might have stimulated carotenogenesis.

Alternately, the secondary inducer might form outside and then translocate into the fruit. Visual observation of halved treated fruit supports this hypothesis. Orange streaks of color emanated from that portion of the albedo just beneath the stem and radiated out through the central core of fruit toward the endocarp. The peel of preharvest treated fruit had red blotches about the stem and along the bottom of the fruit which indicated that lycopene had accumulated in these areas.

The failure of lycopene to accumulate in the endocarp might indicate that the carotenogenic sequence of the endocarp is substantially different from that of other carotenogenic systems.

CONCLUSIONS

Carotenogenesis was induced in orange endocarp by the three synthetic bioregulators tested. Lycopene accumulation was less than normally expected for a postharvest treatment and was not detected on a preharvest basis.

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Relationship between Polyphenols and Browning in Avocado Mesocarp. Comparison between the Fuerte and Lerman Cultivars

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When avocado fruits are cut and exposed to air, the rate of browning of cv. Fuerte is much higher than that of cv. Lerman. A positive correlation was found between the tendency of the fruit to turn brown, total phenols content and polyphenol oxidase (PPO) activity, but not with *o*-dihydroxyphenols (ODHP) content. Reasonable separation of the extracted phenols was achieved by TLC chromatography. Of the large number of spots revealed, leucoanthocyanidine, catechin, and simple phenols (specifically caffeic acid) were identified. Other spots were only partially characterized. Similar phenols were identified in the mesocarp extract of both avocado cultivars. Approximation was made of the endogenous level of ODHP that can potentially be oxidized by avocado PPO. Attempts to identify, in the avocado phenol extract, ODHP which can be specifically oxidized by avocado PPO were unsuccessful, although the sensitivity of the technique was within the estimated range of detection.

We have previously reported that there are pronounced differences in the rate of browning of freshly cut mature ripe fruits of Fuerte and Lerman avocado cultivars (Kahn, 1975; Golan et al., 1977). We also showed that the postharvest behavior of fruits of the two cultivars was the same with regard to ethylene production, respiration, and softening, but that the rate of browning of Fuerte was much higher than that of Lerman throughout the ripening period (Golan et al., 1977).

The browning potential of various fruits, and sometimes in different varieties of the same cultivar, has been shown to be directly related to the phenol level, the polyphenol oxidase (PPO) activity, or a combination of these factors (Mondy et al., 1960; Mapson et al., 1963; Ranadive and Haard, 1971; Chung and Luh, 1972; Mathews and Parpia, 1975). Chubey and Nylund (1969) have demonstrated that the level of total phenols in carrot was more closely associated with the potential browning than was the level of oxidizable *o*-dihydroxyphenols (ODHP). Harel et al. (1970) have shown that both PPO activity and phenol content may determine the rate of browning of apples but that the relative importance of these factors changes at different physiological stages of the fruit. The rate of browning is usually higher in young than in more mature fruit and decreases as the fruit ripens. Likewise, the level of phenolic compounds may change as the fruit matures (Van Buren, 1970; Maier and Metzler, 1962; Ranadive and Haard, 1971).

It has already been shown in our laboratory that the PPO level in commercially mature Fuerte avocado is much higher than that in Lerman (Kahn, 1975). It was interesting to extend this comparison further and to establish whether PPO activity was the sole factor contributing to

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the difference in browning of these two cultivars, or if the level of total phenols and ODHP contributed to these differences.

MATERIALS AND METHODS

Avocado fruits (*Persea americana* Mill.) of two cultivars, Fuerte and Lerman, were obtained from The Volcani Center's orchard and from M. Lerman of Gan Hayyim, Israel, respectively. Uniform mature fruits of each cultivar (about 150 fruits) were hand picked from a single tree in order to minimize variability. The fruits were stored at 20 ± 1 °C. The percentage of oil was determined by the method of Gazit and Spodheim (1970), and the value was used as an approximate index of maturity.

Browning potential was estimated by measuring the change in tristimulus color parameters during 210 min of exposure to air at 20 °C (Golan et al., 1977).

Extraction of Polyphenols. *Preparation of Acetone Powder.* The flesh of the peeled and deseeded avocado was blended for 2 min with cold acetone (−20 °C) in a 1:3 (w/v) ratio. The mixture was filtered through Whatman No. 1 filter paper and the filtrate (F) was saved for phenol analysis (see below). The pellet was reextracted with acetone (−20 °C) three times, yielding a white powder. The white powder was dried in the hood at room temperature for 20 h, pulverized, and stored at 3 °C for the extraction of PPO, catalase, and peroxidase as described below.

Acetone was evaporated (at 36 °C, in vacuo) from filtrate F (see above), and the remaining aqueous fraction was centrifuged (4 °C) at 10 000*g* and any precipitate present was discarded. Lipid material was extracted from the aqueous residue to two volumes of chloroform (1:2). The delipidated aqueous phase was extracted several times into petroleum ether (60–80 °C) until a clear organic phase was obtained. The opaque aqueous phase was clarified by saturation with NaCl, followed by filtration through glass wool. The clear filtrate was designated fraction P.

A portion of fraction P (containing the polyphenols) was extracted with ethyl acetate (in a ratio of 3:1) five times. The ethyl acetate extracts were pooled (designated ethyl acetate fraction), dried over Na_2SO_4, filtrated, and reduced in volume in vacuo to 3 mL; the concentrated ethyl acetate extract was evaporated to dryness with nitrogen and stored at −20 °C. The samples were dissolved in 2 mL of methanol for TLC chromatography.

