Natural Antioxidants Preserve the Lipid Oxidative Stability of Minimally Processed Avocado Purée

PEDRO ELEZ-MARTÍNEZ, ROBERT C. SOLIVA-FORTUNY, SHELA GORINSTEIN, AND OLGA MARTÍN-BELLOSO

ABSTRACT: Lipid oxidation is one of the major phenomena that limit the shelf-life of avocado products. The effects of adding 100 ppm α-tocopherol, 200 ppm ascorbic acid, and 200 ppm ethylenediaminetetraacetic acid (EDTA) on the stability of the lipidic fraction of minimally processed avocado purées were evaluated throughout storage. α-tocopherol, followed by ascorbic acid, reduced oil rancidity processes during storage. Peroxide formation was minimal when α-tocopherol was added to avocado pulp, prolonging the induction stage of oils for at least 12 wk. When preserved in vacuum, iodine and specific extinction coefficient at 270 nm (k_{270}) values were more stable, with changes of 5.87 g I_2/100 g oil and 0.237, respectively, during 24 wk. On the other hand, EDTA was not shown to be effective in preserving the stability of the lipid fraction of avocado preserved by combined methods.

Keywords: minimal processing, avocado oil, oxidative stability

Introduction

Avocado (*Persea Americana* Mill., family Lauraceae) is a fruit of unusually high oil content (15% to 30% depending on the variety) (Werman and Neeman 1987). The cultivar was originally grown in pre-columbine Central America but nowadays can be found in many mild-winter areas such as Southern Europe and United States. In most of these areas, the Hass variety has a considerable commercial importance (Gaydou and others 1987). The ripe fruit is dark-colored with a distinguishing rough and bumpy skin. In most Latin American countries, it is used to make guacamole and is useful for dressings because of its high fat content.

Heat treatments are detrimental to the quality of avocado pulp because they induce several undesirable reactions that turn into browning, flavor damage, and/or nutritional losses. Minimal processing, as a combination of mild preservation techniques, may ensure microbiological stability as well as the sensory quality of most fruits (Chirife 1993; Leistner and Gorris 1995; Alzamora and others 1998; Soliva-Fortuny and others 2004). Indeed, several researchers have proposed this technology to obtain high-quality avocado products (Solomos 1994; Dorantes and others 1998; Soliva and others 2001).

The shelf-life of avocado pulp is severely determined by oxidative processes, which affect both lipidic and aqueous fractions. Avocado is a climacteric fruit with a large increase in protein synthesis at its peak of respiration rate. The protein produced is mostly composed of enzymes that are responsible for most of the changes observed during ripening and also for the rapid spoilage of the fruit (Wong 1989). This decay is known to be produced by enzyme-mediated oxidative reactions, especially in the aqueous fraction, where phenolic substrates are hydroxylated and then oxydized to form brown compounds (Kahn 1977). On the other hand, the changes in the lipid fraction are a consequence of autoxidation.

Like other vegetable oils, avocado oil is sensitive to oxidative processes resulting in rancidity and subsequent production of undesirable flavors and quality losses throughout storage (Gunstone and Norris 1982). Autoxidative processes are greater in avocado oil than in other oils because amounts of natural antioxidants are small and chlorophyll is present in large quantities, thus increasing the rates of photooxidation (Werman and Neeman 1986a). Werman and Neeman (1986b) studied the effectiveness of naturally derived antioxidants such as propyl gallate and α-tocopherol in stabilizing the fatty fraction of avocado. Tocopherols are natural antioxidants containing an unsaturated aromatic ring with an hydroxyl group that acts as hydrogen donor and thus retards the formation of free radicals during the initiation stage of oxidative processes. Their maximum effectiveness has been recorded at levels similar to the naturally occurring amounts in vegetable oils. Higher concentrations may have undesirable prooxidant effects (Fennema 1996). Ascorbic acid acts as a powerful antioxidant in the aqueous fraction of avocado, especially preventing browning reactions (Soliva and others 2001), but it may also have antioxidant properties in the lipid fraction. Fatty acid esters of ascorbic acid enhance the effectiveness of natural antioxidants (Gunstone and Norris 1982). Ethylenediaminetetraacetic acid (EDTA) acts as an antioxidant in lipids by chelating metallic cations, thus reducing autoxidation (Lagunes-Galvez and others 2002), but its popularity has decreased in the last decades due to its synthetic nature. Although analyses have continued to be made on avocado oil stability during the past decades, most studies provide results referred to refined oil, in which prooxidant substances are present in very small quantities. No references in literature undertake the study of the rancidity processes in avocado oil contained in the processed fruit matrix throughout storage.

