The Journal of Supercritical Fluids 190 (2022) 105750

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Hass and Fuerte avocado (*Persea americana sp.*) oils extracted by supercritical carbon dioxide: Bioactive compounds, fatty acid content, antioxidant capacity and oxidative stability

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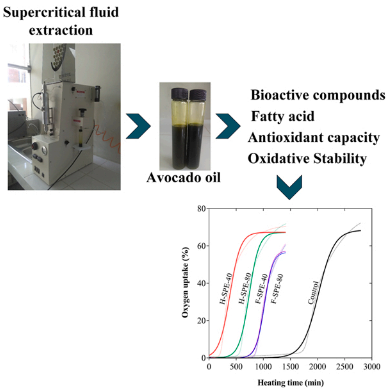
HIGHLIGHTS

- Oil avocado extraction by supercritical CO_2 (AO-SPE) was fitted to the logistic model.
- AO-SPE showed higher total carotenoid and chlorophyll content.
- AO-SPE's at 80 °C and 400 bar showed higher antioxidant capacity.
- Lipid oxidation of AO-SPE was performed using the Oxitest system.
- AO-SPE was more susceptible to lipid oxidation as showed estimated kinetic parameters.

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Avocado oil
Functional and gourmet oils
Green technology extraction

ABSTRACT

Avocado oils (AO) from Hass and Fuerte varieties were extracted by supercritical CO_2 ($scCO_2$) at 40 and 80 °C (400 bar). The yields of the extraction ranged from 36 % to 38 % and AO extraction using $scCO_2$ showed a good fit to the logistic model. Physicochemical, bioactive compounds, fatty acid composition, antioxidant capacity and

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https://doi.org/10.1016/j.supflu.2022.105750
Received 31 May 2022; Received in revised form 8 September 2022; Accepted 8 September 2022
Available online 12 September 2022

Phenolic compounds
Antioxidant capacity
Oxidative stability under accelerated conditions

oxidative stability of the oil were influenced by $scCO_2$ extraction. Compared to commercial product extracted by cold pressing, the AO extracted with $scCO_2$ showed a lower total phenolic content, except for that extracted from the Fuerte variety at 80 °C, as well as higher total carotenoid and chlorophyll contents, unsaturated/saturate fatty acid ratio and antioxidant capacity in that extracted at 80 °C. However, initiation and propagation kinetic parameters, estimated at the first time using accelerated Oxitest system, showed that AO obtained by $scCO_2$ is more susceptible to lipid oxidation.

1. Introduction

The avocado (*Persea americana* Mill.) is a tropical fruit native to the Americas which is cultivated for food and medicinal purposes. It has high nutritional value [1] and contains many bioactive compounds [2]. The avocado contains a significant amount of oil (15–30 g/100 g edible portion) and this avocado oil (AO) is used as a gourmet and health-promoting ingredient in culinary and cosmetic applications [3]. AO consists of 76 % monounsaturated fatty acids (MUFAs), 12 % polyunsaturated fatty acids (PUFAs), and 12 % saturated fatty acids [4]. In addition, AO contains several antioxidant compounds, notably α-tocopherol (70–190 μg/g) and also β-, γ-, and δ-tocopherol (< 10 μg/g of each) [2], carotenoids (11.1–46.9 μg β-carotene/g), chlorophylls (22.3–69.8 μg/g) [3,5] and phenolic compounds such as apigenin 7-glucoside (0.25 μg/g), p-hydroxybenzoic acid (0.21 μg/g), caffeic acid (0.06 μg/g) and luteolin (0.03 μg/g) [6]. Meanwhile, the phytosterol content of AO (3.3–4.5 mg/g) is higher than that of olive oil, with the most abundant being β-sitosterol, and including smaller amounts of sitostanol, cycloartenol, cycloeucalenol and D7-avenasterol [7]. However, the nutritional composition and bioactive compound content of AO could be strongly influenced, among other factors, by the extraction processes used to produce it and by the avocado variety [8–11].

Green technology for edible oil extraction is currently a hot research topic in the food industry because of the potential to reduce energy consumption, carbon and water footprints, and to enhance the quality of the product [12–14]. In this context, extraction with supercritical CO_2 ($scCO_2$) has been used to extract AO at a low temperature (40–80 °C) and pressure (200–400 bar) and over a short extraction time [15–17]. This allows thermally labile components to be retained and the energy cost to be minimized [18]. Characteristics of the $scCO_2$ such as temperature, pressure or co-solvent influence the AO yield and composition [4]. Thus, OA extracted at 40 °C and at 250 bar over 150 min affords the maximum yield for single-step $scCO_2$ extraction (40 %) and the resultant AO showed DPPH scavenging activity of about 65 % [15]. In another study using $scCO_2$ at 200 bar and at 60 °C, the resultant AO yield was significantly improved by the introduction of a second extraction step using a mixture of $scCO_2$ and ethanol. In this case, the AO yield reached about 65 % and was accompanied by an increase in the tocopherol content, which in some cases was similar to that of commercial samples [17]. However, no studies have yet reported the effect of different $scCO_2$ extraction conditions on the antioxidant capacity of AO, the content of mainly antioxidants such as carotenoids, chlorophyll and phenolic compounds, and AO oxidative stability.

Therefore, the aim of the present study was to evaluate the effect of the temperature and pressure of $scCO_2$ on the physical and functional properties of AO from the Hass and Fuerte varieties of avocado. These include the contents of bioactive compounds such as phenolics, carotenoids, chlorophyll and fatty acids; as well as antioxidant capacity measured by single electron transfer and hydrogen atom transfer assays, and lipid oxidation under accelerated conditions determined using a free-reagent Oxitest system.

2. Materials and methods

2.1. Material

Hass and Fuerte avocado varieties were purchased from a local market (Moquegua, Peru) and we selected fruit with good physical integrity. The avocados were transported to the laboratory where the pulp was isolated manually and cut into 4–5 pieces; a sample fruit of each variety was used to measure the moisture content. The rest of the pulp was frozen at − 80 °C for 24 h in an ultra-low-temperature freezer BDF-86V58 (BIOBASE, CA, USA) and then lyophilized for 40 h in a freeze dryer BK-FD10PT (BIOBASE, CA, USA). Finally, the lyophilized pulp was ground using a universal M20 grinder (IKA® WERKER, Staufen, Germany), sieved through No. 50 mesh, packed in plastic bags and stored to − 20 °C until use. A food grade commercial avocado oil obtained by cold-pressing (Marnys®, Cartagena, Spain) was used as control sample.

2.2. Moisture content and drying rate of avocado

The moisture content of the Hass and Fuerte avocados was determined on an AnD MX-50 moisture analyzer (A&D Company Limited, IL, USA) and it was used as a maturity index [19]. Briefly, 5 g of avocado pulp was placed in pre-weighed aluminum dishes and dried at 105 °C; moisture was monitored every 5 s until equilibrium was reached using a computer with an RS232 connection and WinCT-Moisture software (A&D Company Limited, IL, USA). The experimental data were then fitted to the Page model derived from Newton's law (Eq. (1)) and the drying rate parameters were estimated [20]:

$$MR = \exp(-kt^{n}) \tag{1}$$

where MR is the moisture ratio, k is the rate drying (s^{-1}) and n is the constant of the model (dimensionless).

2.3. Avocado oil extraction by supercritical carbon dioxide

AO from the Hass and Fuerte varieties was obtained using $scCO_2$ in a Speed SFE Basic system (Applied Separation, PA, USA) reported by Corzzini et al. [17] with slight modifications. Briefly, the beds were packaged with 5.0 g of ground and lyophilized avocado spread horizontally, and then the $scCO_2$ flowed through the bed at 2.5 g/min. AO was extracted at 400 bar at two temperatures (40 and 80 °C), and collected every 20 min for 4 h. At the different extraction times, the percentage yield of AO (Eq. (2)) was fitted to the adapted logistic model by non-linear regression analysis (Eq. (3)) [21,22].

$$Yield\left(\frac{g \quad oil}{100 \quad g \quad lyophilized \quad avocado}\right) = \frac{\text{mass of avocado oil(g)}}{\text{mass of lyophilized avocado(g)}} x \quad 100 \tag{2}$$

$$Yield(t) = \frac{y_0}{\exp(Ct_m)}\left[\frac{1+\exp(Ct_m)}{1+ \quad \exp(C(t_m-t))} - 1\right] \tag{3}$$

where y_0 is the maximum yield of oil achieved, t_m is the time (min) when the maximum yield rate of oil occurs, and C (min^{-1}) is an adjustable parameter without physical meaning.

2.4. Characterization of avocado oil obtained using $scCO_2$

2.4.1. Color determination

The color of the AO was measured using a CR-400 colorimeter

(Konica Minolta Inc, Japan). One hundred microliters of AO were placed over the lens of the colorimeter. The *L, a* and b** values were measured in triplicated and the chroma (C) and hue angle (h°) were calculated (Eqs. (4) and (5)) [23]. A CRA43 standard white tile (Konica Minolta Inc., Japan) was used as a reference to calibrate the colorimeter.

$$C = \sqrt{a^{*2} + b^{*2}} \tag{4}$$

$$h^{\circ} = tan^{-1}\left(\frac{b^*}{a^*}\right) \tag{5}$$

2.4.2. *Total phenolics*

Phenolic compounds were extracted from the AO following the procedure reported by Köseoğlu et al. [24] with slight modifications. Briefly, 0.25 g of AO was solved in 0.5 mL of hexane; phenolic compounds were extracted using 0.5 mL methanol/water (60:40 v/v) by shaking the mixture at 1400 rpm for 10 min at room temperature. Afterwards, the methanol/water phase was separated by centrifugation at 10,000 g for 5 min at 4 °C, collected and stored at − 20 °C until analysis.

