Role of Lipoxygenases in Soybean Seed Protein Quality

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Soybean seeds contain three lipoxygenase isozymes: lipoxygenase 1 (L-1), lipoxygenase 2 (L-2), and lipoxygenase 3 (L-3). Use was made of recessive mutants lacking L-1 that have been found and the more rapid heat inactivation of L-2 and -3 relative to L-1 to determine the roles of these lipoxygenase isozymes in the generation of volatile carbonyl compounds associated with poor flavor of soybean protein. All lipoxygenase isozymes increased the level of carbonyl compounds and thiobarbituric acid reacting substances in aqueous extracts of whole soybean seeds with the effect of L-2 and -3 being greater than that of L-1. A disproportionate level of free fatty acid hydroperoxides was detected in water extracts of soybeans, indicating that lipase action precedes lipoxygenase-catalyzed lipid oxidation. The evidence indicates that the use of the L-1-deficient mutants of soybeans can reduce the level of volatile carbonyl compounds and TBA-reacting substances with a minimum of heating and protein loss.

Soybeans represent a very abundant and economical source of high-quality vegetable protein. Numerous recent studies demonstrate plasma cholesterol reduction induced by the dietary administration of soy protein to some animal species and man (Bosisio et al., 1981; Gibney, 1982; Goldberg et al., 1982; Huff et al., 1982; Schwandt et al., 1981; Wolfe et al., 1981). Hence, the consumption of soy protein by humans is being encouraged.

The wide use of many soybean protein preparations, particularly soy milk, is limited due to undersirable flavor. The principal causes of the objectionable flavors are short chain length volatile carbonyl compounds that bind to the soy protein, particularly hexanal (Fujimaki et al., 1965; Hsieh et al., 1981; Sasaki et al., 1981). Removal of the bound aldehydes is difficult, but incubation of soybean extract with aldehyde dehydrogenase in the presence of a NAD^+ almost completely removes the undesirable flavors through oxidation of aldehydes into their corresponding acids (Chiba et al. 1979a, b; Takahashi et al., 1979).

Considerable evidence has accumulated implicating soybean seed lipoxygenase catalyzed lipid oxidation as the critical first step in the formation of these undesirable carbonyl compounds (Arai et al., 1970; Grosch and Laskawy, 1975; Sekiya et al., 1982). Inactivation of lipoxygenase eliminates the formation of the undesirable flavors in soybean protein preparations. This can be accomplished by various heat treatments, but the concomitant loss of protein solubility and the impartation of a cooked flavor is a problem (Ashraff and Snyder, 1981; Brown et al., 1982; Mustakas et al., 1969).

Soybean seeds cntain at least three lipoxygenase isozymes. Lipoxygenase 1 (L-1) has a higher pH optimum and is more reactive with free than esterified fatty acid substrates than lipoxygenase 2 (L-2) or lipoxygenase 3 (L-3) (Bild et al., 1977; Christopher et al., 1972; Diel and Stan, 1978). L-1 exhibits far greater heat stability than L-2 or L-3 (Cristopher et al., 1970). A recessive mutation has been found in the soybean germ plasm (lx_1lx_1) resulting in no L-1 activity (Hildebrand and Hymowitz, 1981, 1982). This mutation does not have any apparent effects on soybean growth or yield.

The objective of this study was to determine the utility of this L-1 mutation coupled with differential heat inactivation of the lipoxygenase isozymes for decreasing the levels of objectionable compounds in soybean protein preparations.

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MATERIALS AND METHODS

Materials and Reagents. Seeds of PI 133226 and 408251, which lack L-1, and seeds of experimental lines UFV-1 and ICA-109, which have normal L-1 activity, were grown in adjacent plots in Puerto Rico in 1981. Plants were harvested individually. Ten seeds were checked from each plant of each L-1-deficient mutant for the presence of the L-1 protein (due to outcrossing, etc.) by Ouchterlony double diffusion (Hildebrand and Hymowitz, 1982), and all plant families containing seeds with the L-1 protein were discarded.

