0.2c	6.6 ± 0.2d	8.1 ± 0.3e	1.7 ± 0.1a	4.5 ± 0.1b
Ninth	5.4 ± 0.1b	9.3 ± 0.2d	11.8 ± 0.3e	13.8 ± 0.3f	4.2 ± 0.2a	8.1 ± 0.1c
Tenth	9.8 ± 0.3b	12.1 ± 0.2c	13.8 ± 0.1d	15.3 ± 0.3e	4.5 ± 0.1a	10.2 ± 0.1b
Thermoxidation						
10 h	5.6 ± 0.0b	9.1 ± 0.1d	11.1 ± 0.1e	14.6 ± 0.2f	4.8 ± 0.1a	8.5 ± 0.1c

Abbreviations: VAO, virgin avocado oil; MRAO, minimally refined avocado oil; RAO, refined avocado oil; SO, sunflower oil; HOSO, high-oleic sunflower oil: VOO, virgin olive oil.

Results are expressed as means ± SD (n = 3). Different letters in the same row indicate significant differences according to Tukey's test at $P< 0.05$.

agreement with results obtained previously in our lab (Holgado *et al.*, 2021). Table 3 summarizes the main parameters for linear regression. The relative rates of formation of polymers were calculated assuming value 1 for the oil presenting the lowest level, i.e., HOSO.

Table 3. Kinetic data for the formation of polymers in oils during frying.

Oil	k (%/h)	r	Relative rate
VAO	1.06 ± 0.04	0.967	1.37
MRAO	2.06 ± 0.05	0.998	2.68
RAO	2.48 ± 0.05	0.999	3.22
SO	2.49 ± 0.06	0.997	3.23
HOSO	0.80 ± 0.04	0.969	1.00
VOO	1.68 ± 0.04	0.998	2.29

Abbreviations: k, rate constant; r, linear regression coefficient; VAO, virgin avocado oil; MRAO, minimally refined avocado oil; RAO, refined avocado oil; SO, sunflower oil; HOSO, high-oleic sunflower oil: VOO, virgin olive oil.

Results are expressed as means ± SD (n = 3).

3.3. Tocopherol losses

Figure 1 shows the tocopherol levels in unused oils and after the ninth frying operation and 10 h-thermoxidation. Except for VAO, no significant differences were found between data obtained after the ninth frying operation and 10 h-thermoxidation. In RAO and VOO, only residual amounts were left while the highest amounts remaining were found in VAO and HOSO.

3.4. Groups of polar compounds

Oil samples corresponding to the ninth frying operation and 10 h-thermoxidation were further analyzed to determine the levels of different groups of polar compounds. Results are listed in Tables 4 and 5.

With very few exceptions, no significant differences were found between values at the ninth frying operation and 10 h-thermoxidation for all the groups of compounds quantitated. Data for total polar compounds show that SO and RAO were at or close to the rejection limit (25%), respectively. As concluded for polymer determination (Table 2), the order of frying stability according to polar compounds levels was also HOSO > VAO > VOO > MRAO > RAO > SO. Roughly half of the polar compounds was constituted by polymers (triacylglycerol dimers and higher oligomers) and, as compared to initial values (Table 1), diacylglycerols and free fatty acids remained practically at the same levels, thus indicating that hydrolytic reactions were not significant.

4. DISCUSSION

Frying performance of SO, HOSO and VOO has been extensively studied and comparison between them and other oils thoroughly reviewed (Márquez-Ruiz and Holgado, 2018; Grompone, 2020). Results obtained in the present study for SO, HOSO and VOO are consistent with most of the available published data. Thus, it has been widely reported that VOO shows better frying performance than SO, mainly because of the lower unsaturation degree of VOO and its contents of polyphenols and other minor compounds with antioxidant action. HOSO is obtained from sunflower seeds modified to achieve a similar high-oleic content to that in olive oils, resulting in comparable good frying performance (Grompone, 2020). In the present study, the especially high resistance to alteration of the HOSO tested, apart from his monounsaturated fatty acid profile, is the presence of higher amounts of tocopherols, including γ-tocopherol. These results agree with those showing the outstanding antioxidant action of γ-tocopherol (Marmesat *et al*., 2008). Besides, addition of dimethylpolysiloxane enhanced frying stability since it acts as a strong inhibitor of thermoxidative reactions in discontinuous frying (Márquez-Ruiz *et al*., 2004).

