changes in amounts of lipids present in different lipid fractions, destruction of unsaturated fatty acids and probable degradation of 16:0. The oxidative changes, however, did not increase TBA which decreased during storage.

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Polyphenols in Olive Oils

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ABSTRACT

The levels of total polyphenols and *o*-diphenols were determined in virgin oils and in chloroform/methanol-extracted oils. The solvent-extracted oils were richer in polyphenols than the virgin oils. High polyphenol content was associated with a high resistance to oxidation of the oils. A linear relationship was found between polyphenol content and the oxidative stability of the virgin oils during storage at 60 C. After removal of the polyphenols, the oxidative stability of the oils decreased considerably and seemed to depend on polyunsaturated fatty acid concentration.

INTRODUCTION

In contrast to other crude oils, virgin olive oil produced from olives of good quality is consumed unrefined. Thus, virgin olive oils contain polyphenols which are usually removed from other edible oils in the various refining stages (1,2). Olive oils are low in tocopherols (3); therefore, the presence of other phenolic constituents capable of antioxidant activity is of particular importance.

Several studies concerning the polyphenols in Italian and Spanish olive oils were published (2,4,5). Vazquez Roncero et al. (2) found that oils with high polyphenol content were of good quality. Among the polyphenolic compounds identified in olive oils were caffeic, vanillic, *p*-coumaric, syringic and *p*-hydroxy benzoic acids, 3-hydroxyphenyl-ethanol and 3,4-dihydroxyphenyl-ethanol. The 3,4-dihydroxyphenyl-ethanol seems to be responsible for the high oxidation resistance of the oil (5,6).

Since the levels of phenolic compounds in olives differ widely among varieties and locations (7), it was of interest to determine in this study the amount of polyphenols in oils obtained from locally grown olives. It was also important to investigate the effect of polyphenol content on the oxidative stability of the oils during storage.

EXPERIMENTAL PROCEDURES

Twenty-one samples of virgin olive oils were obtained from Suri variety olives from different locations in Israel. The oils were commercially produced by mechanical processes, i.e., grinding of the olives, pressing of the pomance and separation of the oil from the vegetation water by centrifuging.

The pulp of three samples of Suri and Manzanillo variety olives was extracted with a chloroform/methanol mixture as described previously (8).

The polyphenols were extracted from the oils according to the method described by Vazquez Roncero et al. (2). Ten g of oil was dissolved in 50 mL hexane and the solution was extracted successively with three 20-mL portions of 60% aqueous methanol. The mixture was shaken each time for 2 min. The combined extracts were brought to dryness in a vacuum rotary evaporator at 40 C. The residue was dissolved in 1 mL methanol and stored at -20 C until it was used.

The concentration of total polyphenols in the methanolic extract was estimated with Folin-Ciocalteau reagent (9). The procedure consisted of dilution of 0.1 mL or a suitable aliquot of the extract (up to 0.4 mL methanol) with water to 5 mL in a 10-mL volumetric flask, and addition of 0.5 mL Folin-Ciocalteau reagent. After 3 min, 1 mL of saturated (ca. 35%) Na_2CO_3 solution was added. The content was mixed and diluted to volume with water. The extinction was measured after 1 hr at 725 nm against a reagent blank. Caffeic acid served as a standard for preparing the calibration curve ranging 0-100 μg/10 mL assay solution.

The concentration of *o*-diphenols in the methanolic extract was determined with molybdate (10). The procedure consisted of dilution of 0.2 mL extract to 1 mL with water, addition of 1 mL 0.1 M phosphate buffer (pH=6.5) and 2 mL 5% $Na_2MoO_4 \times 2H_2O$ solution. The content was mixed and the extinction was measured after 15 min at 350 nm against a reagent blank. Caffeic acid served as a standard for preparation of a calibration curve in the range 0-50 μg/4 mL assay solution.

Fatty acid compositions of the oils were determined by gas liquid chromatography on 10% EGSS-X on Gas Chrom

P as described previously (8).

Storage tests of the oils were done at 60 C. One-g samples of the oil were stored in containers 1 cm in diameter and of 1.5 cm height. Peroxide values of the stored oils were periodically determined according to AOCS Official Method Cd 8-53 (11).