Separation and Characterization. Plates of silica gel G (approximate thickness, 0.25 mm) were used. Samples of avocado extract in methanol were spotted on the plates and developed in closed lined jars at 24 °C. Polyphenols were usually separated by one-dimensional TLC using several solvent systems (see Table III). Two-dimensional chromatography, using solvent no. 6 in the first direction and solvent no. 5 in the second direction, was also employed.

The developed plates were dried, and spots were located by their absorption or fluorescence under UV light (254 and 366 nm). The plates were sprayed with specific reagents as listed in Table IV. Plates were also sprayed with a partially purified avocado PPO in 0.05 M phosphate buffer, pH 6.5. As previously shown (Kahn, 1976b), avocado PPO can act on 4-methylcatechol, dopamine, catechol, chlorogenic acid, DL-Dopa, caffeic acid, catechin, and pyrogallol, but cannot act on phloroglucinol, protocatechuic acid, resorcinol, orcinol, *p*-cresol, gallic acid, guiacol, ferulic acid, or *p*-coumaric acid.

Samples of the following phenols (without prior purification) were chromatographed, singly or as a mixture, concurrently with the avocado extract, and served as markers: *trans*-cinnamic acid, protocatechuic acid, *p*-coumaric acid, ferulic acid, chlorogenic acid, caffeic acid, L-Dopa, quercetin, dopamine, tyramine, serotonin (purchased from K & K), and 4-methylcatechol (Fluka).

The reliability of the extraction of ODHP from avocado mesocarp adopted in this work was tested as follows: avocado fruit was cut longitudinally into two approximately equal halves and a mixture of ODHP consisting of 4-methylcatechol, caffeic acid, chlorogenic acid, and DL-catechin (20 mg of each) was added to only one-half of the fruit. Total phenols were extracted and purified from each half-fruit as described above. Aliquots were chromatographed by TLC along with the untreated ODHP. The tests showed that a good recovery of the exogenously added ODHP was achieved and that ODHP retain their original structure throughout the various stages of extraction and purification employed in this study.

Quantitative Estimation of Polyphenols. Total phenolic content was determined with Folin-Ciocalteau reagent essentially as described by Andersen and Todd (1968). A 20-mL sample of fraction P (see above) was adjusted to pH 3.5 with acetic acid and divided into two equal parts (A, B); 250 mg of purified Polyclar AT (high molecular weight, insoluble, cross-linked polyvinylpyrrolidone) was added to sample A. Samples A and B were then treated in parallel: each sample was shaken for 30 min at 20 °C, centrifuged at 3000 rpm for 20 min, and the supernatant was saved. Aliquots of the supernatant were mixed with 5 mL of water and 1 mL of freshly diluted 1 N Folin-Ciocalteau reagent. After 3 min, 1 mL of saturated Na_2CO_3 solution was added and the absorbance at 725 nm was read 3 h later. Chlorogenic acid served as the standard phenol.

A plot of absorbance vs. chlorogenic acid concentration (up to 90 μg/9 mL total volume) in the absence (B) and presence (A) of Polyclar AT yielded two straight lines; the difference in absorbance of lines B − A yielded a linear standard curve. Color formation between the Folin-Ciocalteau reagent and amino acids and other interfering material in the extract was thus excluded (Andersen and Sowers, 1968).

To test the reliability of this procedure, known amounts of chlorogenic acid were added to aliquots of fraction P. Determination of the total phenol in aliquots of the resultant mixture compared with appropriate controls indicated a 90% recovery of exogenously added chlorogenic acid.

Orthodihydroxyphenols (ODHP). ODHP was measured essentially as described by Mapson et al. (1963). In a total volume of 11 mL, the assay mixture included: a sample of fraction P (up to 5 mL), 1 mL of 0.2 M sodium phosphate buffer (pH 7.3), 4 mL of water, and 1 mL of solution of 5% sodium molybdate. The content was mixed, and the absorbance at 350 nm was read after 15 min against an appropriate control. Caffeic acid served as a standard phenol.

Enzymatic Activity. The flesh of the deseeded fruit was homogenized for 2 min with 0.1 M sodium phosphate buffer, pH 6.5 (1:5 w/v) in a blender. The slurry was filtered through four layers of cheesecloth, centrifuged for 15 min at 3000 rpm, and the supernatant was used immediately for PPO, peroxidase, and catalase measurements.

Assays of PPO, Peroxidase, and Catalase. PPO was assayed in a reaction mixture that consisted of 5 mL of freshly mixed 0.1 M sodium phosphate buffer (pH 6.5) and 5 mL of freshly prepared 0.02 M 4-methylcatechol. Peroxidase was assayed in a reaction mixture that contained 10 mL of 0.05 M sodium phosphate buffer (pH 6.5),

Table I. Total Phenols, ODHP and Enzymatic Activity in Fuerte and Lerman Avocado Mesocarp[a,e]

Parameter	Cultivar			
	Fuerte		Lerman	
	Mean	SD	Mean	SD
Weight, g	191.1	28.5	515.3	92.4
Fat, %	7.6	0.5	15.6	1.5
Browning potential, ΔL[b]	16.3	4.0	2.9	1.0
Total phenols,[c] mg/100 g f. wt.	28.7	3.0	2.8	0.8
ODHP,[d] mg/100 g fresh wt	1.4	0.4	1.6	1.1
Total PPO activity, units/g fresh wt	13.7	6.2	1.3	0.6
Specific activity				
PPO	0.82		0.08	
Peroxidase	0.08		0.06	
Catalase	1.4		1.4	

