Separation and Concentration of Natural Antioxidants From the Rape of Olives

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Polyphenols were extracted from the rape of Israeli olive oil using hexane, acetone and ethanol in a simple sequential procedure yielding three fractions (A,B,C). Fraction A (extracted with hexane) contained few polyphenols (0.05%), while Fraction B (extracted with acetone) and Fraction C (extracted with ethanol) contained about 5% polyphenols each. Fractions B and C were also found to contain the highest ortho-di-phenol concentration (about 3%). The addition of purified Fraction B at a level of 100 ppm to refined olive or soybean oils partially inhibited the oxidative deterioration when the oils were stored in the dark at 100 C.

Polyphenols are natural antioxidants in olive leaves and olives (1). Van Buren found that the level of phenolic compounds in olives differs widely among varieties and locations (2). Also, the oil extraction procedure influences the polyphenol content, and oils obtained from healthy, mature olives by mechanical means (virgin oils) are lower in phenols than those obtained by extraction with organic solvents (3). Good quality virgin olive oils have a high polyphenol content (4).

Rape, a major by-product of mechanical extraction, still has little industrial use and is presumed to contain polyphenols. In this paper, a method for polyphenol extraction and purification from rape is reported and the antioxidative property of the purified fraction investigated.

EXPERIMENTAL PROCEDURES

Materials. The refined olive and soybean oils were obtained from the Shemen Company, Haifa, Israel. A Galilee representative rape obtained from Syrian variety olives by mechanical means was received from Shaa'b village, Western Galilee, Israel.

The polyphenol standards, the reagents and the TLC plates were obtained commercially. All solvents used were AR grade.

Determination of water content. A sample (500–1300 g) was weighed and dried at 50 C overnight in a vacuum oven (≤100 mm Hg). The sample was cooled 30 min in a desiccator and reweighed.

Polyphenol extraction. In solvent extraction, the polyphenols were extracted from the rape by acetone:methanol according to the method described by Roncero et al. (5). In sequential extraction, 400–900 g of dried rape were weighed into a cloth sleeve. The polyphenols were extracted sequentially with two l of hexane, acetone and ethyl alcohol for equal periods (4–10 hr) using a Soxhlet extraction apparatus. After every extraction, the solvent in the extraction thimble was allowed to evaporate at room temperature and the dry rape was weighed. The solvent in the receiving flask was evaporated in vacuum at 50 C, and the dried extract was stored at -20 C.

Total polyphenols were determined according to AOAC method 9.098–9.100 (6). Ortho-di-phenols were determined as described by Pridham (7).

Thin-Layer Chromatography (TLC). The polyphenols were separated and identified by their migration by one- and two-dimensional TLC as compared to standards.

The solvents used were chloroform + ethyl acetate + formic acid (50:40:10); benzene + glacial acetic acid + water (60:70:30) (the upper phase); toluene + chloroform + acetone (90:25:35).

Detecting reagents were

I. 1% $FeCl_3$ + 1% $K_3Fe(CN)_6$ (1:1) solution (7).
II. Folin Reagent (8, 9).
III. Diazotized 4-nitroaniline solution (9).
IV. Molybdate solution (10).

Antioxidative activity. Oxidative stability of the oils was determined at 100 C for 120 hr. Polyphenols from purified Fraction B at levels of 0, 100, 200 and 1,000 ppm were added directly to soybean or olive oils which were stirred at ca. 60 C for one hr to ensure complete dissolution of the antioxidant in the oil.

Samples of oil, 10 g each, were then transferred to a series of opened transparent glass bottles of 20 ml volume and 5 cm^2 cross section and stored at 100 C. An entire bottle was used for analysis. Peroxide values and anisidine values were determined according to AOCS Official Method Cd 8-53 (11) and according to Parquot (12), respectively.

Separation of rape extracts containing polyphenols. Samples of 20 mg of Fractions B and C were dissolved in one ml of methanol and then added to 25 ml of ethyl acetate or isobutanol. Then one of the following solvents was added: (25 ml) 0.1M phosphate buffer (pH 7); 0.1M phosphate buffer (pH 6), or 0.1M NaCl brought to pH 4, pH 3 or pH 2 by 0.1M HCl. The containers were tightly closed and rotated overnight. After the separation of the two phases, the total polyphenol content was determined in both phases. The aqueous phase was extracted twice more, and the total polyphenol content was determined as above.