Total phenolic content (TP) was assessed by a rapid and microtiter and Folin Ciocalteau (F-C) assay [25] with slight modifications. Briefly, 50 µL of gallic acid standard solution (0, 6.25, 12.5, 25 and 50 mg/L) or the methanol/water AO fraction and 50 µL of diluted F-C reagent (1:20 v/v) was placed in the Eppendorf tubes, mixed and left to rest for 2 min. Following this, 100 µL of NaOH (0.35 mol/L) was added and the tubes were incubated for 10 min in dark conditions. Afterwards, 100 µL of supernatant, obtained by centrifugation for 2 min at 10,000*g* and 4 °C, was transferred to the 96-well Nunc™ MicroWell™ microplate (Thermo Scientific, Madrid, Spain). The absorbance was read at 760 nm in a Synergy HTX Multi-Mode microplate reader (Biotek, Rochester, VT, USA). Finally, TP was expressed as mg gallic acid equivalent (GAE)/kg of AO.

2.4.3. *Total carotenoids and chlorophyll*

Total carotenoid and chlorophyll contents of the AO were determined following the method described by Lichtenthaler & Buschmann [26]. Briefly, 100 µL of AO was mixed with 1 mL of acetone and absorption spectra were acquired at 400–700 nm every 0.5 nm in a Lambda 1050 UV/Vis/NIR spectrophotometer (PerkinElmer, CA, USA). The spectra were deconvoluted by applying a linear baseline and the Gauss Amp model of the PeakFit v.4.12 software (Systat Software, Inc., USA) following Borello & Domenici [27]. The absorbance of the peaks at 470, 644.8 and 661.6 nm were used for the quantification of total carotenoids, and chlorophyll a and b, respectively, which were calculated (Eqs. 6–8) and expressed as mg/kg of AO [26].

$$\mathrm{Ca} = 11.24A_{661.6\ nm} - 2.04A_{644.8\ nm} \tag{6}$$

$$\mathrm{Cb} = 20.13A_{644.8\ nm} - 4.19A_{661.6\ nm} \tag{7}$$

$$\mathrm{CT} = \frac{1000A_{470\ nm} - 1.91Ca \quad - 63.14Cb}{214} \tag{8}$$

where: C_a, C_b and C_T are chlorophyll a and b, and total carotenoids, respectively, in µg/mL; and A is the absorbance.

2.4.4. *Fatty acid composition*

The fatty acid content of the AO was previously derivatized to methyl esters and measured by gas chromatography according to the official AOAC method 991.39 [28]. Briefly, 0.025 g of AO and 1.5 mL 0.5 M methanolic NaOH were put into glass tubes, blanketed with N_2, capped, mixed and heated to 100 °C for 5 min. The tubes were cooled and then 2 mL BF_3 in methanol was added, they were blanketed again with N_2, capped tightly, mixed, and heated to 100 °C for 30 min. The mixtures were then cooled to 30–40 °C and 1 mL isooctane was added, and again they were blanketed with N_2, capped, and shaken vigorously for 30 s while still warm. Then 5 mL saturated NaCl solution was added, they were blanketed with N_2, capped, mixed thoroughly, and cooled at room temperature. When the isooctane layer was separated from the lower aqueous phase, it was transferred to a clean glass tube, blanketed with N_2, and capped. The aqueous phase was extracted with isooctane again, as above.

The fatty acids were analyzed with gas chromatography using a Shimadzu GC-2010 equipped with an AOC-20Si auto injector and flame ionization detector at 250 °C (Shimadzu, Kyoto, Japan) and a capillary column SP®-2560 (100 m × 0.25 mm i.d., liquid films 0.20 µm; Restek, Bellefonte, PA, USA). As a carrier gas we used helium at 261.5 kPa and a flow rate of 30 mL/min, the oven temperature was programed as follows: 100 °C for 4 min, then increased to 240 °C at a rate of 3 °C/min, and kept at 240 °C for 10 min. The injection (1 µL) was performed in split mode with a ratio of 1/100 and the injector temperature set at 225 °C. Identification of the fatty acids was based on the retention times of the fatty acid methyl esters (FAME), whereas the percentage of relative peak areas with respect to the total area from the chromatograms was performed as qualitative analysis.

2.4.5. *2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) assay*

The ABTS assay adapted to the microplate reader was used to measure the antioxidant capacity of the AO [29]. Firstly, the ABTS cation radical ($ABTS^{\circ+}$) was synthesized by reaction of 7 mM ABTS cation with 2.45 mM potassium persulfate in water, and leaving the mixture in the darkness at room temperature for 16 h [30]. The $ABTS^{\circ+}$ working solution (AWS) was prepared by dilution of stock solution (1:50 v/v with ethanol) and diluted AO was prepared by dilution in acetone (1:100 v/v) and centrifugation at 10,000 g for 5 min at 4 °C. For the ABTS assay, 10 µL of Trolox (0–600 µM) or diluted AO was added to a 96-well Nunc™ MicroWell™ microplate (Thermo Scientific, Madrid, Spain), immediately followed by 100 µL of AWS. Afterwards, the mixture was shaken for 1 min and absorbance was read at 734 nm in triplicate using a Synergy HTX Multi-Mode microplate reader (Biotek; Rochester, VT, USA) after 4 min. Finally, the antioxidant capacity was calculated as Trolox equivalent antioxidant capacity (TEAC) from the Trolox standard curve and expressed as mmol TEAC/kg of AO.

2.4.6. *Oxygen radicals absorbance capacity (ORAC)-pyrogallol red (PGR) assay*

The antioxidant capacity of the AO was also measured by adapted the ORAC-PGR assay [31]. Briefly, 40 µL of diluted AO (1:100 v/v in acetone) was mixed with 170 µL of 75 mM phosphate buffer (pH 7.4) (PB) and centrifuged at 10,000*g* for 2 min at 4 °C to obtain a supernatant. Then, 210 µL of Trolox (0–120 µM) or the supernatant prepared as detailed above, was added to a 96-well Nunc™ MicroWell™ microplate (Thermo Scientific, Madrid, Spain), automatically mixed at medium speed and incubated at 37 °C with 20 µL PGR (62.5 µM in PB) introduced by the automatic injector of the Synergy HTX Multi-Mode microplate reader (Biotek, Rochester, VT, USA). After this, 20 µL 2, 2′-Azobis(2-methylpropionamidine) dihydrochloride (AAPH) (125 mM in PB) was added to each well by another automatic injector on the microplate reader and the absorbance at 540 nm and 37 °C was monitored every 1 min for 60 min. From the absorbance ratios (A/A_0) and incubation times, the area under the curve (AUC) was calculated. Finally, the net AUC values for the diluted AO and Trolox were calculated by subtracting the value of the blank with AAPH; the antioxidant activity of the AO was expressed as µmol Trolox/100 g of AO using the Trolox standard curve.

2.4.7. *Accelerated oxidative stability of avocado oil obtained using $scCO_2$*

The oxidative stability of the AO was also assessed using an Oxidation Test Reactor (Velp® Scientifica, Usmate, Italy) [32]. Briefly, 5.0 g of AO extracted by $scCO_2$ and by cold pressing (control) were distributed homogenously in two hermetically sealed titanium chambers.

Afterwards, O_2 was purged into the chamber up to a pressure of 6 bar and the reactor temperature was set at 90 °C. The oxygen pressure was monitored using the OXISoft™ software (Velp® Scientifica, Usmate, Italy) and the O_2 uptake (%) of the AO during the lipid oxidation was calculated at different times as:

$$O_2 \quad uptake \quad (\%) = \left(1 - \frac{OP_t}{OP_0}\right) x \quad 100 \tag{9}$$

where: OP_t and OP_0 are the oxygen pressure at a given time and initially, respectively.