[a] Fuerte and Lerman fruits were harvested on Oct 13 and Dec 8, 1974, respectively. Enzymatic activities and protein content were measured in freshly prepared homogenates. Protein content in the homogenate of Fuerte and Lerman differed by ± 3%. Total phenols and ODHP were determined in fraction P. [b] Browning potential (ΔL) was determined at the proximal and distal ends of 35 fruits, during 7 days of storage, in each cultivar. For further details, see the Methods section. [c] Calculated as chlorogenic acid equivalent. [d] Calculated as caffeic acid equivalent. [e] Enzymatic activity and phenol content were measured on six fruits of each cultivar.

Table II. Total Phenols, ODHP, PPO Activity, and Browning Potential in the Proximal and Distal Ends of Fuerte Mesocarp before and after Ripening[a]

Days in storage (20 ± 2 °C)	Fruit end	Total phenols,[b] mg (100 g fresh wt)		ODHP,[c] mg (100 g fresh wt)		PPO sp act.		Rel browning potential
		Mean	SD	Mean	SD	Mean	SD	
0	Proximal	7.0	1.0	1.0	0.0	0.67	0.23	Low
	Distal	12.6	1.7	1.0	0.1	1.16	0.35	High
7	Proximal	5.4	3.3	0.6	0.3	0.30	0.12	Low
	Distal	13.4	3.3	1.3	0.6	0.80	0.22	High

[a] The fruits were harvested on March 6, 1975. Data are means of three fruits of approximately the same size. The mean weight of the fruits was 232 ± 42 g and the fat content was 19.5 ± 2%. Total phenols and ODHP were measured in fraction P. PPO activity was determined in freshly prepared homogenate. For details, see the Methods section. [b] Calculated as chlorogenic acid equivalent. [c] Calculated as caffeic acid equivalent.

1 mL of 0.5% guaiacol (in 50% ethanol), and 1 mL of 0.3% H_2O_2. Catalase was assayed in a total volume of 3 mL that included 1 mL of 7×10^{-3} M H_2O_2 and 2 mL of 0.05 M sodium phosphate buffer (pH 6.5).

Each assay was initiated by the addition of aliquots of the avocado supernatant. Absorbance was measured at 410 and 470 nm (for PPO and peroxidase, respectively) using a Gilford Model N-300 spectrophotometer; the activity of catalase was recorded at 240 nm in a Varian Model 635-D spectrophotometer. Reaction velocity was computed from the initial linear slopes of the curves obtained.

Activity is expressed as absorbance (at the specified wavelength) per minute and specific activity as activity/mg of protein.

Protein was determined by the method of Lowry et al. (1951).

Enzymatic Oxidation of Commercial and Avocado-Extracted Phenols. In a total volume of 3 mL, the reaction mixture included: 1.5 mL of 0.1 M sodium phosphate buffer (pH 6.5) and 1.4 mL of solution containing either commercial phenols or avocado phenolic extract (fraction P).

The reaction was initiated by the addition of 0.1 mL of partially purified PPO [40–75% $(NH_4)_2SO_4$ fraction] from Fuerte which was prepared as described previously (Kahn, 1976a). The spectrum changes during incubation of these reaction mixtures at room temperatures were recorded automatically in a Varian Model 635-D spectrophotometer.

RESULTS

Total Phenols, ODHP, and Enzymatic Activity. The extraction of avocado mesocarp with acetone (first step) extracts mainly polar phenolic glycosides and aglycones (the phenolic nonsugar moiety of such glycosides) (Egger, 1969). The chloroform and petroleum ether purification steps extract nonpolar compounds, especially lipids, and usually extract very little phenolic compounds (Seikel, 1964). The final purification step (with ethyl acetate) extracts mostly phenols of low polarity (Singleton and Esau, 1969). It is likely that phenolic glycosides of high polarity that were present in the initial acetone extract are only partially extracted into the final ethyl acetate fraction.

Table I shows that the total phenol content/gram fresh weight is significantly higher in Fuerte than in Lerman, while ODHP content and proteins were comparable in fruits of both cultivars. Table I also demonstrates that the total and specific activity of PPO were much higher in a homogenate of mature ripe Fuerte avocado than of Lerman, as expected from our studies with acetone powder extracts (Kahn, 1975). The specific activity of peroxidase or catalase, on the other hand, was about the same in both cultivars. The mean value of browning potential of Fuerte avocado fruit was higher than that of Lerman.

In the course of this study, large differences were observed in the potential browning of the proximal and distal ends of either Lerman or Fuerte fruits. Table II demonstrates that total phenol content and the specific activity of PPO were higher in the distal end of the fruits before and after ripening, while ODHP level was approximately the same in the two ends of the fruit. After ripening, however, there was a relatively higher ODHP and total phenol content in the distal end compared with the proximal end, a trend which is not very significant. The specific activity of PPO decreased by approximately 50% during 7 days of ripening.

