Flavors Derived from Linoleic and Linolenic Acid Hydroperoxides[1]

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ABSTRACT

Hydroperoxides of linoleic and linolenic acid were prepared using soybean lipoxygenase. After purification by silicic acid chromatography, flavors associated with the hydroperoxides, or their breakdown products, in water were characterized by a trained taste panel. Linoleic acid hydroperoxide (50 p.p.m.) was described as predominantly grassy/beany, musty/stale, and bitter. Linolenic acid hydroperoxide (10 p.p.m.) was described with a variety of terms with the most predominant description being grassy/beany followed by bitter and astringent. Each purified hydroperoxide was stored as a 5 to 6% solution in ethanol at −6°C. During the 10-day storage period, no significant changes occurred in the flavor intensity or description of the hydroperoxides. Dilute solutions of linoleic (~50 p.p.m.) and linolenic (~30 p.p.m.) acids in 0.05N borate buffer, pH 9.0, treated with lipoxygenase were tasted directly. Flavor responses were very similar to the responses of the purified hydroperoxides.

When soybeans are water-soaked and ground in the conventional manner of making soy milk, a rancid, green-beany flavor develops (1,2). Lipoxygenase is at least partially responsible for the formation of that flavor as evidenced by Wilkens et al. (1) and Kon et al. (3) when they improved the flavor of soy milk by

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inactivating lipoxygenase. Similarly, Mustakas et al. (4) improved the odor, flavor, and stability of full-fat soy flour by inactivating lipoxygenase.

Lipoxygenase hydroperoxidation of lipids containing the *cis,cis*-pentadiene system such as linoleic acid has been studied (5). Initial reaction products are 13-hydroperoxyoctadecadienoic acid or 9-hydroperoxyoctadecadienoic acid or both depending on the lipoxygenase used (6). These hydroperoxides can be enzymatically or nonenzymatically decomposed to form a variety of compounds. Many of the compounds thought to be formed have flavor and consequently could be partly responsible for flavor in soy products. Several of these compounds have been shown to be among the flavor constituents of cut or ground cucumbers (7) and to be involved in off-flavor development in unblanched peas during storage (8).

Mattick and Hand (9) identified ethyl vinyl ketone as one of the main components of the raw bean flavor of conventionally prepared soy milk. They also showed the probable pathway for the formation of ethyl vinyl ketone by enzymatic oxidation of linolenic acid. Further proof of enzyme involvement in flavor formation in soy products was given by Badenhop and Wilkens (10). They observed formation of 1-octen-3-ol in soybeans during soaking. The product was found to be optically active, indicating that it was formed enzymatically.

More recently Arai et al. (11) have shown the presence of *n*-hexanal and *n*-pentanol in soy products. Evidence was presented that these two compounds can be formed through the breakdown of linoleic acid hydroperoxide (LOHP) formed by lipoxygenase. They also found that *n*-hexanal and *n*-pentanol could be produced enzymatically in a soy flour extract. Grosch and Schwencke (12) identified pentanal, hexanal, hept-2-enal, oct-2-enal, nona-2,4-dienal, deca-2,4-dienal, and pentanol among the volatiles isolated from a reaction of soy lipoxygenase and linoleic acid.

All the work mentioned here indicates that soy lipoxygenase produces flavor compounds. Since none of the analyses of these products involved sensory studies, we determined the flavors of the hydroperoxides of linoleic and linolenic acids using a trained taste panel. Both crude and purified preparations were analyzed by the taste panel in an effort to determine if the flavors of the hydroperoxides and any decomposition products that might have developed are characteristic of raw soy flour.

