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Effectiveness of Antioxidants in Refined, Bleached Avocado Oil

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The addition of antioxidants propyl gallate (PG), α-tocopherol and ethoxyquin at a level of 250 ppm to refined, bleached avocado oil resulted in the retardation of the oxidative deterioration of the oil when it was stored in the dark at room temperature, exposed to daylight at room temperature (on the shelf) and at 60 C.

The extent of oxidation was followed by measuring the peroxide and anisidine values and oil color. Ethoxyquin and α-tocopherol were relatively ineffective antioxidants, whereas PG greatly improved the stability of avocado oil stored in the dark at 60 C, but not in oil exposed to daylight.

Avocado oil is derived from the mesocarp of the avocado fruit. There are two major methods for producing avocado oil. One is by drying and pressing the fruit at elevated temperatures and extracting the oil with an organic solvent. The second is by centrifugal force separation. Crude avocado oil undergoes refinement, bleaching and deodorization, yielding the edible oil. Avocado oil is used mainly in the cosmetic industry in its crude form. In the future, avocado oil is expected to enter the food industry. Like other well known edible oils, avocado oil is sensitive to oxidative processes resulting in rancidity, production of undesirable flavors and quality losses during storage (1). The primary products of lipid oxidation are hydroperoxides which generally are referred to as peroxides. Therefore, it seems reasonable to determine the concentration of peroxides as a measure of the extent of oxidation. However, this approach is misleading because of the transitory nature of the peroxides (2). Moreover, the iodometric method for peroxide value has inherent errors due to the absorption of iodine at the unsaturated bonds and the generation of iodine from potassium iodide by oxygen in the solution (3).

The effectiveness of various antioxidants in stabilizing edible oils has been studied previously (3-6). Most of the commonly used antioxidant compounds are referred to as phenolic antioxidants (7). These antioxidants contain an unsaturated aromatic ring containing hydroxyl or amine groups that are hydrogen donors, and thus retard the production of free radicals during the initiation stage of oxidative processes (8).

In view of the lack of data on the retarding effect of antioxidants on autoxidation of avocado oil, the present study was set up to evaluate the effectiveness of propyl gallate, α-tocopherol and ethoxyquin under various conditions.

EXPERIMENTAL PROCEDURES

Preparation of samples. Refined, bleached avocado oil was obtained from Avochem, Santa Paula, California. In accordance with the manufacturer's statement the oil is free of added antioxidants and preservatives. The characteristics of the avocado oil are given in Table 1.

The antioxidants were of analytical grade. DL-α-tocopherol was obtained from Fluka AG, Buches, Switzerland. Ethoxyquin (1,2-dihydro-6-ethoxy-2,2,4-trimethyl quinoline) was obtained from ABIC LTD, Ramat-Gan, Israel, and n-propyl gallate from Sigma Chemical Co., St. Louis, Missouri.

Each antioxidant, at a level of 250 ppm, was added directly to the oil, which was stirred at ca. 60 C for one hr to ensure complete dissolution of the antioxidant in the oil. Samples of oil, 10 ml each, were then transferred to a series of transparent bottles of 20 ml volume and 5 cm^2 cross section each. The bottles were loosely closed to enable direct contact between the oil surface and atmospheric air.

The oil samples were exposed to several oxidative conditions. These were (i) complete darkness at room temperature; (ii) "on the shelf," exposed to daylight at ambient temperature, and (iii) opaque laboratory oven at 60 C.

Analysis of samples. Peroxide values were determined periodically in accordance with the AOCS Official Methods (9). Ansidine values were obtained using IUPAC methods(10).

Oil color was evaluated using the Stillman (11) method based upon measurements at 460 and 550 millimicrons for evaluating the color of a bleached oil containing no chlorophyll.

$$\text{Color} = 4.6\ A_{460} + 34\ A_{550}$$

where A is the absorbance of the oil at the specified wavelengths.

The fatty acid composition of the oil was determined by gas liquid chromatography (GLC) in a Packard gas chromatograph. 1% (w/v) of sulfuric acid in absolute methanol was used to prepare methyl esters (12).

