STUDY ON PEANUT LIPOXYGENASE DURING AQUEOUS EXTRACTION

ZUHEID NOOR AND KAPTI RAHAYU

Faculty of Agricultural Product Processing,
Gadjah Mada University, Yogyakarta, Indonesia

ABSTRACT

It is obvious that the presence of lipoxygenase in peanut is a problem that should not be ignored, therefore, it is appropriate to study this particular enzyme in related to aqueous process of peanuts.

Lypoxygenase catalyses the oxidation of fatty acids containing the cis, cis, methylene interupted diene system, forming the conjugated cis, trans, diene hydroperoxide. It has been implicated in off-flavor production in several commodities. Measurement of oxygen uptake by warburg technique or polarographic method, following the rate of conjugated diene formation by measuring the Absorbance at 234 nm, peroxide formation at 480 nm and sensory evaluation of the oxidative off flavor have been used to determine the lipoxygenase activity in the various commodities.

The crude lipoxygenase was extracted at pH 2.0, 7.0 and 9.0 from whole peanut. The assay pH was determined for both unheated and heated at 40°C, 60°C, 80°C and 100°C for 30 minutes, measuring the activity by using trilinolein and linoleic acid as substrates.

The pH profiles of peanut lipoxygenase measured by trilinolein and linoleic acid as substrates showed simular pattern, but lipoxygenase activity measured by trilinolein had a lower activity compared to that of linoleic acid.

The highest activity was obtained in the pH 7.0 extract and followed by the pH 9.0 and 2.0 extracts. The enzyme in the pH 7 extract was completely in activated on heating at 80°C for 30 minutes and in the pH 2.0 and 9.0 extracts retained some residuae activity even after heating at 100°C.

The optimum assay pH was determined for both unheated and heated samples to be pH 6.0, and the activity diminished below pH 7.0.

INTRODUCTION

Lipoxygenase catalyzes the oxidation of fatty acids containing the cis, cis, methylene-interupted diene system, forming the conjugated cis, trans diene hydroperoxide. It has been implicated in off-flavor production in several commodities. Measurement of oxygen uptake by Warburg technique (6) or polarographic method (5, 9), following the rate of conjugated diene formation by measuring the Absorbance at 234 nm (3, 7, 8, and 9), peroxide formation at 480 nm (1, 4) and sensory evaluation of the oxidative off-flavor (2) have been used to determine the lipoxygenase activity in the various commodities.

Results from several investigators indicate that there are various pH optima for peanut lipoxygenase. Dillard *et al.* (1) reported two pH optima for peanut lipoxygenase in whole peanut extracts with linoleic acid as substrate, a

sharp peak at pH 8.1 and a slight hump at pH 6.0. They also found that two pH optima occured at pH 7.5 and 5.5 with trilinolein as the substrate. Sanders *et al.* (5) observed optima at pH 6.2 and 8.3 for partially purified peanut lipoxygenase. St. Angelo and Ory (7), using a partially purified enzyme and Mitchell and Malphrus (4), using a crude lipoxygenase preparation, found the optimum at pH 6.0. Our preliminary study revealed a low lipoxygenase activity at pH 8.1 using either linoleic acid or trilinolein substrates, by measuring the peroxide oxidation of ferrous iron at 480 nm.

Kon *et al.* (2), showed that the oxidized off-flavor as determined by a small untrained panel of judges of raw ground California small white beans and soybeans can be controlled by acidifying the blended material to pH 3.85 and below. Lao (3) found that soymilk extracted at pH 2, whether subsequently neutralized to pH 6.5 or not, did not have any lipoxygenase activity, and on storage at 4°C for 64 hr. did not show any reactivation of the enzyme activity. When extracted at higher pH's, the activity of lipoxygenase decreased from its maximum in the pH 7 extract but even at pH 9 its activity was higher than in the acidic extracts of pH 5 or lower. Lao (3) also found that soymilk extracted at pH 6.5 yielded the most complex mixture of volatiles which may be attributed to lipoxygenase induced oxidation. However, soymilk extracted at pH 9.5 had only a beany flavor and very few volatiles were present in its gas chromatographic patterns.

It is obvious that the presence of lipoxygenase in peanut is a problem that should not be ignored, therefore, it is appropriate to study this particular enzyme in related to aqueous process of peanuts.