RESULTS AND DISCUSSION

Polyphenols extraction. The water content of the rape was 28%. The hexane extract (Fraction A) was a large fraction, about 9% of the dried rape, but its polyphenolic content (0.05%) was negligible (Table 1). Fraction B was smaller than Fraction C, but the polyphenol concentration in both was approximately the same (Table 1). Fraction B had a bland taste and light green color, whereas Fraction C had a bitter taste and dark color. The procedure described by Roncero for polyphenol extraction from olives (5) yielded 23.2% of the dried rape with a polyphenol content of 4.3%, but its dark color and bitter taste made its use as an oil additive

TABLE 1

Characteristics of Extracts Obtained by Successive Soxhlet Extractions or by Acetone + Methanol

Characteristics	Fraction A	B	C	D[a]
Fraction %[b]	9.0	4.0	7.0	23.2
Total polyphenols %	0.05	4.7	5.1	4.3
Ortho diphenols %	0.03	3	2.7	2.5
Polyphenols composition[c]				
Chlorogenic acid	+	+	+	+
Catechol	+	+	+	-
Caffeic acid	-	+	+	+
Gallic acid	-	+	+	+
3,4-hydroxyphenylethanol	-	+	-	-
Tyrosol	-	+	+	+
Kinic acid	-	+	-	-
Syringic acid	+	+	+	+
Vanillic acid	-	-	+	+
Cinnamic acid	-	-	+	+
Unidentified polyphenols	+	+	+	+

[a]Fraction D was obtained according to Roncero (2).
[b]The values are averages of 3 determinations.
[c]Polyphenol found (+) or unfound (-) in specific fraction as determined by TLC.

undesirable. The four fractions contain few common polyphenolic compounds as determined by TLC (Table 1). This may be due to differences in their solubility in the extraction solvent (5,13), or to interference between them and other extracted material.

Polyphenol purification from Fractions B and C. The highest polyphenol concentration was obtained at pH 4 after a single extraction step of either fraction using ethyl acetate or isobutyl alcohol (Table 2). In contrast to other pH's, at pH 4 the polyphenol solubility is maximal in the organic phase while the nonpolyphenolic components had much less solubility. Multiple extraction of both fractions (B,C) increased the yield of polyphenols by 10-20%. The unsuccessful purification of Fraction B with isobutyl alcohol may be due to the good solubility of other components in this solvent: 75% of the matter to be purified was extracted in the first step (data not shown). Fraction D was not subjected to further purification because purification did not remove the dark color and bitterness from Fraction C.

Oil stability usually is determined at accelerated conditions (60 C and more) because ambient conditions demand an excessively long period of time (14,15). The antioxidative potential of Fraction B was evaluated by addition to refined olive and soybean oils.

Primary oxidation. The peroxide value serves as an indicator of oil quality. Although it does not distinguish between the various unsaturated fatty acids that undergo oxidation and does not supply information about the secondary oxidative products formed by hydroperoxide decomposition, it generally can be stated that the peroxide value is an indicator of the primary level of oil oxidation (16). The change in peroxide values vs time exhibits both an induction stage, where no secondary oxidative products are formed, and an oxidative stage, where a steep increase in peroxide value occurs. Low quality oil will have shorter induction periods (17).

Addition of polyphenols from purified Fraction B (in three different concentrations) enhanced primary oxidative stability compared to the controls (Fig. 1 and 3). Although tocopherols are the natural antioxidants in soybean oil (18), olive polyphenols added to this oil improved oil stability (Figs. 1 and 3). This in agreement with a linear relationship found between polyphenol content and the oxidative stability of olive oil by Gutfinger (3).

Secondary oxidation. Although peroxide value does not serve as an absolute indicator of oxidative conditions, the anisidine value (AV) supplies information about the secondary deposition products formed during oil oxidation (12). The rate of change of AV in oils to which polyphenols of purified fraction B were added is lower than that of the controls (Figs. 2 and 4). This might be due to slow peroxides formation rate (Figs. 1 and 3).

This work indicates that the simple extraction, concentration and purification methods studied may be used to obtain an effective antioxidant additive as a new by-product from the mechanical press olive oil

TABLE 2

Effect of Fraction B or C Purification by Extraction with Ethylacetate or Isobutyl Alcohol and Aqueous Solutions on the Polyphenol Concentration and Yield (%)