Thin-Layer Chromatography of the Ethyl Acetate

Table III. R_f Values on TLC of Different Phenols in Six Solvent Systems

No.	Compound	R_f values: Solvent system[a] 1	2	3	4	5	6
1	*trans*-Cinnamic acid	Nt	0.91	Nt	0.62	0.80	Nt
2	4-Methylcatechol	0.79	0.92	Nt	0.53	0.56	Nt
3	Protocatechuic acid	0.77	0.85	Nt	0.43	0.70	Nt
4	*p*-Coumaric acid	0.78	0.86	0.94	0.52	0.71	0.00
5	DL-Catechin	0.83	0.65	Nt	0.22	0.26	0.02
6	L-Epicatechin	0.83	0.68	Nt	0.18	0.32	0.02
7	Ferulic acid	0.77	0.87	Nt	0.53	0.75	Nt
8	Chlorogenic acid	0.47	0.20	0.62	0.06	0.08	0.00
9	Caffeic acid	0.76	0.88	0.92	0.45	0.73	0.00
10	L-Dopa	Nt	0.78	Nt	0.00	0.00	0.00
11	Dopamine	Nt	0.14	Nt	0.03	0.03	Nt
12	Quercetin	0.8	0.89	Nt	0.43	0.69	Nt
13	Tyramine	Nt	Nt	Nt	0.00	0.00	0.00
14	Serotonine	Nt	Nt	Nt	0.00	0.00	0.00
15	Tryptamine	Nt	Nt	Nt	0.02	0.00	0.00

[a] All solvent systems were prepared by volume ratios (v/v/v): 1, Seikel, 1964; 2, Mizelle et al., 1965; 3-6, Stahl and and Schorn, 1961. Nt = not tested. 1, 1-butanol-acetic acid-water 6:1:2; 2, benzene-ethyl acetate-formic acid-water 9:21:6:5; 3, ethyl acetate-methyl ethyl ketone-formic acid-water 5:3:1:1; 4, toluene-ethyl formate-formic acid 5:4:1; 5, chloroform-ethyl acetate-formic acid 5:4:1; 6, toluene-chloroform-acetone 9:2.5:3.5.

Table IV. Color of Different Phenols Developed on TLC (Silica Gel G) with Different Color Reagents and PPO Enzyme[a]

No.	Compound	UV 254	UV 366	Color developed: Color reagent[b] A	B	C	D	E	F	G	PPO Fuerte 40-75% $(NH_4)_2SO_4$
1	*trans*-Cinnamic acid	+	–	bl	–	–	–	–	–	–	–
2	4-Methylcatechol	+	–	bl	pr	da.b	–	–	–	–	l.b
3	Protocatechuic acid	+	–	bl	pr	b	–	–	–	b	l.y
4	*p*-Coumaric acid	+	+	l.bl	v.b	y	–	–	–	b	–
5	DL-Catechin	+	–	bl	pr	l.g	m.m	e	r	m.m	y
6	L-Epicatechin	+	–	bl	pr	l.b	m.m	e	r	m.m	l.y
7	Ferulic acid	+	+	bl	m.m	e	–	–			–
8	Chlorogenic acid	+	+	bl	m.m	s.y	v	–			–
9	Caffeic acid	+	+	bl	l.v	d.b	v	–		b	l.o
10	L-Dopa	–	–	bl	pr	da.b	–	–		a.y	
11	Dopamine	+	+	bl	pr	m.m					
12	Quercetin	+	+	bl	pr	da.b					
13	Tyramine	–	+	bl	bb	y					
14	Serotinine	+	+	bl	bb	da.b					
15	Tryptamine	–	+	bl	bb	y					

[a] Blank spaces, not tested; (–) not detected; (+) different shades of blue visible under UV light. [b] Key for color reagents: A = $FeCl_3/K_3Fe(CN)_6$ [Barton et al. (1952)]; B = 4-toluenesulfuric acid (4TS) [Roux (1957)]; C = 4-nitroaniline, diazotized (DNPA) [Van Sumer et al. (1965)]; D = vanillin-H_2SO_4 [LeRosen et al. (1952)]; E = flavon-3,4-diols [Roux and Maihs (1960)]; F = catechin reagent [Tirimanna and Perera (1971)]; G = bisdiazotized benzidine [Roux and Maihs (1960)]. For key to color developed, see Table V.

Extract. Ramirez-Martinez and Luh (1973) detected 15 phenolic compounds in frozen Fuerte avocado. Of these, the level of epicatechin, *p*-coumaric acid, and isoflavone was relatively high, that of caffeic acid, chlorogenic acid, leucoanthocyanidine, and *p*-coumarylquinic acid intermediate, and that of catechin and *cis*-caffeic acid low. The behavior of some of the phenols reported to be present in avocado (Udenfriend et al., 1959; Ramirez-Martinez and Luh, 1973), and those that might potentially be oxidized by avocado PPO, was studied by TLC (silica gel) in six different solvent systems; the results are summarized in Tables III and IV. The R_f values are listed in Table III and the staining specificity with various stains and with partially purified avocado PPO is shown in Table IV.

Similar analyses were performed with each of the six solvent systems. The most satisfactory analyses were obtained with solvents 4, 5, and 6 of Table III, and these data are presented in Tables V, VI, and VII.

Qualitatively, most of the spots revealed in each solvent system were detected in extracts of both cultivars, with very few exceptions (see last two columns in Tables V–VII). The spots revealed in the Fuerte extract were more intense than those in the Lerman extract.