Bearing this in mind, the influence of different antioxidants on the oxidative stability of the lipid fraction minimally processed avocado purée was examined, and aspects related to the shelf-life of such product are discussed in this article.

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Table 1—Centesimal composition and some physicochemical properties of fresh Hass avocados at the stage of processing

Centesimal composition (%)[a]		Physicochemical characterization[a]	
Water	73.11 ± 0.07	Water activity (a_w)	0.99 ± 0.05
Dry extract	26.9 ± 1.2	pH	6.48 ± 0.05
Lipids	20.1 ± 2.0	Total acidity (g citric acid/100 g fruit pulp)	0.028 ± 0.002
Fiber	3.02 ± 0.06	Soluble solids (°Brix)	9.2 ± 0.2
Protein	0.70 ± 0.08	—	—
Ashes	1.94 ± 0.12	Pulp maximum penetration force (N)	1.0 ± 0.1
Carbohydrates	1.12 ± 0.17	Skin color: L^* = 27.5 ± 1,0; a^* = 5.2 ± 1.2; b^* = 7.0 ± 1.5	

[a]Average value ± standard deviation.

Materials and Methods

Characterization of samples

Avocados (var. Hass) were purchased in a local wholesale distributor. The fruits were ripened to reach an optimal state of maturity. According to preliminary studies (Soliva-Fortuny and others 2002), skin color and pulp firmness were used as objective criteria to evaluate changes in maturity (Table 1). Skin CIELAB values were determined with a Macbeth Color-Eye 3000 colorimeter (Macbeth-Kollmorgen Inst. Corp., Newburgh, N.Y., U.S.A.) equipped with a D_{75} light source and the observer at 10°. Pulp firmness was determined using a TA-XT2 Texture Analyser (Stable Micro Systems Ltd., Surrey, U.K.) by measuring the force required for a 4-mm-dia probe to penetrate to a depth of 5 mm into a pulp portion.

Furthermore, a physicochemical characterization, including an analysis of the pulp centesimal composition, was carried out according to AOAC procedures (Horwitz 2000) (Table 1).

Preparation of avocado purée

Avocados were sanitized in tap water with 200 ppm added chlorine. The fruits were peeled, halved lengthwise, and pitted, and the pulp was cut into pieces and ground to get a homogeneous purée. The pH of the pulp was adjusted to 4.0 with citric acid to avoid proliferation of pathogenic clostridia throughout storage.

Sorbic acid was used in a concentration of 300 ppm to get a microbiologically stable product throughout the period of analysis (Soliva-Fortuny and others 2004). Afterward, 100 ppm of α-tocopherol, 200 ppm of ascorbic acid (AA) or 200 ppm of EDTA were added to the pulp. EDTA was used to provide a reference as a synthesis antioxidant. Finally, control purées were prepared without antioxidant addition. After homogenization, about 105 g of purée was packaged in polyethylene bags of a permeability of 15 cm^3 $O_2/m^2/bar/d$. The bags were vacuum-sealed with a compensated vacuum machine or just sealed to compare the effects on purées stored with and without O_2 available. Samples were kept under refrigeration (4 ± 1 °C) for a period of 24 wk.

Thus, 8 different treatments could be compared: antioxidant {ascorbic acid, EDTA, α-tocopherol, control} × packaging {vacuum-sealed, sealed}.