RESULTS AND DISCUSSION

The results showing the stability of avocado oil with and without antioxidants are presented in Tables 2, 3 and 4. The values that appear in these tables are the average of three determinations. There are no differences in peroxide value or ansidine value and color, before and after addition of antioxidants to fresh oil.

TABLE 1

Characteristics of Refined, Bleached Avocado Oil

Characteristics	
Peroxide value meq/kg	4.41
P-Anisidine value	6.0
Acid value	0.11
Saponification number	195.8
Unsaponifiable %	1.35
Chlorophyll, ppm[a]	1.33
Pheophytin, ppm[a]	8.15
Fatty acid composition, area %	
16:0	11.80
16:1	2.18
18:0	0.68
18:1	70.50
18:2	14.20
18:3	0.50

[a]Chlorophyll and pheophytin content were fluorometrically determined (15) using Perkin-Elmer PMF-44B fluorescence spectrophotometer.

The peroxide value serves as an indicator of the extent of primary oxidation products in the oil, while the ansidine value reflects the degree of secondary oxidation products (3). It can be seen from the peroxide values of oil samples stored in the dark at room temperature that only the addition of PG significantly improved avocado oil stability. The addition of α-tocopherol and ethoxyquin somewhat accelerated peroxide formation. In oils stored in the dark at room temperature, the formation of secondary oxidation products is very slow; accordingly, anisidine values are low. In these oils there is no difference in color deterioration between oil free of additives and oil containing ethoxyquin and α-tocopherol. On the other hand, PG has a stabilizing effect on oil color.

The effect of temperature on primary and secondary oxidation and on color loss is evident from results obtained from oils stored in the dark at 60 C and those stored at room temperature. As expected, the formation of peroxides is more rapid at the elevated temperature. The formation of secondary oxidation products, expressed by the anisidine value, also increased significantly at 60 C compared to storage at room temperature in the dark. The rate of color loss is acutely effected by temperature.

In oil without added antioxidants there is a loss from 5.55 color units to 1.68 units during 6 weeks of storage in the dark at 60 C, but only to 4.33 units in oil stored at room temperature. Only the addition of PG stabilized the oil stored at 60 C; after 4 weeks of storage the peroxide value reached 10.7 meq/kg, while in oil without added antioxidant the peroxide value increased to 93.1 meq/kg. In addition, PG stabilized the color and retarded the formation of secondary oxidation products in oils stored at 60 C.

Compared to PG, a weaker stabilizing effect was obtained by the addition of ethoxyquin. On the other hand, α-tocopherol had an opposite effect because the rate of oxidation of the oil was accelerated.

The values appearing in Table 3 show the effect light has on the oil oxidation processes. The formation of peroxides in oils without added antioxidants exposed to daylight is higher than in oils stored in the dark at

TABLE 2

Peroxide and Anisidine Values and Color Units of Refined, Bleached Avocado Oil Containing Antioxidants and Stored in the Dark at Room Temperature.

Storage in the dark (days)	Peroxide and Anisidine Values and Color Units											
	Without antioxidants			Propyl Gallate			α-Tocopherol			Ethoxyquin		
	PV[a]	AV[a]	CU[a]	PV	AV	CU	PV	AV	CU	PV	AV	CU
0	4.4	6.0	5.55	4.4	6.0	5.55	4.4	6.0	5.55	4.4	6.0	5.55
14	5.4	6.1	4.73	4.7	6.0	5.38	5.4	6.0	4.81	5.8	6.1	4.98
28	6.3	6.1	4.53	4.8	6.0	5.21	7.7	6.1	4.58	7.5	6.1	4.63
42	8.7	6.3	4.33	4.8	6.1	5.07	8.3	6.3	4.47	9.6	6.2	4.56
56	10.0	6.2	4.30	5.1	6.1	4.87	10.5	6.3	4.29	10.2	6.3	4.37
70	11.9	6.4	4.28	5.2	6.1	4.73	13.6	6.6	4.27	12.4	6.5	4.21
87	12.9	6.6	4.23	5.8	6.0	4.75	14.4	6.5	4.18	13.6	6.6	4.18
98	13.2	6.6	4.15	6.5	6.1	4.70	16.0	6.7	4.08	14.4	6.6	4.16

[a]PV, peroxide values, AV, anisidine values; and CU, color units.