	Total polyphenol yield[a] and concentration (%)									
	Ethyl acetate						Isobutyl alcohol			
	Fraction						Fraction			
pH	B after 1st extraction	C after 1st extraction	B after 2nd extraction	C after 2nd extraction	B after 3rd extraction	C after 3rd extraction	B after 1st extraction	C after 1st extraction	B after 2nd extraction	C after 2nd extraction
2	6.6(4.2)[b]	12.5(28.0)	2.0(18.5)	9.4(41.4)	1.6(28.0)	4.0(49.2)	3.7(44.3)	5.8(42.4)	2.7(60.2)	5.3(44.4)
3	8.8(36.6)	13.6(26.8)	4.4(46.2)	2.8(36.7)	3.4(50.4)	1.9(39.2)	4.1(65.5)	10.6(51.9)	4.1(75.7)	6.4(62.4)
4	21.6(51.0)	26.8(27.2)	17.5(63.8)	5.9(50.8)	8.3(71.3)	5.5(54.3)	4.9(78.3)	21.3(51.8)	4.9(90.6)	8.7(64.1)
6	13.6(18.3)	9.9(37.3)	8.4(24.0)	3.6(50.0)	6.7(27.7)	3.5(43.3)	4.3(33.9)	14.1(69.2)	4.4(46.2)	9.1(79.4)
7	7.5(36.8)	10.0(22.2)	7.1(51.7)	10.2(33.7)	3.6(42.6)	3.3(42.6)	4.1(34.0)	7.5(34.9)	3.4(49.6)	8.5(49.2)

[a]Yield (%) = (extracted polyphenol weight in combined extractions/initial polyphenols weight) × 100.
[b]The values are averages of three determinations. Data in brackets indicate yield of polyphenols.

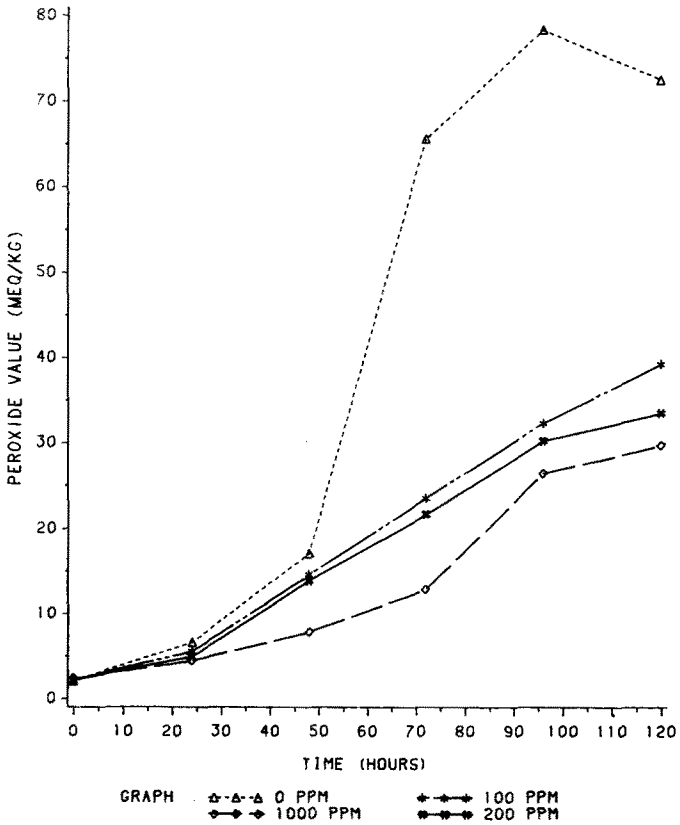

FIG. 1. Peroxide formation in refined soybean oil containing purified Fraction B, during storage at 100 C.

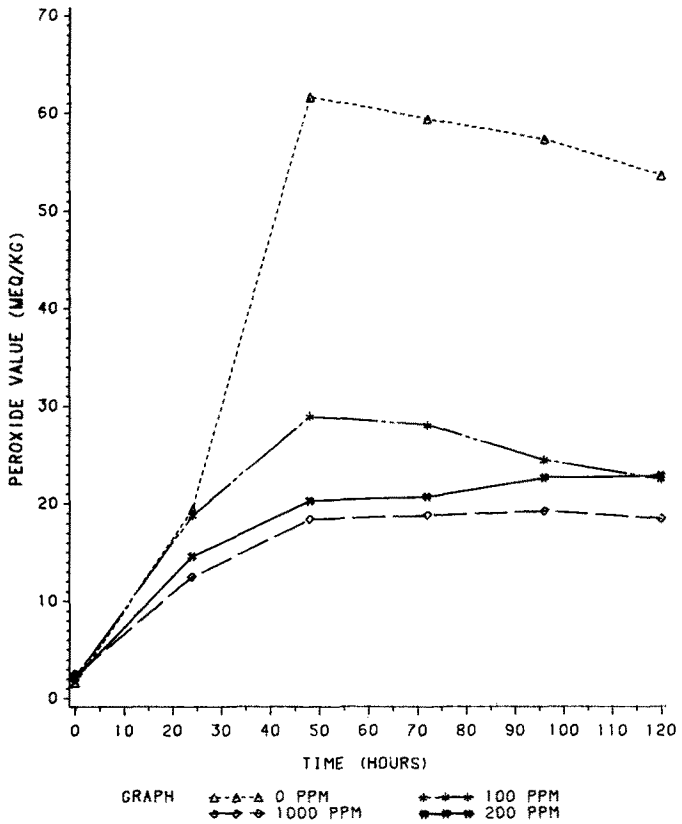

FIG. 3. Peroxide formation in refined olive oil containing purified Fraction B, during storage at 100 C.