Chromatograms were sprayed with different reagents and the following results were obtained: $FeCl_3/K_3Fe(CN)_6$ (reagent A) yielded a blue color with most phenols; DNPA (reagent C), which gives different shades of brownish-yellow color with phenolic acids, yielded faint yellow spots with 12 avocado phenols. Bisdiazotized benzidine (reagent G), considered an efficient and selective phenolic reagent (Roux and Maihs, 1960), yielded very light brown spots. Catechin reagent (reagent F), flavan-3,4-diols (reagent E), 4-toluenesulfuric acid (4TS) (reagent B), and 1% vanillin–H_2SO_4 (reagent D) yielded an intense color which aided in a better identification of the flavonoids.

Flavan-3,4 diols (reagent E) can differentiate between flavonoids with a phloroglucinol-type-A ring (like catechin and leucoanthocyanidine) that give a violet-red color and those with a resorcinol-type-A ring that give a pink color (Roux and Maihs, 1960). Tables V–VII demonstrate that most of the flavonoids in avocado are of the former group. This is supported by the findings with reagent 4TS

Table V. TLC of Fuerte and Lerman Ethyl Acetate Extract in Solvent System 4[a]

Spot no.	R_f	Visible light	UV 254	UV 366	Color developed: Color reagent B	C	D	E	F	Presence in fruit: Fuerte	Lerman
1	0.70	–[b]	–	l.bl	l.g	ar	–	l.v	–	+	+
2	0.62	l.y	–	r	–	–	g	d.bl	s.y	–	+
3	0.58	–	–	–	l.b	–	–	l.b	–	+	+
4	0.53	–	–	–	y	y	g	–	s.y	+	+
5	0.49	–	–	–	m.m	–	b	v.b	o	–	+
6	0.47	l.g	–	l.b	r.v	d.b	o.b	–	–	+	+
7	0.40	l.y	–	l.b	r.v	–	da.b	v.b	o	+	+
8	0.35	l.g	–	r	v	d.b	b	pr	o	+	+
9	0.28	l.g	–	–	e	–	d.gr	–	–	+	+
10	0.25	–	–	–	e	d.b	ar	d.g	l.y	+	+
11	0.21	–	–	l.r	l.g	m.b	d.v	d.v	–	+	+
12	0.15	p	f.bb	f.bl	f.bl	–	d	d.v	l.o	+	+
13	0.10	–	–	–	–	b	–	o.b	l.o	+	+
14	0.05	–	–	–	–	b	p.b	o.b	l.o	+	+
15	0.00	l.y	–	–	d.b	d.b	d.gr	o.b	l.o	+	+

[a] For key to color reagents, see Table IV. Key to color notations: yellow, y; light yellow, l.y; green, g; light green, l.g; dull green, d.g; brown, b; dark brown, d.b; light brown, l.b; dull brown, d.b; mustard brown, m.b; blue, bl; fluorescent blue, f.bl; light blue, l.bl; dull blue, d.bl; baby blue, bb; dull, d; fluorescent baby blue, f.bb; antique red, ar; red violet, r.v; oak buff, o.b; violet, v; prune, pr; red, r; dull violet, d.v; violet black, v.b; light violet, l.v; dull gray, d.gr; orange, o; light orange, l.o; saffron yellow, sy; musk melon, m.m; ember, e; inca gold, i.g; pink, p. [b] (–) not detected.

Table VI. TLC of Fuerte and Lerman Ethyl Acetate Extract in Solvent System 5[a]

Spot no.	R_f	Visible light	UV 254	UV 366	Color developed: Color reagent B	C	D	E	F	Presence in fruit: Fuerte	Lerman
1	0.83	–[b]	–	–	–	–	–	–	–	+	+
2	0.78	–	–	–	bb	ar	bl	d	–	+	+
3	0.73	l.y	–	f.bb	–	–	–	d.b	–	–	+
4	0.68	–	–	l.b	v.b	–	b	pr	l.o	–	+
5	0.62	–	–	–	r.v	y	–	–	–	+	+
6	0.59	l.g	–	–	v.b	ar	da.b	d.bl	l.o	+	+
7	0.54	–	–	–	p	l.y	–	–	–	+	+
8	0.52	–	–	–	–	i.g	–	–	–	+	–
9	0.49	–	–	–	e	ar	b	v.b	l.o	+	+
10	0.44	l.g	–	r	p	–	bb	–	l.o	+	+
11	0.40	–	–	–	–	–	v	l.v	–	–	+
12	0.35	–	–	–	–	l.b	v	–	–	+	–
13	0.32	–	–	–	–	b	l.g	bl	–	+	+
14	0.29	–	–	–	l.b	–	–	–	–	+	+
15	0.25	–	–	l.r	s.y	i.g	d.b	d.g	l.g	+	+
16	0.16	p	+	f.bl	y	–	v.b	d.b	s.y	+	+
17	0.10	–	+	f.bl	o	b	–	d.b	o	+	+
18	0.03	–	–	f.bb	d	b	b	d.b	s.y	+	+
19	0.00	l.b	–	–	–	i.g	d	d.b	–	+	+

[a] For key to color reagents, see Table IV. For key to colors developed on chromatograms, see Table V. [b] (–) not detected.

