Lipoxygenase and Peroxidase Activities of Soybeans as Related to the Flavor Profile During Maturation[1]

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ABSTRACT

Hawkeye and Amsoy soybeans were picked at 15 intervals from 24 to about 66 days after flowering. During maturation, percentage of dry matter increased from 16 to 92 and total dry matter from 5 to 243 mg. per bean. Flavor intensities of each picking were determined by a taste panel. Lipoxygenase activity of beans of similar fresh weight, picked on the same day, was measured by the oxygen uptake method. Beany and bitter were the two predominant flavors in maturing soybeans. Most tasters used the terms green-beany, beany, and raw beany to describe the "beaniness" of soybeans. Grassy was an infrequent response. Beany and bitter flavor responses were recorded as flavor intensity values (FIV) based on a scale of 1 for weak, 2 for moderate, and 3 for strong. FIV with respect to beany varied from 2.0 to 2.7 during maturation, with the average being 2.4; no significant trends were noted. The average FIV for bitter for the two varieties increased threefold from about 0.54 in immature soybeans to 1.9 at maturity. Lipoxygenase activity at pH 6.8 varied from a low of 12 μliters O_2 per min. per mg. dry matter in the early stages to 35 at 34 days after flowering, then down to 23 at 45 days, up to 43 at about 1 week before maturity, and then slowly decreased to about 32 μliters O_2 uptake at maturity; O_2 uptake values of 11 to 23 μliters were recorded for lipoxygenase activity at pH 9.0. The FIV for beany did not correlate with changes in lipoxygenase activity; however, a correlation ($r = 0.73$) exists between lipoxygenase activity and the increase in FIV for bitter flavor as beans mature. An active peroxidase capable of utilizing linolenic hydroperoxide was also present. Peroxidase activity remained relatively constant throughout most of the maturation period, except for a large decrease at maturity.

The major objectionable flavors in raw, full-fat, and defatted soy flours in order of decreasing intensity are: beany, bitter, and green (1). Flavor scores above 7 are obtained with soy flakes extracted with 80% ethanol or isopropanol (1). After raw soy flour was steamed 10 to 40 min., flavor scores increased from 1.5 to a maximum of about 6.0 to 6.3 and the green flavor disappeared (1). The predominant flavors in commercially manufactured soy flours, concentrates, and isolates have been described as beany and bitter (2). Honig et al. (3) showed that residual lipids and most of the flavor in raw defatted flakes can be readily extracted with hydrogen-bond breaking solvents, such as hexane-alcohol azeotropes and hot 95% ethanol. Although bitter and other objectionable flavors occur in the various fractions isolated from the azeotrope extracts, no beany flavor was detected (3). Flavor scores of hexane-alcohol azeotrope-extracted flakes and of isolates prepared from these extracted flakes improve significantly; however, the two predominant soybean flavors, beany and bitter, remain (4). Two bitter components, ethyl-α-D-galactopyranoside and L-tryptophan, have been isolated from

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hexane-ethanol azeotrope extracts of full-fat and defatted flakes (5). The galactoside is an artifact formed during extraction. Ethyl galactoside and L-tryptophan do not contribute to the bitterness of soy flakes since the amounts present are well below their bitter threshold level (5).

The triglycerides of soybeans undergo considerable transformation in composition during maturation (6). Linolenic acid decreases from 34.2 to 11.7%; the percentages of linoleic and oleic acids increase (6). Composition and concentration of phospholipids and glycolipids also change during maturation (7).

This study was undertaken to determine by taste-panel evaluation at which maturation stage beany and bitter flavors develop, and to ascertain whether the development of these characteristic soybean flavors can be correlated with lipid transformation known to occur during maturation. Changes in the flavor profile of soybeans and their relationship to lipoxygenase activity during maturation were also determined.

