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Fatty Acids, Sterols, and Antioxidant Activity in Minimally Processed Avocados during Refrigerated Storage

LUCÍA PLAZA,* CONCEPCIÓN SÁNCHEZ-MORENO, SONIA DE PASCUAL-TERESA, BEGOÑA DE ANCOS, AND M. PILAR CANO*

Department of Plant Foods Science and Technology, Instituto del Frío, Consejo Superior de Investigaciones Científicas (CSIC), José Antonio Novais 10, Ciudad Universitaria, E-28040 Madrid, Spain

Avocado (*Persea americana* Mill.) is a good source of bioactive compounds such as monounsaturated fatty acids and sterols. The impact of minimal processing on its health-promoting attributes was investigated. Avocados cut into slices or halves were packaged in plastic bags under nitrogen, air, or vacuum and stored at 8 °C for 13 days. The stabilities of fatty acids and sterols as well as the effect on antioxidant activity were evaluated. The main fatty acid identified and quantified in avocado was oleic acid (about 57% of total content), whereas β-sitosterol was found to be the major sterol (about 89% of total content). In general, after refrigerated storage, a significant decrease in fatty acid content was observed. Vacuum/halves and air/slices were the samples that maintained better this content. With regard to phytosterols, there were no significant changes during storage. Antioxidant activity showed a slight positive correlation against stearic acid content. At the end of refrigerated storage, a significant increase in antiradical efficiency (AE) was found for vacuum samples. AE values were quite similar among treatments. Hence, minimal processing can be a useful tool to preserve health-related properties of avocado fruit.

KEYWORDS: Minimal processing; fatty acids; phytosterols; antioxidant activity; avocado; *Persea americana* Mill.; refrigerated storage

INTRODUCTION

The avocado (*Persea americana* Mill.) is an important oleaginous fruit cultivated in many tropical and subtropical countries. One of its outstanding characteristics is its high lipid content. Monounsaturated fatty acids (mainly oleic acid) are the principal components of the lipid fraction, representing about 71% of total fatty acids (*1*). High dietary intake of these fatty acids has been related to a decreased risk of cardiovascular disease. Ledesma et al. (*2*) reported that an avocado-enriched diet can improve the lipid profile in healthy and especially in mildly hypercholesterolemic patients by reducing total and LDL cholesterol and triglycerides and increasing HDL cholesterol. In this sense, a recent study has shown that avocado oil is of the same order of atherogenicity as corn oil and olive oil and that the consumption of this oil increases the percentage of serum HDL cholesterol when compared with corn or coconut oils (*3*). In addition, avocados are a rich source of bioactive phytochemicals such as vitamin E, some carotenoids, vitamin C, phenols, and sterols (mainly β-sitosterol), among others (*4, 5*). Phytosterols (or plant sterols) have a chemical structure similar to that of cholesterol but different side-chain configuration. Numerous studies have shown that dietary intake of phytosterols effectively reduces serum cholesterol levels, offering protection from cardiovascular diseases, and also may have a protective role in the development of several cancers (*6*−*10*). The intake of plant sterols and the identification of the major dietary sources of plant sterols in Finnish and Spanish diets have been recently established. The per capita per day intake in these countries were estimated to be around 271 mg (*11*) and 276 mg (*12*), respectively. The contribution of the fruit group to the total plant sterol intake is around 10.4 and 12.2%, respectively, the main food sources being cereals and vegetable fats in the Finnish diet and vegetable oils and cereals in the Spanish diet.

Nowadays, consumer demand for healthy, safe, natural, and fresh-like foods that require only a minimum effort and time for their preparation has increased. This has led to the development of ready-to-eat foods, minimally processed foods that are prepared for consumption. Minimal processing of fruits and vegetables involves the use of a combination of procedures such as washing, peeling, and slicing or shredding that may cause an increase in respiration, biochemical changes, and microbial spoilage and, as a consequence, reduce the shelf life of these products. Modified atmosphere packaging (MAP) in combination with refrigerated storage is one of the most used methods to extend shelf life. MAP involves altering the gases surrounding

* Corresponding authors [telephone +34 915492300; fax +34 915493627; e-mail (L.P.) lucia.plaza@if.csic.es, (M.P.C.) pcano@if.csic.es].

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Table 1. Initial Characteristics of Avocado

characteristic	value[a]
titratable acidity (g of citric acid/100 g of fw[b])	0.07 ± 0.01
pH	6.87 ± 0.18
soluble solids (°Brix at 20 °C)	14.10 ± 0.08
total solids (g/100 g of fw)	34.23 ± 0.08
peroxide index (mequiv of O_2/kg of oil)	0.74 ± 0.01
CIELab color parameters	
L^*	58.15 ± 0.55
a^*	−9.86 ± 0.39
b^*	29.28 ± 0.24
h [$\tan^{-1}(b^*/a^*)$]	71.39 ± 0.68
C $(a^{*2} + b^{*2})^{1/2}$	30.90 ± 0.27

[a] Values are the mean of three independent determinations ± standard deviation. [b] fw, fresh weight.

a commodity, in general, decreasing levels of O_2 and increasing levels of CO_2 to reduce the respiration rate (*13*−*15*).