The kinetic curves of O_2 uptake against time were fitted to the linear (Eq. (10)) and sigmoidal (Eq. (11)) models over the whole range of the kinetic data points in the initiation and propagation phases of O_2 uptake. The equations were adapted from Farhoosh [33,34]. Different kinetic parameters of lipid oxidation were calculated (Eqs. (12)–(18)).

$$Uptake \quad O_2 = \quad k_{IP}(t) + Uptake \quad O_{20} \tag{10}$$

$$Uptake \quad O_2 = \quad \frac{k_c}{exp[k_c(C - t)] + k_d} \tag{11}$$

$$Uptake \quad O_2 \quad _{max} = \quad \frac{k_c}{k_d} \tag{12}$$

$$R_{max} = \quad \frac{k_c^2}{4k_d} \tag{13}$$

$$R_n = \quad \frac{R_{max}}{Uptake \quad O_{2max}} \tag{14}$$

$$IP = \quad \frac{k_c(2 - k_c C + lnk_d) - 4(Uptake \quad O_{20} \quad k_d)}{4k_{IP}k_d - k_c^2} \tag{15}$$

$$O_i = \quad \frac{IP}{k_{IP}} \tag{16}$$

$$Uptake \quad O_{2IP} = \quad k_{IP}(IP) + Uptake \quad O_{20} \tag{17}$$

$$t_p = \quad \frac{4k_d R_{max} - k_c R_n(2 - k_c C + lnk_d)}{Uptake \quad O_{2max}} - IP \tag{18}$$

where: k_{IP} (% of O_2 uptake min^{-1}) is the pseudo zero-order rate constant of the initiation phase and $O_{2\ 0}$ uptake is the % of O_2 uptake at $t = 0$; IP (min) is the duration of the induction period; O_i is the initiation oxidizability parameter (% of O_2 $uptake^{-1}$ min^2); k_c (min^{-1}) and k_d (% of O_2 $uptake^{-1}$/min) are the pseudo first- and second-order composite and decomposition rate constants of O_2 uptake in the propagation phase, respectively; C (% of O_2 uptake $^{-1}$) is an integration constant; O_2 uptake $_{max}$ is the maximum O_2 uptake (%) and R_n (min^{-1}) is a parameter that measures the propagation oxidizability of lipids and t_p (min) is the time of the propagation phase.

2.5. Statistical analysis

Non-linear regression analysis, to model the drying rate of avocado and AO extraction by a supercritical fluid, Student's t-test, one-way ANOVA, Tukey's comparison test and AUC calculations were performed using Prism GraphPad 6.1 (GraphPad Prism, San Diego, CA, USA).

3. Results and discussion

3.1. Moisture content and drying rate of avocado

In the present study, the moisture of the mesocarp of Hass avocados (69.5 % ± 2.7 % wet weight) (Supplementary Material 1) was similar to values previously published for mature Hass avocados: 67.1–69.4 % wet weight [35] and for unripe avocados (70.14 % ± 0.04 % wet weight) [36]. In fact, we harvested the avocado to allow for transportation to the supercritical fluid laboratory before they reached eating ripeness, 4–5 days later. Similarly, the moisture of Fuerte avocados (72.7 % ± 2.3 % wet weight) (Supplementary Material 1) was comparable to values previously published for unripe (70.3 % ± 0.1 % wet weight) and ripe (71.3 % ± 0.1 % wet weight) samples [36]. The maturity of an avocado depends on the moisture content of the pulp. In the Hass variety, the moisture reduces with maturity [35,36]; whereas in Fuerte avocado, increased moisture is found in ripe samples [37]. The maturity of an avocado influences on the flavor, taste, texture, ripeness for eating [38], phenolic compound and fatty acid content, and antioxidant capacity [35,37,39]. Finally, moisture content of our Hass and Fuerte avocados did not show any statistically difference ($p > 0.05$), as was the case for the drying rates (k) and n parameters of the Page model (Supplementary Material 1 and Supplementary Material 2). However, the k and n parameters may be better predictors of the maturity of avocados because they are calculated from all the weight data.

3.2. Avocado oil extraction by supercritical carbon dioxide

The yields of AO extraction from Hass and Fuerte varieties by $scCO_2$ at 40 and 80 °C (400 bar) ranged from 36 to 38 g oil/100 g lyophilized avocado (Table 1). However, under similar extraction conditions, a higher oil yield (57–61 g oil/100 g lyophilized avocado) was obtained for Hass avocados [16,17]. In other work, an oil yield of 40 g oil/100 g oven-dried avocado was obtained for Fortune avocados using $scCO_2$ at 40 °C and 250 bar for 150 min [15]. A lower overall extraction yield (12–39 g oil/100 g lyophilized avocado) was achieved at 200 bar and different temperatures (40, 60 and 80 °C) by $scCO_2$ [17]. However, in none of these studies was the degree of maturity of the samples used in the $scCO_2$ extraction characterized. It is well known that avocado maturity influences the physical structure of samples and their chemical composition. The degradation of parenchyma cells due to the activity of cellulase and polygalacturonase increases during ripening; in turn, this

Table 1
Kinetic parameter of the logistic model fitted to the experimental data for avocado oil extracted by supercritical CO_2 at 40 and 80 °C, and at 400 bar.

Avocado oil samples	Logistic model parameters			Goodness of fit	
	y_o (g oil/100 g lyophilized avocado)	t_m (min)	C (min^{-1})	R^2	RSME
H-SPE-40	36.82 (34.79–39.09)	89.59 (83.62–96.14)	0.058 (0.040–0.089)	0.988	1.741
H-SPE-80	36.19 (33.63–39.10)	84.59 (76.80–93.38)	0.061 (0.037–0.110)	0.980	2.257
F-SPE-40	37.32 (35.45–39.38)	89.88 (84.45–95.78)	0.058 (0.042–0.085)	0.990	1.612
F-SPE-80	36.42 (34.07–39.09)	84.47 (77.41–92.39)	0.061 (0.038–0.104)	0.983	2.083

y0 is the maximum yield (%) of oil achieved, tm is the time (min) when the maximum yield (%) rate of oil occurs, and C (min-1) is an adjustable parameter without physical meaning. H and F are Hass and Fuerte avocado varieties, SPE = supercritical extraction and 40 and 80 are the extraction temperature in °C. (95 % confidence interval), R2 is the coefficient of determination and RSME is the Root Mean Square Error.

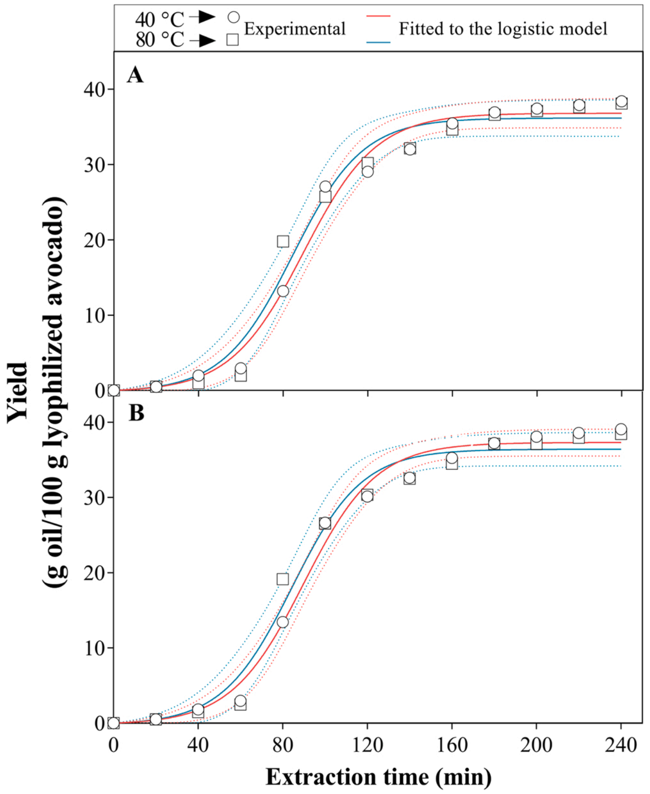

Fig. 1. Experimental and model extraction curves for supercritical CO_2 extraction of avocado oil at 40 and 80 °C, and at 400 bar. A: Hass avocado; B: Fuerte avocado. The values are the means of three replicates.

enhances the oil extraction yield [40,41]. In addition, the particle size after grinding and meshing may influence the oil yield from extraction by $scCO_2$ [17]. Meshes ranging from 8 to 48 have been used to avoid a reduction in AO yield due to the formation of agglomerates when extracting with $scCO_2$ [17]. In the present study, a logistic curve behavior was in evidence during AO extraction using $scCO_2$ at different temperatures (Fig. 1) because freeze-dried avocado was sieved in a No. 50 mesh and agglomeration was not observed. Moreover, the temperature was not statistically significant for the oil yield, as reported for the extraction AO using $scCO_2$ by Abaide et al. [15]. Finally, a positive effect on extraction yield has been reported with $scCO_2$ at 40 and 60 °C, and a negative one at 80 °C [17]. The Table 2 showed the comparison of the experimental condition and yield of avocado oil extracted by $scCO_2$ of the present study and literature.

The kinetics of oil extraction from avocado by $scCO_2$ has been characterized by two stages: a constant extraction rate and a falling extraction rate [15,17]. The former stage is characterized by a straight line, where mass transfer by convection is predominant; while in the latter stage both convection and diffusion are important [15,17]. However, in the present study, the kinetics of oil extraction from Fuerte and Hass avocado varieties showed a good fit to the logistic model (RSME < 2.2 and $R^2 > 0.98$, Table 1), revealing an initial lag phase (Fig. 1). This model has been used to fit experimental kinetic data for oil extraction from shiitake [42] and ginger oleoresin [22]. From this logistic model, the maximum extraction ($y_0 = 36$–37 g oil/100 g lyophilized avocado) was reached at about 160 min for all $scCO_2$ conditions (Table 1), which is similar to values previously published for shiitake oil extraction [42] and lower than that found for ginger oleoresin [22]. Moreover, the maximum rate of oil extraction from avocado by $scCO_2$ was achieved between 84 and 89 min (t_m) (Table 1). This is longer than found for shiitake oil by $scCO_2$ at 200 bar (63 min) [42] and oleoresin from *Capsicum annum* at 250 bar (32 min) [21] but lower than that reported for ginger oleoresin extracted at 250 bar (133–151 min) [22]. Finally, the C parameter values ranged between 0.05 and 0.061 min^{-1} (Table 1) and were similar to those obtained for oleoresin from *Capsicum annum* [21] and shiitake oil [42].