NAD and $NADH_2$ were obtained from Nakarai Chemical, Ltd., Kyoto. Yeast aldehyde dehydrogenase and 1-hexanal were obtained from Sigma Chemical Co. The other reagents were of analytical grade.

Lipoxygenase Measurements. L-1 and L-2 and L-3 activity was determined by the increase in conjugated dienes measured at 234 nm as described (Hildebrand and Hymowitz, 1981). BHT, routinely included in the extraction solution and substrate at 0.01%, was found to have no significant effect on lipoxygenase activity.

Protein Measurement. The protein content of the soybean extracts was determined by using the Bio-Rad protein assay with bovine serum albumin as the standard.

Carbonyl Measurement. The analysis of volatile carbonyl compounds was performed by a modification of the technique of Henick et al. (1954) (Figure 1). Five milliliters of the benzene solution (TB) [5% trichloroacetic acid (TCA), 0.01% 2,4-dinitrophenylhydrozone (DNPH)] is added to tubes with ground glass stoppers. Aliquots of aqueous extracts of soybean seeds and/or aliquots of aqueous 1-hexanal standards in a total volume of 1 mL are mixed with the benzene solutions. The tubes are immediately stoppered and incubated at 60 °C in a water bath for 1 h. The tubes are then cooled in an ice bath, 1 mL of 4% KOH (in ethanol) is added, the solutions are mixed. The tubes are then centrifuges at 100g for 5 min, and after another 5 min, the absorbance of 430 and 460 is recorded (Henick et al., 1954).

This technique was standarized by adding known quantities of aqueous 1-hexanal from a saturated hexanal solution to extracts of soybean seeds that had been heated 2 h at 70 °C (used to zero the spectrophotometer with no addition of 1-hexanal). An 1-hexanal standard curve was included with every set of carbonyl analyses, and the standardized carbonyl value was read off the least-squares regression fitted standard curve as the 1-hexanal equiva-

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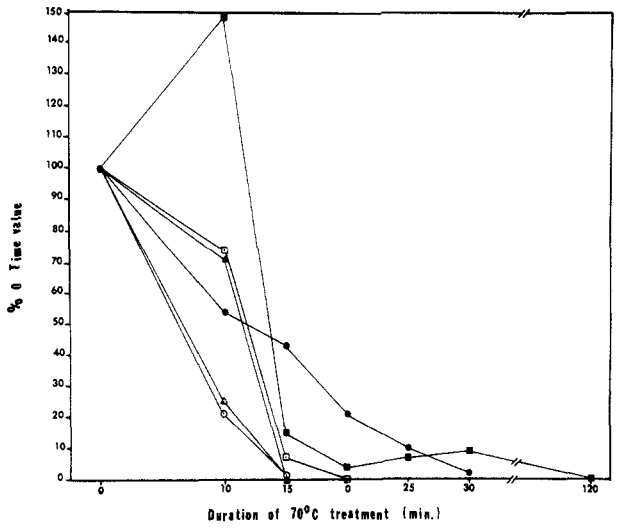

Figure 1. Effects of the duration of the 70 °C treatment on activities of lipoxygenase isozymes. Values for no heat treatment set at 100%. Carbonyl and TBA values adjusted for TCA controls. (●) L-1 activity. (○) L-2 and -3 activity. (▲) Carbonyl value (1-hexanal equivalent) for means of all four genotypes tested. (△) Carbonyl value for means of PI 133226 and 408251. (■) TBA value (malonaldehyde equivalent) for means of all genotypes tested. (□) TBA value for means of PI 133226 and 408251.

lent. The quantity of 1-hexanal present in the aqueous 1-hexanal standards was determined by the coupled reduction of NAD by yeast aldehyde dehydrogenase (Sasaki et al., 1981).

TBA Measurements. The thiobarbituric acid (TBA) value of the soybean seed extracts was determined as described by Asakawa and Matsushita (1979) except no iron catalysts were added.