RESULTS AND DISCUSSION

The contents of total polyphenols and *o*-diphenols in the oils are given in Table I. It can be seen that the method of oil extraction from the fruit affects the amount of polyphenols in the oils. Solvent-extracted oils were richer in polyphenols than oils obtained by pressing (321-574 vs 44-157, μg/g oil). Also, *o*-diphenol content in solvent-extracted oils was considerably higher than in the virgin oils (62-194 vs 5-15 μg/g oil). It is reasonable to assume that the polar polyphenols would dissolve better during laboratory extraction of oil in a chloroform/methanol mixture than in the apolar triglycerides during pressing of the olives in the mill. Total polyphenol content was within the range reported for Italian and Spanish oils (2,5).

Fresh olive oils, in spite of having high peroxide values (ca. 8-30 μeq/g oil, Table I and ref. 12) have a high degree of oxidative stability. It is notable that the same phenomenon was also observed in solvent-extracted avocado oils. This is in contrast to common behavior of oils that are known to oxidize fairly rapidly at such high peroxide values. It is difficult to explain the stability of olive oils. In the literature, this phenomenon was explained (4) by assuming that the measured peroxide value of the olive oil reflected not only lipid peroxides but also oxidation products of phenolic compounds that did not affect the oxidation rate of the oils. Some testimony to the validity of that assumption was found in the fact that high phenol contents in the oils were associated with low peroxide values and vice-versa (4). Such a relationship was not found in this investigation and the two parameters were found to be unrelated (Table I).

Oxidative stability evaluations were done by storage of the oils at 60 C and periodical determination of peroxide value. The number of storage days required to obtain peroxide value 70 μeq/g oil was taken as a measure of the oils' antioxidative stability. From the results summarized in Table I, it can be seen that the oxidative stability of the extracted oils was markedly higher than that of the virgin oils.

The storage time required for the virgin oils to reach a peroxide value of 70 μeq/g was in the range of 10-53 days, whereas, the solvent-extracted oils, after 75 days of storage, exhibited peroxide values of less than 20 μeq/g oil. The high resistance to oxidation of the solvent-extracted oils can probably be attributed to their high polyphenol content, in particular to that of *o*-diphenols. *ortho*-Diphenols

TABLE I

Contents of Total Polyphenols and *o*-Diphenols, Peroxide Value and Oxidative Stability of Several Olive Oils[a]

Sample number	Total polyphenols (μg/g oil)	*o*-Diphenols (μg/g oil)	Peroxide value (μeq/g oil)	Oxidative stability[b] (days)
		Virgin oils		
1	76	12	10.1	30
2	84	11	8.6	24
3	50	5	16.3	15
4	107	8	12.1	27
5	83	8	10.4	20
6	70	10	8.7	25
7	61	6	12.0	18
8	106	6	9.5	26
9	140	8	13.3	37
10	58	10	12.8	19
11	136	9	8.2	36
12	83	7	11.8	32
13	80	9	13.9	25
14	143	9	9.8	53
15	44	5	29.7	10
16	83	7	12.6	20
17	157	10	13.4	30
18	127	15	12.0	39
19	68	13	23.4	15
20	118	11	9.3	30
21	55	–	16.7	–
		Solvent extracted oils		
22	321	62	6.9	–
23	361	75	17.7	75(18.7)[c]
24[a]	574	194	9.1	75(8.9)[c]

[a]Suri variety olives, except for sample 24, which was Manzanillo.

[b]Defined as number of storage days at 60 C to obtain peroxide value 70 μeq/g oil.

[c]In brackets are given the peroxide values of the oils after 75 storage days at 60 C.

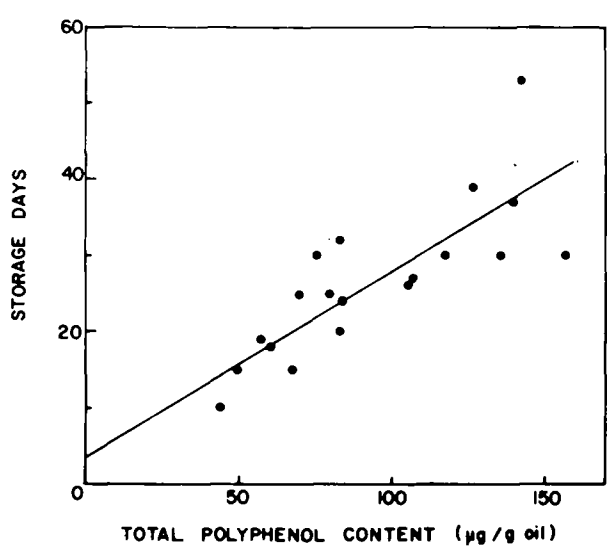

FIG. 1. Number of storage days at 60 C required to obtain a peroxide value of 70 μeq/g oil vs the polyphenol content of the oil.