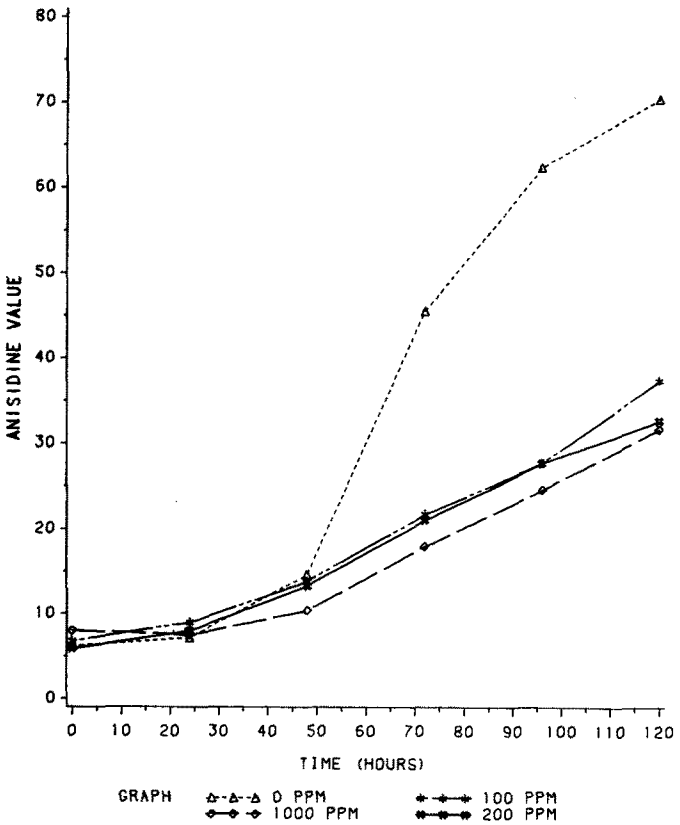

FIG. 2. Development of anisidine value in refined soybean oil containing purified Fraction B, during storage at 100 C.

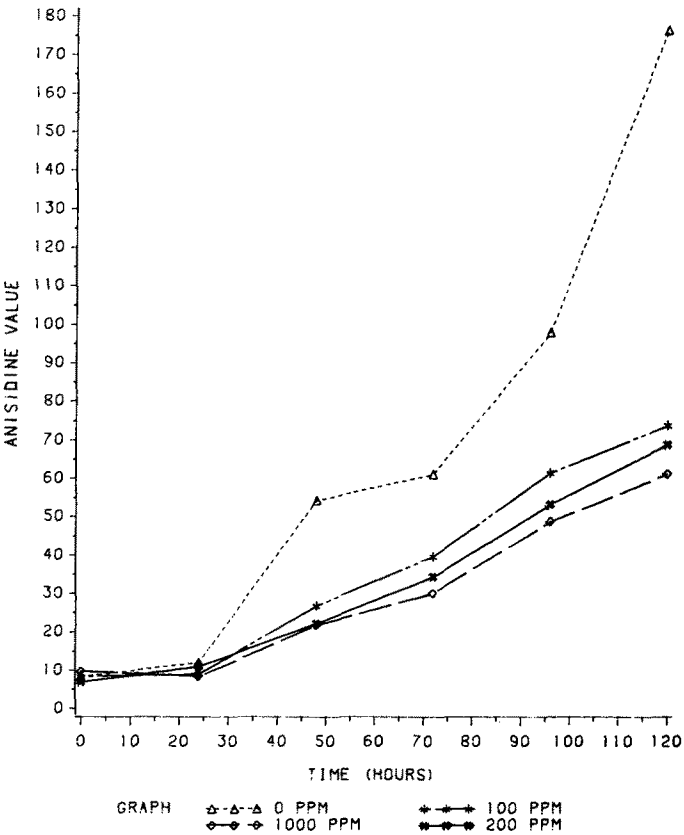

FIG. 4. Development of anisidine value in refined olive oil containing purified Fraction B, during storage at 100 C.

extraction industry. However, testing must be carried out in order to prove that the antioxidant preparation is safe from a toxicological standpoint. In addition, further experiments for excluding bitterness and unwanted colors from Fraction C, which has relatively high polyphenol concentration, are needed.

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