Technical

Storage Effect on Selected Characteristics and Lipids of Defatted Soy Flours

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ABSTRACT

Three defatted soy flours (fully toasted, white and enzyme active) 92 days after production date were stored at 23 C for periods of 0, 60, 120 and 180 days. At each storage period, lipoxygenase activity, thiobarbituric acid number (TBA), Nitrogen Solubility Index (NSI) and total lipid were determined in 5 replications of each flour. The lipid was separated into 3 fractions by silicic acid column chromatography and the fatty acid composition of each fraction was determined by GLC. Lipids in each fraction were identified by TLC procedures. Toasted flour had the greatest amount of total lipid and lowest TBA of the flours and no lipoxygenase activity. Enzyme active soy flour had the highest NSI and the greatest lipoxygenase activity. During storage, lipoxygenase activity of enzyme active flour and TBA and total lipid of each flour decreased. Percentages of lipids in fraction I, II or III were different among the soy flours, and the percentages of lipids in fractions I and II changed during storage. In each lipid fraction during storage, linoleic acid decreased in toasted and white flours but remained constant in enzyme active flour. Linoleic acid in any lipid fraction of enzyme active flour was lowest of the soy flours.

INTRODUCTION

Rackis et al. (1) pointed out that objectionable flavor is the most important factor limiting the use of soy protein products in food. Wolf (2) reported that a major source of compounds responsible for the objectionable flavor in soybean protein products is from lipoxygenase-catalyzed oxidation of soy lipids, particularly under relatively high moisture conditions encountered in present-day soybean processing. Brockmann and Acker (3) found that lipoxygenase also can cause oxidation of lipids at low moisture content (less than 5%) to be much faster than autoxidation.

Today, a wide range of defatted soy flours with less than 10% moisture and ranging from enzyme active to fully toasted are available to the baking industry (4), but few investigations have been reported concerning oxidation of their residual lipids during storage. Maga and Johnson (5) found that storage of defatted flakes at room temperature for up to 12 months decreased the unsaturated fatty acid content of the residual lipids; however, they did not assay for lipoxygenase activity. The objective of this experiment was to investigate what happens during storage to lipids and selected characteristics of defatted soy flours with different enzyme activities.

EXPERIMENTAL PROCEDURES

Materials

Three different defatted soy flours were obtained from Central Soy, Inc., which were described as: (a) a fully toasted soy flour, (b) a white soy flour with minimal heat treatment and (c) an enzyme active soy flour. Each type of soy flour represented a single treatment. Five samples were obtained for each treatment and were produced over a 6-month period from July to November 1975. Each treatment sample was produced on a different date and represented a replication. The oldest sample, when received, was 92 days past production date. So that all samples would be the same age prior to the storage investigation, they were kept in original packaging at room temperature until they were 92 days past production date.

At 92 days past production date, each sample was divided into 4 equal portions (about one kg/portion). Each portion was put into a sealed plastic bag inside a brown paper bag and randomly assigned a storage period of 0, 60, 120 or 180 days. All samples were stored at 23 C. At the end of each storage period, the sample portion was removed and stored at -20 C until analyses could be completed.

Chemical Analyses

At the end of each storage period, analyses were run on each sample. The Nitrogen Solubility Index (NSI) was determined by AOCS method BA 11-65 (6). Lipoxygenase activity was determined by the method of Ben-Aziz et al. (7) on a water extract of the flour (50 mg/mL) and reported as the change in absorbance/min at 234 nm. For the enzyme active flour, the original water extract of the flour was diluted to contain 1 mg/mL for readable results. The thiobarbituric acid number (TBA) was determined by the Rhee and Watts (8) modification of the Tarladgis et al. method (9) and reported as mg malonaldehyde/kg flour. Lipoxygenase activity and TBA were determined on a Perkin Elmer Model 202 spectrophotometer equipped with a constant temperature cell.

Lipids were extracted and quantitated by the method of Melton et al. (10). Lipid extracts were stored in a chloroform solution under nitrogen at -20 C until concentrated and analyzed. Each lipid extract was quantitatively separated into 3 fractions by the silicic acid column chromatographic method of Hornstein et al. (11). Each lipid fraction was expressed as percentage of total lipid fractionated and the recovery of lipids from the column ranged from 95 to 100%.