MATERIALS AND METHODS

Preparation of Purified Hydroperoxides

LOHP and linolenic acid hydroperoxides (LNHP) were prepared with some modifications as described by Gardner (13). Fatty acid (500 mg.) (Applied Science Laboratories, Inc., State College, Pa.) was emulsified with 6 to 8 ml. water and 0.5 ml. Tween 20, then converted to the potassium salt with 0.5M K_2CO_3. Substrate was mixed with 20 mg. of salt-free soy lipoxygenase (Nutritional Biochemical Corp., Lot 4892) in 100 ml. 5.0mM potassium borate buffer pH 9. The mixture (110 ml.) was aerated with pure O_2 in a gas-washing bottle (Fisher Scientific Co., No. 3-037) with Dow AF antifoam added as necessary to reduce foaming. Ultraviolet absorption at 234 nm. was used to follow formation of hydroperoxide. After about 1 hr. the reaction mixture was acidified with 1N HCl, extracted with $CHCl_3$-CH_3OH 2:1 (v./v.), and evaporated to dryness to yield the crude hydroperoxide.

For purification, crude hydroperoxide was chromatographed on a 2.3 X 20 cm. column, packed with 50 g., 100-mesh Mallinckrodt silicic acid. Crude hydroperoxide was slurried in hexane and a small amount of ether for application to the column. Column elution was conducted with stepwise gradient with the following solvents: 70 ml. 10% anhydrous ether in redistilled hexane, 200 ml. 20% ether, 250 ml. 30% ether, 250 ml. 40% ether, and 600 ml. 50% ether. Flow rate was about 2 ml. per min. and 10 to 15 ml. fractions were collected. Absorbance at 234

NAME_____ DATE_____

Please indicate the score by placing a check mark (✓) in the space opposite the proper intensity for Odor (O) and Flavor (F).

1. Smell all the samples
2. Taste all samples, tasting the sample with least odor first

		Sample 1		Sample 2		Sample 3		Sample 4	
		O	F	O	F	O	F	O	F
Bland	10								
Weak	9								
	8								
	7								
Moderate	6								
	5								
Strong	4								
	3								
Very Strong	2								
	1								

Describe predominant odors and flavors by placing a check mark (✓) opposite proper flavors and odors and/or writing description in "other" space

ODOR AND FLAVOR	Sample 1		Sample 2		Sample 3		Sample 4	
	O	F	O	F	O	F	O	F
Bland								
Cereal/Grain								
Grassy/Beany								
Chalky								
Musty/Stale								
Bitter								
Astringent								
Other								

Fig. 1. Score sheet used for flavor evaluation of purified fatty acid hydroperoxides. For crude hydroperoxides the same score sheet was used without the preprinted descriptions.

nm. was read on each fraction before it was evaporated to dryness at room temperature and weighed. Fractions of the hydroperoxide peak were dissolved in absolute ethanol, pooled, and stored at -6°C. The concentration of the stored samples varied from 3.6 to 5.1%. For taste panel work, the hydroperoxides were diluted each day to the appropriate concentration using charcoal-filtered tap water.

Peroxide values (14) were determined on fractions from the hydroperoxide peaks and also on samples of the pooled fraction.

Preparation of Corn Germ Lipoxygenase

Corn germ lipoxygenase had to be partially purified before use primarily because LOHP isomerase (13) interfered with production of hydroperoxide. Hexane-defatted corn germ flour was prepared from a hybrid corn dried at harvest time with ambient air and subsequently stored at 0°F. The flour (9 g.) was stirred with 100 ml. 0.1M phosphate buffer, pH 6.8, for about 1 hr at 0°C. The mixture was adjusted to pH 4.5, centrifuged, and the supernatant carefully filtered through glass wool to eliminate all traces of precipitate. The supernatant was then adjusted to pH 6.5 to 7.0. Lipoxygenase was precipitated between 40 and 50% $(NH_4)_2SO_4$ saturation. The precipitated lipoxygenase was dissolved in 0.1M phosphate buffer, pH 6.8, and stored at -6°C.

Preparation of Crude Hydroperoxides

Purified fatty acid (10 to 50 mg.) was diluted to 500 ml. with 0.05M borate buffer which had been flushed with nitrogen. The mixture was stirred for about 16 hr. with continuous nitrogen bubbling. Two hours before tasting, the solution was divided in half. One half had no further treatment, while the other half was flushed with air. To the aerated half, 1 to 3 mg. of commercial soy lipoxygenase was added, stirring continued for about 45 min., and the sample then given to the panel. Formation of hydroperoxide was evident by increased absorbance at 234 nm. Mixtures of 50 p.p.m. linoleic and 10 p.p.m. linolenic acids were prepared in the same manner.