In the open literature, the impact of several combined methods of preservation (such as MAP, controlled atmosphere storage, edible coatings, reduction of pH, and cold storage) on quality attributes (mainly color and firmness), enzymatic activity, and microbiological shelf life of avocado has been studied (*16*−*21*). Nevertheless, to date, no research has integrated the study of the effect of minimal processing and storage on health-related compounds of fresh-cut avocado. Therefore, the aim of this work was to study the influence of minimal processing on the fatty acid and sterol contents and antioxidant activity of avocado fruit during cold storage.

MATERIALS AND METHODS

Chemicals. Campesterol, 5α-cholestane, 2,2-diphenyl-1-picrylhydrazyl (DPPH•), linoleic acid, γ-linolenic acid, methyl heptadecanoate, oleic acid, palmitic acid, palmitoleic acid, β-sitosterol, stearic acid, stigmasterol, and Tween 20 were obtained from Sigma (St. Louis, MO). Citric acid monohydrate, ethanol 96%, hydrochloric acid (HCl) 35%, potassium hydroxide (KOH) 85%, and sodium hydroxide (NaOH) 0.1 N were purchased from Panreac Química (Barcelona, Spain). Methanol, *n*-hexane 95%, and tetrahydrofuran (THF) were obtained from Labscan Ltd. (Dublin, Ireland).

Plant Material and Processing. Avocados (*P. americana* Mill. cv. Hass) were obtained from a local market at commercial maturity. Fruits free of visual defects and uniform in color and size were selected. Avocados were cleaned, peeled, cored, and cut into 1−1.5 cm thick slices or cut in half with a sharp stainless steel knife. Approximately 200 g of avocado (slices or halves) was packaged in plastic bags (AMCOR P-Plus premade bags, antimist coated oriented polypropylene film, AMCOR Flexibles Hispania S.A., Granollers, Barcelona, Spain) of medium oxygen permeability [5200 cm^3/ (m^2 24 h bar) at 23 °C and 0% relative humidity (RH)] under different atmosphere conditions (100% N_2 and air) and sealed by a Multivac vacuum machine (Wolferchweden, Germany). The relationship between the amount of product and the injected gas mixture was 1:2. A third set of samples was prepared by applying vacuum to avocado slices or halves previously introduced in plastic bags (BB4L, CRYOVAC Europe, Grace S.A., Sant Boi de Llobregat, Barcelona, Spain) of low oxygen permeability [20 cm^3/ (m^2 24 h bar) at 23 °C and 0% RH]. Bags were stored at 8 °C in darkness for 13 days. Initial characteristics of avocado are shown in **Table 1**.

Color, Peroxide Index, pH, Titratable Acidity, and Soluble and Total Solids. The color of avocado was measured using a tristimulus reflectance colorimeter (HunterLab, model D25 A9, Hunter Associates Laboratory, Inc., Reston, VA), as described in Plaza et al. (*22*). The peroxide index was determined according to the AOAC method (*23*) and was expressed as milliequivalents of active oxygen per kilogram of oil. For pH and titratable acidity, avocado (10 g) was blended with 20 mL of deionized water in an ultrahomogenizer (Omni mixer, model ES-207, Omni International Inc., Gainesville, VA). The mixture was heated to 100 °C, and then 20 mL of deionized water was added, and the resulting mixture was cooled to 20 °C. The pH was measured at this temperature with a pH-meter (Microph 2000, Crison, Barcelona, Spain). After determination of the pH, the solution was titrated with 0.1 N NaOH to pH 8.1, and the results were expressed as percentage of citric acid (grams of citric acid per 100 g of fresh weight). Soluble solids of avocado were determined using a digital refractometer (ATAGO, Tokyo, Japan) at 20 °C, and results were reported as degrees Brix. Total solids were measured as described in Plaza et al. (*24*), and results were expressed as grams of total solids per 100 g of fresh weight.

Fatty Acid Analysis. The analysis of fatty acids in avocado was carried out by gas chromatography−mass spectrometry (GC-MS) after oil extraction and conversion of the fatty acids into their methyl esters. For oil extraction, the avocado sample (10 g) was ground in an Ultraturrax for 5 min and extracted in a Soxhlet apparatus with *n*-hexane at 140 °C for 2 h. The solvent was evaporated and the oil recovered. Then, the fatty acid methyl esters were prepared by diluting an aliquot of oil in *n*-hexane (1:10) and adding 50 μL of methanolic 2 M KOH. Methyl heptadecanoate (5 μL from a standard solution of 1 mg/mL) was added as internal standard. After 30 min at room temperature, the upper layer was selected. For GC-MS analysis, a Hewlett-Packard 5890 series II gas chromatograph equipped with an on-column injection system and a Hewlett-Packard 5972 series mass selective detector was used. Separation was performed on a fused-silica capillary column (50 m × 0.25 mm i.d., coating CP-selec CB, Varian Inc., Palo Alto, CA), using helium as the carrier gas (column pressure = 1 MPa). The oven temperature was programmed as follows: starting from 125 °C (10 min) and increasing to 165 °C at a rate of 10 °C/min and then increasing to 200 °C at a rate of 2 °C/min. Injector and detector temperatures were maintained at 280 °C. A 2 μL sample was injected. Individual fatty acids were identified and quantified by comparison of their retention times and peak areas to external standards (linoleic acid, γ-linolenic acid, oleic acid, palmitic acid, palmitoleic acid, and stearic acid). Results were expressed as grams per 100 g of dry weight. Ratios of polyunsaturated (linoleic acid, γ-linolenic acid) to saturated (palmitic acid, stearic acid) fatty acids and polyunsaturated plus monounsaturated (oleic acid, palmitoleic acid, *cis*-vaccenic acid) to saturated fatty acids were calculated.