MATERIALS AND METHODS

Soybeans

Hawkeye soybeans were tested in 1969, and the Amsoy variety was evaluated in the 1970 crop year. Plants were obtained from the same farm. At 24 days after flowering, plants were pulled about 7:00 a.m. on the day of testing, they were brought to the laboratory, and the pods were shucked by hand. Fragments of endocarp, which adheres to the early green seeds, were removed by gently rubbing the seeds. Varying amounts of whole beans of uniform fresh weight were given to the taste panel. The number of beans given was estimated to approximate as nearly as possible the amount of dry matter present in a single mature bean. The beans were placed in a beaker and covered with a damp cloth until the taste-panel evaluation was made between 9:00 and 9:30 a.m. Beans of comparable fresh weight were also collected for determination of dry matter and lipoxygenase activity. Stages of maturation were characterized by fresh-weight data, descriptions of the seed appearance, and days after flowering.

Dry matter is given as a percentage of the fresh weight. Dry weights were determined by drying seeds in a forced-draft oven for 2 hr. at 130°C. There was no additional loss of weight of seeds when dried for 3 hr. Larger seeds picked 40 days or later after flowering were split into halves to facilitate drying.

Taste-Panel Evaluation

The number of tasters varied between 12 and 15 throughout the tests. They were asked to describe the predominant flavors and to list other flavors detected. A flavor intensity value (FIV) served to evaluate changes in predominant flavors that occurred during maturation (8). FIV equals the intensity-weighted summations of the flavor responses divided by the number of tasters: FIV = [(number of weak responses) + 2 (number of moderate responses) + 3 (number of strong responses)] /n, where n is the number of tasters. Limits of the flavor-intensity values will, of course, be from 0.0 to 3.0. This formula allows for those tasters showing no response.

Extracts for Enzyme Assay

A mortar and pestle containing 50-mesh white sea sand was cooled in an ice

bath. Soybeans were then added and ground until a smooth paste formed. A calculated amount of buffer was added to give an approximate solvent:bean ratio of 20:1, taking into account the amount of water in the beans based on previously determined moisture analyses. This approximation of the ratio was corrected when the moisture content of the beans for that day was determined. For lipoxygenase assay, the soybean samples were ground in 0.165M phosphate containing 0.59 mM Ca^{+2}. To assay for peroxidase, the extracts were prepared in pH 6.5 citrate-phosphate buffer (9).

The bean paste, in added buffer, was reground until a smooth green-milky mixture was produced. After this mixture was centrifuged in a refrigerated centrifuge at 5°C. for 10 min. (RCF = 17,000 X g), the milky top layer containing the soybean oil globules (spherosomes) was carefully sucked off with a capillary tube attached to a water aspirator. The supernatant was recentrifuged (RCF = 12,100 X g) for 5 min., and any spherosomes remaining were removed.

Lipoxygenase Assay

Linoleic acid (The Hormel Institute, Austin, Minn.) substrate was prepared by slowly adding 0.1N KOH to 50 μliters linoleic acid and 50 μliters Tween 20 until the solution became clear, and then diluting to 10 ml. with distilled water.

Aliquots of the soybean extract were diluted with phosphate buffer, pH 6.8, or with 0.165M borate buffer, pH 9.0, since soybeans contain two distinct isoenzymes of lipoxygenase which differ in their pH activity profile (10). Enzyme activity was determined by measuring O_2 uptake in a Gilson Differential Respirometer at 15°C. Several controls were used: a) soybean lipoxygenase (salt-free; 20,000 units per γ) purchased from Pierce Chemical Co., Rockford, Ill., was used with each analysis to determine whether significant changes in O_2 uptake occurred as new buffers and substrates were prepared; lipoxygenase solutions were made up fresh; b) the substrate control vessel contained linoleic acid and buffer to determine O_2 uptake resulting from autoxidation of the substrate; and c) the endogenous soybean control consisted of the diluted buffered extract of the immature beans without added substrate.

Lipoxygenase activity in the soybean extracts was determined from the O_2 uptake calculated from the initial slope of the plotted data (μliter O_2 vs. time) after correction for O_2 uptake occurring in the substrate control vessel and the endogenous control vessel at the same dilution and over the same time period. The substrate-control data were also used to correct for the O_2 uptake occurring with the lipoxygenase standard. Enzyme activity was calculated in terms of μliter O_2 per min. per mg. dry matter.