Lipid Hydroperoxide Measurements. Quantification of the lipid hydroperoxide (LHPO) molecular species resulting from lipid oxidation in soybean extracts was attempted by the following procedures: LHPO were isolated by mixing the whole soybean extracts with 4 volumes of acetic acid–$CHCl_3$ (3:2 v/v) containing 0.01% BHT in a separatory funnel. The $CHCl_3$ layer was separated and filtered, the solutions were washed twice with $CHCl_3$ containing 0.01% BHT, and the $CHCl_3$ layers wer filtered and pooled. Excess $CHCl_3$ was removed in vacuo. LHPO were seperated by using Merck silica gel 60 TLC plates with ethyl ether–hexane–acetic acid (50:50:5 v/v/v) as the solvent. LHPO standards were prepared by the photosensitized oxidation of linoleic acid to a mixture of linoleic acid and linoleic acid hydroperoxide and of trilinolein to a mixture of trilinolein and triglyceride mono-, di-, and trihydroperoxides (Terao and Matsushita, 1981).

The LHPO separated by TLC were analyzed in the following 4 ways: (1) The fluoroescent quenching of LHPO spots on plates sprayed with 0.1% dichlorofluorescein in ethanol viewed under UV light was used for qualitative analysis. (2) The peroxide value of the LHPO was determined of the corresponding bands removed from the TLC plates by the iodometric technique of Wills (1971) using benzoyl peroxide as the standard. (3) The TLC plates with the separated LHPO were scanned at 234 nm on a Shimadzu dual-wavelength TLC scanner, CS-910. (4) LHPO were separated on silica gel rods as with the TLC plates and the bands analyzed by flame ionization detection using an Iatroscan TH-10 coupled to a Shimadzu Chromatopac E1A integrator. It was not possible to distinguish phospholipid hydroperoxides (PLPO) from PL with technique 4 since, with the developing solvent used, both PLPO and PL remained at the origin on the silica gel rods and this technique does not distinghish LHPO from lipids.

Experimental Design. Soybean seeds were scarified and soaked 24 h at 4 °C in 20 volumes of water. The soaked seeds were then ground in 20 volumes of water with a Polytron PCU-2 at a setting of 3 with two 3-s bursts while keeping the tubes in an ice bath. The soybean homogenates were then centrifuged at 1000*g* for 10 min, and the supernatant (soybean seed extract) was used for all analyses. An aliquot of the seed extracts was kept at 0 °C for lipoxygenase activity and protein determinations. Seed extracts were incubated at 37 °C for 2 h in a shaking water bath for carbonyl, TBA, and LHPO analysis. For the spectrophotometric determination of lipoxygenase activity, the soybean extracts were clarified by mixing with an equal volume 0.1 M Tris, pH 9.1, saturated with sucrose.

The analysis of variance of the data was calculated as a two-factor factorial with heating time and genotypes the factors. The *F* test of the effects of heating time, genotypes, and heating time × genotypes was calculated for L-1 activity, L-2 and -3 activity, soluble protein, carbonyl level, and TBA values. The restricted least significant difference (Carmer and Swanson, 1973) was calculated for soluble protein, saturated carbnyl level, and TBA value. Heating time had a highly significant effect (0.1% level) on all parameters examined. The presence of the L-1 gene product had a significant effect (5% level) on the carbonyl value and TBA value.

RESULTS

The technique for the measurement of volatile carbonyls described in this paper is effective for rapid, quantitative determination of volatile carbonyl compounds present in aqueous solutions providing that the readings are made from a standard curve included in each set of analyses. The correlation coefficients of 1-hexanal vs. the carbonyl value reading ranged from $r = 0.81$ to $r = 0.96$ for the standard curves. The standard deviation of the carbonyl value for fixed 1-hexanal quantity varied from $Sy{\cdot}x = 1$ to $Sy{\cdot}x = 6$.

Lipoxygenase Inactivation. The in situ inactivation of L-2 and -3 is much more rapid than that of L-1 (Figure 1; Table I). No L-2 and -3 activity was detectable after 20 min at 70 °C. L-1 activity was readily detectable after 20 min at 70 °C.