Samples to be reacted with corn lipoxygenase had to be prepared somewhat differently because of the lower pH optimum of the corn enzyme. Fatty acid (10 to 50 mg.) was converted to the potassium salt with potassium hydroxide, and dissolved in a few drops of ethanol. After substrate preparation the procedure was the same except that 0.01M sodium phosphate buffer, pH 6.8, was used instead of borate buffer.

Flavor Evaluation

Sixteen panel members were first tested for their taste acuity using caffeine for bitter (42 to 1,350 p.p.m.), lima bean extract for beany (0.002 to 0.25%), *cis*-3-hexenol for grassy (0.05-3.2 p.p.m.), white corn grits for cereal/grain (8 to 32%), calcium carbonate for chalky (0.25 to 2%), and tannic acid for astringent (0.025 to 0.2%). Then they were trained to recognize the flavor categories used on the score sheet in Fig. 1. Some difficulty arose during the initial testing and training of panel members. With the standards used for grassy and beany, most of the panel could not consistently differentiate the two flavors at low concentrations; hence, they were combined into one category.

Panel was conducted with 7 to 14 members from 9:00 to 9:30 a.m. daily in a room specifically designed for sensory evaluation. Ten-milliliter portions of samples

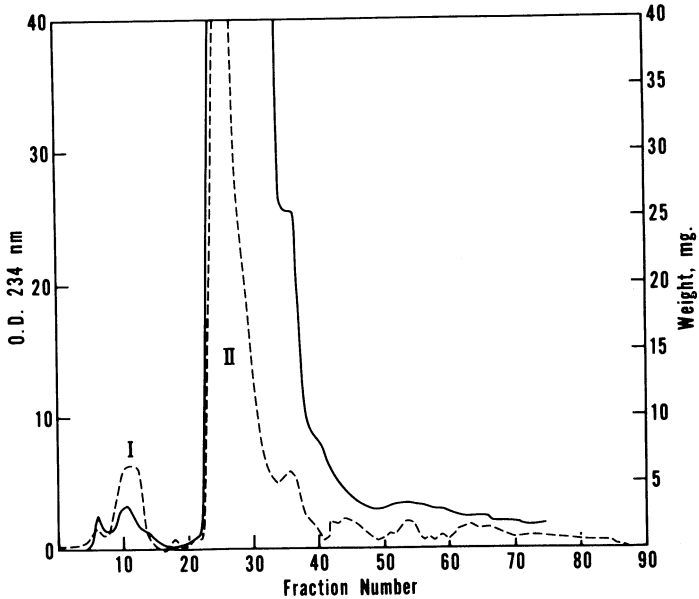

Fig. 2. Elution diagram of crude linolenic acid hydroperoxide from a silicic acid column. Peak I represents unreacted fatty acid and Peak II is the hydroperoxide.

in 50-ml. beakers with glass covers were given at room temperature. Two samples were presented each day in a random order. A laboratory-prepared hexane-defatted soy flour (0.25%) was used as a control for all tastings. It has been observed (15) that this concentration is above the threshold level for beany and bitter flavors. Charcoal-filtered tap water was provided for rinsing between samples. The score sheet shown in Fig. 1 was used to evaluate the purified hydroperoxides. For the crude hydroperoxides, the same score sheet was used without the preprinted flavor descriptions.

RESULTS AND DISCUSSION

Preliminary tastings of the fatty acids, buffers, lipoxygenase, and Tween 20 in dilute solution indicated that chromatographic separation of the reaction mixture was necessary because Tween 20 had a very bitter taste. After observing the concentrations of LOHP and LNHP, which gave grassy/beany responses by the taste panel, we were able in later studies to prepare and taste the hydroperoxides in dilute solutions in the absence of Tween 20.