Peroxidase Assay

For the quantitative estimation of peroxidase activity, a modified procedure of Vetter et al. (9) was employed. The extracts were diluted to about 1:100,000, and 5 ml. of diluted extract was mixed with 0.2 ml. of 1% aqueous *o*-phenylene diamine and 0.2 ml. of 0.3% H_2O_2. Absorbance at 430 nm. was measured in a Beckman DB spectrophotometer. To determine optimum pH of soybean peroxidase, the initial extract in citrate-phosphate pH 6.5 was diluted with citrate-phosphate buffer of various pH values.

RESULTS AND DISCUSSION

Weight and Color Changes in Maturing Soybeans

Analyses of fresh weight, percent dry matter, and color of soybeans in relation to days after flowering, for soybeans grown in 1969 and 1970, are given in Tables I and II, respectively. Although two different soybean varieties are compared, the relationship between fresh weight and percent dry matter and the onset of the yellowing stage were in good agreement with each other. At every subsequent picking, percent dry matter for beans of fresh weight comparable to those which were analyzed in the previous test was always redetermined. The results indicated that the percent dry matter did not differ more than ±1%, even when the beans were picked as much as a week apart. These analyses indicated that fresh weight and percent dry matter, along with days after flowering, may be used as indexes of degree of maturity.

Flavor Evaluation of Maturing Soybeans (1969 Crop Year)

The FIV's of maturing Hawkeye soybeans in relation to days after flowering and fresh weight are shown in Table III.

The tasters were instructed to record the predominant flavors and to record the intensity of each. Only two predominant types of flavors, beany and bitter, were given throughout the test. All of the tasters, except one, described the "beaniness" of maturing soybeans as: green-beany, beany, and raw beany. Other flavors recorded infrequently included: grassy, raw pea shells, and raw peas.

Attempts were made to equalize the amount of dry matter tasted throughout the test period by decreasing the number of beans per taster as the soybeans matured. In the early stages of maturity, the beans were so small that enough of them could not be easily harvested. At 24 days after flowering, a beany FIV below 2.0 could have resulted from the much smaller amount of dry matter tasted;

TABLE I. FRESH WEIGHT, DRY MATTER, AND COLOR CHARACTERISTICS OF MATURING SOYBEANS (1969 CROP YEAR)

Days After Flowering[a]	Average Fresh Weight mg./seed	Dry Matter %	Color of Beans	
			Pods	Seeds
22	30[b]	16.0	Green	Green
24	59	22.2	Green	Green
27	131	23.0	Green	Green
29	220	23.2	Green	Green
29	295	27.6	Green	Green
31	313	28.0	Green	Green
35	384	30.0	Green	Green
40	498	32.4	Green	Green
44	568[c]	39.0	Yellow	Light green
49	523	42.7	Yellow	Yellow-green
52	440	49.6	Brown	Yellow
55	331	71.2	Brown	Buff-brown
59[d]	253	84.1	Brown	Buff-brown
64[d]	209	91.6	Brown	Buff-brown

[a]Flowering date, July 14, 1969; Hawkeye soybeans.

[b]Range in weight within ±5%.

[c]Maximum fresh weight occurs during the yellowing stage.

[d]Mature beans after harvest.

TABLE II. FRESH WEIGHT AND DRY MATTER OF MATURING SOYBEANS (1970 CROP YEAR)[a]

Days After Flowering[b]	Average Fresh Weight mg./seed	Dry Matter %
24	73	19.0
25	88	20.0
28	157	22.8
31	265	26.9
34	380	32.4
38	415	35.1
41	460	35.9
45	510	38.4
47	555	39.1
49[c]	570	40.0
53[c]	577	40.4
56	473	45.6
59	466	47.7
63	359	59.6
66(mature)	300	81.0

[a]Color changes same as shown in Table I.

[b]Flowering date, July 17, 1970; Amsoy soybeans.

[c]Maximum fresh weight at yellowing stage.

TABLE III. FLAVOR EVALUATION OF MATURING SOYBEANS (HAWKEYE VARIETY, 1969 CROP)

Days After Flowering	Dry Matter Tasted mg.	Beany[a] FIV[b]	Bitter %[c]	Bitter FIV
24	21	1.6	25	0.40
24	26	1.7	33	0.42
27	60	2.0	21	0.29
27	102	2.1	36	0.43
29	104	2.5	29	0.57
29	152	2.4	29	0.43
31	175	2.5	42	0.65
33	115[d]	2.0	25	0.50
35	232[d]	2.4	43	0.57
40	169	2.4	36	0.64
44	222	2.2	38	0.54
49	223	2.0	69	1.10
52	219	2.0	50	0.94
55	236	2.1	57	1.10
59	216	2.7	77	1.60

[a]Includes all "beaniness" responses: green-beany, beany, and raw beany. Except for one taster, all the others recorded a positive beaniness response.