When the L-1-deficient mutants and the differential heat inactivation of the different lipoxygenase isozymes were used, the following combinations of lipoxygenase activities present could be set up: L-1 activity and L-2 and -3 activity present in extracts of UFV-1 and ICA-109 seeds with no heat treatment, L-2 and -3 activity present in extracts of unheated seeds of PI 133226 and 408251, L-1 activity present in extracts of UFV-1 and ICA-109 seeds heated for 20 min at 70 °C, and no lipoxygenase activity in extracts of PI 133226 and 408251 seeds heated at 70 °C for 20 min or UFV-1 and ICA-109 seeds heated at 70 °C for 120 min.

Saturated Carbonyl Levels. There is no diference in saturated carbonyl levels of extracts of unheated seeds of all four genotypes (Table II). After 10 min at 70 °C, extracts of seeds of the two genotypes lacking L-1 show significantly lower carbonyl levels than extracts of the two genotypes possessing L-1. After heat treatment of 15 min or longer, the carbonyl levels of extracts of seeds of all genotypes were not different from those of the TCA control (Table II).

TBA Values. As with carbonyl levels, the TBA values of extracts of unheated seeds of all four genotypes were not different (Table III). After 10 min at 70 °C, extracts

Table I. Effects of Heat Treatment of Soybean Seed on L-1 and L-2 and -3 Activity of Seed Extracts of Two Normal and Two Accssions Lacking L-1[a]

minutes of heating at 70 °C	lipoxygenase activity, ΔA_{234} min^{-1} (mg of protein)$^{-1}$					
	PI 133226,[b] L-2 and L-3[c]	PI 408251, L-2 and L-3	UFV-1		ICA-109	
			L-2 and L-3	L-1	L-2 and L-3	L-1
0	35 ± 2	23 ± 1	38 ± 2	253 ± 9	35 ± 2	240 ± 7
10	3 ± 1	9 ± 1	1.5 ± 0.1	113 ± 9	1.2 ± 0.1	154 ± 7
15	0.4 ± 0.1	0.3 ± 0.2	0.8 ± 0.1	101 ± 6	0.5 ± 0.1	112 ± 8
20	0	0	0	49 ± 8	0	56 ± 6
25	0	0	0	20 ± 5	0	27 ± 5
30	0	0	0	5 ± 0.3	0	4 ± 1
120	0	0	0	0	0	0

[a] Zero lipoxygenase activity was considered the level of extracts made with 10% TCA. Scarified seeds were soaked in water for 24 h at 4 °C and then heated in a water bath at 70 °C for the indicated durations. Data presented as a means of six determinations ± SE. [b] 133226 and 408251 have no detectable L-1 activity. Experimental lines UFV-1 and ICA-109 have normal L-1 activity. [c] Assays for the different lipoxygenase isozymes are as previous reported (Hildebrand and Hymowitz, 1981).

Table II. Effects of Heat Treatment of Soybean Seeds on Saturated Carbonyl Levels of Aqueous Extracts[a]

minutes of heating at 70 °C	saturated carbonyl levels, μmol of 1-hexanal/mg of protein[b]			
	PI 133226	PI 408251	UFV-1	ICA-109
0	14 ± 2 A[d]	13 ± 1 C	14 ± 1 E	13 ± 1 G
10	7 ± 0.3 B	5 ± 1 D	11 ± 1 E	12 ± 0.3 G
15	3 ± 0.5 B	3 ± 0.4 D	6 ± 1 F	7 ± 2 H
20	5 ± 1 B	3 ± 1 D	5 ± 1 F	7 ± 1 H
25	4 ± 0.6 B	5 ± 2 D	4 ± 2 F	5 ± 1 H
30	2 ± 1 B	2 ± 1 D	6 ± 1 F	6 ± 2 H
120	3 ± 1 B	2 ± 0.6 D	4 ± 1 F	5 ± 2 H
TCA control[c]	4 ± 1 B	3 ± 1 D	6 ± 1 F	7 ± 1 H

[a] Conditions as in Table I. Data presented as means of six 1-hexanal equivalent determinations ± SE calculated from the 1-hexanal standard curve. [b] mL of soybean extract analyzed. The extracts generally contained 3–14 mg of protein mL^{-1}. [c] TCA control represents extract aldehyde level of soybean seeds extracted in 10% TCA. [d] Means not followed by the same letter are significantly different at the 5% level as determined by the restricted least significant difference method (Carmer and Swanson, 1973).