Each fraction was separated further by thin layer chromatography (TLC) on 250 μM Absorbosil 5 thin layer plates (Applied Science, State College, PA). Fraction I was developed by petroleum ether/ethyl ether/acetic acid, 70:20:4, v/v/v (solvent A) to show mono-, di-, and triglycerides and by chloroform/methanol/water, 65:35:4, v/v/v (solvent B). Fractions II and III did not contain any neutral glycerides; therefore, they were developed in solvent B system only. Specific lipids were identified as described by Melton et al. (10).

Fatty acids (C12-C20) in each lipid fraction were determined by gas liquid chromatographic (GLC) analysis of their methyl esters according the AOCS method Ce-66 (6). Fatty acid analysis was done by a Bendix Model 2600

chromatograph equipped with FID and disc integrator. The column was 1.83 m x 6.35 mm od stainless steel packed with 15% DEGS on 60/80 mesh Chromosorb W/AW (Applied Science). A fatty acid methyl ester standard containing the approximate composition of fatty acids in soybean oil was used to check accuracy and precision of GLC analysis.

Moisture of soy flour was determined by AOAC method 14.076 (12). All analyses except NSI are reported on a dry matter basis.

Statistical Methods

The experimental design was a split-plot design with 5 recpliations. The whole plot was the soy flours previously described and the split-plot was the storage time. When the storage and/or flour x storage (F x S) interaction was significant for a dependent variable, the sum of squares for storage plus F x S were partitioned by orthogonal polynomials into linear, quadratic, or cubic effects for each flour. When storage effect, but not F x S interaction, was significant for a dependent variable, the effects of storage across flours were partitioned by orthogonal polynomials. Significant storage effects on fatty acids were partitioned by orthogonal polynomials for each flour whether or not F x S was significant. When flour effect was significant, flour means either across storage or within each storage time were separated by a multiple range test (13).

Mean squares from analysis of variance of each variable are given in tabular form to show significant effects. Means of each variable during storage also are presented in tabular form.

RESULTS AND DISCUSSION

Total lipid content of defatted soy flours was significantly different among flours and decreased linearly during storage (Tables I and II). Toasted flour had more lipid than white or enzyme active flours and total lipid averaged across flour (storage means, Table II) decreased from 2.99 to 2.80%

TABLE I

Mean Squares from Analysis of Variance for Total Lipid of Defatted Soy Flours and Fractionation of That Lipid

Source	Degrees freedom	Total lipid	Fraction I lipids	Fraction II lipids	Fraction III lipids
Flour (F)	2	1.04[c]	444.25[d]	320.25[d]	62.05[d]
Storage (S)	3	0.13[c]	13.92[c]	25.38[d]	3.10
Linear	1	0.33[d]	7.83[c]	15.41[d]	–
Quadratic	1	0.06	30.01[d]	38.53[d]	–
Cubic	1	0.01	3.92	22.20[d]	–
F x S	6	0.06	2.70	5.31	–
Error 1[a]	12	0.2195	59.53	31.67	3.22
Error 2[b]	36	0.03	3.45	4.63	2.51

[a]Used to test flour for significance.

[b]Used to test storage and F x S for significance.

[c]Significant at the $p<0.05$ level.

[d]Significant at the $p<0.01$ level.

TABLE II

Total Lipid in Defatted Soy Flours and Fractionation of That Lipid

Item	Soy flour[a]	Days storage 0	60	120	180	Flour means[b]
		% of flour (as is)				
Total lipid	1	3.18	3.01	3.20	3.10	3.12[e]
	2	2.94	2.81	2.64	2.68	2.77[d]
	3	2.85	2.80	2.51	2.62	2.70[d]
Storage means		2.99	2.87	2.78	2.80	
		% of total lipid				
Fraction I lipids	1	34.7	34.0	34.8	35.6	34.8[d]
	2	25.7	24.6	24.8	27.4	25.6[e]
	3	28.7	28.6	26.4	29.6	28.3[e]
Storage means		29.7	29.1	28.7	30.9	
Fraction II lipids	1	53.7	53.0	53.0	52.6	53.1[d]
	2	60.7	61.4	63.1	58.8	61.0[e]
	3	56.2	56.3	58.2	53.4	56.0[d]
Storage means		56.8	56.9	58.1	54.9	
Fraction III lipids	1	11.6	13.0	12.3	11.8	12.1[d]
	2	13.7	14.0	12.1	13.8	13.4[d]
	3	15.1	15.1	15.3	16.9	15.6[e]
Storage means		13.5	14.0	13.2	14.2	

[a]1 = toasted, 2 = white and 3 = enzyme active.