To the best of our knowledge, this work is the first to evaluate avocado oils in frying. Virgin and two refined avocado oils of different refining grade were selected. Fatty acid composition was very similar for all of them but frying performance differed significantly. VAO showed an excellent behavior, even better than VOO, attributable to higher amounts of initial tocopherols and possibly also to the presence of other antioxidant compounds characteristic of virgin avocado oils, like carotenoids (lutein, α-, β-carotenes), phenolic and polyphenolic compounds (Woolf *et al.*, 2009; Cervantes-Paz and Yahia, 2021).

MRAO also exhibited noteworthy potential as a frying oil since results of both polar compounds and polymers showed that among refined oils, except for HOSO, MRAO showed superior performance. This

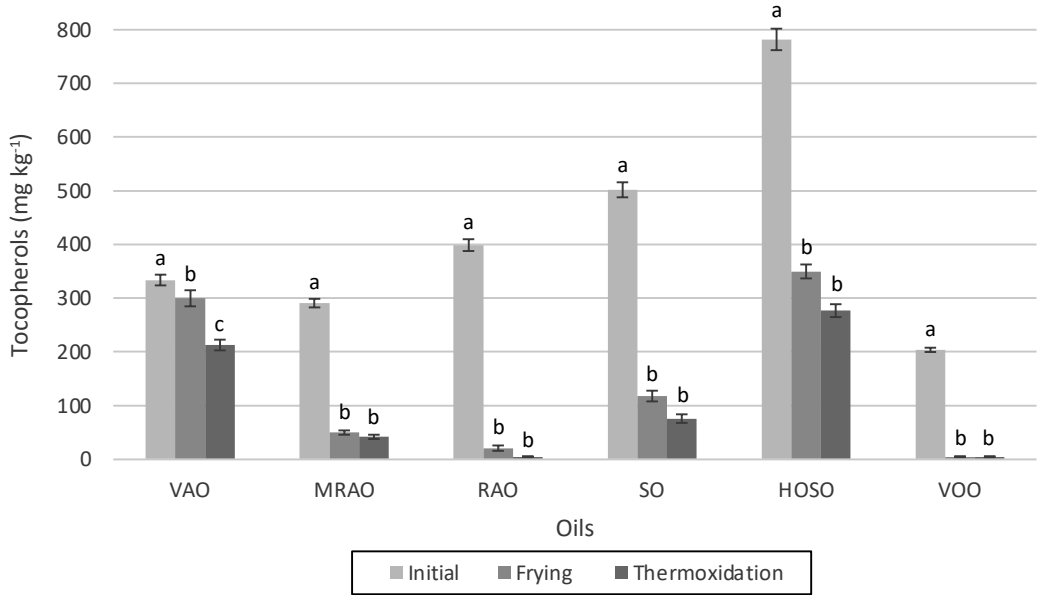

Figure 1. Tocopherol levels in unused oils, after 9 frying operations and after 10 h-thermoxidation. Abbreviations: VAO, virgin avocado oil; MRAO, minimally refined avocado oil; RAO, refined avocado oil; SO, sunflower oil; HOSO, high-oleic sunflower oil: VOO, virgin olive oil. Results are expressed as means ± SD (n = 3). Different letters in the same oil indicate significant differences a ccording to Tukey's test at $P< 0.05$.

finding is important to note since refined oils, and not virgin oils, are preferred for frying applications for their lower costs. In contrast, the performance of RAO, although acceptable, fell short of expectations, marginally outperforming SO despite the latter's primarily unsaturated fatty acid composition that makes it more susceptible to oxidation. This divergence may be partially attributed to the initially higher tocopherol levels in SO in comparison to RAO. Additionally, potential impacts stemming from the different refining processes applied to MRAO and RAO might have contributed to their distinct frying performance.

It is important to remark that the refining process serves to effectively eliminate or diminish undesired components such as free fatty acids, pigments, gums, and phospholipids to acceptable levels. However, it also results in the removal of beneficial minor constituents like phytosterols, tocopherols, carotenoids, and polyphenolics. Opting for a milder approach to refining holds the potential to safeguard these health-promoting elements. In the quest to minimize oil loss while maximizing refined oil stability and preserving essential nutrients, the concept of minimal refining has emerged as an innovative alternative to traditional refining techniques (García-González *et al.*, 2021).