EXPERIMENTAL

Preparation of Crude Lypoxygenase

Flowsheet for preparing the crude lipoxygenase is presented in Fig. 1.

Procedure for Lipoxygenase Determination.

The procedure adopted is as follow:

a. To 100 ml of glass distilled deionized water in 250 ml Erlenmeyer flasks, add 5 ml of 0.1 M phosphate buffer.
b. Add to each flask 1 ml of the substrate solution, followed by 1 ml enzyme extract and allow to react for 5 minutes at room temperature.
c. Add 10 ml concentrated HCl to stop the reaction and to provide an acid medium for oxidation of the ferrous iron by the hydroperoxide.
d. Add 1 ml of a 5% solution of ferrous ammonium sulfate in 3% HCl, then pipet 10 ml of this solution into a 50 ml stoppered test tube.

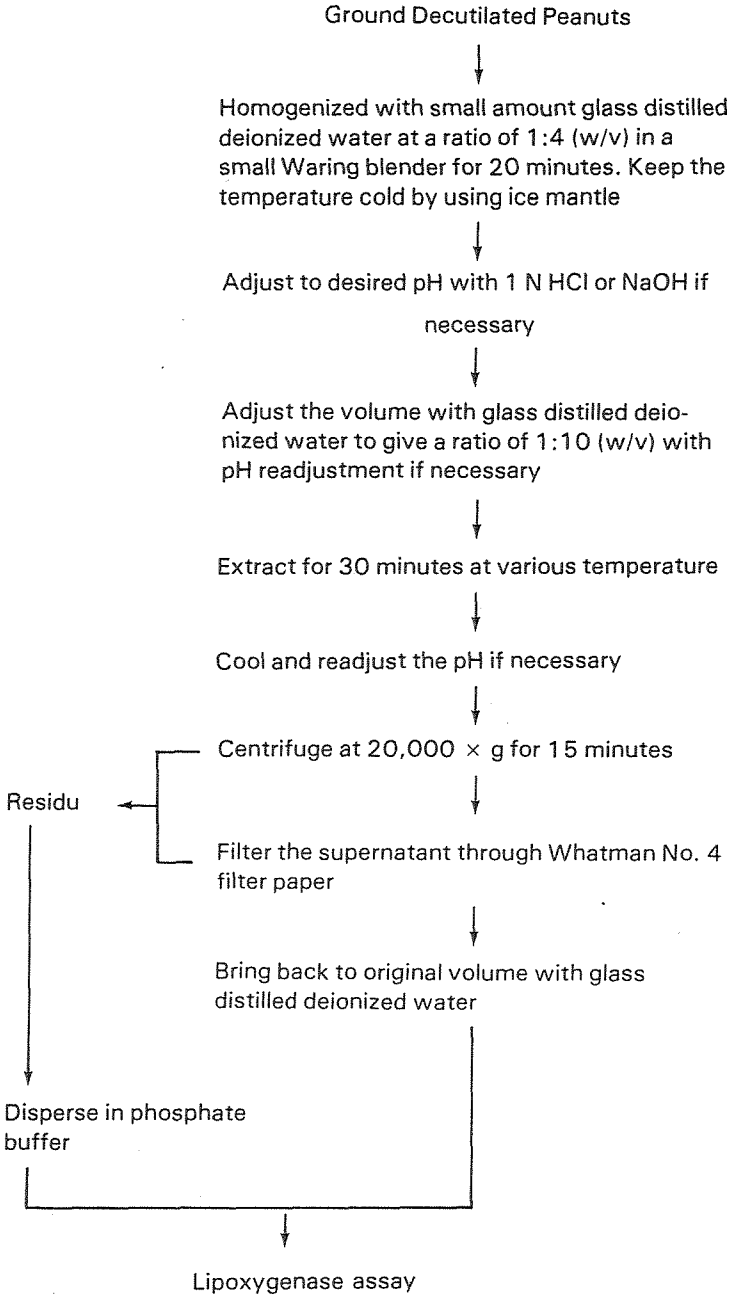

Fig. 1. Flowsheet for preparing crude peanut lipoxygenase.

e. Exactly 5 minutes after the addition of ferrous salt, 1 ml of 20% ammonium thiocyanate is added.
f. Add 20 ml of 95% ethanol, mix thoroughly and filter rapidly through Whatman No. 4 filter paper.
g. Read the absorbance at 480 nm against a blank that is prepared by adding the enzyme extract after the reaction mixture is acidified.