[b]n = 20, across storage days.

[c]N = 15, across flours.

[d,e]For each item in a column, means bearing the same superscripts are not significantly different ($p<0.05$).

during storage.

Lipids present in fractions I and II of total lipids in defatted flours at 0 and 180 days storage are shown in Figure 1. Fraction I lipids were composed of minor amounts of glycolipids (regions A and G), a sterol glycoside (E), a sterol (F), a lipid-containing carbohydrate and phosphorus (C) and mono-, di- and triglycerides (H). Lipids B and D did not react with any specific detection tests and were not identified. The lipids in H were identified by TLC solvent A system.

Fraction II lipids shown in Figure 1 were composed of lipids which reacted positively to carbohydrate and sterol tests (K through O and T), carbohydrate tests only (J, P and V) and sterol test (S). Positive identifications were made for phosphatidylcholine (R), phosphatidylethanolamine (U) and phosphatidylinositol (Q). Lecithin (R), found in fraction II lipids, was the main lipid found in fraction III lipids (Fig. 2). The elution scheme from silicic acid used in this investigation did not completely separate all lecithin in the total lipid extract as previously reported (10).

Lipids of defatted soy flours underwent degradation during storage. Besides the amount of total lipids extracted from the flours decreasing during storage, significant changes occurred to the percentages of these lipids found in fractions I and II (Tables I and II). The percentage of defatted soy flour lipids in fraction I (storage means, Table II) decreased from 29.7% at 0 day to 28.7% at 120 days' storage and then increased to 30.9% at 180 days' storage. Percentages of lipids in fraction II increased from 56.8% at 0 day to 58.1% at 120 days and then decreased to 54.9% at 180 days' storage. There was no significant change in percentage lipids in fraction III (Tables I and II) during storage; however, since the total amount of extractable lipids decreased, the quantity of fraction III lecithin (Fig. 2) in each soy flour decreased during storage.

Nitrogen Solubility Index was significantly different among soy flours and when it was averaged across flours, NSI did not change significantly during storage (Tables III and IV). However, there was a significant F × S interaction for NSI (Table III), which when partitioned, showed that the NSI of the white flour decreased linearly from 69.5 to 64.3% during storage (Tables III and IV). The decrease in the NSI of white flour agrees with findings of other investigators. Smith and Circle (14) reported that the NSI of defatted soy meal decreased about 1.1%/month during the first few months at room temperature, and Jones and Gersdorff (15) reported decreases in dispersibility in 10% salt solution of nitrogenous compounds of defatted soy meal stored at room temperature for up to 6 months.

The lipoxygenase activity of enzyme active soy flour was far greater than in the other 2 soy flours and decreased at an increasing rate during storage (Tables III and IV). Even though there was some measurable lipoxygenase activity in the white flour, it was not significantly different than that of toasted flour which had no activity at all (Table IV).

At 0 days' storage, enzyme active soy flour had the largest TBA of the flours and toasted soy flour had the lowest TBA throughout storage (Tables III and IV). The TBA values of all 3 flours were affected by storage (Table III). TBA of toasted flour decreased as a decreasing rate up to 120 days storage and then increased (Tables II and IV). TBA of white soy flour decreased linearly during storage and TBA of enzyme active soy flour decreased up to 120 days storage and then increased slightly. The greatest change in TBA during storage, even though significant for each soy flour, was a decrease of 1.5 units in TBA of enzyme active soy flour. Sessa et al. (16) reported that