Oil extraction

Avocado pulp was heated to 60 °C for 30 min and periodically stirred to improve mechanical and enzymatic destruction of oil cells. Next, avocado paste was centrifuged at 22100 × *g* for 30 min at 4 °C (Centrifuge AVANTI™ J-25, Beckman Instruments Inc., Fullerton, Calif., U.S.A.). It has been demonstrated that this technique yields lower amounts of unsaponificables compared with solvent extraction methods (Werman and Neeman 1987). The supernatant phase was separated from the aqueous phase, and oil samples were filtered to remove impurities.

Oil samples analysis

Peroxide value. Peroxide value was assayed according to a modification of the method proposed by García and others (1996) for olive oil. A 2 g avocado oil sample from each replicate was placed in a 250-mL Erlenmeyer flask, which was previously purged with nitrogen. The sample was shaken and dissolved in 25 mL of an acetic acid:chloroform solution (3:2, v/v). Next, 1 mL of saturated potassium iodide (KI) solution was added, and the flask was placed in darkness for 5 min. After that period, 75 mL of distilled water was added, and the mixture was titrated with 0.005 *N* sodium thiosulphate with a 1% (w/v) starch indicator solution. Results were expressed in milliequivalents of oxygen per kilogram of oil.

Specific extinction coefficient at 270 nm (k_{270}). An oil sample of 100 mg was diluted to 10 mL in a graduated flask with cyclohexane (spectrophotometry grade). The sample was homogenized and absorbance at 270 nm was determined in a spectrophotometer (Cecil Instruments Ltd., Cambridge, U.K., model CE 1010) with a 1-cm-path quartz cuvette using pure cyclohexane as a blank. The sample was diluted conveniently and the measure repeated when absorbance values fell out of the range of 0.2 and 0.8.

Iodine value. Iodine index was assessed using Wijs method (ISO 3961:1996 or ASTM D 1959). Accurately weighed 200 mg of sample were dissolved in a 300-mL glass-stoppered flask containing 15 mL of carbon tetrachloride, and 25.0 mL of Wijs reagent was pipetted into the flask. The flask was swirled and put in darkness for 1 h. Subsequently, 20 mL of 10% potassium iodide (w/v) and 150 mL of distilled water were added. The excess iodine was titrated with 0.1 *N* sodium thiosulfate using a 1% (w/v) starch indicator solution. Results were expressed in grams I_2 per 100 g of oil.

Statistical analysis

Analytical procedures were carried out for triplicate samples. In addition, all the analyses of each replicate sample were done in triplicate. Statistical analysis was carried out using a Statgraphics v.3.0 statistical package (Statistical Graphics Co., Rockville, Md., U.S.A., 1994 to 1997). A multifactor analysis of covariance (ANCOVA) examining antioxidant treatment and packaging atmosphere was performed on each of the indexes measured (least significant difference [LSD] type III). The analyses were performed with a 95% confidence interval. In addition, a Pearson correlation test was performed on all combinations of 2 indexes (correlation matrix) to assess whether there were any significant relationships between the variables.

Results and Discussion

Rancidity processes in minimally processed avocado purée can be monitored by studying the oxidative stability of crude oil. Peroxide index determination serves as an indicator of the oil quality, thus giving information about the oxidative stability of stored

purées. This method does not distinguish between the various unsaturated fatty acids that undergo oxidation, nor does it supply information about the secondary oxidative products formed by hydroperoxide decomposition. A rapid hydroperoxide formation unleashes the oxidative mechanisms that curb oil quality, whereas prolonged induction stages indicate higher stability of the lipidic fraction (Werman and Neeman 1986a).