RESULTS AND DISCUSSION

The optimum assay pH was determined for both unheated and heated samples to be pH 6.0, as seen in Tables 1, 2, 3, 4, and Figure 2. This optimum

Table 1. Effect of assay pH and substrate on lipoxygenase activity of crude enzyme extract from whole peanuts.

Assay pH	Activity, absorbance units/g[1] Substrate	
	Trilinolein	Linoleic acid
3.0	0.423 ± 0.013	0.520 ± 0.024
4.0	4.579 ± 0.353	9.327 ± 0.143
5.0	7.972 ± 0.309	15.274 ± 0.505
5.5	6.664 ± 0.318	12.689 ± 0.279
6.0	8.874 ± 0.095	17.928 ± 0.103
7.0	2.516 ± 0.345	17.387 ± 0.336
8.0	1.123 ± 0.149	4.089 ± 0.305
9.0	1.103 ± 0.084	2.396 ± 0.432

[1] Average of duplicate analyses of two aliquot of the coarsely ground peanut samples ± std. deviation.

Table 2. Effect of assay pH and heat treatment on the lipoxygenase activity of the crude enzyme extracted at pH 2.0 from whole peanuts (linoleic acid used as substrate).

Assay pH	Activity, absorbance units/g[1]				
	Unheated	40°C	60°C	80°C	100°C
3.0	1.487	1.679	0.942	1.166	1.282
4.0	1.421	1.551	1.340	1.026	1.322
5.0	2.122	2.769	1.628	1.561	1.272
5.5	0.635	0.864	0.814	0.971	0.546
6.0	3.438	2.900	1.483	1.978	0.896
7.0	1.718	1.291	1.715	1.567	1.442
8.0	2.103	2.435	2.054	2.241	1.581
9.0	1.442	1.379	1.502	1.025	1.961

[1] Average of duplicate analyses.

Table 3. Effect of assay pH and heat treatment on the lipoxygenase activity of the crude enzyme extracted at pH 7.0 from whole peanuts (linoleic acid used as substrate).

Assay pH	Activity, absorbance units/g[1]				
	Unheated	40°C	60°C	80°C	100°C
3.0	0.485	0.593	0.269	0	0
4.0	8.611	8.749	0.647	0	0
5.0	14.767	14.198	2.578	0	0
5.5	11.943	12.282	1.889	0	0
6.0	17.875	17.174	2.808	0	0
7.0	10.147	15.970	1.269	0	0
8.0	3.377	3.269	0.600	0	0
9.0	2.018	0.862	0	0	0

[1] Average of duplicate analyses.

Table 4. Effect of assay pH and heat treatment on the lipoxygenase activity of the crude enzyme extracted at pH 9.0 from whole peanuts (linoleic acid used as substrate).

Assay pH	Activity, Absorbance units/g[1]				
	Unheated	40°C	60°C	80°C	100°C
3.0	2.091	1.889	1.619	1.282	1.674
4.0	1.929	1.991	1.686	1.349	1.930
5.0	5.019	2.968	2.140	1.754	2.020
5.5	3.400	2.631	1.889	2.091	2.079
6.0	6.813	4.789	2.226	2.159	2.249
7.0	1.538	1.282	1.646	2.083	1.968
8.0	2.105	1.673	1.821	2.024	1.969
9.0	1.619	1.889	2.064	1.848	1.757

[1] Average of duplicate analyses.

assay pH confirms what was reported by Mitchell and Malphrus (4) and St. Angelo and Ory (7). Apparently, heating did not change the optimum assay pH (Tables 2, 3, and 4).

The pH profiles of peanut lipoxygenase measured by trilinolein and linoleic acid as substrates showed similar patterns (Fig. 2), except that the lipoxygenase activity measured by trilinolein had a lower activity compared to that of linoleic acid. The difference in magnitude was probably due to the nature of these two substrates. Linoleic acid is probably more readily attacked by lipoxygenase than trilinolein; therefore it gives a higher activity in a given time. However, there is also the possibility that more than one enzyme is present with different substrate specificities, as reported by others (1, 5, and 6). On the other hand, the similarity of the patterns does not seem to indicate this.