Sterol Analysis. Sterols were extracted from avocado according to the method of Toivo et al. (*25*) with minor modifications. At the beginning of the determination, 1 mL of 5α-cholestane 0.1% in ethanol was added as internal standard to the fresh homogenized sample (1 g). Avocado samples were subjected to acid hydrolysis with HCl in ethanol at 82 °C for 60 min and alkaline hydrolysis with KOH at the same temperature for 30 min to liberate sterols from conjugates. Thereafter, 12 mL of water was added, and unsaponifiable lipids were extracted twice with 20 mL of *n*-hexane. Organic phases were pooled and dried under vacuum conditions and redissolved in 0.5 mL of *n*-hexane. The analysis of sterols was carried out by GC-MS as the trimethylsilyl (TMS) ether derivates using the same chromatographic system as for fatty acids. Samples were injected in the hot splitless mode (2 μL, 280 °C, splitless time = 1 min) in an ultra-low-bleed 5%-phenyl column based on diphenyl methylsiloxane chemistry (HP-5MS column, 30 m × 0.25 mm i.d., 0.25 μm film thickness, Hewlett-Packard, Palo Alto, CA). The column temperature was programmed from 75 °C (1 min) to 275 °C at a rate of 30 °C/min and then maintained for 25 min. Injector and detector temperatures were 280 °C. Sterols were identified and quantified by comparison of their retention times and peak areas to external standards (campesterol, β-sitosterol, and stigmasterol). Results were expressed as grams per 100 g of dry weight.

Antioxidant Activity Determination. The antioxidant activity was determined using the stable radical DPPH•. The method is described extensively elsewhere (*26*). Briefly, triplicates of each sample (30 g) were extracted three times with 20 mL of THF and centrifuged at 10000*g* for 10 min at 4 °C. Supernatants were combined to yield an organic fraction. The solvent was evaporated to dryness, and the organic residue was dissolved in 3 mL of a Tween 20 solution (10% THF). The radical scavenging capacity was evaluated by employing a reaction mixture constituted by aliquots of organic extract and a DPPH• methanolic solution. The parameter EC_{50}, linked to the concentration

Table 2. Fatty Acid Composition of Minimally Processed Avocado during Storage at 8 °C (Grams per 100 g of Dry Weight)[a]