Table III. Effects of Heat Treatment of Soybean Seeds on TBA Values of Aqueous Extracts[a]

minutes of heating at 70 °C	TBA values, nmol of malonaldehyde/100 mg of protein[b]			
	PI 133226	PI 408251	UFV-1	ICA-109
0	52 ± 3 A[c]	62 ± 5 C	60 ± 2 E	58 ± 6 G
10	44 ± 4 B	55 ± 12 C	75 ± 3 E	81 ± 13 G
15	23 ± 1 B	38 ± 6 D	28 ± 5 F	26 ± 4 H
20	18 ± 3 B	27 ± 1 D	15 ± 4 F	31 ± 6 H
25	16 ± 1 B	35 ± 4 D	19 ± 6 F	29 ± 7 H
30	11 ± 1 B	26 ± 3 D	16 ± 4 F	34 ± 6 H
120	14 ± 2 B	29 ± 7 D	17 ± 5 F	18 ± 4 H
TCA	21 ± 1 B	36 ± 11 D	22 ± 6 F	21 ± 5 H

[a] Conditions as in Table I. Data presented as means of malonaldehyde equivalent ± SE (six determinations). TBA values determined after Askawa and Matsushita (1979) by using 1,1,3,3-tetraethoxypropane as the standard. [b] See Materials and Methods for analytical details. [c] As in Table II, footnote *d*.

of seeds of the two genotypes possessing L-1 activity show significantly higher TBA values. With 15 min or longer heat treatment, extracts of seeds of all four genotypes showed TBA values that were not different from those of the TCA controls (Table III).

Effects of Lipoxygenase Activity on Carbonyl and TBA Levels. Heat treatment of imbibed soybeans prior to grinding had a large effect on lipoxygenase activity, carbonyl level, and TBA value without having a large effect on protein solubility (Table IV). Heat treatment for 10 min resulted in a greater than 80% reduction in L-2 and -3 activity and less than 50% reduction in L-1 activity.

Table IV. Relationship of Carbonyl Production (1-Hexanal Equivalent) and TBA Value (Malonaldehyde Equivalent) with Genetic and/or Heating Removal of Lipoxygenase Isozymes

lipoxygenase[a] activity present	carbonyl level, μmol of 1-hexanal/ mg of protein	TBA value, nmol of malonaldehyde/ 100 mg of protein	protein, mg/mL
–[b]	5 ± 2 A[g]	18 ± 5 C	8 ± 1 E
–[c]	4 ± 1 A	23 ± 3 C	9 ± 2 E, F
L-1[d]	6 ± 1 A	23 ± 6 D	11 ± 2 E, F
L-2 and -3[e]	14 ± 2 B	57 ± 5 D	13 ± 1 F
L-1, L-2 and -3[f]	14 ± 1 B	59 ± 6 D	13 ± 2 F

[a] See Table I. [b] Mean values of extracts from seeds of UFV-1 and ICA-109 heated at 70 °C for 120 min. [c] Mean values of extracts from seeds of PI 133226 and 408251 heated for 20 min. [d] Mean values of extracts from UFV-1 and ICA-109 heated for 20 min. [e] Mean values of unheated extracts of PI 133226 and 408251. [f] Mean values of unheated extracts of UFV-1 and ICA-109. [g] As in Table II, footnote *c*.

Table V. Hydroperoxide Analysis of Enzymatic Oxidation of Lipids in Whole Soybean Extracts

hydroperoxide molecular species	% of total lipid peroxides for hydroperoxide analysis technique[a]		
	peroxide value analysis	TLC scanning	Iotron scanning
triglyceride hydroperoxide	55 ± 14	47 ± 10	35 ± 12
free fatty acid hydroperoxide	45 ± 16	40 ± 11	65 ± 18
phospholipid hydroperoxide	0	13 ± 7	–[b]

[a] See Materials and Methods. [b] It is not possible to distinguish PLPO from PL with Iotron scanner since PLPO and PL both remain at the origin on the silica gel rods.