(reagent B), which yielded (at UV 366) shades of reddish-yellow color with most of the spots, suggesting the presence of leucoanthocyanidines containing the hydroxyl group at position 5 (spots 5, 9, 10, Table V; spots 7, 9, 10, 14, 15, Table VI; spots 7, 10, 12, 13, 14, 16, 17, 19, 20, Table VII). Spots which yielded a reddish-violet color with reagent B (at UV 366) suggest the presence of leucoanthocyanidines that lack the hydroxyl group at position 5 (spots 6, 7, Table V; spot 5, Table VI; spots 8, 15, Table VII). Spots stained blue-violet with reagent B (at UV 366) indicate the presence of catechin (spot 8, Table V; spots 4, 6, Table VI; spots 11, 18, Table VII). The presence of catechin was supported further by the fact that color developed in spots of similar R_f when the chromatograms were sprayed with reagent F, which is specific for catechins (Tirimanna and Perera, 1971), or with 1% vanillin–H_2SO_4.

Since there was considerable overlap of various compounds (see Tables V–VII), it was of interest to carry out the analysis by two-dimensional chromatography. Better separation was then achieved (21 spots revealed with reagent A) using toluene–chloroform–acetone (9:2.5:3.5 v/v/v; solvent 6) in the first direction and chloroform–ethyl acetate–formic acid (5:4:1 v/v/v; solvent 5) in the second direction. Again, it should be stressed that essentially the same phenolic compounds were detected in extracts of Fuerte and Lerman avocados.

Because of the relatively large number of spots revealed in the avocado extract and their tendency to overlap even in two-dimensional chromatography, it was difficult to identify ODHP in spite of the fact that they had been identified in frozen avocados (Ramirez-Martinez and Luh, 1973).

Attempts to reveal avocado ODHP by spraying the chromatogram with partially purified avocado PPO failed, although commercial ODHP (4-methylcatechol, protocatechuic acid, DL-catechin, L-epicatechin, caffeic acid) were detected as faint yellowish-brown spots (a minimum of 10 μg of ODHP was required) immediately after spraying (Table IV).

Effect of PPO on the Phenol Extract. Scanning a

Table VII. TLC of Fuerte and Lerman Ethyl Acetate Extract in Solvent System 6[a]

Spot no.	R_f	Visible light	UV 366	Color developed: Color reagent A	B	C	D	E	Presence in fruit: Fuerte	Lerman
1	0.79	f.bl	f.bl	l.b	–	–	–	–	+	+
2	0.75	g	m.m	–	l.b	–	d.v	–	+	+
3	0.71	v.b	–	bl	bb	–	–	d	+	+
4	0.69	f.bl	f.bl	bl	l.b	–	–	–	+	+
5	0.64	d	m.m	bl	–	ar	v	pr	+	+
6	0.51	l.g	p	bl	bb	–	–	–	+	–
7	0.54	–[b]	–	bl	s.y	–	–	–	+	+
8	0.48	–	–	–	r.v	y	r	v.b	+	+
9	0.44	–	–	–	–	–	m.m	bl	–	+
10	0.40	–	–	bl	m.m	d.b	–	pr	+	–
11	0.38	–	–	bl	v	–	m.m	l.bl	+	+
12	0.34	d	d	bl	s.y	–	pr	l.bl	+	+
13	0.29	–	–	–	pp	–	pr	l.bl	–	+
14	0.24	–	l.r	bl	s.y	y	e	d.v	+	+
15	0.19	–	–	bl	r.v	–	r.v	l.bl	+	+
16	0.14	–	l.v	bl	p	b	–	–	+	+
17	0.10	d	g	bl	s.y	–	pr	l.bl	+	+
18	0.05	–	–	–	r.v	–	–	–	+	+
19	0.03	–	r	bl	e	m.b	–	–	+	+
20	0.00	l.g	f.bb	bl	o	m.b	m.m	d.b	+	+

[a] For key to color reagents, see Table IV. For key to color developed on chromatograms, see Table V. [b] (–) not detected.

reaction mixture (from 330–500 nm) consisting of Fuerte or Lerman avocado phenol extracts (fraction P) and a partially purified avocado PPO at known intervals showed an increase in absorbance throughout the spectrum (read against the appropriate control), with a peak around 350–360 nm (Figure 1). The spectrum obtained when 4-methylcatechol, caffeic acid, or a combination thereof was incubated with PPO under similar conditions is shown for comparison (Figure 1, parts 1, 2, and 3, respectively). While the maximum absorption with these known ODHP was reached at the end of about 2 h of incubation, that of PPO and avocado phenol extract increased continuously for about 24 h, with black color finally being formed (data not shown). These findings indicate that fraction P constitutes a mixture of phenolic compounds that include some ODHP. The final color formed is probably a result of a reaction occurring nonenzymatically between oxidized ODHP and other phenolic compounds (Wong et al., 1971; Kahn, 1976b).