sample	storage days	palmitic acid	palmitoleic acid	stearic acid	oleic acid	*cis*-vaccenic acid (as oleic acid)	linoleic acid	γ-linolenic acid	total content
vacuum									
slices	0	4.99 ± 0.06 e	1.96 ± 0.01 c	0.12 ± 0.01 d	18.70 ± 0.29 e	2.93 ± 0.03 d	3.96 ± 0.11 d	0.35 ± 0.04 d	33.00 ± 0.41 e
	1	4.34 ± 0.08 dB	1.86 ± 0.04 cB	0.10 ± 0.00 cC	14.95 ± 0.23 dC	2.63 ± 0.02 cC	3.36 ± 0.09 cB	0.27 ± 0.02 cB	27.51 ± 0.40 dBCD
	3	2.92 ± 0.20 aC	1.13 ± 0.08 aB	0.04 ± 0.00 aA	8.86 ± 0.70 aA	1.89 ± 0.13 aC	2.31 ± 0.17 aB	0.15 ± 0.02 aA	17.31 ± 1.29 aB
	6	3.99 ± 0.18 cdB	1.62 ± 0.08 bC	0.07 ± 0.01 bB	12.01 ± 0.53 cD	2.24 ± 0.08 bC	2.94 ± 0.13 bC	0.21 ± 0.02 bBC	23.07 ± 1.01 cD
	9	3.09 ± 0.03 abB	1.21 ± 0.02 aB	0.05 ± 0.00 abB	9.84 ± 0.20 bB	1.88 ± 0.02 aB	2.48 ± 0.04 aB	0.19 ± 0.00 abB	18.76 ± 0.31 bB
	13	3.50 ± 0.84 bcA	1.24 ± 0.34 aA	0.07 ± 0.03 bAB	11.71 ± 0.75 cB	1.92 ± 0.32 aA	2.54 ± 0.52 aA	0.18 ± 0.05 abA	21.15 ± 1.06 cB
halves	0	4.99 ± 0.06 c	1.96 ± 0.01 cd	0.12 ± 0.01 d	18.70 ± 0.29 d	2.93 ± 0.03 d	3.96 ± 0.11 d	0.35 ± 0.04 d	33.00 ± 0.41 e
	1	4.67 ± 0.17 cC	1.83 ± 0.09 cB	0.09 ± 0.01 cC	14.51 ± 0.57 cC	2.42 ± 0.07 cB	3.24 ± 0.10 cB	0.25 ± 0.01 cB	27.02 ± 1.02 cBC
	3	2.38 ± 0.50 aAB	0.98 ± 0.23 aAB	0.03 ± 0.01 aA	8.11 ± 0.33 aA	1.43 ± 0.21 aAB	1.76 ± 0.31 aA	0.13 ± 0.03 aA	14.82 ± 0.50 aAB
	6	3.67 ± 0.30 bB	1.37 ± 0.13 bB	0.07 ± 0.01 bB	11.09 ± 0.88 bC	2.08 ± 0.12 bB	2.73 ± 0.19 bB	0.19 ± 0.02 bAB	21.20 ± 1.66 bC
	9	3.66 ± 0.20 bC	1.46 ± 0.09 bC	0.07 ± 0.01 bC	11.86 ± 0.60 bC	2.09 ± 0.10 bC	2.77 ± 0.13 bC	0.22 ± 0.01 bcC	22.12 ± 1.12 bC
	13	5.76 ± 0.37 dC	2.16 ± 0.15 dD	0.13 ± 0.01 dC	15.37 ± 0.97 cD	2.43 ± 0.13 cC	3.79 ± 0.23 dD	0.33 ± 0.02 dC	29.96 ± 1.89 dD
air									
slices	0	4.99 ± 0.06 e	1.96 ± 0.01 c	0.12 ± 0.01 c	18.70 ± 0.29 d	2.93 ± 0.03 e	3.96 ± 0.11 e	0.35 ± 0.04 d	33.00 ± 0.41 d
	1	2.75 ± 0.13 abA	1.12 ± 0.05 aA	0.04 ± 0.01 aA	8.70 ± 0.35 aA	1.88 ± 0.05 bA	2.46 ± 0.11 bA	0.18 ± 0.01 abA	17.13 ± 072 aA
	3	2.51 ± 0.45 aABC	1.07 ± 0.22 aAB	0.04 ± 0.01 aAB	7.93 ± 1.78 aA	1.55 ± 0.25 aB	1.91 ± 0.44 aAB	0.14 ± 0.06 aA	15.14 ± 3.22 aAB
	6	2.94 ± 0.09 bA	1.12 ± 0.05 aA	0.04 ± 0.00 aA	7.87 ± 0.26 aA	1.79 ± 0.04 bA	2.34 ± 0.07 bA	0.21 ± 0.01 bcB	16.32 ± 0.51 aA
	9	3.60 ± 0.21 cC	1.59 ± 0.10 bD	0.08 ± 0.01 bCD	11.71 ± 0.81 bC	2.22 ± 0.12 cD	2.91 ± 0.21 cC	0.25 ± 0.03 cD	22.36 ± 1.49 bC
	13	4.36 ± 0.13 dB	1.74 ± 0.06 bBC	0.09 ± 0.01 bB	14.10 ± 0.42 cC	2.44 ± 0.05 dC	3.31 ± 0.08 dC	0.25 ± 0.04 cB	26.28 ± 0.77 cC
halves	0	4.99 ± 0.06 d	1.96 ± 0.01 d	0.12 ± 0.01 d	18.70 ± 0.29 e	2.93 ± 0.03 e	3.96 ± 0.11 d	0.35 ± 0.04 d	33.00 ± 0.41 e
	1	4.76 ± 0.20 dC	2.12 ± 0.07 eD	0.10 ± 0.01 cC	15.20 ± 0.65 dC	2.70 ± 0.08 dC	3.80 ± 0.16 dC	0.35 ± 0.02 dC	29.03 ± 0.77 dD
	3	2.17 ± 0.11 aA	0.87 ± 0.05 aA	0.04 ± 0.00 aA	6.53 ± 0.35 aA	1.26 ± 0.05 aA	1.78 ± 0.10 aA	0.14 ± 0.02 aA	12.79 ± 0.67 aA
	6	2.99 ± 0.07 bA	1.31 ± 0.04 bB	0.05 ± 0.00 aA	9.77 ± 0.29 bB	1.98 ± 0.04 bB	2.34 ± 0.06 bA	0.18 ± 0.01 bA	18.62 ± 0.50 bB
	9	6.12 ± 0.15 eE	2.61 ± 0.07 fF	0.14 ± 0.01 eE	18.30 ± 0.34 eD	3.04 ± 0.03 fE	4.26 ± 0.05 eE	0.36 ± 0.00 dF	34.83 ± 0.48 fD
	13	3.90 ± 0.31 cAB	1.79 ± 0.15 cC	0.07 ± 0.01 bAB	10.88 ± 0.82 cB	2.22 ± 0.12 cBC	2.73 ± 0.17 cAB	0.27 ± 0.02 cB	21.85 ± 1.60 cB
nitrogen									
slices	0	4.99 ± 0.06 c	1.96 ± 0.01 c	0.12 ± 0.01 d	18.70 ± 0.29 d	2.93 ± 0.03 d	3.96 ± 0.11 b	0.35 ± 0.04 d	33.00 ± 0.41 d
	1	4.69 ± 0.32 cC	1.99 ± 0.12 cC	0.10 ± 0.01 cdC	14.86 ± 1.04 cC	2.63 ± 0.13 cC	3.75 ± 0.30 bC	0.33 ± 0.04 cdC	28.36 ± 1.95 cCD
	3	5.06 ± 0.35 cD	2.17 ± 0.22 dC	0.10 ± 0.01 bcC	15.54 ± 1.19 cA	2.90 ± 0.17 dD	3.82 ± 0.36 bC	0.30 ± 0.03 bcC	29.89 ± 2.71 cC
	6	4.82 ± 0.46 cC	1.89 ± 0.04 bcD	0.09 ± 0.00 bC	12.74 ± 0.26 bD	2.39 ± 0.04 bD	3.12 ± 0.05 aD	0.23 ± 0.00 aC	25.27 ± 0.73 bE
	9	4.21 ± 0.11 bD	1.80 ± 0.05 bE	0.08 ± 0.00 abD	11.62 ± 0.30 aC	2.15 ± 0.05 aCD	3.17 ± 0.07 aD	0.28 ± 0.01 bE	23.31 ± 0.56 abC
	13	3.73 ± 0.08 aA	1.61 ± 0.04 aBC	0.07 ± 0.01 aAB	10.98 ± 0.28 aB	2.19 ± 0.03 aB	2.92 ± 0.07 aB	0.26 ± 0.01 abB	21.77 ± 0.50 aB
halves	0	4.99 ± 0.06 e	1.96 ± 0.01 d	0.12 ± 0.01 e	18.70 ± 0.29 d	2.93 ± 0.03 e	3.96 ± 0.11 e	0.35 ± 0.04 d	33.00 ± 0.41 e
	1	4.71 ± 0.10 dC	2.01 ± 0.05 deCD	0.07 ± 0.00 cB	13.52 ± 0.30 cB	2.74 ± 0.06 dC	3.43 ± 0.07 dB	0.26 ± 0.01 cB	26.74 ± 0.59 dB
	3	2.83 ± 0.32 bBC	1.13 ± 0.13 bB	0.06 ± 0.01 bB	14.07 ± 0.65 aA	1.50 ± 0.16 bAB	2.18 ± 0.25 bAB	0.22 ± 0.02 bB	16.29 ± 1.88 bB
	6	5.10 ± 0.19 eC	2.12 ± 0.09 eE	0.10 ± 0.01 dC	8.38 ± 0.99 cE	2.66 ± 0.10 dE	3.27 ± 0.13 dD	0.26 ± 0.02 cD	27.56 ± 1.18 dF
	9	2.34 ± 0.07 aA	0.93 ± 0.02 aA	0.04 ± 0.00 aA	6.60 ± 0.22 bA	1.33 ± 0.04 aA	1.82 ± 0.06 aA	0.16 ± 0.01 aA	13.21 ± 0.42 aA
	13	3.33 ± 0.16 cA	1.49 ± 0.08 cB	0.07 ± 0.00 cA	8.80 ± 0.40 bA	1.86 ± 0.05 cA	2.50 ± 0.09 cA	0.20 ± 0.01 bA	18.25 ± 0.79 cA