In this work, the influence of refining on the tocopherol content of the unused VAO, MRAO and RAO was clearly noted. Although MRAO showed the lowest overall content, it showed higher γ-tocopherol concentration, as did VAO, both with similar tocopherol profiles. However, RAO had the lowest γ-tocopherol content, one tenth of the content of VAO and MRAO, and the highest α-tocopherol content, which became the dominant component, probably added to compensate for the loss of antioxidant minor compounds during intensive refining. This additional α-tocopherol is more susceptible than γ-tocopherol to degradation during the frying process (Marmesat *et al.*, 2008). Our findings could indicate that minimally processed oils not only retain a higher nutritional value, making them healthier alternatives to extensively refined oils, but also exhibit greater resistance to thermoxidative processes.

Results obtained in this study showed that levels of triacylglycerol polymers closely correlated with polar compound levels, in agreement with previous results obtained by Marmesat *et al.* (2007) based on the analysis of 105 samples of used frying fats and

Table 4.- Distribution of polar compounds (%) in oils after 9 frying operations

	VAO	MRAO	RAO	SO	HOSO	VOO
OxTAG	3.4 ± 0.1a	7.5 ± 0.2b	9.0 ± 0.2c	10.3 ± 0.4d	3.4 ± 0.2a	7.5 ± 0.3b
TAGD	3.9 ± 0.1b	7.2 ± 0.2c	9.0 ± 0.2d	9.4 ± 0.2d	3.1 ± 0.1a	6.5 ± 0.2c
TAGO	1.5 ± 0.1b	2.1 ± 0.1d	2.8 ± 0.1e	4.4 ± 0.1f	1.1 ± 0.0a	1.6 ± 0.1c
DAG	2.1 ± 0.1a	2.6 ± 0.2bc	3.0 ± 0.1c	1.8 ± 0.1a	2.0 ± 0.1a	2.5 ± 0.1b
FFA[a]	0.5 ± 0.1a	0.3 ± 0.1a	0.3 ± 0.1a	0.6 ± 0.2a	0.3 ± 0.1a	0.4 ± 0.1a
Total	11.4 ± 0.4b	19.7 ± 0.5d	23.9 ± 0.2e	25.1 ± 0.3f	9.9 ± 0.3a	18.5 ± 0.5c

Abbreviations: VAO, virgin avocado oil; MRAO, minimally refined avocado oil; RAO, refined avocado oil; SO, sunflower oil; HOSO, high-oleic sunflower oil: VOO, virgin olive oil; oxTAG, oxidized triacylglycerols; TAGD, triacylglycerol dimers; TAGO, triacylglycerol oligomers; DAG, diacylglycerols; FFA, free fatty acids.
Results are expressed as means ± SD (n = 3). Different letters in the same row indicate significant differences according to Tukey's test at $P< 0.05$.
[a]It also includes polar unsaponifiable matter.

oils from various sources, including domestic and industrial fryers, restaurants, and catering services. They reported that 25% of polar compounds corresponded to 13.7% of triacylglycerol polymers. The results obtained in the present study fit well into that correlation. For example, after nine frying operations, RAO showed contents of 23.9% polar compounds and 11.8% polymers, and calculated polymers would give 12.9 % polymers. In the case of SO, which showed contents of 25.1% polar compounds and 13.8% polymers, calculated polymers would be likewise 13.8%.

The results obtained in this study show that hydrolytic compounds were not formed during frying. In fact, there were no significant differences between results of diacylglycerols and free fatty acids in frying and thermoxidation experiments, the former with the added moisture coming from potatoes.

Regarding the few studies published on thermoxidation experiments using avocado oils, our results are consistent with those reported by Machado da-Costa *et al.* (2021), who showed similar values of *p*-anisidine index in VOO acquired in a local market and VAO extracted in their laboratory, heated at 180°C. Unfortunately, the indexes used in that study (acidity, peroxide value, iodine value, specific extinction coefficients) were not appropriate to evaluate oil alteration at high temperature, they did not provide quantitative data and, at best, evaluated only a partial aspect of the alteration. For example, *p*-anisidine is a spectrophotometric measurement of aldehydes. In the study undergone by Berasategi *et al.* (2012), a mixture of virgin and refined avocado oils and extra virgin olive oils heated at 180°C showed similar stability as determined by thiobarbituric acid-reactant substances (TBARS). TBARS is another spectrophotometric index not recommended for frying, shows limited specificity and ruggedness, and poor sensitivity for monounsaturated oils. Only in the study of De Alzaa *et al.* (2012), polar compound determination was used, among other parameters, to evaluate oil alteration after heating at 180°C for 6 hours. Different commercial oils were tested including avocado, conventional sunflower and olive oils. In the case of avocado oil, the authors did not specify whether it was virgin or refined. In case it was virgin avocado oil, our results would agree with those obtained in that study since they indicated similar behavior of virgin olive and avocado oils, both being more stable that sunflower oil.