[b]Flavor intensity value (FIV) = [(number of weak responses) + 2 (number of moderate responses) + 3 (number of strong responses)]/n, where n is the number of tasters.

[c]Percent of tasters giving a positive bitter response; total number of tasters ranged between 12 and 15.

[d]Repeat of taste test at twice the level of intake of beans at the same maturity level.

otherwise, the beany intensity did not change much with increasing maturity as indicated by the small differences in FIV. To about 44 days after flowering, 71 to 92% of the tasters recorded green-beany; after 44 days, 50 to 62% of the tasters recorded green-beany.

A different pattern was observed for the bitter flavor. The average FIV for bitter was 0.39 in the early stages of maturity (range 0.29 to 0.43). As soybeans matured however, the FIV for bitter increased (about fourfold) to a maximum value of about 1.6 at maturity. This increase in bitterness may represent an increase in the concentration of the bitter principle, since the number of positive responses also increased from 25 to 77%.

There appears to be maximum development in the intensity of the beany flavor even in the early stages of maturation, since the FIV for beaniness of a single bean did not vary significantly from that obtained with two of the same beans (see Table III, footnote d). On the other hand, the bitter FIV at about 35 days after flowering is very low compared to that of beans at maturity, even at comparable levels of dry matter tasted.

Immature soybeans at a level of maturity comparable to those evaluated 31 days after flowering were also analyzed for possible changes in beany and bitter flavors in relation to days after flowering. For green soybeans of about 300 mg. (295 to 308 mg.) fresh weight and 27.6% dry matter picked 29 and 33 days after flowering, beany FIV's were 2.2 and 2.3, respectively. A bitter FIV of 0.43 was obtained.

Flavor Evaluation of Mature Soybeans

Before the 1970 series was conducted, harvested Hawkeye soybeans from the 1969 series were given to the panel members several times to determine variability in FIV and to ascertain whether tasters could consistently differentiate between the beaniness terms recorded in the 1969 series. To eliminate any differential responses because of the hardness of mature soybeans, the beans were passed through cracking rolls, and the split cotyledons were then dehulled and given to the panel in two forms: dry cotyledons (8.5% moisture) and soaked cotyledons (25% moisture). The soaked cotyledons were prepared by putting the required amount of dry cotyledons into a beaker containing enough distilled water to increase moisture content to 25%. The beakers were swirled occasionally until the water was absorbed in about 40 to 45 min. The types of beaniness responses reported by the taste panel are listed in Table IV.

The most predominant responses were: green-beany, raw beany, and beany. Most of the tasters consistently recorded only one of these descriptions, whereas some tasters used these various beaniness responses interchangeably, much like that observed in the 1969 tests with maturing soybeans (Table III). All tasters, however, recorded a beany response.

On the basis of these results, it was concluded that some of the tasters were recording various descriptions of beaniness without actually being able to discriminate between these terms from one test to another. As a result, the tasters were instructed to record the intensity of beaniness (green-beany, beany, and raw beany) under one collective term; namely, beany. Both beany and bitter FIV's of mature soybeans are summarized in Table V.

Based on four evaluations, the beany FIV's of Hawkeye soybeans harvested in 1969 averaged 2.5 for both dry and soaked cotyledons. Some variability in the degrees of bitterness was observed in these tests; however, the differences in bitterness between dry and soaked cotyledons were small. The bitter FIV of mature soybeans was about 1.0. At the levels given, 50% of the taste panel gave a positive response for bitter flavor.

TABLE IV. TYPES OF BEANINESS FLAVOR RESPONSE TO MATURE SOYBEANS[a]

Flavor Response	Number of Positive Responses in Cotyledons			
	Dry		Soaked	
	A	B	A	B
Green-beany	2	5	5	5
Beany	6	3	4	3
Raw beany	5	4	5	4
Raw pea shells	1	1	1	0
Grassy	0	0	0	1
Green peas	0	0	0	1
Total number of tasters	13	12	13	12

[a]Hawkeye soybeans, 1969 crop year.