The same heat treatment resulted in little reduction in carbonyl or TBA levels with a larger effect in genotypes lacking L-1 (Table II and III). Heat treatments for 20 min, which resulted in complete loss of detectable L-2 and -3 activity with greater than 20% of the original L-1 activity, also reduced the carbonyl and TBA levels to their lowest values. Similarly, extracts from unheated seeds of the genotypes without L-1 activity produced the same levels of carbonyls and TBA-reacting substances as extracts from unheated seeds of genotypes with normal L-1 activity (Table IV).

Hydroperoxide Molecular Species. Of the three lipid hydroperoxide molecular species that were separated and analyzed, most of the lipid hydroperoxides were found in

the triglyceride and free fatty acid hydroperoxide classes (Table V). Fluorescent quenching by LHPO separated by TLC indicated that lipid peroxidation had occurred in all three lipid classes.

DISCUSSION

The results for the in situ inactivation of lipoxygenase corroborate those of Christopher et al. (1970) for the greater heat stability of purified L-1 relative to L-2 and -3. The reduction in carbonyl levels and TBA values with increasing duration of heating was less rapid than the reduction in L-2 and -3 activity but more rapid than that of L-1 activity. Likewise, the reduction in carbonyl levels and TBA values with increased duration of heating was more rapid in the genotypes that lacked L-1 than those with L-1 activity. Complete loss of L-2 and -3 activity with significant L-1 activity remaining (20 min at 70 °C) resulted in maximal reduction of carbonyl levels with further reduction of L-1 activity having no effect. The results indicate that L-1 can affect the rate of production of carbonyl compounds and TBA-reacting substances, but the effect is much less than that of L-2 and -3 and that L-1 has little independent effect. The lack of L-1 activity after 20-min heat treatment may be due to the inactivation of lipase(s). Previous work has indicated that purified L-1 has little activity with esterified fatty acid substances (Bild et al., 1977). Free fatty acids are present in very low levels in biological tissues (sonntag, 1979). It is therefore likely that L-1-catalyzed lipid oxidation must follow lipase action. This is consistent with work on purified lipoxygenase isozymes indicating that L-1 has little reactivity toward esterified fatty acid substrates (Bild et al., 1977; Christopher et al., 1972).

Attempts to quantify the hydroperoxide species resulting from action of lipoxygenase and other components of water extracts of whole soaked soybeans resulted in highly variable data. The values reported here are at best only approximations. The lipids of the mature soybean seeds used in this study consist of greater than 90% storage triglycerides with the remainder of the lipids composed primarily of phospholipids. Undamaged mature soybean seeds contain very low amounts of free fatty acids (Chapman et al., 1976; List et al., 1977; Sonntag, 1979). In cauliflower bud mitochondria, Dupont (1981) has reported that lipoxygenase can catalyze oxidation of phosphatidic acid. In the aqueous extracts of the soybeans of this study, peroxidation of the major lipid triglycerides occurs, but equivalent levels of free fatty acid hydroperoxides were detected by all three quantitative techniques employed and very little phospholipid hydroperoxides were detected. This indicates that the general scheme of lipid oxidation outlined by Gardner (1980) of sequential action of lipases releasing free fatty acids from glycerides followed by oxidation of the free fatty acids by lipoxygenases is occurring in this system. The greater effects of L-2 and -3 in this system relative to the L-1 may therefore be due to the pH of the water extracts (near neutrality) being closer to the optima of L-2 and -3 than of L-1 (Christopher et al., 1972) rather than due to differences in substrate specificity. The presence of TGPO may be the result of autoxidation catalyzed by FAPO or due to direct lipoxygenase attack.

Lipases are necessary for the utilization of storage triglycerides by germinating soybeans; thus, its genetic removal would not be feasible. Genetic removal of all lipoxygenase isozymes would be ideal, but until that objective is accomplished, processors can capitalize on the use of the L-1-deficient mutants and the facile heat inactivation of L-2 and -3.