Primary oxidation

The results showing peroxide values of oil extracted from avocado purées with addition of different antioxidants are presented in Figure 1. Initial peroxide values ranged from 5.90 to 7.01 mEq O_2/kg oil. Comparison of peroxide values for each experimental condition suggests apparent differences between antioxidant treatments. The addition of α-tocopherol clearly delayed peroxide formation. Avocado purée with addition of α-tocopherol stored in vacuum was stable during 20 wk, whereas peroxide values were significantly increased from the 12th wk of storage in air-packaged purée (Figure 1). The rate of increase in peroxide values under those conditions suggests that rancidity processes were still in the induction stage. In contrast to these results, Werman and Neeman (1986b) reported a negative effect of α-tocopherol addition in avocado oil, leading to accelerated peroxide formation. However, the amounts of α-tocopherol used in their study were higher (250 ppm) and were added directly to the refined, bleached oil. When found in high quantity, the antioxidant effect of α-tocopherol may be overwhelmed due to photosensitized oxidation and other pro-oxidative conditions such as the presence of transition metal ions, thus entailing an active participation in autoxidation (Psomiadou and Tsimidou 2002). Because chlorophyll is present in considerable amounts in avocado, it may act as a strong pro-oxidant agent of the lipid fraction. Crude avocado oil has a very high chlorophyll content in contrast to other oils such as olive oil (Werman and Neeman 1986a). In addition, lipolytic enzymes such as lipases and lipoxygenases, released from the fruit cells, could also contribute to oxidation and could be the reason for the relatively high initial peroxide values. These factors and the effect of the antioxidants had a direct influence on the oxidative stability of avocado purée throughout storage, which was visually noticed through changes in the color of oil. These changes can be attributed to the formation of colorless chlorophyll compounds, produced by the interaction between chlorophyll and hydroperoxides, and were evident in control purées and in purées with addition of EDTA. In addition, these antioxidants interacted with the aqueous phase of avocado and acted as inhibitors of browning reactions. In previous studies, AA and especially EDTA had been found to effectively reduce color changes in minimally processed avocado purée (Soliva and others 2001; Soliva-Fortuny and others 2002). However, no changes in the color of the extracted oils could be visually appreciated in this study between purées with addition of AA and α-tocopherol.

As expected, the availability of oxygen in the package headspace had a significant ($P < 0.05$) effect on oxidation (Table 2). Samples stored in vacuum were more stable, thus producing less peroxides after prolonged storage (Figure 1). In our experiments, samples were stored in darkness. Exposure to light is well known to cause a marked acceleration in the deterioration of unsaturated oils (Carlson and others 1976). Analysis of variance (Table 2) indicated that vacuum storage had a more significant effect on secondary oxidation than on primary oxidation. However, this effect was relatively weak if compared with the influence of the antioxidant, indicating that autoxidation was the main process responsible for lipid rancidity.

A progressive increase in peroxide formation can be observed for oil obtained from avocado purées with addition of AA (Figure 1).

Table 2—Multifactor analysis of covariance of experimental data. F-test results are expressed as a ratio of the variance explained by a factor in contrast to the unexplained variance

	Peroxide index	k_{270}[a]	Iodine value
Time (covariate)	13.11[b]	312.31[b]	200.31[b]
Factor A: antioxidant	19.04[b]	254.48[b]	340.64[b]
Factor B: storage atmosphere	10.89[b]	46.16[b]	178.53[b]
Interaction Factor A × factor B	2.26[c]	0.45[c]	25.52[b]

[a] k_{270} = specific extinction coefficient at 270 nm
[b] $P < 0.01$.
[c] $P > 0.05$.

Peroxide values were 20.25 and 23.45 mEq O_2/kg oil at 24 wk of storage for samples stored in vacuum and air, respectively. Although it is water soluble, ascorbic acid is added to vegetable oils because of its complex multifunction, acting as a hydrogen donor, as a metal inactivator, and as a peroxide destroyer (Frankel 1989). This fact would explain why alone, AA could not stop hydroperoxide formation but could reverse these reactions for some time. Therefore, a combination of AA and α-tocopherol could be synergistic because AA may have a regenerative effect over oxidized tocopherol molecules (Yin and others 1993).

Eventually, peroxide values in control purées and purées with addition of EDTA underwent a dramatic increase during the 1st wk of storage, describing a typical curve for lipid oxidation proposed by Mehlenbacher (1960). Maximum values of 16.57 and 20.29 mEq O_2/kg oil were achieved at 4 and 6 wk for control purées and purées with addition of EDTA, respectively (Figure 1). These strong increases were followed by a rapid decrease during successive weeks, given that the rate of hydroperoxide degradation is proportional to the overall extent of a lipid oxidation (Matissek and others 1998). EDTA, which exhibits a powerful action in delaying browning of avocado purée (Soliva and others 2001), is a chelator of metallic cations in food systems. Nevertheless, the blocking effect on metal ions was not sufficient to stop oxidative reactions in the lipidic phase.