Table 2
Experimental condition and yield of avocado oil extracted by supercritical CO_2 of the present study and literature.

Experimental condition	Yield (g oil/100 g avocado)	Reference
$T = 40 - 80$ °C, $P = 400$ bar, $scCO_2$ flow = 2.5 g/min, extraction time = 2 h	38.12–$39.07^{(1)}$	Present study
$T = 40 - 60$ °C, $P = 150$–250 bar, $scCO_2$ flow = 4.0 g/min, extraction time = 2.5 h	10.1–$39.8^{(2)}$	[15]
$T = 50$ °C, $P = 400$ bar, $scCO_2$ flow = 2.5 g/min, extraction time = 3.5 h	$57^{(3)}$	[16]
$T = 40 - 80$ °C, $P = 200$–400 bar, scCO2 flow = 2.5 g/min, extraction time = 3.0 h	12 ± 1–$62 \pm 1^{(4)}$	[17]

T = Temperature, P = Pressure, (1) lyophilized Hass and Fuerte avocado varieties, (2) Oven-dried Hass avocado variety, (3) lyophilized Hass avocado variety, (4) lyophilized ripe avocado.

Table 3
Bioactive compounds and fatty acid composition of avocado oil extracted by cold pressing and supercritical CO_2 at 40 and 80 °C, and at 400 bar.

Composition	Avocado oil				
	Cold pressure extraction	Supercritical fluid CO_2 extraction			
	Control	H-SPE-40	H-SPE-80	F-SPE-40	F-SPE-80
Bioactive compounds					
Total phenolics (mg GAE/kg oil)	27.7 ± 0.4^a	15.9 ± 1.0^c	19.1 ± 1.1^b	10.9 ± 0.9^d	27.1 ± 0.7^a
Total carotenoids (mg/kg oil)	169.2 ± 10.9^d	243.6 ± 6.8^c	446.8 ± 5.2^b	435.7 ± 2.5^b	521.0 ± 2.6^a
Total chlorophyll a (mg/kg oil)	45.3 ± 4.5	36.1 ± 4.7^d	55.1 ± 2.1^b	52.0 ± 0.2^b	85.5 ± 2.4^a
Fatty acids (normalized peak area, %)					
Palmitic acid	18.8 ± 0.0^c	15.6 ± 0.9^e	27.4 ± 0.0^a	25.6 ± 0.0^b	17.0 ± 0.2^d
Palmitoleic acid	7.3 ± 0.0^c	4.2 ± 0.1^e	14.1 ± 0.0^a	13.3 ± 0.0^b	4.7 ± 0.1^d
Oleic acid	60.7 ± 0.0^c	69.1 ± 0.3^a	40.7 ± 0.0^e	42.6 ± 0.0^d	66.1 ± 0.2^b
Linoleic acid	13.3 ± 0.0b	11.1 ± 1.3^c	15.4 ± 0.0^a	14.7 ± 0.0^a	10.5 ± 0.2^c
Linolelaidic acid	ND	ND	2.5 ± 0.0^b	3.8 ± 0.0^a	1.7 ± 0.1^c

ND no detected, H and F are Hass and Fuerte avocado varieties, SPE = supercritical extraction and 40 and 80 are the extraction temperature in °C. The values are the means of three replicates and different superscript letters mean that values are statistically significant ($P < 0.05$).

3.3. *Characterization of avocado oil obtained by $scCO_2$*

3.3.1. *Total phenolics, carotenoids and chlorophyll*

The total phenolic content (TP) of the control AO extracted by cold pressing was lower than reported for the Hass variety when extracted by expeller-pressing (31.5 mg GAE/kg) [43] or by malaxation and pressing (43–57 mg GAE/kg) [5] (Table 3). It is well established that the TP of AO can be strongly influenced by the extraction process and avocado variety [5,9,11,44]. In the present work, the TP of the AO extracted by $scCO_2$ was lower than for control sample, except for F-SPE-80 (Table 3). In fact, phenolic compounds are efficiently obtained from different sources by use of $scCO_2$ and a co-solvent such as ethanol [45]. Moreover, for both avocado varieties, an increase in temperature from 40° to 80°C during $scCO_2$ extraction improved the TP of the AO, markedly for the Fuerte variety (> 2-fold) (Table 3). Previous studies carried out on several food and vegetable matrixes have shown that the solubility of phenolic compounds in $scCO_2$ increased with increasing temperature [46]. The addition of ethanol co-solvent in supercritical CO_2 extraction significantly improved the total phenolic content (53–110.7 mg/kg) in strawberry leave extracts although in absence of ethanol it is also possible to extract phenolic compounds about 6.7 mg/kg [46]. However, some compounds such as phospholipids interfere in the total phenolic determination of oils using Folin-Ciocalteu reagent, resulting in the overestimation of phenolic contents [10].

The total carotenoid value in the control AO was higher than reported previously (11.1–46.9 mg/kg) [5,41] (Table 3). It is well known that bioactive compounds present in vegetable oils are influenced by the processing conditions and by the type and variety of the source [5,9–11,44]. In this work, the AO extracted by $scCO_2$ showed higher total carotenoids than control sample (Table 3), and this was associated with the hue angle h° ($r = 0.8294$, $p = 0.0824$, Supplementary material 3). Extraction using $scCO_2$ has been demonstrated to be an efficient technology to obtain carotenoids from different food matrixes due to their non-polar chemical nature [47,48]. Likewise, when extracting phenolic compounds using $scCO_2$, an increase in temperature promoted the extraction of carotenoids from avocado by improving their solubility in $scCO_2$ (Table 3). The extraction of carotenoids by CO_2 supercritical have been reported increasing with the temperature for different matriz such as *Scenedesmus obliquus* [49] and pelletized microalgae [48].

The control AO and that obtained by $scCO_2$ at different temperatures and 400 bar contain only chlorophyll a (Table 3). The total chlorophyll of the control AO was 22.3–69.8 mg/100 g [5,41]: higher than Hass AO extracted using an Abencor® system (17 mg/kg) [50]. Likewise, the use of $scCO_2$ allowed us to obtain AO (H-SPE-80, F-SPE-40 and F-SPE-80) with more chlorophyll than the control (55.1–85.5 mg/kg) (Table 3), which is related to the $a*$ color parameter (Supplementary material 3). Moreover, an increase in chlorophyll a content was obtained at 80 °C for both avocado varieties (Table 3). Chlorophyll and the carotenoid lutein have been extracted by $scCO_2$, optimized at 500 bar, from spinach with the inclusion of 10 % ethanol as a co-solvent, due to the more polar nature of chlorophyll [51]. Moreover, increasing the extraction temperature resulted in major chlorophyll extraction [51]. Conversely, there was a reduction of chlorophyll extraction from olive husk [52] and *Scenedesmus obliquus* microorganism [49] by $scCO_2$ at 350 bar and 250 bar, respectively. Therefore, the source matrix could influence the effect of temperature on the $scCO_2$ extraction process.

3.3.2. *Fatty acids*

The fatty acid composition of the control AO was similar to previously published data. The percentage of palmitic acid (18.8 %), palmitoleic acid (7.3 %), oleic acid (60.7 %) and linoleic acid (13.3 %) (Table 3) were similar to those reported for Hass and Fuerte AO extracted by expeller-pressing and cold pressing [4,8,41]. Moreover, the comparison of F-SPE-80 and control AO showed that monounsaturated fatty acid (MUFA) content was higher in AO extracted by $scCO_2$ (70.8 % versus 68 %) (Table 3). This figure was also higher than reported previously for Hass and Fuerte [53] and Fortuna [15] avocado oils extracted by $scCO_2$. In addition, MUFA/saturated fatty acid (SFA) ratio was also higher in the F-SPE-80 than in the control sample (4.2 versus 3.6) (Table 3). MUFA consumption has been suggested as treatment or prevention of atherosclerosis [54]. Furthermore, the comparison of F-SPE-80 and control AO revealed that polyunsaturated fatty acid (PUFA) content was higher in the control sample (10.5 % versus 13.3 %) but this content was comparable to the those found for Fortuna AO extracted by $scCO_2$ (12.1–13 %) at 80 °C and 250 bar [15] (Table 3). Finally, comparison of F-SPE-80 and control AO showed that the MUFA+PUFA/SFA ratio was higher in the former (4.8 versus 4.3) (Table 3). In contrast, the PUFA/SFA ratio was slightly lower in the F-SPE-80 sample, which has been strongly associated with a greater hypocholesterolemic effect [54,55].