Purified Hydroperoxides

Figure 2 shows a typical elution pattern from silicic acid chromatography of LNHP. Peak I represents the unreacted fatty acid while Peak II represents purified hydroperoxide. The same elution pattern was obtained when LOHP was chromatographed. Presence of hydroperoxide was confirmed by 234 nm. absorbance and peroxide analysis. Yields were 20 to 40%. Initial yields were low, but yields can be increased by using a more dilute reaction mixture.

Throughout this paper, we refer to purified LNHP and LOHP even though there is undoubtedly continual breakdown of the hydroperoxides with storage. The amount of decomposition and change was not measured, as the main interest was in the flavor of the hydroperoxides and any products arising from them. After several weeks of storage at -6°C., however, 234 nm. absorbance was decreased only slightly (~10%); peroxide value (PV) was at maximum theoretical value. PV analysis in this concentration range has a large error factor but is indicative of the concentration.

Christopher and Axelrod (6) have reported that the Theorell enzyme (L_1) forms only the 13-hydroperoxydecadienoic acid whereas the isozyme, L_2, produces a 50:50 mixture of 9 and 13 isomers. In this study, the commercial enzyme was used exclusively for soy lipoxygenase. The enzyme sample probably is a mixture of L_1 and L_2, but at pH 9 the major product would be the 13 isomer. Corn germ lipoxygenase, which Gardner and Weisleder (16) have reported yields predominantly the 9-hydroperoxide, was used for comparative purposes and also as an indication of the flavors which would be produced by lipoxygenase L_2. Purified LNHP and LOHP prepared using soy lipoxygenase (LNHPs and LOHPs) and LNHP formed with corn lipoxygenase (LNHPc) were tasted by the panel 1, 3, 6, 8, and 10 days after purification. Comparison of the flavor scores of the hydroperoxides with the scores of the soy flour control showed no significant change in flavor scores for each 10-day series. Trials I and II of the LNHPs were also tasted after 26 days of

TABLE I. FLAVOR EVALUATION OF THE PURIFIED LINOLEIC AND LINOLENIC ACID HYDROPEROXIDES TASTED 1, 3, 6, 8, AND 10 DAYS AFTER PREPARATION, STORED AT -6° C.

Hydroperoxides	LNHPs(I)[a]	LNHPs(II)	LOHPs	LNHPc	Soy Flour, 0.25%
Concentration, p.p.m.	10	10	50	10	...
Average flavor score[b,c]	5.5	5.2	5.5	4.6	5.6
Range of flavor scores	5.1-6.0	5.1-5.5	5.1-5.9	4.3-4.9	5.1-6.3
Flavor description			% Tasters		
Grassy/beany	98	90	80	85	94
Bitter	18	19	16	20	30
Astringent	17	19	19	10	34
Raw vegetable flavors[d]	12	16	20	14	...
Fruity flavors[e]	13	...	5	18	...
Chalky	4	5	6	...	23
Cereal/grain	2	...	8	...	20
Musty/stale	5	...	35	6	8
Rancid oil	...	5	44	...	3
Other[f]	8	18	15	35	7

[a]LNHPs(I) = Linolenic acid hydroperoxide prepared with soy lipoxygenase, first trial; LNHPs (II) = linolenic acid hydroperoxide prepared with soy lipoxygenase, second trial; LOHPs = linoleic acid hydroperoxide prepared with soy lipoxygenase; LNHPc = linolenic acid hydroperoxide prepared with corn lipoxygenase.

[b]Average of flavor scores from 1-, 3-, 6-, 8-, and 10-day tastings.

[c]Standard deviation of flavor scores was 0.38.

[d]Represents a variety of descriptions including peas, cabbage, cucumber, onion, pumpkin, rutabaga, water cress, and raw vegetable.

[e]Represents a variety of descriptions including watermelon, watermelon rind, melon, blackberries, and fruity.

[f]"Other" = sum of descriptions given by less than 10% of the panel.