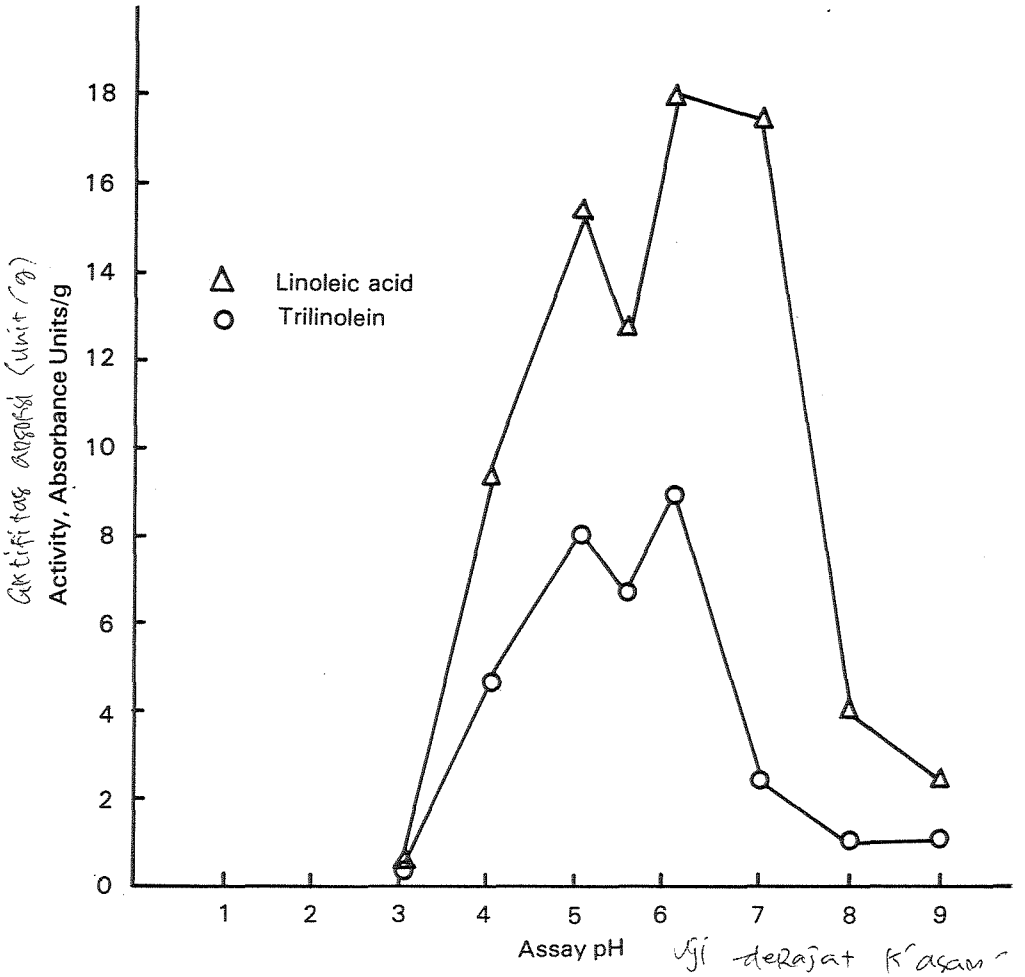

Fig. 2. Lipoxygenase activity of whole peanut extract (trilinolein and linoleic acid as substrates).

Tables 2, 3, and 4 give the lipoxygenase activity of the crude enzyme extracted at pH 2, 7, and 9 from whole peanuts. Although the extraction pH did not change the optimum assay pH, the level of lipoxygenase activity was not the same. Highest activity was obtained in the pH 7 extract followed by the pH 9 and pH 2 extracts. The enzyme in the pH 7 extract was completely inactivated on heating at 80°C for 30 minutes, regardless of the assay pH used. On the other hand, enzymes in the pH 2 and 9 extracts retained some residual activity even after heating at 100°C for 30 minutes. Athough the residual activity was low (about 2 Absorbance units per gram), there is no

ready explanation for it. In the absence of other supporting evidence it can only be speculated that exposures to pH 2 and pH 9 modified the enzyme so as to confer a greater heat resistance to it or that different lipoxygenase enzymes are extracted. Whatever the case, the pH profiles remained unchanged, which implies that the residual enzyme is a related lipoxygenase.