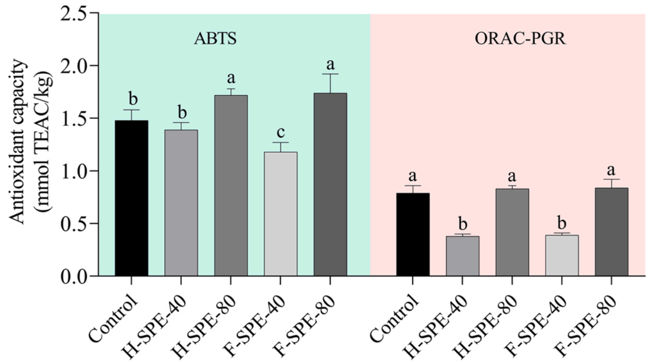

Fig. 2. Antioxidant capacity measured by ABTS and ORAC-PGR assays of avocado oil extracted by cold pressing (control) and supercritical CO_2 at 400 bar. H and F are the Hass and Fuerte avocado varieties; SPE: supercritical extraction; 40 and 80 are the extraction temperature in °C. The values are the means of three replicates and different superscript letters indicate that the values are statistically significant ($P < 0.05$).

3.3.3. *Antioxidant capacity*

The antioxidant capacity measured by the ABTS assay ranged from 1.18 to 1.74 mmol TEAC/kg for control AO and that extracted by $scCO_2$ (Fig. 2). These TEAC antioxidant capacity values are comparable to previous reports for AO extracted by cold pressing (1.5–2.0 mmol TE/kg) [43] but lower than for AO obtained using $scCO_2$ (4.52 mmol TE/kg) [10]. Moreover, AO extracted by $scCO_2$ at 80 °C (H-SPE-80 and F-SPE-80) showed a higher antioxidant capacity than control AO (Fig. 2). Meanwhile, to the best of our knowledge, this is the first time that the antioxidant capacity of AO has been measured by an ORAC-PGR assay, which is a HAT methodology that is different from the ABTS assay. The ORAC-PGR antioxidant capacity of the all the samples ranged from 0.38 to 0.84 mmol TEAC/kg; and we found H-SPE-80 and F-SPE-80 to have equal antioxidant capacity to that of control AO (Fig. 2). The antioxidant capacities measured by both ABTS and ORAC-PGR assays increased significantly with increasing extraction temperature. This is in agreement with values previously published for $scCO_2$ extraction of Fortune AO: an increased antioxidant capacity, from 17.4 % to 82.5 %, for inhibition of DPPH was demonstrated when the extraction temperature was increased from 40 to 80 °C [15]. The antioxidant capacity of avocado oil extracted by CO2 supercritical was increase with the temperature in agreement with the higher carotenoids and phenolic compounds of the samples (Fig. 2 and Table 3). Finally, the ABTS and ORAC-PGR values showed marginal correlation ($r = 0.869$; $p = 0.055$).

3.4. *Accelerated oxidative stability of avocado oil*

The lipid oxidation of the control AO and that extracted by $scCO_2$ was evaluated under accelerated conditions using the Oxitest system. The oxygen uptake in all the AO samples followed a sigmoidal curve

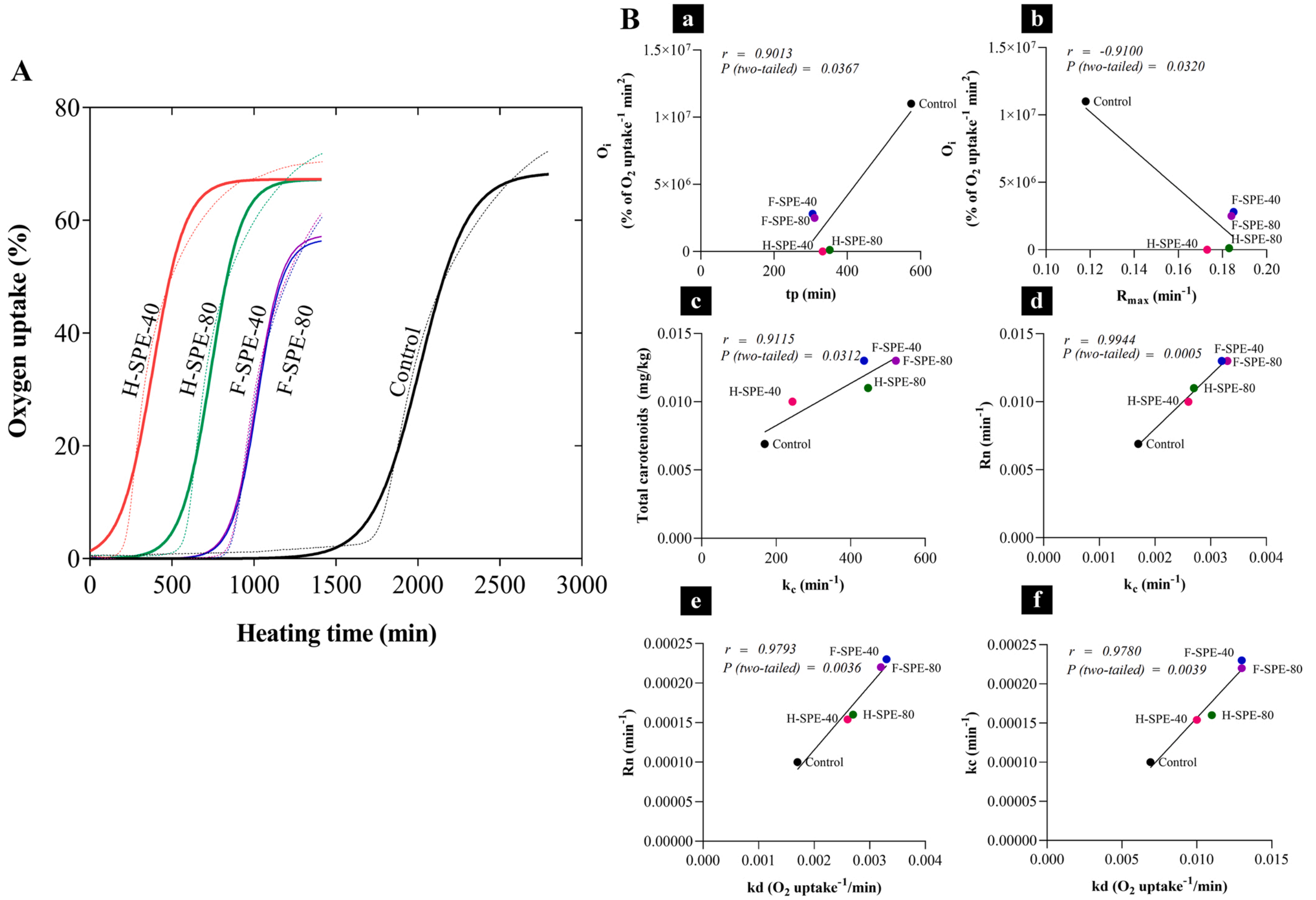

Fig. 3. Kinetic curves of the oxygen uptake (%) obtained in the Oxitest system during the lipid oxidation of the avocado oil extracted by cold pressing and supercritical CO_2 at 400 bar. H and F are the Hass and Fuerte avocado varieties; SPE: supercritical extraction; 40 and 80 are the extraction temperature in °C. The experimental data (__) were fitted to the linear and sigmoidal model over the initiation phase and the whole range (- - - -), respectively.

with initiation, propagation and termination phases (Fig. 3A). Similar behavior has been found for canola, sesame, fish, sunflower, soybean, corn, olive and rice bran at 60 °C [33,34]. In the present work, experimental data from an Oxitest were transformed and relativized to fit the sigmoidal model and estimate lipid oxidation kinetic parameters.

3.4.1. Initiation phase of lipid oxidation

The initiation phase of lipid oxidation can be characterized by IP, k_{IP}, formation of the oxidation product hydroperoxide or oxygen consumption, and the oxidizability parameter O_i [33,34]. The control AO showed an IP at 90 °C (28.8 h) that was similar to that found for olive oil at 60 °C (29.5 h) [34] and higher than the AO extracted by $scCO_2$. However, F-SPE-40 and F-SPE-80 samples showed IP values (around 14.5 h) higher than those found for oil from soybean (10.3 h), corn (9.8 h), canola (10.7 h), sunflower (4 h) and sesame (4.4 h) at 60 °C [33,34]. Moreover, AO from Fuerte showed IP values higher than those found for the Hass variety when were extracted by $scCO_2$ (Table 3). In the present study, lipid oxidation was measured using the Oxitest system under accelerated conditions by monitoring oxygen uptake, while Farhoosh [33,34] measured hydroperoxide content.

The control AO showed O_i values higher than those of the oil samples extracted by $scCO_2$; while F-SPE-40 and F-SPE-80 AO showed higher O_i values than H-SPE-40 and H-SPE-80 (Table 4). It is well known that O_i (the IP/k_{IP} ratio) is the initiation oxidizability parameter related to the resistance of oil to the formation of hydroperoxides. Thus, oil with a high SFA content shows an O_i value higher than oil rich in MUFAs and PUFAs [33]. In this way, O_i values for soybean, corn, canola and olive oils were higher (5- to 188-fold) than for sunflower oil [33,34]. Moreover, a higher O_i value was measured for canola oil than sesame oil at 60 °C, associated with lower calculated oxidizability (Cox) (4.53 vs 5.68) [33]. However, Cox values for the control AO and those for AO extracted by $scCO_2$ were around 2.0 (calculated from Table 3); and therefore, this does not explain the difference in the O_i values (Table 4). Another plausible explanation is that AO extracted by $scCO_2$ contains HAT antioxidant and prooxidant compounds, which could also influence the O_i values. Indeed, a very interesting positive correlation was found between total carotenoids and the pseudo first-order composite rate constant k_c (Fig. 3B-c). This has been related with a prooxidant effect of carotenoids on lipid oxidation in AO, which had previously been reported [56,57]. In fact, IP was lower for AO extracted by $scCO_2$, which contains higher total carotenoids, than for control sample (Table 3). A tendency towards a negative correlation was observed between the two parameters ($r = -0.358$, $p = 0.554$), although it was not significant.