Secondary oxidation

To give a complete view of the oxidative changes, k_{270} and iodine

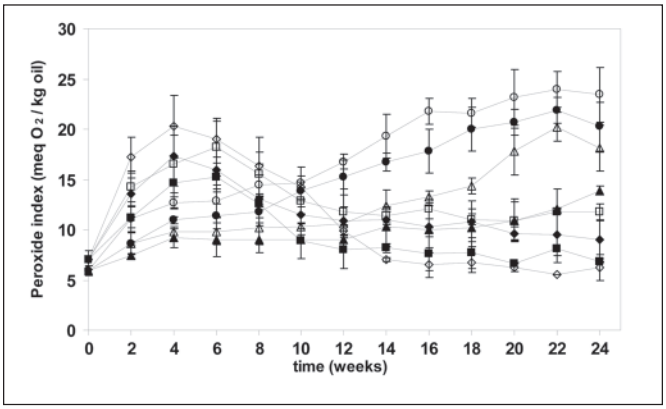

Figure 1—Peroxide index of avocado oil samples obtained from minimally processed avocado pulp with addition of different antioxidants. Bold symbols correspond to vacuum-packaged purées, whereas empty symbols stand for air-packaged control samples. Triangles = 100 ppm α-tocopherol; circles = 200 ppm ascorbic acid; squares = 200 ppm ethylenediaminetetraacetic acid (EDTA); diamonds = control samples.

value were determined. Because of the peroxide's transitory nature, information about the secondary deposition products formed during oil oxidation is required. Changes in UV extinction coefficient at 270 nm are a good indicator of secondary oxidation because they can be related to the amount of trienes or unsaturated carbonyl compounds, such as aldehydes and ketones, that are produced in the final steps of lipid oxidation (Gertz and Klostermann 2002) and were responsible for the appearance of rancidity odors, specially in control purées and in purées with addition of EDTA. The iodine value determination is used to ascertain the extent to which the bonds of the oil can be regarded as unsaturated. It provides relatively exact values with samples not containing a very large proportion of conjugated double bonds, but otherwise the results serve more as a guide of the overall oxidation of an oil. Although the fatty acid composition of avocado oil varies with different cultivars, stages of ripening, anatomical region of the fruit, and geographic growing location, the major fatty acid is always oleic, followed by linoleic, palmitic, and palmitoleic. Trace amounts of linolenic and stearic also are present (Swisher 1988). This composition and that of olive oil overlap in their high contents of monounsaturated and polyunsaturated fatty acids, which are now considered highly desirable to be included in healthy diets.

The specific extinction coefficient at 270 nm (k_{270}) of avocado oil increased with different intensity, depending on the kind of antioxidant added to the pulp (Figure 2). The k_{270} values in oils obtained from just processed avocados ranged from 0.370 to 0.451. Contrasting with our results, Martínez-Nieto and others (1988) reported a slightly lower value of 0.327 for k_{270} in oil extracted from Hass avocados. Among the tried antioxidants, α-tocopherol was the most effective to get stable purées. K_{270} values were 0.607 and 0.741 at 24 wk of storage when α-tocopherol was added to the processed fruit under vacuum and air, respectively. Although differences between purées with addition of α-tocopherol and AA were significant ($P < 0.05$), avocado purées were preserved from secondary oxidation with similar intensity. When AA was added to the purée, k_{270} increments during 24 wk of storage were 0.717 and 0.877 (Figure 2). Iodine values correlated well with these changes in k_{270}. Thus, k_{270} increased as iodine values decreased (Figure 2 and 3) with a Pearson's product-moment correlation of 0.9091. Hence, changes in iodine values of oil from purées stored in vacuum with added α-tocopherol were minimal (3.7% of the initial values) compared with control purées, which underwent a decrease of 27.0%. As displayed in Figure 2 through k_{270} values, EDTA appeared to exert a poor effect in delaying the formation of products from secondary oxidation. As shown for control purées, k_{270} values increased exponentially during the 1st 10 to 12 wk of storage and then maintained steady values during the following weeks. Therefore, it can be deduced that, because of its method of action, EDTA did not contribute with remarkable significance to protect the lipid fraction of avocado purées from oxidation. The chelation effect of metallic ions was not sufficient to stop autoxidative processes that were mainly responsible for oil rancidity. Unlike EDTA, α-tocopherol and AA had a determinant influence on the primary oxidation process, thus inverting the production of hydroperoxides that are the substrate of subsequent steps resulting in oil rancidity.