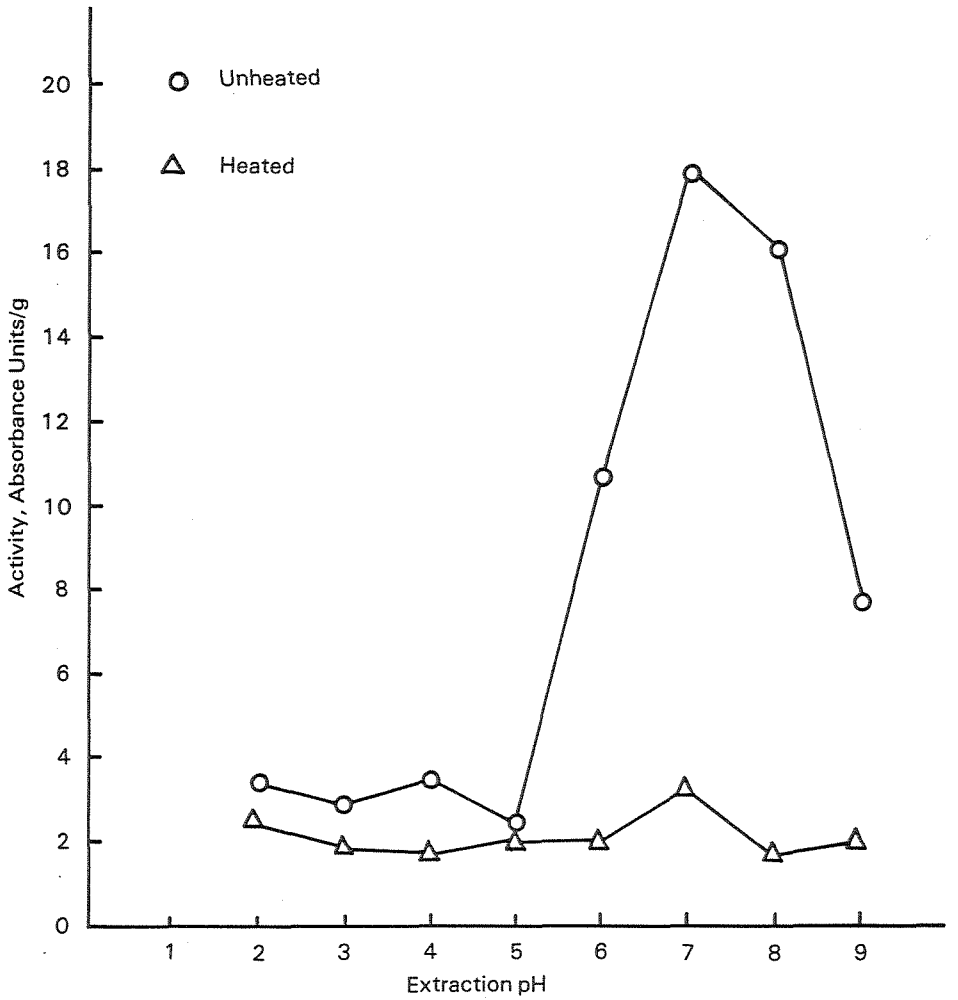

Fig. 3. Lipoxygenase activity of whole peanut extracts before and after heating.

Results in Table 5 and Fig. 3 indicate that lipoxygenase activity diminishes below pH 7. This agrees with the report of Kon *et al.* (2) for California small white beans lipoxygenase, and Lao (3) for soymilk.

Since Lao (3) indicated that on storage at 4 °C for 64 hrs did not show any reactivation of the enzyme activity of soymilk extracted at pH 2, we think that the absence of lipoxygenase activity after storage is simply due to insolubility of this enzyme at that pH. To clarify this, we performed another experiment. The result presented in Fig. 4, suggest that the absence of lipoxygenase activity in that pH and also pH 7 and higher, is due to inactivation of the enzyme. However, further study is still needed in this particular area.

Table 5. Effect of extraction pH and heat treatment on the lipoxygenase activity.

Sample number	Activity, absorbance units/g[1]
11	18.001 ± 0.336
21	3.367 ± 0.377
22	2.403 ± 0.113
31	2.766 ± 0.083
32	1.764 ± 0.159
41	3.400 ± 0.314
42	1.670 ± 0.219
51	2.386 ± 0.126
52	1.996 ± 0.066
61	10.702 ± 0.169
62	1.985 ± 0.092
71	17.853 ± 0.361
72	3.169 ± 0.297
81	16.138 ± 0.351
82	1.696 ± 0.264
91	7.702 ± 0.570
92	1.953 ± 0.118

[1] Average of duplicate analyses of two aliquots of the coarsely ground peanut samples ± standard deviation.