TABLE V. BEANY AND BITTER FIV'S OF MATURE SOYBEANS[a]

Flavor	FIV[b]	
	Dry cotyledons	Soaked cotyledons
Beany[c]	2.38	2.54
	2.58	2.25
	2.60	2.57
	2.53	2.60
Average	2.5	2.5
Bitter	0.90	0.85
	0.90	1.02
	1.07	1.0
	1.27	1.13
Average	1.0	1.0

[a]Hawkeye soybeans, 1969 crop; 120 mg. dry matter tasted.

[b]See footnote b, Table III.

[c]To include all beaniness responses: green-beany, beany, and raw beany.

TABLE VI. FLAVOR EVALUATION OF MATURING SOYBEANS (AMSOY VARIETY, 1970 CROP YEAR)

Flowering	Dry Matter Tasted mg.	Beany[a]		Bitter	
		%[b]	FIV[c]	%	FIV
24	42	100	2.4	42	0.67
25	53	100	2.5	54	0.69
28	107	100	2.0	25	0.42
31	142	100	2.3	64	0.91
34	246	90	2.3	50	0.90
38	146	100	2.5	87	1.1
41	151	100	2.1	80	1.2
45	197	100	2.4	69	1.2
47	217[d]	92	2.3	77	1.5
49	228[d]	100	2.5	82	1.4
53	233[d]	100	2.4	82	1.5
56	216	100	2.2	86	1.9
59	222	92	2.5	69	1.5
63	213	100	2.7	100	2.2
66	243	92	2.5	100	2.1

[a]Includes all beaniness responses: beany, green-beany, and raw beany.

[b]Percent of tasters giving a positive response.

[c]See footnote b, Table III.

[d]Repeat at same fresh weight (555 to 577) but different days after flowering.

Flavor Evaluation of Maturing Soybeans, 1970 Series

Taste-panel results of tests on Amsoy soybeans grown in 1970 are given in Table VI. In this series, the tasters were instructed to record intensities (weak, moderate, and strong) of bitter and beany flavors. The latter was considered a collective term for green-beany, beany, and raw-beany responses. There was very little change in the beany flavor of maturing soybeans; the average beany FIV was 2.4 (range 2.0 to 2.7). The bitter FIV increased with maturation, with an overall increase of about threefold compared with a fourfold increase for Hawkeye soybeans, 1969 crop year. The FIV for bitter of beans picked 34 days after flowering is less than half that of mature beans 66 days after flowering even when dry matter tasted is the same. As shown in Table VI, there was no significant change in FIV for beany and bitter for soybeans at the same yellowing stage when picked between 47 and 53 days after flowering.

Except for a beany FIV below 2 for the small beans picked 24 days after flowering in the 1969 series (Table III), the data on the beany and bitter FIV's of beans in the two series of taste evaluations are in good agreement (Tables III and VI). The taste-panel results indicate that the beany flavor factor(s) and the bitter principle(s) are detected during all stages of development from the small immature bean to the fully developed seed.

Lipoxygenase Activity in Maturing Soybeans

Changes in lipoxygenase activity of Amsoy soybeans, 1970 crop year, are shown in Table VII.

Values were calculated from data obtained with aliquots of extracts that gave the highest net rate of O_2 uptake. In all analyses, greatest O_2 uptake per mg. dry matter occurred with extracts diluted 1:400 or 1:625.

TABLE VII. LIPOXYGENASE ACTIVITY IN SOYBEANS DURING MATURATION (AMSOY VARIETY, 1970 CROP YEAR)

Days After Flowering	Fresh Weight mg.	Activity[a] pH 6.8	pH 9.0
24	103	18.9	...
25	110	18.9	...
28	179	11.8	17.0
31	253	28.8	...
34	340	35.3	10.6
38	420	33.4	13.1
41	477	25.7	14.3
45[b]	500	22.5	11.0
49(59)[c]	566 (533)	38.9 (31.6)	23.2
53	588	36.3	...
56[d]	476	42.9	16.4
63	365	35.1	...
66 (mature)	293	32.4	...