It is well established that bulk oils are water/oil nano- and/or microemulsions (commercial oil contains 0.02 %–0.05 % water) comprised of lamellar structures and/or reverse micelles that provide an interface between the oil and traces of water where lipid peroxidation occurs [58]. For this reason, many studies have shown that hydrophilic Trolox is a more effective antioxidant than lipophilic α-tocopherol in bulk oils [58]. In the present study, AO (H-SPE-40 and H-SPE-80) showed higher oxygen uptake than the control AO and Fuerte AO extracted by $scCO_2$, with these latter samples showing similar values (Table 4). The concentration of hydroperoxide, equivalent to the oxygen uptake parameter, depends on the level of amphiphilicity, size, shape and degree of saturation of the fatty acids and their triacylglycerol composition [59]. The AO samples showed similar fatty acid composition (Table 3); and

Table 4
Oxidation parameters resulting from the linear and sigmoidal functions fitted to the kinetic curves for oxygen uptake obtained in the Oxitest system during the lipid oxidation of the avocado oils extracted by cold pressing and supercritical CO_2 at 40 and 80 °C, and at 400 bar.

Oxidation parameters	Avocado oil				
	Cold pressure extraction	Supercritical fluid extraction			
	Control	H-SPE-40	H-SPE-80	F-SPE-40	F-SPE-80
Initiation phase					
IP (min)	1728.7 ± 33.4^a	245.1 ± 0.6^d	570.7 ± 6.9^c	871.3 ± 16.9^b	862.2 ± 16.6^b
K_{IP} (% of O_2 uptake. min^{-1})	$1.6 \times 10^{-4} \pm 6.7 \times 10^{-6a}$	$4.2 \times 10^{-2} \pm 8.8 \times 10^{-4b}$	$4.9 \times 10^{-4} \pm 1.3 \times 10^{-4a}$	$3.7 \times 10^{-4} \pm 1.1 \times 10^{-4a}$	$3.5x10^{-4} \pm 1.7x10^{-5a}$
O_i (% of O_2 uptake^{-1}. min^2)	$1.1 \times 10^7 \pm 2.4 \times 10^{5a}$	$5.8 \times 10^3 \pm 1.4 \times 10^{2c}$	$1.2 \times 10^5 \pm 4.7 \times 10^{3c}$	$2.8 \times 10^6 \pm 2.1 \times 10^{5b}$	$2.5x10^6 \pm 1.6x10^{5b}$
O_2 uptake $_{IP}$ (%)	0.278 ± 0.017^a	10.302 ± 0.192^b	2.767 ± 0.043^c	0.273 ± 0.010^a	0.303 ± 0.009^a
Propagation phase					
tp (min)	574.3 ± 18.4^a	332.5 ± 4.7^b	351.7 ± 9.6^b	305.3 ± 2.6^b	310.4 ± 13.0^b
R_{max} (% of O_2 uptake. min^{-1})	0.118 ± 0.006^a	0.173 ± 0.005^b	0.183 ± 0.007^b	0.185 ± 0.002^b	0.184 ± 0.008^b
O_2 uptake $_{max}$ (%)	68.4 ± 1.2^a	68.1 ± 1.1^a	67.3 ± 0.7^a	56.6 ± 1.1^b	57.5 ± 0.1^b
R_n (min^{-1})	$1.7 \times 10^{-3} \pm 5.6 \times 10^{-5a}$	$2.6 \times 10^{-3} \pm 3.5 \times 10^{-5b}$	$2.7 \times 10^{-3} \pm 7.4 \times 10^{-5b}$	$3.3 \times 10^{-3} \pm 2.8 \times 10^{-5c}$	$3.2 \times 10^{-3} \pm 1.3 \times 10^{-4c}$
k_c (min^{-1})	$6.9 \times 10^{-3} \pm 2.3 \times 10^{-4a}$	$1.0 \times 10^{-2} \pm 1.4 \times 10^{-4b}$	$1.1 \times 10^{-2} \pm 3.0 \times 10^{-4b}$	$1.3 \times 10^{-2} \pm 1.1 \times 10^{-4c}$	$1.3 \times 10^{-2} \pm 5.4 \times 10^{-4c}$
k_d (% of O_2 uptake^{-1}/min)	$1.0 \times 10^{-4} \pm 1.6 \times 10^{-6a}$	$1.5 \times 10^{-4} \pm 3.5 \times 10^{-7b}$	$1.6 \times 10^{-4} \pm 2.7 \times 10^{-6b}$	$2.3 \times 10^{-4} \pm 6.4 \times 10^{-6c}$	$2.2 \times 10^{-4} \pm 9.6 \times 10^{-6c}$

H and F are Hass and Fuerte avocado varieties, SPE= supercritical extraction and 40 and 80 are the extraction temperature in °C. The values are the means of three replicates and different superscript letters mean that values are statistically significant ($P < 0.05$).

therefore, the presence of hydrophilic and hydrophobic antioxidants and prooxidants could influence the lipid peroxidation. As previously discussed, carotenoids in the AO can act as prooxidants. Moreover, the AO chlorophyll content (in H-SPE-80, F-SPE-40 and F-SPE-80) was higher than in the control sample (Table 3), which may have a slight influence on the oxidative stability of lipids through photosensitization mechanisms [60]. In the present work, total AO chlorophyll content showed a marginal positive correlation with k_c ($r = 0.602$, $p = 0.283$).

3.4.2. *Propagation phase of lipid peroxidation*

The duration of the propagation phase (t_p) was less than IP for control AO and AO extracted by $scCO_2$, except for H-SPE-40 (Table 4). The t_p value was around 5 h for AO obtained by $scCO_2$ (Table 4), which allows for differentiation of the types of oil, as previously reported by Farhoosh [33]. Moreover, t_p showed a positive correlation with O_i (Fig. 3B-a). On the other hand, the maximum rate R_{max}, a measurement of the rate of formation of hydroperoxide or oxygen uptake in the Oxitest system, was higher than k_{IP} in our AO samples (Table 3). Moreover, R_{max} showed positive correlation with O_i (Fig. 3B-b). This reflects domination of hydroperoxide decomposition during the initiation phase of lipid peroxidation, producing reactive radicals for the propagation phase [34]. Finally, the maximum oxygen uptake, equivalent to the hydroperoxide maximum, is different for each AO sample (Table 4), and this is affected by the rate of hydroperoxide formation (k_c) and decomposition (k_d) [33].

R_n has been reported as a measure of propagation oxidizability, demonstrating the propensity of the triacylglycerol compositions for lipid peroxidation [33]. The AO extracted by $scCO_2$ showed R_n values 1.5– 1.9-fold higher than for the control sample (Table 4). In a similar way, k_c as a measure of oxidizability propagation, also showed a higher value for AO extracted by $scCO_2$ than for control AO (1.4 – 1.9-fold) (Table 4). A significant positive correlation was found between R_n and k_c (Fig. 3B-d) similar to a previous report for vegetable oils [33]. R_n and k_c were similar for the AO extracted by $scCO_2$ (Table 4). Moreover, the rate of hydroperoxide decomposition, k_d, was lower in the control AO than in to AO extracted by $scCO_2$ (Table 4) and showed a positive correlation with R_n and k_c (Figs. 3B-e and 3B-f). The formation of hydroperoxide (or oxygen uptake) exceeds decomposition ($k_c >> k_d$) (Table 4) in agreement with findings for other vegetable oils [33,34].

4. Conclusion

The kinetics of avocado oil extraction from Fuerte and Hass avocado varieties by $scCO_2$ fits the logistic model well. The extraction of AO by $scCO_2$ influences the physicochemical, bioactive compounds, fatty acid composition, antioxidant capacity and oxidative stability of the oil. Thus, total phenolic content of AO extracted by $scCO_2$ was lower than in control sample, except for F-SPE-80. A higher total carotenoid and chlorophyll content was found in AO extracted by $scCO_2$, which is associated with the hue angle h° and *a** color parameters. The MUFA content, (the MUFA/SFA ratio only of F-SPE-80) and the (MUFA+PUFA)/SFA value were higher in AO extracted by $scCO_2$ than in control AO. TEAC antioxidant capacity was higher in F-SPE-80 and H-SPE-80 than in control AO. Finally, the oxidative stability under accelerated conditions using an Oxitest system, reflected by initiation and propagation kinetic parameters, showed that AO extracted by $scCO_2$ is more susceptible to lipid oxidation. Therefore, appropriate storage and packaging conditions must be used to guarantee the shelf life of the gourmet AO obtained by green technology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

Acknowledgments

The authors are indebted to Scientific Analyst & Consultant E.I.R.L., Lima, Peru for the funding and support provided.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.supflu.2022.105750.

References

[1] S.S. Ranade, P. Thiagarajan, A review on Persea americana Mill. (Avocado)- Its fruit and oil. ⟨https://www.sphinxsai.com/2015/ph_vol8_no6/1/(72–77)V8N6PT.pdf⟩.