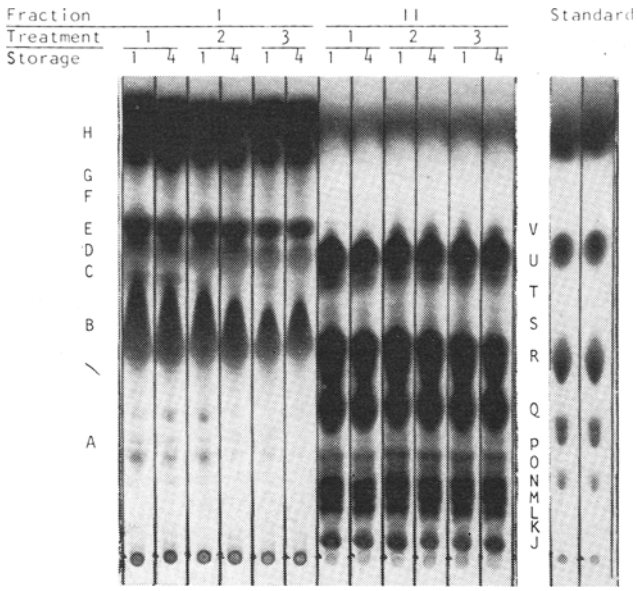

FIG. 1. Chromatograms of fractions I and II lipids of defatted soy flours during storage. Treatments 1, 2 and 3 are toasted, white and enzyme active soy flours, respectively. Storage periods 1 and 4 are 0 and 180 days, respectively.

toasted defatted soy flakes had lower TBA of raw soybean flakes did not change more than 3 units during storage for 7 months at room temperature, but they did not report the change as an increase or decrease. Honig et al. (17) reported that volatiles of defatted soybean flakes decreased during a 6-month storage at 24.4 C compared with defatted soybean flakes stored at -17 C. The decrease in TBA determined in the present investigation at 532 nm would most likely be due to a reduction in 2,4-alkadienals (18).

Mean squares from analysis of variance and means for fatty acids of fraction I lipids are shown in Tables V and VI. In fraction I lipids up to 120 days' storage, toasted and white soy flours had higher levels of 18:2 and lower levels of 18:0 than enzyme active soy flour. Throughout storage, enzyme active soy flour had higher levels of 18:1 than toasted or white. The lower quantities of 18:2 acid in

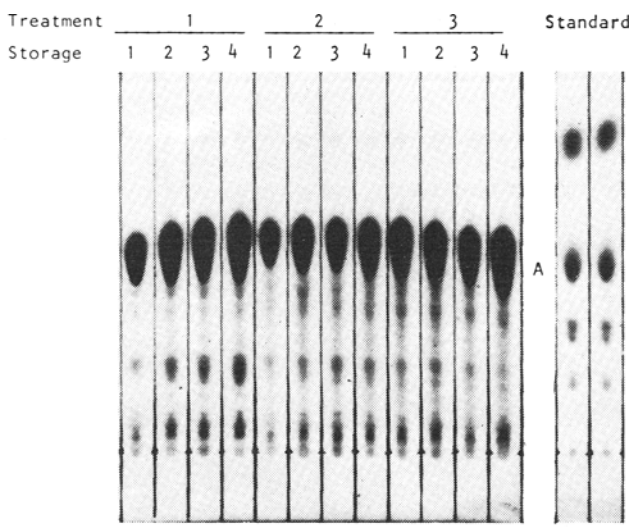

FIG. 2. Chromatograms of fraction III lipids of defatted soy flours during storage. Treatments 1, 2 and 3 are toasted, white and enzyme active soy flours, respectively, and storage periods 1, 2, 3 and 4 are 0, 60, 120 and 180 days, respectively. A = lecithin.

enzyme active soy flour are easily explained, since lipoxygenase catalyzes oxidation of polyunsaturated fatty acids and esters containing a 1,4-pentadiene system (1).

Storage significantly affected levels of all acids except 16:0 and there were significant F × S interactions for all fatty acids of fraction I lipids (Table V). The most significant storage effect was a decrease in 18:2 percentage in fraction I lipids of toasted and white soy flours from ca. 60% at 0-day storage to 54-55% at 180-day storage (Table VI). There was not any significant change in the percentage of 18:2 in fraction I lipids of enzyme active soy flour during storage. Since fatty acid composition was determined on a 100% basis of 16:0, 18:0, 18:2, 18:2 and 18:3, the decrease in the percentage of 18:2 for toasted and white soy flours resulted in increases in the percentages of other fatty acids during storage. For toasted soy flour, 18:0 and 18:1 increased linearly with increasing storage time and 18:3 increased in a quadratic curve with increasing storage time. In fraction I lipids of white soy flour, 18:3 increased linearly from 0 to 180 days' storage and percentages of 18:0 and 18:1 increased from 0 to 60 days' storage, decreased from days 60 to 120 and then increased to the highest percentage present at any storage time at 180 days. During 180 days' storage, the sum of the increases of 18:0, 18:1 and 18:3 in fraction I lipids of toasted or white soy flours approximately equals the decrease in the percentage of 18:2 for each respective flour. In fraction I lipids of enzyme active soy flour, the 18:3 percentage decreased linearly from 7.2 to 6.4% during 180 days' storage. This decrease was offset by an overall increase in 18:0 from 4.7 to 5.5% during the 120 days storage, and the linear increase in 16:0 from 16.4 to 17.6% during 180 days' storage. The percentage of 18:1 of enzyme active soy flour fraction I lipids had maximal per-