TABLE 3

Peroxide and Anisidine Values and Color Units of Refined, Bleached Avocado Oil Containing Antioxidants "On the Shelf" at Room Temperature

"On the shelf" (days)	Peroxide and Anisidine Values and Color Units											
	Without antioxidants			Propyl Gallate			α-Tocopherol			Ethoxyquin		
	PV[a]	AV[a]	CU[a]	PV	AV	CU	PV	AV	CU	PV	AV	CU
0	4.4	6.0	5.55	4.4	6.0	5.55	4.4	6.0	5.55	4.4	6.0	5.55[b]
7	13.5	6.2	4.20	12.3	6.1	4.15	11.0	6.2	4.33	10.1	6.2	
14	20.8	6.5	3.35	23.2	6.3	3.28	19.5	6.4	3.48	19.4	6.1	
21	29.7	6.4	3.30	31.5	6.2	3.21	29.6	6.3	3.26	33.5	6.6	
28	38.5	6.8	3.05	40.7	6.5	3.07	34.8	6.6	3.04	41.5	6.5	
35	43.5	7.0	2.84	48.2	6.4	2.90	44.2	6.7	2.98	56.1	6.8	
42	51.3	7.3	2.68	58.5	6.7	2.54	49.2	6.9	2.63	61.3	6.9	
49	56.5	7.2	2.39	66.5	6.9	2.26	56.9	7.1	2.45	76.8	7.0	
56	62.8	7.3	2.30	79.2	7.1	2.17	62.6	7.3	2.27	68.2	7.3	
63	77.0	7.7	2.21	86.3	7.2	7.09	76.3	7.6	2.15	86.6	7.8	
70	80.4	8.0	2.16	91.1	7.6	2.08	83.5	7.9	2.12	93.3	7.9	

[a]PV, peroxide value; AV, anisidine value, and CV, color units.

[b]Oil samples containing ethoxyquin were brownish-yellow colored after a week of exposure to daylight.

TABLE 4

Peroxide and Anisidine Values and Color Units of Refined, Bleached Avocado Oil Containing Antioxidants and Stored in the Dark at 60 C

Storage at 60 C (days)	Peroxide and Anisidine Values and Color Units											
	Without antioxidants			Propyl Gallate			α-Tocopherol			Ethoxyquin		
	PV[a]	AV[a]	CU[a]	PV	AV	CU	PV	AV	CU	PV	AV	CU
0	4.4	6.0	5.55	4.4	6.0	5.55	4.4	6.0	5.55	4.4	6.0	5.55
3	7.8	6.3	5.23	5.0	6.0	5.29	12.7	6.4	4.99	7.6	6.3	4.83
7	16.6	6.7	4.83	5.7	6.1	4.98	25.4	6.9	3.87	15.5	6.7	4.78
10	19.6	7.1	4.53	6.0	6.1	4.71	48.3	9.7	2.93	19.7	7.2	4.49
14	26.3	7.8	4.37	6.7	6.2	4.53	99.8	15.6	2.66	30.7	7.9	4.03
17	48.7	10.1	3.54	7.5	6.2	4.41	120.9	27.8	2.12	39.0	8.8	3.80
21	69.6	14.3	2.91	9.6	6.2	4.30	149.3	41.3	1.82	47.4	9.5	3.63
28	93.1	23.4	2.33	10.7	6.3	4.27	164.1	46.8	1.54	63.0	11.2	3.41
31	139.8	35.7	1.95	13.4	6.4	4.19	153.1	61.7	1.12	90.1	16.3	3.06
35	147.6	42.9	1.84	13.8	6.5	4.15	124.5	70.15	1.10	94.7	18.7	2.94
38	153.0	53.5	1.73	14.3	6.8	4.10	120.4	79.9	1.09	109.3	24.5	2.68
42	160.2	65.8	1.68	15.0	7.0	4.07	119.9	90.2	1.07	122.0	30.8	2.48

[a]PV, peroxide value; AV, anisidine value, and CU, color units.

room temperature. After 10 weeks of storage, the oils stored in the dark had a peroxide value of 11.9 meq/kg, while the oils exposed to daylight had a peroxide value of 80.4 meq/kg.