[2] C.X. Tan, Virgin avocado oil: An emerging source of functional fruit oil, J. Funct. Foods 54 (2019) 381–392, https://doi.org/10.1016/j.jff.2018.12.031.

[3] A. Woolf, M. Wong, L. Eyres, T. McGhie, C. Lund, S. Olsson, Y. Wang, C. Bulley, M. Wang, E. Friel, C. Requejo-Jackman, Avocado oil, in: Gourmet Health-Promot. Spec. Oils, AOCS Press, 2009, pp. 73–125, https://doi.org/10.1016/B978-1-893997-97-4.50008-5.

[4] S. Satriana, M.D. Supardan, N. Arpi, W.A. Wan Mustapha, Development of methods used in the extraction of avocado oil, Eur. J. Lipid Sci. Technol. 121 (2019), 1800210, https://doi.org/10.1002/ejlt.201800210.

[5] M. Flores, J. Ortiz-Viedma, A. Curaqueo, A. Rodriguez, G. Dovale-Rosabal, F. Magaña, C. Vega, M. Toro, L. López, R. Ferreyra, B.G. Defilippi, Preliminary studies of chemical and physical properties of two varieties of avocado seeds grown in Chile, J. Food Qual. 2019 (2019) 1–11, https://doi.org/10.1155/2019/3563750.

[6] V. da S. Santos, G.D. Fernandes, Chapter 37 - Cold pressed avocado (Persea americana Mill.) oil, in: M.F. Ramadan (Ed.), Cold Press. Oils, Academic Press, 2020, pp. 405–428, https://doi.org/10.1016/B978-0-12-818188-1.00037-2.

[7] I. Berasategi, B. Barriuso, D. Ansorena, I. Astiasarán, Stability of avocado oil during heating: comparative study to olive oil, Food Chem. 132 (2012) 439–446, https://doi.org/10.1016/j.foodchem.2011.11.018.

[8] M. Flores, C. Saravia, C. Vergara, F. Avila, H. Valdés, J. Ortiz-Viedma, Avocado oil: characteristics, properties, and applications, Molecules 24 (2019) 2172, https://doi.org/10.3390/molecules24112172.

[9] F.D. Krumreich, C.D. Borges, C.R.B. Mendonça, C. Jansen-Alves, R.C. Zambiazi, Bioactive compounds and quality parameters of avocado oil obtained by different processes, Food Chem. 257 (2018) 376–381, https://doi.org/10.1016/j.foodchem.2018.03.048.

[10] C.X. Tan, G.H. Chong, H. Hamzah, H.M. Ghazali, Characterization of virgin avocado oil obtained via advanced green techniques, Eur. J. Lipid Sci. Technol. 120 (2018), 1800170, https://doi.org/10.1002/ejlt.201800170.

[11] C.X. Tan, C.G. Hean, H. Hamzah, H.M. Ghazali, Optimization of ultrasound-assisted aqueous extraction to produce virgin avocado oil with low free fatty acids, J. Food Process Eng. 41 (2018), e12656, https://doi.org/10.1111/jfpe.12656.

[12] F. Chemat, N. Rombaut, A. Meullemiestre, M. Turk, S. Perino, A.-S. Fabiano-Tixier, M. Abert-Vian, Review of green food processing techniques. preservation, transformation, and extraction, Innov. Food Sci. Emerg. Technol. 41 (2017) 357–377, https://doi.org/10.1016/j.ifset.2017.04.016.

[13] M.F. Ramadan, in: M.F. Ramadan (Ed.), Chapter 1 - Introduction to Cold Pressed Oils: Green Technology, Bioactive Compounds, Functionality, and Applications, Cold Press. Oils, Academic Press, 2020, pp. 1–5, https://doi.org/10.1016/B978-0-12-818188-1.00001-3.

[14] I.J.B. Ferreira, E.M.C. Alexandre, J.A. Saraiva, M. Pintado, Green emerging extraction technologies to obtain high-quality vegetable oils from nuts: a review, Innov. Food Sci. Emerg. Technol. 76 (2022), 102931, https://doi.org/10.1016/j.ifset.2022.102931.

[15] E.R. Abaide, G.L. Zabot, M.V. Tres, R.F. Martins, J.L. Fagundez, L.F. Nunes, S. Druzian, J.F. Soares, V. Dal Prá, J.R.F. Silva, R.C. Kuhn, M.A. Mazutti, Yield, composition, and antioxidant activity of avocado pulp oil extracted by pressurized fluids, Food Bioprod. Process. 102 (2017) 289–298, https://doi.org/10.1016/j.fbp.2017.01.008.

[16] H.D.F.Q. Barros, R. Grimaldi, F.A. Cabral, Lycopene-rich avocado oil obtained by simultaneous supercritical extraction from avocado pulp and tomato pomace, J. Supercrit. Fluids 120 (2017) 1–6, https://doi.org/10.1016/j.supflu.2016.09.021.

[17] S.C.S. Corzzini, H.D.F.Q. Barros, R. Grimaldi, F.A. Cabral, Extraction of edible avocado oil using supercritical CO_2 and a CO_2/ethanol mixture as solvents, J. Food Eng. 194 (2017) 40–45, https://doi.org/10.1016/j.jfoodeng.2016.09.004.

[18] Y.N. Manaf, A.P. Rahardjo, Y.A. Yusof, M.N.M. Desa, B.P. Nusantoro, Lipid characteristics and tocopherol content of the oils of native avocado cultivars grown in Indonesia, Int. J. Food Prop. 21 (2018) 2758–2771, https://doi.org/10.1080/10942912.2018.1564761.

[19] R.J. Blakey, Evaluation of avocado fruit maturity with a portable near-infrared spectrometer, Postharvest Biol. Technol. 121 (2016) 101–105, https://doi.org/10.1016/j.postharvbio.2016.06.016.

[20] D.I. Onwude, N. Hashim, R.B. Janius, N.M. Nawi, K. Abdan, Modeling the thin-layer drying of fruits and vegetables: a review, Compr. Rev. Food Sci. Food Saf. 15 (2016) 599–618, https://doi.org/10.1111/1541-4337.12196.

[21] A.C. Fornereto Soldan, S. Arvelos, É.O. Watanabe, C.E. Hori, Supercritical fluid extraction of oleoresin from Capsicum annuum industrial waste, J. Clean. Prod. 297 (2021), 126593, https://doi.org/10.1016/j.jclepro.2021.126593.

[22] J. Martínez, A.R. Monteiro, P.T.V. Rosa, M.O.M. Marques, M.A.A. Meireles, Multicomponent model to describe extraction of ginger oleoresin with supercritical carbon dioxide, Ind. Eng. Chem. Res. 42 (2003) 1057–1063, https://doi.org/10.1021/ie020694f.

[23] J.G. Waramboi, M.J. Gidley, P.A. Sopade, Carotenoid contents of extruded and non-extruded sweetpotato flours from Papua New Guinea and Australia, Food Chem. 141 (2013) 1740–1746, https://doi.org/10.1016/j.foodchem.2013.04.070.

[24] O. Köseoğlu, D. Sevim, P. Kadiroğlu, Quality characteristics and antioxidant properties of Turkish monovarietal olive oils regarding stages of olive ripening, Food Chem. 212 (2016) 628–634, https://doi.org/10.1016/j.foodchem.2016.06.027.

[25] M. Abderrahim, S.M. Arribas, L. Condezo-Hoyos, A novel high-throughput image based rapid Folin-Ciocalteau assay for assessment of reducing capacity in foods, Talanta 152 (2016) 82–89, https://doi.org/10.1016/j.talanta.2016.01.051.

[26] H.K. Lichtenthaler, C. Buschmann, Chlorophylls and carotenoids: measurement and characterization by UV–VIS spectroscopy, Curr. Protoc. Food Anal. Chem. 1 (2001) F4.3.1–F4.3.8, https://doi.org/10.1002/0471142913.faf0403s01.

[27] E. Borello, V. Domenici, Determination of pigments in virgin and extra-virgin olive oils: a comparison between two near UV-Vis spectroscopic techniques, Foods Basel Switz. (2019), https://doi.org/10.3390/foods8010018.

[28] Official Methods of Analysis, 21st Edition (2019), AOAC Int. (n.d.). ⟨https://www.aoac.org/official-methods-of-analysis-21st-edition-2019/⟩ (Accessed 19 March 2022).

[29] L. Condezo-Hoyos, F. Abderrahim, M.V. Conde, C. Susín, J.J. Díaz-Gil, M. C. González, S.M. Arribas, Antioxidant activity of liver growth factor, a bilirubin covalently bound to albumin, Free Radic. Biol. Med. (2009), https://doi.org/10.1016/j.freeradbiomed.2008.12.002.

[30] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (1999) 1231–1237, https://doi.org/10.1016/S0891-5849(98)00315-3.

[31] R. Ortiz, M. Antilén, H. Speisky, M.E. Aliaga, C. López-Alarcón, S. Baugh, Application of a microplate-based ORAC-pyrogallol red assay for the estimation ofantioxidant capacity: first action 2012.03, J. AOAC Int. 95 (2012) 1558–1561, https://doi.org/10.5740/jaoacint.cs2012_03.