TABLE III

Mean Squares from Analysis of Variance of Selected Characteristics of Defatted Soy Flours

Source	Degrees freedom	NSI	Lipoxygenase activity	TBA
Flour (F)	2	21887.99[d]	160.21[d]	8.89[d]
Storage (S)	3	3.75	0.97[d]	1.22[d]
F × S	6	16.76[c]	0.96[d]	0.35[d]
Partition[a]				
Toasted				
linear	1	0.01	0.00	0.09
quadratic	1	2.16	0.00	0.78[d]
cubic	1	0.38	0.00	0.04
White				
linear	1	69.98[d]	0.00	0.54[d]
quadratic	1	14.11	0.00	0.03
cubic	1	0.92	0.00	0.01
Enzyme active				
linear	1	13.01	7.88[d]	2.85[d]
quadratic	1	8.81	0.77[c]	1.32[d]
cubic	1	2.44	0.02	0.11
Error 1[b]	12	12.35	16.27	0.11
Error 2[b]	36	7.38	0.12	0.06

[a]Storage and F× S sum of squares were partitioned by orthogonal polynomials for each type of flour.

[b]Flour was tested for significance by error 1 and storage, F × S and partition by error 2.

[c]Significant at the $p<0.05$ level.

[d]Significant at the $p<0.01$ level.

TABLE IV

Selected Characteristics of Defatted Soy Flours

Characteristic	Soy flour[a]	Days storage 0	60	120	180
		% soluble nitrogen			
NSI	1	21.2[b]	21.6[b]	21.9[b]	21.0[b]
	2	69.5[c]	65.8[c]	64.7[c]	64.3[c]
	3	84.5[d]	85.9[d]	87.5[d]	86.3[d]
		ΔA_{234} nm/min			
Lipoxygenase activity	1	0.000[b]	0.000[b]	0.000[b]	0.000[b]
	2	0.044[b]	0.036[b]	0.041[b]	0.030[b]
	3	5.580[c]	5.360[c]	4.873[c]	3.870[c]
		mg malonaldehyde/kg flour			
TBA	1	2.61[b]	2.24[b]	2.06[b]	2.48[b]
	2	3.74[c]	3.64[c]	3.53[c]	3.28[c]
	3	4.25[d]	3.26[d]	3.12[d]	3.17[c]

[a]1 = toasted, 2 = white and 3 = enzyme active.

[b,c,d]For any one characteristic in a column, means bearing the same superscripts are not significantly different ($p<0.05$).

centage at 120 days' storage followed by a decrease to 18.0% at 180 days' storage (Table VI).

Mean squares from the analysis of variance and means for fatty acid percentage in fraction II lipids are shown in Tables VII and VIII, respectively. In these lipids up to 120 days' storage, toasted and white soy flours had more 18:2 and less 18:1 than enzyme active soy flour (Table VIII). Significant storage effects for all fatty acids and F × S interactions for all except 18:2 were found in fraction II lipids (Table VII). The greatest change during storage was the decrease in the level of 18:2 for toasted and white soy flours. The percentage of 18:2 decreased from 61.9% for toasted flour at 0 day to 58.0% at 120 days' storage and from 61.6% for white flour at 0-day to 57.2% at 120 days (Table VIII). The 18:2 level in fraction II lipids of enzyme active soy flour did not change during storage. The percentages of 16:0 in fraction II lipids of toasted and white soy flours also decreased linearly during storage. The decreases in 18:2 and 16:0 resulted in linear increases during storage for 18:0, 18:1 and 18:3 of toasted soy flour fraction II lipids and in linear increases for 18:0 and 18:3 and a curvilinear increase in 18:1 of white soy flour fraction II lipids (Tables VII and VIII). The percentages of 18:0, 18:1 and 18:3 in enzyme active soy flour fraction II lipids increased from 0 to 60 days' storage, remained unchanged or decreased slightly from 60 to 120 days' storage and decreased from 120 to 180 days' storage to about the same or slightly less than the percentage present at 0-day. The percentage of 16:0 in these same lipids decreased from 23.8 to 22.5% during the first 60 days' storage and then increased to 25.0% at 180 days' storage (Table VIII).