[a] Values are the mean of three independent determinations ± standard deviation. Different lower case letters in the same fatty acid and treatment indicate significant differences ($P < 0.05$). Different capital letters in the same fatty acid and storage day indicate significant differences ($P < 0.05$).

of the sample able to scavenge 50% of the radical, and the time needed to reach the steady state at EC_{50} concentration (t_{EC50}) were calculated. The antiradical efficiency ($AE = 1/EC_{50} \times t_{EC50}$), a parameter that combines both factors, was also calculated.

Statistical Analysis. Results were collected as mean ± standard deviation of three independent determinations. Significant differences between means were calculated by one-way analysis of variance (ANOVA). Differences at $P < 0.05$ (95% confidence level) were considered to be significant. All statistical analyses were performed using Statgraphics Plus 5.1 (Statistical Graphics Corp., Inc., Rockville, MD).

RESULTS AND DISCUSSION

Fatty Acid Content. The fatty acid composition of avocado is shown in **Table 2**. Oleic (C18:1) acid was found to be the major fatty acid in avocado, representing around 57% of total fatty acids quantified. Also, avocados were rich in palmitic (C16), linoleic (C18:2), *cis*-vaccenic (C18:1), and palmitoleic (C16:1) acids (15, 12, 9, and 6% of total fatty acids quantified, respectively), whereas γ-linolenic (C18:3) and stearic (C18) acids were presented in small amounts. Monounsaturated, polyunsaturated, and saturated fatty acids represented about 71.5, 13, and 15.5%, respectively, of the total fatty acids quantified. The ratios of polyunsaturated to saturated and polyunsaturated plus monounsaturated to saturated fatty acids were calculated (0.84 and 5.47, respectively), because they can be considered to be indicators of nutritional value (**Figure 1**). These results are in agreement with those reported by other authors for avocado (*27, 28*).

In general, there was a significant decrease in avocado fatty acid content during the 13 days of cold storage at 8 °C, being related to the oxidative degradation of fatty acids. Only the vacuum/halves sample showed an increase of palmitic acid content (15.5%) with regard to the initial value and no significant differences for palmitoleic, stearic, linoleic, and γ-linolenic acids. After 1 day of cold storage, the air/slices sample showed the lowest content for all fatty acids, whereas, at the end of the storage period, it presented one of the highest levels. The rest of the samples showed a strong decrease in the fatty acid content at 3 days of cold storage and then an increase at day 6, except for the N_2/slices sample that presented a continuous decrease throughout the storage period. Although the air/halves sample showed the highest level at day 9, it presented one of the lowest levels at the end of storage. After 13 days of cold storage, the sample that maintained better the fatty acid content was vacuum/halves. However, taking into account the two different ratios calculated (polyunsaturated to saturated and polyunsaturated plus monounsaturated to saturated fatty acids), N_2/slices and air/slices samples presented the highest values, whereas vacuum/halves showed the lowest one (**Figure 1**). For both ratios, samples cut into thick slices showed higher values than those cut in halves. Cutting could release natural antioxidants (such as α-tocopherol and ascorbic acid) from the avocado matrix as