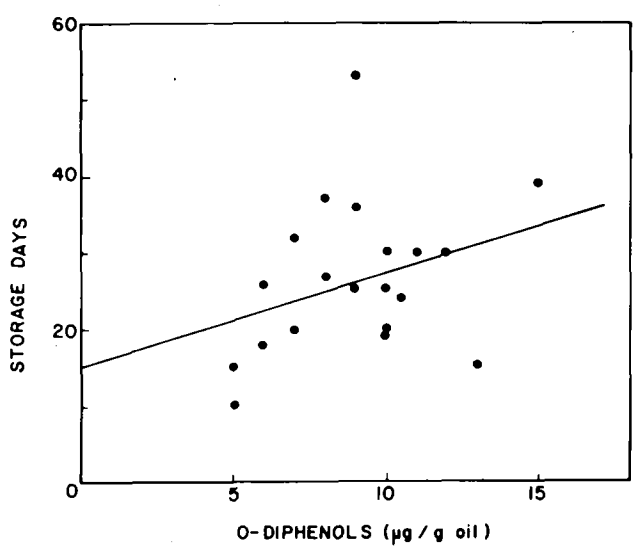

FIG. 2. Number of storage days at 60 C required to obtain a peroxide value of 70 μeq/g oil vs the *o*-diphenol content of the oil.

TABLE II

Polyphenol Content, Fatty Acid Composition and Oxidative Stability before and after Polyphenol Extraction for Several Olive Oils

	Sample number			
	14	18	20	23
Polyphenol content (μg/g oil)	143	127	118	361
Fatty acid composition (%)				
Palmitic	12.5	13.3	9.3	15.2
Palmitoleic	0.4	0.4	0.7	0.8
Stearic	3.5	3.5	3.0	4.0
Oleic	72.3	70.8	74.3	62.7
Linoleic	11.0	11.5	12.3	16.6
Linolenic	0.3	0.5	0.4	0.7
Polyunsaturated fatty acid (%)	11.3	12.0	12.7	17.3
Oxidative stability[a] (days)				
Before extraction of polyphenols	53	39	30	90[b]
After extraction of polyphenols	20	15	12	12

[a]See footnote b in Table I.

[b]This number was calculated by extrapolation from Equation I.

are considered better antioxidants. Emanual and Lyaskovskaya (13) have shown that insertion of a second or third hydroxy group on the aromatic ring of the phenol increases antioxidant activity.

In a linear least-square regression analysis, the relationship between oxidative stability of the virgin oils and total polyphenol and *o*-diphenol content resulted in the following equations, respectively,

$$Y = 0.24X + 3.74 \quad (r = 0.979) \quad [I]$$

$$Y = 1.2\ Z + 15.7 \quad (r = 0.933), \quad [II]$$

where Y is the number of storage days at 60 C to obtain peroxide value 70 μeq/g oil; X and Z are the polyphenol and *o*-diphenol content in μg/g oil, respectively; and r is the correlation coefficient. The two correlations with their corresponding experimental data are given in Figures 1 and 2. The first correlation gives a better fit, as reflected by higher coefficient of correlation. Vazquez Roncero et al. (2), who worked on virgin oils of Spanish origin, obtained also a linear relationship between total polyphenol content and oxidative stability using the Active Oxygen Method.

Comparison of the oxidative stability of the oils during storage before and after the polyphenol extraction, as summarized in Table II, showed that removal of the polyphenols increased markedly the oxidation rate of the oils. Surprisingly, sample 23, which had the highest oxidative stability, became one of the least resistant to oxidation. Examination of the fatty acid composition revealed that the concentration of polyunsaturated fatty acids was the highest in sample 23 (17.3 vs 11.3 - 12.7%, Table II). Thus, the effect of fatty acid composition on oil oxidation rate increased in the absence of polyphenols. We may, therefore, conclude that phenol content can be used as one of the measures for the quality of virgin oil.

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