TABLE V

Mean Squares from Analysis of Variance for Fatty Acid Composition of Fraction I Lipids

Source	Degrees freedom	16:0	18:0	18:1	18:2	18:3
Flour (F)	2	29.87	12.59[d]	62.25[d]	111.16[d]	19.12[d]
Storage (S)	3	0.06	4.63[d]	2.55[d]	36.44[d]	5.96[d]
F × S	6	1.34[d]	0.71[d]	1.94[d]	8.64[d]	4.01[d]
Partition[a]						
Toasted						
linear	1	2.30[d]	6.07[d]	4.34[d]	64.43[d]	24.99[d]
quadratic	1	0.37	0.15	0.85	6.13[c]	3.18[d]
cubic	1	0.01	0.37	0.17	1.74	0.15
White						
linear	1	0.18	7.72[d]	7.80[d]	68.97[d]	10.02[d]
quadratic	1	0.93[c]	0.30	1.11	10.18[d]	0.40
cubic	1	0.19	2.03[d]	1.72[c]	5.59[c]	0.65
Enzyme active						
linear	1	3.47[d]	0.59	0.72	0.09	2.21[c]
quadratic	1	0.70	0.71[c]	0.36	1.39	0.34
cubic	1	0.04	0.23	2.19[c]	2.62	0.02
Error 1[b]	12	16.46	1.34	5.38	7.96	1.15
Error 2[b]	36	0.23	0.16	0.33	1.36	0.38

[a]See footnote a, Table III.

[b]See footnote b, Table III.

[c]Significant at the $p<0.05$ level.

[d]Significant at the $p<0.01$ level.

TABLE VI

Fatty Acid Composition of Fraction I Lipids

Fatty acid	Soy flour[a]	Days storage 0	60	120	180
			%		
16:0	1	15.1[b]	14.5[b]	14.2[b]	14.2[b]
	2	16.2[c]	16.7[c]	16.4[c]	16.0[c]
	3	16.4[c]	16.5[c]	16.8[c]	17.6[d]
18:0	1	2.7[b]	3.6[b]	3.7[b]	4.3[b]
	2	2.8[b]	4.1[b]	3.8[b]	4.7[b,c]
	3	4.7[c]	5.0[c]	5.5[c]	5.0[c]
18:1	1	15.8[b]	16.8[b]	17.0[b]	17.2[b]
	2	13.9[c]	15.4[c]	15.2[c]	15.8[c]
	3	18.9[d]	18.4[d]	19.1[d]	18.0[d]
18:2	1	59.5[b]	56.2[b]	55.4[b]	54.4[b,c]
	2	60.4[b]	56.4[b]	56.1[b]	54.9[c]
	3	52.8[c]	52.9[c]	51.8[c]	53.0[b]
18:3	1	6.9[b]	8.9[b]	9.7[b]	10.0[b]
	2	6.8[b]	7.4[c]	8.5[c]	8.5[c]
	3	7.2[b]	7.2[c]	6.9[d]	6.4[d]

[a]1 = toasted, 2 = white and 3 = enzyme active.

[b,c,d]For any one fatty acid in a column, means bearing the same superscripts are not significantly different ($p<0.05$).

Mean squares from analysis of variance and means of fatty acid percentages in fraction III lipids are shown in Tables IX and X, respectively. Toasted soy flour lipids throughout storage had higher levels of 18:2 and lower levels of 18:1 than enzyme active soy flour lipids; from 60 to 180 days, had lower percentage of 16:0 than either white or enzyme active soy flour lipids; and up to 60 days' storage had less 18:0 than white or enzyme active soy flour lipids (Table X).