TABLE II. FLAVOR EVALUATION OF CRUDE LINOLEIC AND LINOLENIC ACID HYDROPEROXIDES

Fatty Acid/Enzyme	LO[a]	LO+SL	LN	LN+SL	LO+LN	LO+LN+SL	LO	LO+CL	LN	LN+CL	LO+LN	LO+LN+CL	LO+LN	LO+LN+SL+CL	Soy Flour, 0.25%
Concentration-fatty acid, p.p.m.	51		33		50,10		50		50		50,10		50,10		
Flavor score	5.4	4.9	5.0	2.4	3.4	3.5	6.1	4.7	5.1	3.6	6.7	4.8	6.3	4.3	5.4[b]
Flavor description							% Tasters								
Grassy/beany	...	80	23	84	...	89	...	22	36	36	18	45	17	92	98[b]
Bitter	50	40	54	54	88	63	44	22	...	27	45	27	25	25	36
Fishy	...	...	46	23	25	...	...	...	18	18	...	...	...	...	...
Soapy	30	...	...	...	...	...	...	...	...	...	18	18	...	...	...
Melon							...	11	27	45	36	36	...	8	...
Oily									...	18	18	27	17	25	...
Rancid	20				...	...	...	...							
Musty							22	22							
Chalky							22					18			
Astringent							22						18		
Paint							22								

[a]LO = Linoleic acid; SL = soy lipoxygenase; LN = Linolenic acid; CL = corn lipoxygenase.

[b]Average value from seven trials.

storage with no significant change in flavor score. Consequently, only the average flavor scores of each 10-day series and the range of flavor scores are reported. Soy flour scores are an average of 15 days of tasting (three series). Results of tastings are shown in Table I. In general, for a difference in flavor score to be significant at the 95% level, there must be a difference of one unit between tastings. Actual intensity score of the samples has little significance since the concentrations of the samples were adjusted to the middle range of the score sheet.

Even though the scores for all the hydroperoxides were about the same, the LNHPs samples were tasted at a concentration of 10 p.p.m., five times lower than the LOHPs sample at 50 p.p.m. This would indicate that the flavor intensity of LNHP from both corn and soy is about five times more intense than LOHP.

No change in flavor descriptions with time of storage was evident so the results of each series were averaged for Table I. A variety of descriptions were given by less than 10% of the panel so they were grouped into the "Other" category. Two exceptions to this were the categories of raw vegetable flavors and fruity flavors, which also represent a variety of flavors that were usually reported by only one or two panel members each day.

The reason for combining grassy and beany into one category was discussed briefly in Materials and Methods. As stated previously, the panel could not consistently distinguish the two flavors with the standards used. Although the two flavors do not appear to be identical, the category is intended to describe a type of flavor including such descriptions as green beany, raw beany, green, pea shells, grassy, and beany. If good standards were available, the panel trained intensively, and flavors of the samples presented not complex mixtures, it might be possible for the panel to consistently distinguish these flavors. For the present work, we feel justified in combining these flavors into one category and feel that it is a meaningful description.

The descriptions of LNHPs trials I and II agree well except in the raw vegetable and fruity categories. The panel's difficulty in defining the flavor present in these samples accounts for their inconsistency. Moreover, these categories were formed after the testing was completed for clarity in presenting the results. The flavor descriptions of LNHPs and LOHPs were similar with the exception of musty/stale and rancid oil which were predominant responses for LOHPs. Descriptions of LNHPs and LNHPc were almost identical, although there were a greater number of "Other" notations for LNHPc. Soy flour (0.25%) was defined as bitter, astringent, chalky, and cereal/grain by 10 to 15% more panel members than any of the hydroperoxides. In contrast, it had none of the raw vegetable or fruity flavors reported for all of the hydroperoxides and only a small percentage of musty/stale and rancid oil responses typical of LOHPs. Although the flavor responses for the hydroperoxides are not identical to those of uncooked soy flour, they are similar for the major categories of grassy/beany, bitter, and astringent. However, when the LOHP and LNHP were purified some of the flavor descriptions, which resemble raw soy, may have been removed.