None of the tested antioxidants stabilized the oils under these conditions. Light especially affected primary oxidation and the oil color, while its effect on secondary oxidation is less compared to the effect temperature had. In all samples, the formation of peroxides and color deterioration were rapid, while anisidine values were lower than those obtained in storage at 60 C. Similar results for light catalyzed oxidation have been reported by other workers (13,14). Carlsson (14) reported that the photo-oxidation of unsaturated oils, for example soybean, corn, peanut and olive oils, is not prevented by known free radical scavengers, but is retarded by chelates which quench singlet oxygen. During photo-oxidation, the natural antioxidant, α-tocopherol, exposed to light undergoes rapid peroxidation and thus has no effect on inhibiting oil oxidation (14). Oil samples containing ethoxyquin when exposed to daylight developed a brownish-yellow color during the first days of storage. This is a known phenomenon when amine antioxidants are used and is one of the reasons why the use of amine antioxidants is not widespread in the food industry (8).

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♣Oxidative Stability of Avocado Oil

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This study is concerned with the extent of oxidative deterioration and oil stability as determined by measuring peroxide and conjugable oxidation products (COP) values and AOM time of refined bleached avocado oil in comparison with refined soybean and olive oil. The formation of peroxides in avocado oil exposed to daylight at room temperature is similar to that of soybean oil but greater than that of olive oil. No differences were found in peroxide formation, oxodiene values and COP values between the tested oil stored in the dark, at 60 C and at room temperature. The COP ratio in oils stored at 60 C is similar for avocado and olive oil, but differs from that of soybean oil.

The AOM stability time both for refined avocado and soybean oil was approximately 14 hr, and for refined olive oil was 15 hr.

The extent of oxidative stability of crude avocado oil was determined by measuring peroxide value compared with crude olive oil. Crude avocado oil is very sensitive to oxidation when exposed to daylight and fluorescent light, in contrast to its stability in the dark at room temperature. The chlorophyll content in crude avocado oil is reduced rapidly on exposure to daylight and fluorescent light.

The large oil content, 15–30%, is one of the distinguishing features of the avocado fruit. In fact, of all fruits, only the olive and palm fruit can rival the avocado in oil content. The oil is unsaturated and the predominant fatty acid is oleic. Mazliak (1) reported that the edible avocado oil contained 13–16.7% palmitic acid, 3–5.1% palmitoleic acid, 67-72% oleic acid, 10.4–12% linoleic acid and traces to 1.5% linolenic acid.

Large surpluses of avocado are expected in the near future. One way of utilizing these surpluses is by extracting the oil from the fruit. Crude avocado oil is utilized mainly in the cosmetic industry. Refined avocado oil has only recently been introduced in the food industry and the world food market. Thus, there are few reports, if any, dealing with the oxidative stability of avocado oil.

The oxidative deterioration of edible oils and fats is a complex process leading to varied decomposition products (2). These oxidative processes, which occur slowly at normal ambient temperature, are known as autoxidation. Several mechanisms are possible, yet it is known that the oxidation process is initiated by the formation of radicals as a result of homolytic splitting-off of hydrogen atoms in the α-position with respect to the double bond (3). For this reason oils and fats containing unsaturated fatty acids are susceptible to oxidation. Because crude avocado oil contains small amounts of natural antioxidants (4-5) and large amounts of chlorophyll, the rate of its photo-oxidation is greater than that of other oils.

The susceptibility of an oil or fat to autoxidative degeneration can be assessed in terms of oxidative stability. The quality control of oils and fats, in the food industry, can be carried out by either static or dynamic methods. In the static methods, analytical determinations are made of various characteristics (such as peroxide value and the COP assay) relating to the degree of oxidation which already has taken place. In the dynamic methods, the oil is subjected to a stream of air at elevated temperatures. This method is the AOM stability test. Autoxidation can be inhibited by natural or synthetic antioxidants, whose effectiveness may be enhanced still further by synergistic agents such as ascorbic and citric acids (6).

In this work, oxidative stability of avocado oil was determined in comparison with olive and soybean oil, at several oxidative conditions.

EXPERIMENTAL PROCEDURES

Materials. Refined and crude avocado oil was obtained from Avochem (Santa Paula, California), refined olive