[32] R. Romeo, A. De Bruno, V. Imeneo, A. Piscopo, M. Poiana, Impact of stability of enriched oil with phenolic extract from olive mill wastewaters, Foods 9 (2020) 856, https://doi.org/10.3390/foods9070856.

[33] R. Farhoosh, A reconsidered approach providing kinetic parameters and rate constants to analyze the oxidative stability of bulk lipid systems, Food Chem. 327 (2020), 127088, https://doi.org/10.1016/j.foodchem.2020.127088.

[34] R. Farhoosh, Critical kinetic parameters and rate constants representing lipid peroxidation as affected by temperature, Food Chem. 340 (2021), 128137, https://doi.org/10.1016/j.foodchem.2020.128137.

[35] J.A. Villa-Rodríguez, F.J. Molina-Corral, J.F. Ayala-Zavala, G.I. Olivas, G. A. González-Aguilar, Effect of maturity stage on the content of fatty acids and antioxidant activity of 'Hass' avocado, Food Res. Int. 44 (2011) 1231–1237, https://doi.org/10.1016/j.foodres.2010.11.012.

[36] M.S. Alkaltham, N. Uslu, M.M. Özcan, A.M. Salamatullah, I.A. Mohamed Ahmed, K. Hayat, Effect of drying process on oil, phenolic composition and antioxidant activity of avocado (cv. Hass) fruits harvested at two different maturity stages, LWT 148 (2021), 111716, https://doi.org/10.1016/j.lwt.2021.111716.

[37] F. Al-Juhaimi, N. Uslu, M.M. Özcan, E.E. Babiker, K. Ghafoor, I.M. Ahmed, O. N. Alsawmahi, Effects of drying process on oil quality, the bioactive properties and phytochemical characteristics of avocado (Fuerte) fruits harvested at two different maturity stages, J. Food Process. Preserv. 45 (2021), e15368, https://doi.org/10.1111/jfpp.15368.

[38] O.O. Olarewaju, I. Bertling, L.S. Magwaza, Non-destructive evaluation of avocado fruit maturity using near infrared spectroscopy and PLS regression models, Sci. Hortic. 199 (2016) 229–236, https://doi.org/10.1016/j.scienta.2015.12.047.

[39] J.A. Villa-Rodriguez, E.M. Yahia, A. González-León, I. Ifie, R.E. Robles-Zepeda, J. A. Domínguez-Avila, G.A. González-Aguilar, Ripening of 'Hass' avocado mesocarp alters its phytochemical profile and the in vitro cytotoxic activity of its methanolic extracts, S. Afr. J. Bot. 128 (2020) 1–8, https://doi.org/10.1016/j.sajb.2019.09.020.

[40] M.E. Mostert, B.M. Botha, L.M.D. Plessis, K.G. Duodu, Effect of fruit ripeness and method of fruit drying on the extractability of avocado oil with hexane and supercritical carbon dioxide, J. Sci. Food Agric. 87 (2007) 2880–2885, https://doi.org/10.1002/jsfa.3051.

[41] X. Qin, J. Zhong, A review of extraction techniques for avocado oil, J. Oleo Sci. 65 (2016) 881–888, https://doi.org/10.5650/jos.ess16063.

[42] C.S.G. Kitzberger, R.H. Lomonaco, E.M.Z. Michielin, L. Danielski, J. Correia, S.R. S. Ferreira, Supercritical fluid extraction of shiitake oil: Curve modeling and extract composition, J. Food Eng. 90 (2009) 35–43, https://doi.org/10.1016/j.jfoodeng.2008.05.034.

[43] I. Santana, V.N. Castelo-Branco, B.M. Guimarães, L. de, O. Silva, V.O.D.S. Peixoto, L.M.C. Cabral, S.P. Freitas, A.G. Torres, Hass avocado (Persea americana Mill.) oil

enriched in phenolic compounds and tocopherols by expeller-pressing the unpeeled microwave dried fruit, Food Chem. 286 (2019) 354–361, https://doi.org/10.1016/j.foodchem.2019.02.014.

[44] C.X. Tan, G.H. Chong, H. Hamzah, H.M. Ghazali, Comparison of subcritical CO_2 and ultrasound-assisted aqueous methods with the conventional solvent method in the extraction of avocado oil, J. Supercrit. Fluids 135 (2018) 45–51, https://doi.org/10.1016/j.supflu.2017.12.036.

[45] Y.M. Monroy, R.A.F. Rodrigues, A. Sartoratto, F.A. Cabral, Influence of ethanol, water, and their mixtures as co-solvents of the supercritical carbon dioxide in the extraction of phenolics from purple corn cob (Zea mays L.), J. Supercrit. Fluids 118 (2016) 11–18, https://doi.org/10.1016/j.supflu.2016.07.019.

[46] T. Sato, Y. Ikeya, S. Adachi, K. Yagasaki, K. Nihei, N. Itoh, Extraction of strawberry leaves with supercritical carbon dioxide and entrainers: antioxidant capacity, total phenolic content, and inhibitory effect on uric acid production of the extract, Food Bioprod. Process. 117 (2019) 160–169, https://doi.org/10.1016/j.fbp.2019.07.003.

[47] D. Kostrzewa, A. Dobrzyńska-Inger, R. Reszczyński, Pilot scale supercritical CO_2 extraction of carotenoids from sweet paprika (Capsicum annuum L.): influence of particle size and moisture content of plant material, LWT 136 (2021), 110345, https://doi.org/10.1016/j.lwt.2020.110345.

[48] S. Millao, E. Uquiche, Extraction of oil and carotenoids from pelletized microalgae using supercritical carbon dioxide, J. Supercrit. Fluids 116 (2016) 223–231, https://doi.org/10.1016/j.supflu.2016.05.049.

[49] A.C. Guedes, M.S. Gião, A.A. Matias, A.V.M. Nunes, M.E. Pintado, C.M.M. Duarte, F.X. Malcata, Supercritical fluid extraction of carotenoids and chlorophylls a, b and c, from a wild strain of Scenedesmus obliquus for use in food processing, J. Food Eng. 116 (2013) 478–482, https://doi.org/10.1016/j.jfoodeng.2012.12.015.

[50] L.M.B. Resende, V.R. de Souza, G.M.D. Ferreira, C.A. Nunes, Changes in quality and phytochemical contents of avocado oil under different temperatures, J. Food Sci. Technol. 56 (2019) 401–408, https://doi.org/10.1007/s13197-018-3501-7.

[51] M. Derrien, M. Aghabararnejad, A. Gosselin, Y. Desjardins, P. Angers, Y. Boumghar, Optimization of supercritical carbon dioxide extraction of lutein and chlorophyll from spinach by-products using response surface methodology, LWT 93 (2018) 79–87, https://doi.org/10.1016/j.lwt.2018.03.016.

[52] I. Gracia, J.F. Rodríguez, A. de Lucas, M.P. Fernandez-Ronco, M.T. García, Optimization of supercritical CO_2 process for the concentration of tocopherol, carotenoids and chlorophylls from residual olive husk, J. Supercrit. Fluids 59 (2011) 72–77, https://doi.org/10.1016/j.supflu.2011.05.019.

[53] M. Reddy, R. Moodley, S.B. Jonnalagadda, Fatty acid profile and elemental content of avocado (*Persea* americana Mill.) oil –effect of extraction methods, J. Environ. Sci. Health Part B 47 (2012) 529–537, https://doi.org/10.1080/03601234.2012.665669.

[54] N.W. Chang, P.C. Huang, Effects of the ratio of polyunsaturated and monounsaturated fatty acid to saturated fatty acid on rat plasma and liver lipid concentrations, Lipids 33 (1998) 481–487, https://doi.org/10.1007/s11745-998-0231-9.

[55] J. Chen, H. Liu, Nutritional indices for assessing fatty acids: a mini-review, Int. J. Mol. Sci. 21 (2020) 5695, https://doi.org/10.3390/ijms21165695.

[56] D. Ribeiro, M. Freitas, A.M.S. Silva, F. Carvalho, E. Fernandes, Antioxidant and pro-oxidant activities of carotenoids and their oxidation products, Food Chem. Toxicol. 120 (2018) 681–699, https://doi.org/10.1016/j.fct.2018.07.060.

[57] A. Subagio, N. Morita, Instability of carotenoids is a reason for their promotion on lipid oxidation, Food Res. Int. 34 (2001) 183–188, https://doi.org/10.1016/S0963-9969(00)00150-2.

[58] M. Laguerre, J. Lecomte, P. Villeneuve, 14 - The use and effectiveness of antioxidants in lipids preservation: beyond the polar paradox, in: F. Shahidi (Ed.), Handb. Antioxid. Food Preserv., Woodhead Publishing, 2015, pp. 349–372, https://doi.org/10.1016/B978-1-78242-089-7.00014-2.

[59] S. Ghnimi, E. Budilarto, A. Kamal-Eldin, The new paradigm for lipid oxidation and insights to microencapsulation of omega-3 fatty acids, Compr. Rev. Food Sci. Food Saf. 16 (2017) 1206–1218, https://doi.org/10.1111/1541-4337.12300.

[60] T.S. Kim, E.A. Decker, J. Lee, Effects of chlorophyll photosensitisation on the oxidative stability in oil-in-water emulsions, Food Chem. 133 (2012) 1449–1455, https://doi.org/10.1016/j.foodchem.2012.02.033.