Crude Hydroperoxides

Since only very dilute solutions of the LOHP and LNHP are needed for flavor responses, we were able to prepare hydroperoxides in dilute solution without Tween 20, which is bitter.

In this second method, dilute solutions of fatty acids were reacted with enzyme in buffer. Since the products were tasted directly without purification, they will be referred to as crude LNHP and LOHP. Untreated fatty acids in buffer and 0.25% soy flour were given to the panel as controls. Results of tasting the crude hydroperoxides are shown in Table II.

Yields were 50 to 95% as determined by 234 nm. absorbance and thin-layer chromatography (13). Because of the variation in yields, flavor scores cannot be compared except in the same test, i.e., the fatty acid and enzyme-treated fatty acid in each test. Again, for two flavor scores to be significantly different, there must be a difference of about one unit. The score sheet used for the crude hydroperoxides was slightly different from that in Fig. 1. The descriptions were omitted so the panel members could use any description they desired. The main reason was to see how the panel would describe the grassy/beany-type flavors, because some doubts were raised on the validity of this category. Since many of the panel members continued to use the grassy/beany description and those who did not were not consistent, all responses of this type were combined into the grassy/beany category for Table II. The flavor score and percentage of taster values for soy flour are averages of the seven tastings.

There is a significant decrease in flavor score of the samples treated with lipoxygenase except for linoleic acid (LO) treated with soy enzyme and LO-linolenic acid (LN) combination treated with soy lipoxygenase. The changes occurring in flavor of LN and LO with lipoxygenase addition can best be observed in the flavor descriptions. In Table II any description not reported by 20% or more of the panel was deleted. Increases in the percentage of responses are obviously due to enzyme action; however, decreases may be due to masking rather than to a true reduction in flavor.

Although there was no significant change in flavor score of LO with soy lipoxygenase treatment, there is a marked change in flavor description. Grassy/beany response rose from 0 to 80% with decreases in response of bitter, soapy, and rancid. When LN was treated with soy, the grassy/beany response increased, the fishy response decreased, and bitter response remained constant. Also, a significantly lower flavor score indicates an increase in flavor intensity with formation of LOHPs. Similarly, when a LO and LN mixture was treated with soy lipoxygenase an increase from 0 to 89% in grassy/beany response can be seen with decreases in bitter and fishy. Again, as with LO alone, there was little change in flavor score.

When LO was reacted with corn lipoxygenase the flavor score dropped significantly. There were increases in grassy/beany and melon responses, and responses for bitter decreased. While there was a decline in flavor score of LN when treated with corn enzyme, changes in description were principally enhanced bitter, melon, and oily responses. Corn lipoxygenase treatment of a LO and LN mixture resulted in more grassy/beany response compared with the fatty acids, and also slight gain in oily and chalky descriptions. Flavor score was lowered when LN and LO were mixed with corn and soy lipoxygenases; grassy/beany response increased by 75% and bitter remained constant. Oily and melon responses became slightly greater, but astringent declined.

In general, when soy lipoxygenase was added to LO and LN, the grassy/beany response increased greatly and flavor score either decreased or remained constant.

One reason that there was not a greater decline in flavor score could be that the fatty acid was partially autoxidized and already had a flavor of its own; e.g., principally bitter. The effect of adding corn lipoxygenase to the fatty acids was consistently a lowering of flavor score. However, the flavor description changes are not as distinct as with the soy enzyme-treated samples, which had definitely more grassy/beany responses.

SUMMARY AND CONCLUSIONS

Flavors reported by the panel for the purified hydroperoxides show them to be similar to soy flour especially in respect to the grassy/beany, bitter, and astringent responses. The predominant response for both soy flour and the purified hydroperoxides was grassy/beany. Results of taste panel evaluation of the crude hydroperoxides show perhaps more clearly the effect of lipoxygenase on fatty acids in producing flavor. Soy lipoxygenase invariably augmented the grassy/beany response. Corn lipoxygenase always increased the flavor intensity but a variety of flavors were produced instead of one major flavor as with soy lipoxygenase.