Significant storage effects were observed for all fatty acids of fraction III lipids and significant F × S interactions for 18:0, 18:1 and 18:2 (Table IX). In toasted soy flour fraction III lipids, 18:2 did not change during the first 60 days of storage, decreased from 64.4% at 60 days to 59.7% at 120 days and then increased to 60.8% at 180 days' storage (Table X). In white soy flour fraction III lipids, 18:2 decreased from 61.7% at 0 day to 57.2% at 120 days and then increased to 59.3% at 180 days' storage. The levels of 18:0, 18:1 and 18:2 of fraction III lipids of enzyme active soy flour did not change significantly during storage (Tables IX and X). In fraction III lipids of toasted and white soy flours, 18:0 and 18:1 increased to a maximal percentage at 120 days' storage and decreased during storage up to 180 days. In fraction III lipids of all 3 soy flours, 16:0 decreased linearly and 18:3 increased linearly during storage (Tables IX and X).

Decreases in 18:2 levels in lipid fractions of toasted and white defatted soy flours during storage were due to autoxidation. The enzyme active soy flour had the lowest percentage 18:2 in the lipid fraction of all 3 soy flours throughout storage. This indicates that lipoxygenase activity during processing or 92 days after production (prior to the start of the storage investigation) reduced the 18:2 content. However, since there was no further decrease in the level of 18:2 of enzyme active soy flour during storage, there appears to be an inhibitor in the soy

TABLE VII

Mean Squares from Analysis of Variance for Fatty Acid Composition of Fraction II Lipids

Source	Degrees freedom	16:0	18:0	18:1	18:2	18:3
Flour (F)	2	15.70	2.38	12.73[c]	54.14[c]	3.04
Storage (S)	3	3.94[c]	3.33[d]	3.20[d]	29.34[d]	15.27[d]
F × S	6	6.47[d]	0.68[c]	2.05[d]	4.21	4.12[d]
Partition[a]						
Toasted						
linear	1	18.34[d]	4.87[d]	5.76[d]	41.73[d]	37.67[d]
quadratic	1	0.89	0.15	0.31	7.29	0.50
cubic	1	0.05	0.59	0.06	0.77	0.01
White						
linear	1	12.84[d]	6.25[d]	9.68[d]	51.12[d]	26.13[d]
quadratic	1	0.81	0.56	2.44[c]	9.94[c]	0.00
cubic	1	0.59	0.25	0.16	0.20	0.10
Enzyme active						
linear	1	4.67[c]	0.00	1.96[c]	0.77	0.00
quadratic	1	0.30	0.01	0.04	0.11	0.07
Error 1[b]	12	4.10	0.98	2.33	10.17	1.17
Error 2[b]	36	1.04	0.26	0.36	2.22	0.75

[a]See footnote a, Table III.
[b]See footnote b, Table III.
[c]Significant at the $p<0.05$ level.
[d]Significant at the $p<0.01$ level.

TABLE VIII

Fatty Acid Composition of Fraction II Lipids

Fatty acid	Soy flour[a]	Days storage 0	60	120	180
16:0	1	22.9[b]	22.6[b]	21.6[b]	20.4[b]
	2	23.6[b]	23.6[b]	22.4[b,c]	21.6[b]
	3	23.8[b]	22.5[b]	23.2[c]	25.0[c]
18:0	1	3.8[b]	4.1[b]	5.0[b]	5.0[b]
	2	4.2[b,c]	4.8[c]	5.6[b]	5.6[b]
	3	4.8[c]	5.4[c]	5.4[b]	4.9[b]
18:1	1	5.6[b]	6.2[b]	6.8[b]	6.9[b]
	2	5.1[b]	6.2[b]	7.1[b]	6.9[b]
	3	7.9[c]	8.1[c]	7.9[c]	7.0[b]
18:2	1	61.9[b]	59.8[b]	58.0[b]	58.2[b]
	2	61.6[b]	58.9[b]	57.2[b]	57.4[b]
	3	56.8[c]	56.3[c]	55.8[b]	56.4[b]
18:3	1	5.8[b]	7.4[b,c]	8.6[b]	9.5[c]
	2	5.6[b]	6.5[b]	7.7[b]	8.6[c]
	3	6.6[b]	7.8[c]	7.7[b]	6.7[b]

[a]1 = toasted, 2 = white and 3 = enzyme active.
[b,c]For any one fatty acid in a column, means bearing the same superscripts are not significantly different ($p<0.05$).

flour which prevented oxidation of 18:2 below a given level.