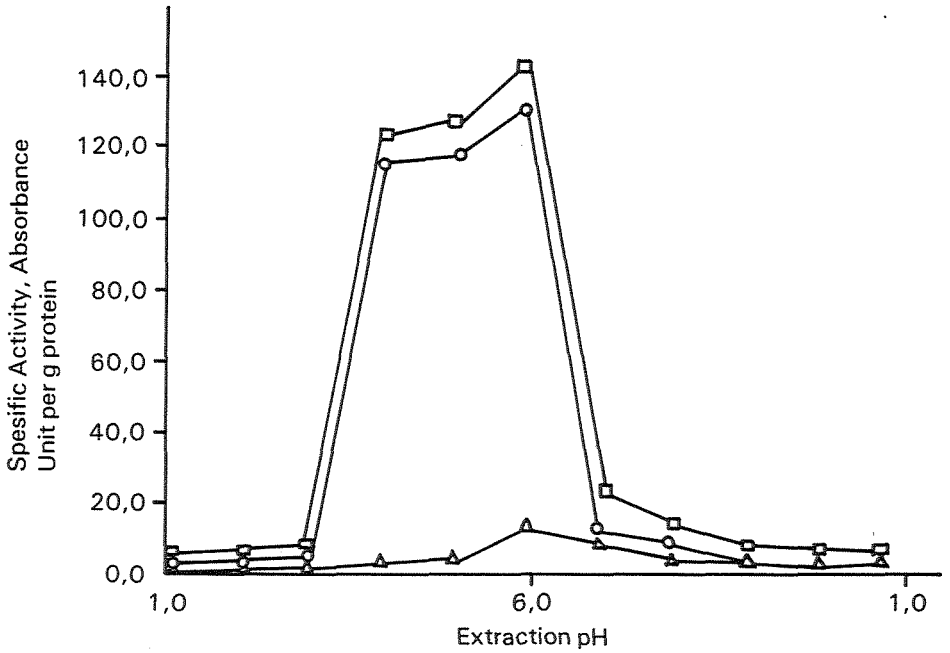

Fig. 4. Lipoxygenase activity of whole peanut extracts and their residues.

REFERENCES

1. Dieckert, J.W., Snowden, J.E. Jr., Moore, A.T., Heinzelman, D.C., and Altschul, A.M. (1962). Composition of Some Subcellular Fractions from Seeds of *Arachis hypogaea*. J. Food Sci., 27 (3): 321.
2. Kon, S., Wagner, J.R., Guadagni, D.C., and Horvat, R.J. (1970). pH Adjustment Control of Oxidative Off-Flavors During Grinding of Raw Legume Seeds. J. Food Sci., 35 (4): 343.
3. Lao, T.B. (1971). A Study of the Chemical Changes Relating to Flavor of Soybean Extracts. Ph.D. thesis, University of Illinois at Urbana-Champaign.
4. Mitchell, J.H. Jr. and Malphrus, R.K. (1977). Lipid Oxidation in Spanish Peanuts: The Effect of Moist Heat Treatments. J. Food Sci., 42 (6): 1457.
5. Sanders, T.H., Pattee, H.E., and Singleton, J.A. (1975). Lipoxygenase Isozymes of Peanut. Lipids, 10 (11): 681.
6. Siddiqi, A.M. and Tappel, A.L. (1957). Comparison of Some Lipoxidases and Their Mechanism of Action. J. Amer. Oil Chem. Soc., 34 (12): 529.
7. St. Angelo, A.J. and Ory, R.L. (1972). Investigation on Lipoxygenase and Associated Lipid-Oxidizing Systems in Dormant Peanuts. In Symposium: Seed Proteins, C.E. Inglett, ed. Westport, Conn.: The AVI Publishing Co., Inc., p. 284.
8. St. Angelo, A.J., Ory, R.L., and Brown, L.E. (1975). Comparison of Methods for Determining Peroxidation in Processed Whole Peanut Products. J. Amer. Oil Chem. Soc., 52 (2): 34.
9. Yoon, S. (1978). Purification and Characterization of Pea Seed Lipoxygenase Isoenzymes. Ph.D. Thesis, Univ. of Ill. at Urbana-Champaign.