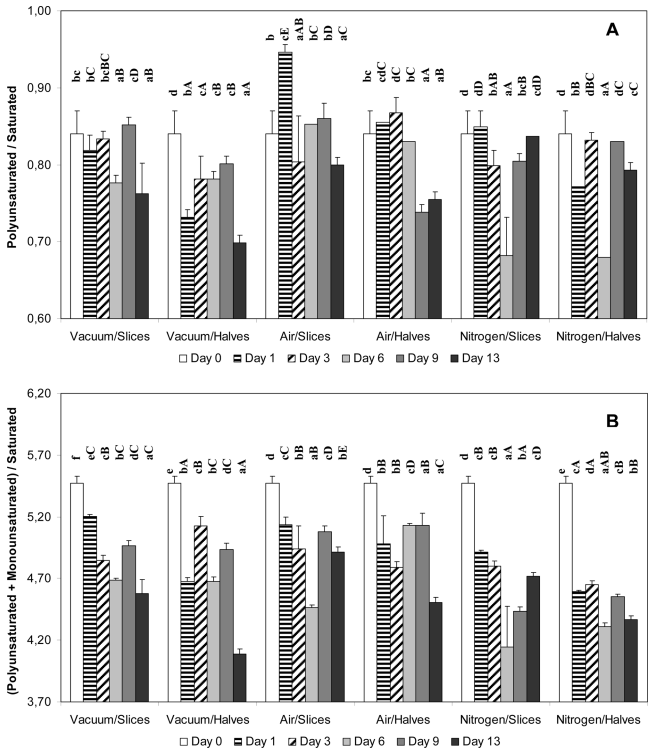

Figure 1. Ratio of (**A**) polyunsaturated to saturated fatty acids and (**B**) polyunsaturated plus monounsaturated to saturated fatty acids in minimally processed avocado during storage at 8 °C. Different lower case letters in the same treatment indicate significant differences ($P < 0.05$). Different capital letters in the same storage day indicate significant differences ($P < 0.05$).

a response to an external stress. The addition of those antioxidants to avocado puree has been related to a higher stability of the fatty acid fraction (*29*). During the cold storage period, a significant decrease of both ratio values for all samples, with the exception of polyunsaturated to saturated fatty acids ratio for nitrogen/slices sample, was observed. Zhuang et al. (*30*) studied the influence of storage temperature (2, 13, and 23 °C) on fatty acid content of broccoli florets packaged in microperforated polymeric film bags. They reported that increased temperatures resulted in significantly reduced contents over storage. In fact, there is a relationship between the degree of unsaturation and chilling resistance (*31*). An increase in the degree of unsaturation has been described as a mechanism of acclimation to low temperatures (*32, 33*).

Sterol Content. Avocado fruit was found to be a good source of 4-desmethylsterols, β-sitosterol being the most abundant (21.29×10^{-2} g/100 g of dry weight, around 89% of total sterol content). Other predominant phytosterols were campesterol (2.32×10^{-2} g/100 g of dry weight) and stigmasterol (0.24×10^{-2} g/100 g of dry weight), representing about 10 and 1% of total content, respectively (**Table 3**). Values obtained in this work were in agreement with those published by Piironen et al. (*9*) for avocado (24.04×10^{-2}, 1.59×10^{-2}, and 0.12×10^{-2} g/100 g of dry weight, respectively).

With regard to the effect of processing (cutting and packaging under air, N_2, or vacuum) and cold storage on sterol content, in general, there were no significant changes (**Figure 2**). At the end of storage at 8 °C, only stigmasterol content showed a significant decrease for all slices samples and for air/halves samples. However, taking into account campesterol, β-sitosterol, and total sterol content, air/halves and N_2/halves samples presented the highest values. There are few studies in relation

Table 3. Sterol Composition of Minimally Processed Avocado during Storage at 8 °C ($\times 10^{-2}$ Grams per 100 g of Dry Weight)[a]

sample	storage days	campesterol	stigmasterol	β-sitosterol
vacuum				
slices	0	2.32 ± 0.79 ab	0.24 ± 0.05 c	21.29 ± 4.55 ab
	1	2.72 ± 0.65 bAB	0.17 ± 0.02 bAB	25.05 ± 2.48 bB
	6	2.80 ± 0.58 bAB	0.34 ± 0.04 dBC	24.47 ± 5.64 bAB
	13	1.48 ± 0.43 aA	0.05 ± 0.01 aA	14.78 ± 3.40 aA
halves	0	2.32 ± 0.79 a	0.24 ± 0.05 a	21.29 ± 4.55 a
	1	2.39 ± 0.83 aAB	0.64 ± 0.09 bD	25.18 ± 3.76 aB
	6	2.25 ± 0.95 aA	0.13 ± 0.09 aA	19.02 ± 7.26 aA
	13	1.82 ± 0.68 aAB	0.18 ± 0.02 aC	15.99 ± 6.06 aAB
air				
slices	0	2.32 ± 0.79 a	0.24 ± 0.05 b	21.29 ± 4.55 a
	6	3.43 ± 0.92 aAB	0.29 ± 0.03 bB	27.91 ± 7.21 aAB
	13	2.37 ± 0.66 aAB	0.03 ± 0.01 aA	23.45 ± 4.11 aBC
halves	0	2.32 ± 0.79 a	0.24 ± 0.05 c	21.29 ± 4.55 a
	1	3.62 ± 0.90 bB	0.32 ± 0.02 dC	33.13 ± 8.93 bB
	6	3.13 ± 0.59 abAB	0.17 ± 0.03 bA	28.88 ± 4.02 abBC
	13	3.65 ± 0.24 bC	0.10 ± 0.01 aB	31.13 ± 5.55 abD
nitrogen				
slices	0	2.32 ± 0.79 a	0.24 ± 0.05 b	21.29 ± 4.55 a
	1	2.19 ± 0.28 aA	0.11 ± 0.05 aA	23.37 ± 6.35 aAB
	6	3.95 ± 0.80 bB	0.42 ± 0.07 cC	38.41 ± 4.07 bC
	13	2.10 ± 0.58 aAB	0.11 ± 0.01 aB	18.42 ± 1.30 aABC
halves	0	2.32 ± 0.79 a	0.24 ± 0.05 a	21.29 ± 4.55 ab
	1	1.65 ± 0.79 aA	0.25 ± 0.05 aBC	14.87 ± 3.42 aA
	6	2.88 ± 0.84 aAB	0.39 ± 0.05 bBC	20.22 ± 3.84 abAB
	13	2.83 ± 0.76 aBC	0.19 ± 0.03 aC	23.88 ± 3.57 bCD