New products containing soy protein are appearing with great frequency. Methods are available for the production of Domiati (Metwalli et al., 1982) and mozzarella (Yang, 1982) cheeses and yogurt-like products (Peng, 1982) utilizing soy protein with the main limitation to commercialization being flavor. The results of this study indicate that the use of the L-1-deficient soybeans might help accomplish this objective with a minimum of heat treatment, thus enabling consumers to benefit more fully from the health attributes and economics of soybean protein consumption. However, further studies are needed to determine the possible benefits to food processors of using protein from L-1-deficient soybean lines.

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Registry No. Lipoxygenase, 9029-60-1; lipase, 9001-62-1; 1-hexanal, 66-25-1; malonaldehyde, 542-78-9.

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Relationship of Tannin Levels and Trypsin Inhibitor Activity with the in Vitro Protein Digestibilities of Raw and Heat-Treated Winged Bean (*Psophocarpus tetragonolobus*)

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Three varieties of winged bean were heat processed in five different ways to determine the relationship among in vitro protein digestibility (IVPD), trypsin inhibitor activity (TIA), and assayable tannin. The IVPD of raw winged bean ranged from 68.8 to 72.9%. Heat treatments increased the IVPD to 76.0–90.7% and reduced both TIA and tannin. In terms of energy costs, 5-min autoclaving and quick cooking were the two most effective methods of improving IVPD. Dry heat (200 °C, 30 min) and direct boiling (20 min, no soaking) were least effective. Reductions in tannins and TIA did not necessarily result in proportional increases in IVPD.

The problem of plant protein digestibility has been extensively studied. Trypsin inhibitors, tannins, and other "antiphysiological factors" have been suggested as factors responsible for the low digestibility of some plant proteins (Jaffe, 1968; Bressani et al., 1975; Featherstone and Rogler, 1975).

No direct evidence, however, has been produced to show which is the reason for the poor digestibility of certain legume proteins. Recently, Elias et al. (1979) suggested that in legumes, tannins might play an important role in the reduction of protein digestibility. Subsequently, in studying the effects of winged bean (*Psophocarpus tetragonolobus*) meals on broiler performance, de Lumen et al. (1982) suggested that the hull of winged bean, with its high tannin content and indigestible fiber, may be the main factor responsible for the lower metabolizable energy of the winged bean diets that led to the poorer response of the broilers. The present work was undertaken to determine the possible relationship of the tannin levels and trypsin inhibitor activity with the in vitro protein digestibilities in several varieties of raw and heat-treated winged beans.

MATERIALS AND METHODS

Winged beans of varieties V1 (yellowish seed coat), 243 (brown seed coat), and C33PB (dark brown seed coat) were grown locally at the Experimental Farm of Agricultural University of Malaysia, Serdang, Malaysia. Winged bean meals were prepared by grinding the mature beans manually with a pestle and mortar, and the fine powder was stored below 0 °C in a glass container before use. All chemicals are of analytical reagent grade and were purchased from Sigma Chemical Co. or Merck.

Preparation of the Heat-Treated Winged Bean Meals. *Dry Heat Treated Winged Bean Meals.* These were prepared by heating 10 g of finely ground winged bean meals, in a beaker at a thickness not exceeding 1 cm, in an oven maintained at 200 ± 2 °C for 30 min.

Autoclaved Winged Bean Meals. The autoclaved winged bean meals were prepared by autoclaving 10 g of the finely ground bean meals in a beaker at a thickness not exceeding 1 cm at 120 °C, 1.05 kg/cm^2 for 5 or 10 min, after the desired temperature had been reached. The autoclave was preheated before use to minimize the time required to reach the desired temperature (approximately 7 min).

Boiled Winged Bean Meals. One gram of whole winged beans was treated by boiling in distilled water (10 mL) for 20 min, their cooking broths were drained off, and the beans were dried in an oven at a temperature of 50 °C for 2 h. The beans were then ground to yield the boiled winged bean meals.

Cooked Winged Bean Meals (Normal Cooking). The cooking of winged beans was carried out as described by Rockland et al. (1979). Ten grams of the whole beans was soaked in 30 mL of distilled water at room temperature (32 °C) for 24 h, followed by cooking in 6 volumes of boiling distilled water for 240 min with a loose cover to minimize water loss. The cooked beans were drained and dried in

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