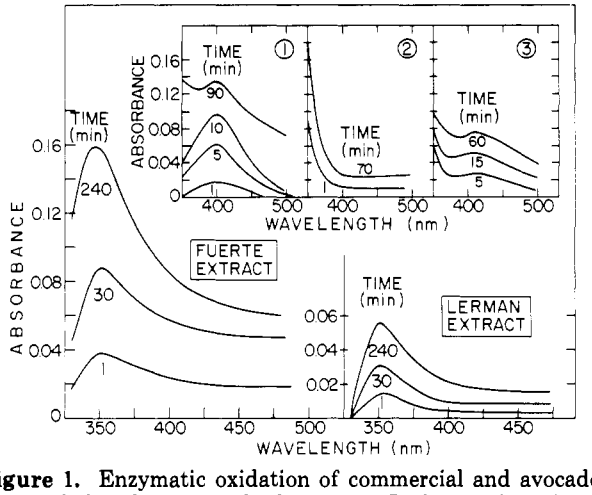

Figure 1. Enzymatic oxidation of commercial and avocado-extracted phenols: In a total volume of 3 mL, the reaction mixture included 1.4 mL of 0.1 M sodium phosphate buffer (pH 6.5), 15 mL of solution of either A [commercial phenols (60 μg of 4-methylcatechol; 87 μg of caffeic acid; or 30 μg of 4-methylcatechol and 43.5 μg of caffeic acid)] or B [phenolic extract (fraction P) from equivalent weights of Fuerte or Lerman avocado mesocarp]. The reaction was initiated by the addition of 0.1 mL of partially purified PPO [40–75% $(NH_4)_2SO_4$ fraction from Fuerte], containing 0.06 and 3.3 activity units, to samples in group A or B, respectively.

DISCUSSION

Our studies show that the high rate of browning of freshly cut Fuerte avocado fruits compared with that of Lerman was positively correlated with total phenol content and PPO activity but not with ODHP content or with the presence of any specific phenolic compound in one cultivar as opposed to the other. In avocado of either cultivar, the rate of browning of the distal end was higher than that of the proximal end (Table II); this, too, was positively correlated with total phenol content and PPO activity, while the level of ODHP was approximately the same in both ends of the fruit.

Various procedures have been employed for the extraction of phenols from plant tissues (Khanna et al., 1968; Egger, 1969; Wrolstad and Heatherbell, 1974). Of these, our choice of procedure was extraction with acetone, since it has the advantage that the extraction of the phenols is conducted at low temperature (–20 °C), thus largely eliminating oxidation of phenols. Following this step, several organic solvent extractions were performed as proposed by Ramirez-Martinez and Luh (1973). These authors have pointed out that the extraction of phenols from avocado mesocarp is particularly difficult due to the high lipid content of the tissue and because of apparently unavoidable losses of some phenols into the organic solvents used during the isolation and purification of the sample.

The phenolic content of ripe avocado fruit was found to be fairly low compared with that of most ripe fruits (Goldstein and Swain, 1963; Van Buren, 1970). This could be due to the fact that losses of phenols might have occurred during the analytical steps required to remove the lipids from the tissue. The low level detected is, however, in good agreement with the findings of Udenfriend et al. (1959), who detected in avocado similar low levels of dopamine, serotonin, and tyramine (5, 10, and 23 μg/g fresh weight, respectively) by a fluorometric assay.

Reasonable separation of the ethyl acetate extract was achieved by one- and two-dimensional TLC. Of the large number of spots revealed by various color reactions (Tables

V–VII), leucoanthocyanidines (flavan-3,4-diols), catechins (flavan-3-ols), simple phenols, and a very small amount of ODHP were detected. Similar phenolic compounds were present in the extract of both Lerman and Fuerte avocado mesocarp.

The capacity of specific naturally occurring phenols to turn brown can be demonstrated by chromatographing the extracted phenols and then spraying the chromatogram with a PPO preparation of the same tissue (Griffiths, 1957; Chubey and Nylund, 1969). Our failure to detect in avocado specific PPO-oxidizable components by the same technique is not yet understood. It is conceivable that no color developed because of a possible overlap of several compounds on the plate, a fact which could have prevented the activity of the enzyme. For example, we mentioned above that there was an overlap of simple phenols and flavonoids. The low level of ODHP in avocado (Tables I and II) raises the possibility that the level of each OHDP on the chromatogram was too low for detection with PPO. The possibility that in the course of extraction of the phenols from avocado mesocarp an interaction occurred between the ODHP and other phenols present can be excluded in view of our findings that exogenous ODHP, added to the mesocarp in the first step of extraction, did appear on the chromatogram subsequent to spraying with DNPA (reagent C).

The spectra obtained for the reaction mixtures of avocado PPO and phenolic extract (fraction P) of either Lerman or Fuerte are very similar in the visible light region (Figure 1). Both the rate of change in absorbance and the color obtained after 24 h of incubation were higher in Fuerte than in Lerman. This is compatible with our results (Table I) showing higher total phenols in Fuerte compared with Lerman, but similar ODHP level in the two cultivars. Makower and Schwimmer (1957) observed that the addition of Dopa to the surface of avocado slices had no effect on the rate of color formation or on the final color developed, indicating that the level of ODHP was not the rate-limiting step in the browning of avocado fruit. The spectra (Figure 1) indicate that ODHP, which can serve as substrates for the PPO enzyme, are present in the avocado flesh. The endogenous level of ODHP in the avocado flesh was found to be very low (Table II). This is compatible with the findings shown in Figure 1, where the action of PPO (0.06 activity units) on a mixture of 4-methylcatechol and caffeic acid (30 and 43.5 μg, respectively, in the final reaction mixture of 3 mL) resulted in an increase of 0.06 OD units after 30 min of incubation, while the action of PPO, that was 50-fold more active (3.3 activity units) on the phenolic extract from Fuerte avocado (fraction P) (in the same volume of reaction mixture), resulted, after the same time interval, in an increase of 0.09 OD units only.