Overall, the results show that while soy lipoxygenase does not produce the identical flavors of raw soy flour, the hydroperoxides and their decomposition products have similar flavors especially of the grassy/beany type. Therefore, these compounds must certainly contribute to the flavor of soy products. Further work on identification of the decomposition products and also the effect of other enzymes on the flavor of the hydroperoxides will undoubtedly show more clearly the extent lipoxygenase plays in producing flavor in soy.

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Literature Cited

1. WILKENS, W. F., MATTICK, L. R., and HAND, D. B. Effect of processing method on oxidative off-flavors of soybean milk. Food Technol. 21: 1630 (1967).
2. WILKENS, W. F., and LIN, F. M. Gas chromatographic and mass spectral analysis of soybean milk volatiles. J. Agr. Food Chem. 18: 333 (1970).
3. KON, S., WAGNER, J. R., GUADAGNI, D. G., and HORVAT, R. J. pH adjustment control of oxidative off-flavors during grinding of raw legume seeds. J. Food Sci. 35: 343 (1970).
4. MUSTAKAS, G. C., ALBRECHT, W. J., McGHEE, J. E., BLACK, L. T., BOOKWALTER, G. N., and GRIFFIN, E. L., Jr. Lipoxidase deactivation to improve stability, odor, and flavor of full-fat soy flours. J. Amer. Oil Chem. Soc. 46: 623 (1969).
5. DOLEV, A., ROHWEDDER, W. K., and DUTTON, H. J. Mechanism of lipoxidase reaction. I. Specificity of hydroperoxidation of linoleic acid. Lipids 2: 1 (1967).
6. CHRISTOPHER, J., and AXELROD, B. On the different positional specificities of peroxidation of linoleate shown by two isozymes of soybean lipoxygenase. Biochem. Biophys. Res. Commun. 44: 731 (1971).
7. GROSCH, W., and SCHWARZ, J. H. Linoleic and linolenic acid as precursors of the cucumber flavor. Lipids 6: 351 (1971).
8. GROSCH, W. Linoleic and linolenic acids as substrates for the enzymic formation of volatile carbonyl compounds in peas. Z. Lebensm. Unters. Forsch. 137: 216 (1968).
9. MATTICK, L. R., and HAND, D. B. Identification of a volatile component in soybeans that contributes to the raw bean flavor. J. Agr. Food Chem. 17: 15 (1969).
10. BADENHOP, C. F., and WILKENS, W. F. The formation of 1-octen-3-ol in soybeans during soaking. J. Amer. Oil Chem. Soc. 46: 179 (1969).

11. ARAI, S., NOGUCHI, M., KAJI, M., KATO, H., and FUJIMAKI, M. Studies on flavor components in soybean. Part V. *n*-Hexanal and some volatile alcohols. Their distribution in raw soybean tissues and formation in crude soy protein concentrate by lipoxygenase. Agr. Biol. Chem. 34: 1420 (1970).
12. GROSCH, W., and SCHWENCKE, D. Soybean lipoxygenase: volatile aldehydes and alcohols from linoleic acid. Lebensm. Wiss. Technol. 2: 109 (1969).
13. GARDNER, H. W. Sequential enzymes of linoleic acid oxidation in corn germ: lipoxygenase and linoleate hydroperoxide isomerase. J. Lipid Res. 11: 311 (1970).
14. AMERICAN OIL CHEMISTS' SOCIETY. Official and tentative methods (3rd ed.). The Society: Chicago (1965).
15. KALBRENER, J. E., ELDRIDGE, A. C., MOSER, H. A., and WOLF, W. J. Sensory evaluation of commercial soy flours, concentrates, and isolates. Cereal Chem. 48:595 (1971).
16. GARDNER, H. W., and WEISLEDER, D. Lipoxygenase from Zea mays: 9-D-Hydroperoxy-*trans*-10,*cis*-12-octadecadienoic acid from linoleic acid. Lipids 5: 678 (1970).

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