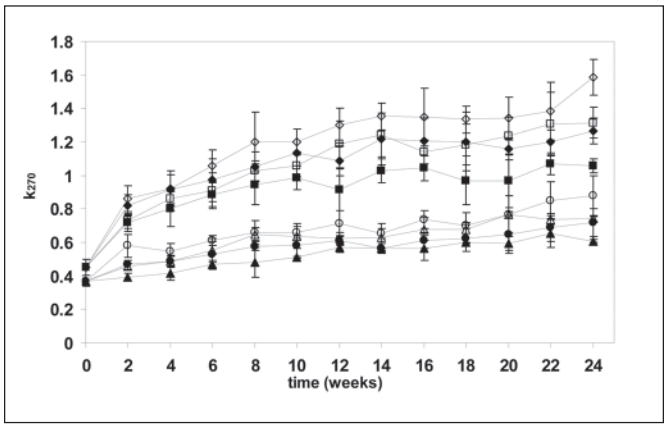

Figure 2—Ultraviolet (UV) spectrophotometric absorbance (k_{270}) of avocado oil samples obtained from minimally processed avocado pulp with addition of different antioxidants. Bold symbols correspond to vacuum-packaged purées, whereas empty symbols stand for air-packaged control samples. Triangles = 100 ppm α-tocopherol; circles = 200 ppm ascorbic acid; squares = 200 ppm ethylenediaminetetraacetic acid (EDTA); diamonds = control samples.

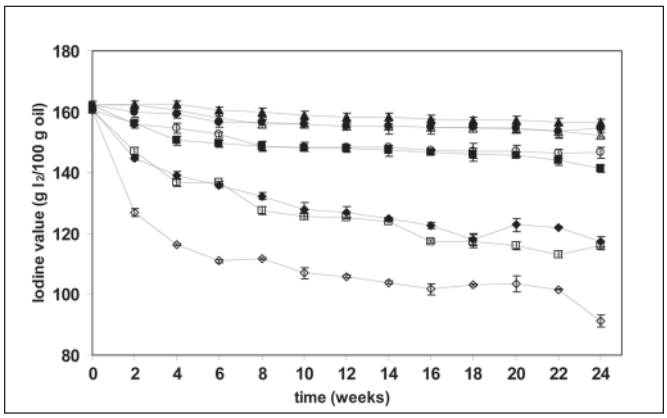

Figure 3—Iodine values of avocado oil samples obtained from minimally processed avocado pulp with addition of different antioxidants. Bold symbols correspond to vacuum-packaged purées, whereas empty symbols stand for air-packaged control samples. Triangles = 100 ppm α-tocopherol; circles = 200 ppm ascorbic acid; squares = 200 ppm ethylenediaminetetraacetic acid (EDTA); diamonds = control samples.

Conclusions

The shelf-life of minimally processed avocado purée greatly depends on the oxidative stability of the lipid fraction. Addition of 100 ppm α-tocopherol or 200 ppm ascorbic acid could stabilize the products from the point of view of rancidity for at least 24 wk of refrigerated storage. α-tocopherol was the most effective antioxidant, but ascorbic acid is advantageous because it also has a beneficial effect on the aqueous fraction of avocado by retarding browning. On the contrary, the metal chelating effect of EDTA had little impact on the preservation of the oxidative stability of avocado oil. Future investigations about the combined effect of ascorbic acid and α-tocopherol would be of interest for prolonged storage times.

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