The decreases which occurred in unsaturated fatty acids (18:1, 18:2 and 18:3) during storage of defatted soy flours were because of oxidation. The increase in any fatty acid during storage of defatted soy flours most likely was because of other fatty acid decreases. It is hard to explain significant decreases in 16:0 in fraction II and III lipids of toasted and white soy flours and in fraction III lipids of enzyme active soy flour during storage (Tables VI, VIII and X). Decreases in the level of 16:0 in any lipid fraction during storage could result in increases in the level of 16:0 in other lipid fractions if oxidation of lipids containing high levels of 16:0 and unsaturated fatty acids caused those lipids to elute in lipid fractions other than the original fraction. This possibility might be the cause of the decrease in the 16:0 level in fraction III lipids of enzyme active soy flour during storage since the 16:0 level in fraction I and II lipids of this flour increased during the same period. However, this possibility does not exist for toasted and white soy flours since 16:0 in fraction I lipids tended to decrease in toasted soy flour and remained unchanged in white soy flour (Table VI). Another possibility that could cause a decrease in the 16:0 level during storage is the reaction of 16:0 with free radicals from oxidizing unsaturated fatty acids to produce saturated products at normal storage temperatures as has been reported in milk and corn lipid oxidation (19,20).

The NSI of white soy flour and lipoxygenase activity of enzyme active soy flour decreased and oxidation of lipids of defatted soy flours occurred during 180 days of storage at 23 C. The oxidation resulted in decreased lipid extracted,

TABLE IX

Mean Squares from Analysis of Variance for Fatty Acid Composition of Fraction III Lipids

Source	Degrees freedom	16:0	18:0	18:1	18:2	18:3
Flour (F)	2	22.83[c]	8.52[d]	29.04[d]	116.59[d]	2.81
Storage (S)	3	9.65[d]	4.64[d]	8.08[d]	32.63[d]	12.50[d]
F X S	6	1.48	0.81[d]	1.82[d]	7.20[d]	0.89
Partition						
Toasted						
linear	1	12.16[d]	8.83[d]	10.69[d]	53.33[d]	23.18[d]
quadratic	1	0.45	0.05	1.74	0.77	0.40
cubic	1	3.84	2.35[d]	5.20	28.72[d]	0.25
White						
linear	1	7.82[c]	5.24[d]	7.72[d]	28.89[d]	9.75[d]
quadratic	1	6.57[c]	0.83	2.79[d]	12.85[d]	2.42
cubic	1	0.06	1.08[c]	6.36[d]	14.86[d]	0.29
Enzyme active						
linear	1	6.45[c]	0.24	0.33	0.44	6.38[d]
quadratic	1	0.00	0.17	0.08	1.26	0.08
cubic	1	0.50	0.00	0.29	0.00	0.10
Error 1[b]	12	4.07	0.91	3.26	6.50	1.66
Error 2[b]	36	1.26	0.17	0.27	1.72	0.75

[a]See footnote a, Table III.

[b]See footnote b, Table III.

[c]Significant at the $p<0.05$ level.

[d]Significant at the $p<0.01$ level.

TABLE X

Fatty Acid Composition of Fraction III Lipids

Fatty acid	Soy flour[a]	Days storage 0	60	120	180
				%	
16:0	1	21.4[b]	19.7[b]	20.1[b]	19.0[b]
	2	22.4[b]	23.0[d]	22.3[c]	20.7[c]
	3	22.4[b]	21.5[c]	21.5[c]	20.7[c]
18:0	1	3.4[b]	3.5[b]	5.0[b]	4.9[b]
	2	4.4[c]	4.9[c]	6.0[c]	5.6[c]
	3	5.1[d]	5.4[c]	5.5[b,c]	5.4[b,c]
18:1	1	6.2[b]	6.5[b]	8.6[b]	7.7[b]
	2	6.4[b]	6.6[b]	8.7[b,c]	7.5[b]
	3	9.1[c]	9.5[c]	9.3[c]	9.5[c]
18:2	1	64.1[b]	64.4[b]	59.7[c]	60.8[c]
	2	61.7[c]	60.6[c]	57.2[b]	59.3[c]
	3	57.9[d]	57.2[d]	57.1[b]	57.5[b]
18:3	1	4.9[b]	5.8[b]	6.6[b]	7.7[b]
	2	5.1[b]	4.8[b]	5.8[b]	6.9[b]
	3	5.5[b]	6.3[b]	6.6[b]	7.0[b]

[a]1 = toasted, 2 = white and 3 = enzyme active.

[b,c,d]For any one fatty acid in a column, means bearing the same superscripts are not significantly different ($p<0.05$).

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