It is known that various phenolic compounds that do not serve as a substrate for PPO can act as synergists or inhibitors of some PPO from plants (Robb et al., 1966; Johnson and Pratt, 1969; Wong et al., 1971; Pratt, 1972). Using avocado PPO, it has been specifically shown in our laboratory, for example, that *p*-coumaric acid, protocatechuic acid, and *p*-cresol arrested the development of dark-colored *o*-quinones produced from the action of PPO on 4-methylcatechol, while phloroglucinol, orcinol, and resorcinol markedly increased their formation (Kahn, 1976b). It can thus be suggested that ODHP do not determine the extent of browning; their contribution to browning is probably in the initial stages of the process, due to their oxidation to quinones. Other types of phenols, endogenously present in the avocado, might contribute to browning by their interaction and subsequent polymerization with these quinones.

It can therefore be concluded that both high PPO and total phenol content contribute to the high browning potential of the flesh of the Fuerte cultivar, compared with that of the Lerman.

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Amino Acid Composition of Red Pepper

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The amino acid composition of the Greek "sweet" and "hot" red pepper (powdered whole fruit) and that of the dehydrated pericarp, seeds, and stem of the "sweet" and "hot" red fruit of *Capsicum* was obtained by an automatic amino acid analyzer. Data not previously reported are presented. The amino acid composition of the seeds with respect to that of the pericarp and stem showed characteristic differences. No characteristic differences in amino acid composition between "sweet" and "hot" corresponding species were noticed. Comparison of the amino acid composition of the "sweet" pericarp with that of the pericarp of a similar Italian variety showed several differences, whereas the amino acid compositions of the seeds of this pepper and those of a pepper of American origin were approximately similar with a few exceptions. Three unidentified peaks eluted between cysteic acid and hydroxyproline (aspartic acid) with maximum absorbance at 440 nm are discussed.

In a previous paper (Tsatsaronis and Kehayoglou, 1964), the protein (N × 6.25) content of the Greek "sweet" (paprika) and "hot" red pepper (powdered whole fruit) as well as that of the dehydrated components (pericarp, seeds, stem) of the "sweet" and "hot" red fruit of *Capsicum* was reported among other analytical constants. In this paper the amino acid composition of these products was determined by an amino acid analyzer to obtain data not previously reported and to examine possible differences among the various parts of the fruit and between "sweet" and "hot" species. Knowledge of the amino acid composition of these products is also of interest in evaluating their nutritional value even though their contribution to the protein content of the diet is not at a high level. However, the use of their by-products (e.g., defatted seeds) as an animal feed might enhance their contribution to protein intake.

The amino acid composition of "sweet" and "hot" red pepper (powdered whole fruit) and that of the dehydrated stem with calyxes of the "sweet" and "hot" fruit of *Capsicum* is originally reported in this study, as well as some supplementary data for the amino acid composition of pericarp and seeds.

Previous studies of the amino acid composition of the fruit of *Capsicum* were only for the fresh pericarp of various Italian varieties and did not include hydroxyproline and tryptophan (Bottazzi et al., 1968), or were for seeds of *Capsicum frutescens* of American origin and did not include cystine and tryptophan (Van Etten et al., 1963), whereas the amino acid composition of Spanish paprika was partly determined by paper chromatography (Navarro et al., 1962).

The results of this study are also compared with those in literature.

EXPERIMENTAL SECTION

Samples. Several representative samples of the Greek "sweet" and "hot" red pepper (powdered whole fruit) and those of the dehydrated components (pericarp, seeds, stem with calyxes) of the "sweet" and "hot" red fruit of *Capsicum* were obtained from large quantities from the two main processing plants in Greece located in the Almopia region. The products were composites of typical varieties of Almopia, from the 1972 crop, and recently processed. The fruit components were ground to pass through a 30-mesh (0.5 mm) screen. The pericarps were dried at 60 °C for grinding.

Methods. The weight loss at 65 °C for 48 h was taken as the samples' moisture content to calculate the amino acid content on a dry weight basis.

Hydrolysis with Hydrochloric Acid. Hydrolysis was essentially as described by Tkachuk and Irvine (1969) and Robbins and Pomeranz (1972): 5 mL 6 N HCl was added to 40–50 mg of sample and heated at 110 ± 1 °C for 24 h in a forced-draft oven. The hydrolysate was filtered (Jamalian and Pellet, 1968) through a sintered glass disk and was concentrated to dryness. The residue was redissolved in water, concentrated to dryness three times, and then redissolved in a pH 2.2 citrate buffer (0.2 N Na^+), and the solution was made up to 25 mL.

Performate Oxidation. Samples (40 mg) were oxidized with performic acid according to the procedure of Moore (1963) and then hydrolyzed with 4 mL of 6 N HCl as reported above.

Alkaline Hydrolysis. Hydrolysis of a 100–mg sample with octahydrate barium hydroxide (1.5 g) and 1 mL of distilled water was carried out in an evacuated, sealed tube at 110 ± 1 °C in a forced-draft oven for 16 h. The cooled hydrolysate was neutralized (Miller, 1967) with 6 N HCl until colorless to phenolpthalein. The solution was transferred by washing with 10 mL of H_2O to a centrifuge tube, and barium ions were precipitated by a solution of 10% sodium sulfate. The tube was centrifuged, and the supernatant was decanted to a 25-mL volumetric flask. The residue was twice resuspended in 5 mL of water and then recentrifuged, and the washings were added to the flask and then made up to volume. The solutions were kept in a deep-freeze and used for determination of tryptophan as soon as possible.

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