[a] Values are the mean of three independent determinations ± standard deviation. Different lower case letters in the same sterol and treatment indicate significant differences ($P < 0.05$). Different capital letters in the same sterol and storage day indicate significant differences ($P < 0.05$).

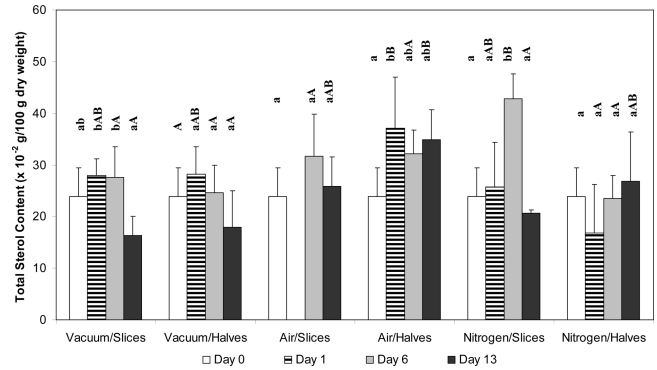

Figure 2. Total sterol content in minimally processed avocado during storage at 8 °C. Different lower case letters in the same treatment indicate significant differences ($P < 0.05$). Different capital letters in the same storage day indicate significant differences ($P < 0.05$).

to the stability of plant sterols during storage. Makhlouf et al. (*34*) studied the effect of low temperature and controlled atmosphere or air storage on the sterol content of broccoli flower buds, reporting that storage and atmosphere had little influence. Also, Lurie et al. (*35*) reported that apple fruits showed no change in sterol content after 8 weeks of storage under air or controlled atmosphere at 0 °C. Another study evidences a low sterol oxidation level in ready-to-eat infant foods during 9 months of storage at 25 °C (*36*). It seems that no significant changes take place in total sterol content in most common conditions, although some oxidation products may be found after prolonged storage (*37*).

Antioxidant Activity. Radical scavenging activities of different minimally processed avocado samples were evaluated during refrigerated storage at 8 °C. **Table 4** shows EC_{50} and t_{EC50} values, whereas antiradical efficiency (AE) values are shown in **Figure 3**.

Table 4. Radical Scavenging Parameters of Minimally Processed Avocado during Storage at 8 °C[a]

sample	storage days	EC_{50} (g of dw[b]/g of DPPH•)	t_{EC50} (min)
vacuum			
slices	0	820.41 ± 21.08 b	37.61 ± 3.18 ab
	1	837.30 ± 30.42 bA	38.96 ± 6.43 bA
	3	1476.25 ± 21.5 eE	35.38 ± 6.92 abA
	6	1330.69 ± 39.88 dE	50.51 ± 2.95 cC
	9	1104.11 ± 11.73 cD	33.81 ± 2.11 abB
	13	766.37 ± 38.58 aA	28.83 ± 8.16 aA
halves	0	820.41 ± 21.08 a	37.61 ± 3.18 bc
	1	788.00 ± 32.25 aA	44.94 ± 5.57 dA
	3	818.23 ± 16.31 aA	36.63 ± 3.58 bA
	6	1074.04 ± 15.97 cBC	43.53 ± 2.64 cdB
	9	902.34 ± 51.41 bB	31.39 ± 4.31 abAB
	13	836.74 ± 20.66 aA	28.95 ± 1.61 aA
air			
slices	0	820.41 ± 21.08 a	37.61 ± 3.18 b
	1	813.65 ± 70.50 aA	46.32 ± 1.07 cA
	3	1327.95 ± 58.82 cD	49.52 ± 6.57 cC
	6	1004.90 ± 39.63 bAB	27.35 ± 1.08 aA
	9	785.67 ± 20.58 aA	30.20 ± 4.36 abAB
	13	920.29 ± 74.67 bA	24.84 ± 5.69 aA
halves	0	820.41 ± 21.08 a	37.61 ± 3.18 b
	1	823.00 ± 29.13 aA	45.70 ± 3.88 cA
	3	1022.59 ± 71.35 bB	45.44 ± 3.71 cBC
	6	1187.76 ± 22.09 cD	48.26 ± 6.48 cBC
	9	749.65 ± 32.87 aA	25.73 ± 3.45 aA
	13	1529.33 ± 199.87 dB	25.57 ± 4.14 aA
nitrogen			
slices	0	820.41 ± 21.08 a	37.61 ± 3.18 bc
	1	867.25 ± 21.76 abA	40.12 ± 8.58 cA
	3	1180.96 ± 66.67 dC	38.98 ± 2.50 cAB
	6	961.54 ± 34.41 cA	30.15 ± 2.39 abA
	9	921.58 ± 29.42 bcBC	29.00 ± 3.13 aAB
	13	872.38 ± 13.33 abA	44.27 ± 1.91 cB
halves	0	820.41 ± 21.08 a	37.61 ± 3.18 b
	1	834.75 ± 100.90 aA	47.53 ± 7.39 cA
	3	959.24 ± 38.09 bB	38.09 ± 2.28 bAB
	6	1116.47 ± 65.94 cC	47.76 ± 4.02 cBC
	9	966.17 ± 49.58 bC	25.84 ± 4.64 aA
	13	780.08 ± 14.36 aA	31.97 ± 2.40 abA