[a]Lipoxygenase control, average activity at pH 6.8 throughout test: 13.7 ±1 μliters O_2/min./mg. dry matter.

[b]Beginning of the yellowing stage.

[c]Comparison of activity of beans of similar wet weight picked 10 days apart.

[d]End of yellow bean stage.

Lipoxygenase activity at pH 6.8 varied during maturation but tended to increase with maturation. Activity increased from a low of 12 μliters O_2 per min. per mg. during early stages of maturity to a value of about 35 μliters O_2 at 34 days after flowering, dropped appreciably when yellowing began, and then rose again to a maximum value of 43 μliters O_2 uptake at the end of the yellowing stage (56 days after flowering). From this stage, lipoxygenase activity decreased somewhat as beans reached maturity. Lipoxygenase activity at pH 9.0 remained relatively constant during maturation. Level of activity was always below that at pH 6.8.

Peroxidase Activity in Maturing Soybeans

Peroxidase activity in maturing Harosoy soybeans, 1971 crop, and in raw, dehulled, defatted soy flakes prepared from Amsoy soybeans, 1970 crop, is shown in Table VIII. The pH optimum for peroxidase activity as determined in maturing soybeans was pH 5.0. However, measurements were also made at pH 6.5, since cloudy solutions were encountered in the reaction vessels with pH 5.0 extracts of maturing soybeans at the yellowing stage. In addition, protein precipitation occurs in extracts of defatted flakes below pH 6.5.

The level of peroxidase activity in maturing soybeans is relatively constant throughout most of the maturation period. Peroxidase activity decreased greatly at maturity (72 days after flowering). Activity in raw flakes is very low. The relative amounts of lipoxygenase and peroxidase in maturing soybeans were not determined. It appears, however, that peroxidase activity was greater since

TABLE VIII. PEROXIDASE ACTIVITY IN MATURING SOYBEANS[a]

Days After Flowering	Fresh Weight mg.	Dry Matter %	Activity[b] pH 5.0	Activity[b] pH 6.5
35	283	25.8	...	14.2
40	376	31.3	46.2	16.4
42	452	35.3	40.5	13.5
44	470	36.8	42.1	18.9
48	516	37.4	45.2	17.4
51	577[b]	39.6	48.5	15.6
55	500	44.6	48.5	16.1
68	300	74.5	44.0	12.1
72	235	89.0	19.1	8.0
Dehulled,defatted flakes[c]			0.67	0.22

[a]Harosoy soybeans, 1971 crop year.

[b]Absorption units per g. dry matter X 10^{-3}

[c]Amsoy soybeans, 1970 crop year.

TABLE IX. THIOBARBITURIC ACID (TBA) NUMBER OF MATURING SOYBEANS[a]

Days After Flowering	Fresh Weight mg.	TBA Number[b] Without acid	TBA Number[b] With acid
29	105	20.2	1.4
29	128	24.7	1.4
32	219	28.8	1.1
32	318	19.7	1.1
48	389	22.5	...
49	509	23.2	...
52	419	13.7	1.1
55	290	11.9	...
59 (mature)	254	12.0	...
Full-fat flakes[c]		83.8	10.5

[a]Hawkeye, 1969 crop year.

[b]Reported as: mg. malonaldehyde per kg. sample (for procedure, see Ref. 7).

[c]Prepared from Amsoy soybeans.

peroxidase assays were carried out on extracts diluted 1:100,000 per gram sample compared with dilutions of 1:400 to 1:625 for lipoxygenase analysis.

Soybean peroxidase from maturing soybeans can effectively utilize highly purified linolenic hydroperoxide (peroxide value 6,848). Linoleic acid hydroperoxide can also be used as a substrate. Quantitative determination of the relative peroxidase activity with the lipohydroperoxides as substrates could not be made, because cloudy solutions formed in the reaction vessel containing the lipohydroperoxides.

Thiobarbituric Acid Analysis

For a measure of lipid oxidation, the absorbance of steam-distillable

thiobarbituric acid (TBA)-reactive substances in maturing soybeans was determined at 532 nm. by the procedure of Sessa et al. (11). The analyses were carried out with and without acid to inactivate lipoxygenase because Rhee and Watts (12) suggested that a comparison of TBA values by such a procedure might measure "lipid oxidation potential". TBA analyses of green soybeans are shown in Table IX.