In the present study, it is important to remark the excellent correlation found between the results obtained from frying experiments and those from the thermoxidation test simulating frying. Ten hours of thermoxidation were equivalent to nine discontinuous frying operations under the frying conditions used. Application of the Rancimat equipment, which is widely used by the edible and pharmaceutical oils industries to determine oxidative stability, could be also used to simulate frying following the procedure proposed. This would avoid the intensive labor involved in frying experiments and would enable

to compare easily the results found between labs, thereby thwarting the influence of uncontrolled variables involved in frying (Machado *et al.*, 2007).

5. CONCLUSIONS

The novel contribution of this work was to evaluate the performance of avocado oils in frying for the first time. Comparison with oils commonly used for frying showed that the order of frying stability was HOSO > VAO > VOO > MRAO > RAO > SO. Among avocado oils, VAO showed an excellent frying behavior followed by MRAO while RAO was just slightly better than SO. Taking into account the lower cost of refined oils as compared to virgin oils, minimally refined avocado oil can be considered a good alternative for frying.

It is also important to remark that results obtained through the thermoxidation test used in this study came to the same conclusions than did the frying experiments. Therefore, the proposed test could be used as an excellent alternative to frying experiments to improve comparison of the results obtained between laboratories.

In the context of avocado oils, initial tocopherol levels of avocado oils clearly reflected the effect of refining conditions, which could have contributed to their distinct frying performance. Therefore, future research should focus on the development and implementation of reduced cost, minimal refining processes, to minimize losses of antioxidants and bioactive compounds, and obtain competitively priced avocado oils for use in frying.

DECLARATION OF COMPETING INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

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AUTHORSHIP CONTRIBUTION STATEMENT

F Holgado: Formal analysis, Investigation, Methodology, Writing – review & editing. M Martínez-Ávila: Formal analysis, Investigation, Writing – review & editing. MV Ruiz-Méndez, Funding acquisition, Investigation, Methodology, Writing – review & editing. G. Márquez-Ruiz: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft.

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Table 5.- Distribution of polar compounds (%) in oils after 10 h-thermoxidation

	VAO	MRAO	RAO	SO	HOSO	VOO
OxTAG	4.1 ± 0.1a	8.1 ± 0.2b	9.1 ± 0.3d	8.1 ± 0.2c	3.6 ± 0.2a	8.0 ± 0.3bcd
TAGD	4.5 ± 0.1b	7.0 ± 0.2c	8.1 ± 0.1d	11.1 ± 0.2e	4.0 ± 0.1a	6.6 ± 0.1c
TAGO	1.1 ± 0.0b	2.1 ± 0.1d	3.0 ± 0.1e	3.5 ± 0.1f	0.8 ± 0.0a	1.9 ± 0.0c
DAG	2.0 ± 0.1a	2.7 ± 0.2bc	3.0 ± 0.2c	1.6 ± 0.1a	1.8 ± 0.1a	2.4 ± 0.1b
FFA[a]	0.3 ± 0.1ab	0.2 ± 0.0a	0.2 ± 0.1ab	0.5 ± 0.1b	0.2 ± 0.0a	0.5 ± 0.1b
Total	12.0 ± 0.3b	20.1 ± 0.4d	23.7 ± 0.2e	24.7 ± 0.2f	10.4 ± 0.3a	19.4 ± 0.3c

Abbreviations: VAO, virgin avocado oil; MRAO, minimally refined avocado oil; RAO, refined avocado oil; SO, sunflower oil; HOSO, high-oleic sunflower oil: VOO, virgin olive oil; oxTAG, oxidized triacylglycerols; TAGD, triacylglycerol dimers; TAGO, triacylglycerol oligomers; DAG, diacylglycerols; FFA, free fatty acids.

Results are expressed as means ± SD (n = 3). Different letters in the same row indicate significant differences according to Tukey's test at $P < 0.05$.

[a]It also includes polar unsaponifiable matter.

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