[a] Values are the mean of three independent determinations ± standard deviation. Different lower case letters in the same parameter and treatment indicate significant differences ($P < 0.05$). Different capital letters in the same parameter and storage day indicate significant differences ($P < 0.05$). [b] dw, dry weight.

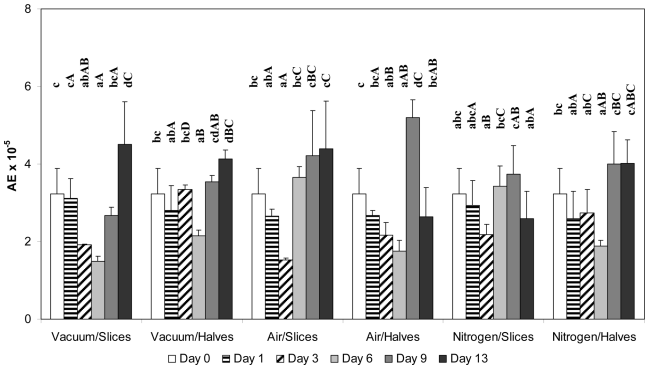

Figure 3. AE values in minimally processed avocado during storage at 8 °C. AE is expressed as 1/[EC_{50} (g of dw/g of DPPH•) × t_{EC50} (min)]. Different lower case letters in the same treatment indicate significant differences ($P < 0.05$). Different capital letters in the same storage day indicate significant differences ($P < 0.05$).

The extract of avocado presented initial EC_{50}, t_{EC50}, and AE values of 820.41 g of dry weight/g of DPPH•, 37.61 min, and 3.23×10^{-5} 1/[(g of dw/g of DPPH•) × min], respectively. For tomato juice (organic fraction), Sánchez-Moreno et al. (*26*) reported similar values (462 g of dry weight/g of DPPH•, 41 min, and 5.3×10^{-5} 1/[(g of dw/g of DPPH•) × min], respectively). Several authors have evaluated the antioxidant activity (mainly hydrophilic) of avocado by using different methods such as DPPH•, $ABTS^{•+}$ (2,2′-azinobis(3-ethylbenzthiozoline-6-sulfonic acid), or FRAP (ferric reducing antioxidant power) assays. They reported medium or low activity for this fruit (*38, 39*). García-Alonso et al. (*40*) studied the antioxidant activity of extracts from 28 fruits in lipid and aqueous phases by TBARS method, reporting that avocado fruit presented the lowest antioxidant activity value. These works confirm the low antioxidant activity found by us in the present study.

At the end of refrigerated storage, vacuum/slices and vacuum/halves samples showed a significant increase in antioxidant activity with regard to their initial value (AE increased around 40 and 28%, respectively), whereas there were no significant changes for the rest of the samples. It seems that cutting and/or absence of air could induce stress in the samples, leading to the release of an additional amount of antioxidants, revealed in the increased value of AE at the end of the storage. When we compare the antioxidant activity of different processed samples after 13 days of storage at 8 °C, AE average values were quite similar, vacuum samples being slightly out of the norm. Thus, we could affirm that the storage temperature is an important factor affecting the antioxidant response with respect to different gaseous environments around the avocado pieces. According to Ayala-Zavala et al. (*41*), storage temperatures can significantly affect the antioxidant activity. In this sense, they found that values changed very little during storage at 0 °C but significantly increased at 5 or 10 °C for strawberries. However, the effect of temperature will depend on the vegetable matrix.

To relate the antioxidant activity of avocado samples to the bioactive compounds studied in this work (fatty acids and sterols), regression analyses were carried out. No correlations were found except for stearic acid, EC_{50} ($r^2 = 0.3673$, $P = 0.0421$) and AE ($r^2 = 0.3898$, $P = 0.0302$). These results seem to indicate that other phytochemicals, not measured in the present work (for example, vitamin E and carotenoids), might contribute to the antioxidant activity of avocado. Also, it is necessary to take into account the possible synergic and/or antagonistic effects of those compounds.

In conclusion, minimal processing could be a good option to preserve potentially health-promoting attributes of avocado. However, further studies are necessary to clarify the effect of these treatments on the bioavailability of avocado bioactive compounds.

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