The TBA number for maturing soybeans homogenized without acid remained relatively constant up to about 49 days after flowering, after which the TBA number decreased to one-half at maturity. A part of this decrease in TBA number may be attributed to the greater difficulty in disrupting the cellular structure to release soybean oil for reaction with lipoxygenase. The formation of TBA-reactive substances did not correlate with lipoxygenase activity. For example after 49 days after flowering, the TBA number for maturing soybeans decreases greatly; whereas, lipoxygenase activity increases. In contrast to intact seeds, raw full-fat flakes, in which more than 99% of the oil can readily be extracted, have a much higher TBA number of about 84 (11). When blended with acid to inactivate lipoxygenase, maturing soybeans have a TBA number of less than 2, which indicates little preformed TBA-reactive substances were present. In spite of large differences in lipid oxidation potential between mature soybeans and raw full-fat flakes, no flavor changes were noted when whole soybeans were processed into full-fat and defatted flakes (13).

CONCLUSIONS

Patterns of fresh- and dry-weight accumulation in maturing soybeans were similar to those reported previously (14,15). Our studies indicate that flavors and lipoxygenase activities of immature soybeans of the same fresh weight do not change appreciably when these seeds are picked a week to 10 days apart.

Whether the same constituents or precursors to flavor constituents are responsible for the beany and bitter flavors in immature soybeans and in soy protein products is speculative. There appears to be little correlation between lipoxygenase activity and the presence of the beany factor(s) in maturing soybeans. However, Roehm and Privett (6) have shown that major changes in fatty acid composition of the triglycerides, particularly in linolenic acid content, occurred during early stages of maturation. This occurrence suggests that even minimal oxidation of the highly unsaturated lipids by the low levels of lipoxygenase in green soybeans (24 to 29 days after flowering) is enough to initiate the formation of the characteristic beaniness flavor that was detected throughout the entire maturation period. Pattee et al. (16) reported that there is an increase in lipoxygenase and alcohol dehydrogenase activities in maturing peanuts, and that increased activity of these enzymes is associated with the formation of acetaldehyde, ethanol, pentane, and hexanal. No analyses were made in their studies to determine whether the large fluctuations in acetaldehyde, ethanol, pentane, and hexanal contents during maturation were associated with flavor changes that may or may not have occurred.

An analysis of our data shows that there is a correlation ($r = 0.73$), significant at the 1% level, between lipoxygenase activity and the increase in bitter FIV in maturing soybeans. Specific enzymatic changes are associated with bitterness in cereal products (17). Rothe (18) visualized the formation of bitter material in oats to be as follows: a) formation of peroxides by lipoxygenase, b) destruction of peroxide by the antioxidant complex aided by peroxidase enzymes, and c)

subsequent polymerization of the radicals that form in the complex. Possibly such a mechanism occurs in maturing soybeans, since a very active peroxidase capable of utilizing a lipohydroperoxide was found in extracts of soybeans picked at 4, 44, and 51 days after flowering and in extracts of defatted flakes.

Soybean phospholipids also develop a bitter flavor after ultraviolet irradiation (11). The bitter flavor appears to be associated with the formation of a nonvolatile constituent(s), since TBA numbers of the irradiated phosphatides increased sevenfold before bitterness was detected. Rothe's mechanism (18) may also explain the lack of correlation between lipid oxidation and the formation of objectionable flavors in irradiated soybean phospholipids (11), in full-fat and defatted soy flakes (11,13), and in maturing soybeans, when the TBA assay was used to determine the extent of lipid oxidation. The TBA assay, as employed in our laboratory, measures the formation of steam-distillable and other volatile degradation products from oxidized lipids.

Based on our studies of maturing soybeans, we conclude that beany and bitter constituents preexist in immature soybeans. Beany, bitter, and other objectionable flavors are also formed under certain processing conditions, such as in wet grinding and blending of raw full-fat soybeans (19,20). As a result, development of processes for the preparation of bland soy-protein products needs to take into account two factors: a) effective removal or preexisting flavors, and b) prevention or removal, or both, of derived flavor constituents.

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