Mikami, N.; Takahashi, N.; Hayashi, K.; Miyamoto, J. *J. Pestic. Sci.* **1980**, *5*, 225.
Miller, G. C.; Zepp, R. G. *Residue Rev.* **1983**, *85*, 89.
Miyamoto, J.; Mikami, N. *Int. Congr. Pestic, Chem., 5th* **1983**, *2*, 193.
Nash, R. G. *Residue Rev.* **1983**, *85*, 199.
Quistad, G. B.; Staiger, L. E. *J. Agric. Food Chem.* **1984**, *32*, 1134.
Ruzo, L. O.; Holmstead, R. L.; Casida, J. E. *J. Agric. Food Chem.* **1977**, *25*, 1385.
Savage, K. E.; Jordan, T. N. *Weed Sci.* **1980**, *28*, 105.
Steel, R. G. D.; Torrie, J. H. "Principles and Procedures of Statistics"; McGraw-Hill: New York, 1980.
Swann, R. L.; Eschenroeder, A.; Eds. "Fate of Chemicals in the Environment"; American Chemical Society: Washington, D.C., 1983; ACS Symp. Ser. No. 225.
Walker, A. *J. Environ. Qual.* **1974**, *3*, 396.
Walker, A.; Barnes, A. *Pestic. Sci.* **1981**, *12*, 123.
Williams, J. H.; Eagle, D. *J. Weed Res.* **1979**, *19*, 315.
Zimdahl, R. L.; Gwynn, S. M. *Weed Sci.* **1977**, *25*, 247.

Received for review February 28, 1985. Accepted June 14, 1985. Parts of this paper were presented at the 188th National Meeting of the American Chemical Society, Division of Pesticide Chemistry, Philadelphia, PA.

Polyphenols in Mung Bean (*Vigna radiata* (L.) Wilczek): Determination and Removal

Charlene F. Barroga, Antonio C. Laurena, and Evelyn Mae T. Mendoza*

Ten cultivars of mung bean (*Vigna radiata* (L.) Wilczek) were analyzed for polyphenol content by three methods: modified vanillin, Prussian blue, and protein precipitation. Polyphenols in mung bean had low protein precipitating capacity, relatively high flavanol levels, and were concentrated in the seed coat. Soaking seeds in water reduced assayable polyphenol content from 24 to 50%. Boiling for 30 min and roasting for 10 min resulted in 73% and 17% reduction of polyphenols, respectively. The lowering of polyphenols was significantly positively correlated with the decrease in protein-precipitable phenols (+0.95**). Mung bean sprouts had 36% less polyphenols after 48 h germination than after longer germination in which polyphenol content increased.

INTRODUCTION

Leguminous seeds constitute one of the richest and cheapest sources of proteins and are consequently becoming an important part of the people's diet in many parts of the world. Unfortunately, although the protein content of legume food is high (20–25%), its protein quality is low. This has been attributed to two factors: the deficiency of sulfur-containing amino acids (Elias et al., 1964; Bressani et al., 1973; Elias and Bressani, 1974) and the presence of antiphysiological and toxic factors such as trypsin inhibitors, hemagglutinins, cyanogenic glycosides, saponins, flatulence factors, and phytates (Jaffe, 1968; Liener, 1980; Elkowicz and Sosulski, 1982). Moreover, the presence of tannins (polyphenols) in sorghum and several legumes which could lower their protein digestibility has been reported. These legumes include the common beans *Phaseolus vulgaris* L. (Elias et al., 1979), winged beans *Psophocarpus tetragonolobus* L. (De Lumen and Salamat, 1980), broad and tick beans *Vicia faba* L., and maple beans *Pisum sativum* L. (Griffiths, 1981; Griffiths and Moseley, 1980), chick peas *Cicer arietum* L., green bean or mung bean *Vigna radiata* (L.) Wilczek, soybean *Glycine max* L., hyacinth bean *Dolichos lablab*, pigeon pea *Cajanus cajan* (L.) Millsp. (Narasinga Rao and Prabhavathi, 1982), horse gram *Macrotyloma uniflorum* (Lam.) Verdc., moth bean *Phaseolus aconitifolia* (Jacq.) Marechal (Satwadhar et al., 1981), and cowpea *Vigna unguiculata* (L.) Walp. (Laurena et al., 1984a,b).

In the Philippines, one of the most popular legume foods is mung bean (*Vigna radiata* (L.) Wilczek). Boiled whole mature seeds are utilized in native delicacies such as "hopia", "butse-butse", and "halo-halo"; mung bean flour can be processed to a noodle "sotanghon", or it can be used as a vegetable dish (boiled whole beans or sprouted seeds known as "togue") in combination with shrimp and meat. Because of the increasing importance of mung bean, it is imperative to study the role of protein-precipitable phenols on the nutritional quality of this legume. This paper reports on the determination and localization of polyphenols and the effects of soaking, heating, and germination on polyphenol content of mung bean. Related studies deal with the purification and characterization of condensed tannins from mung bean and their effect on its in vitro protein digestibility (Barroga et al., 1985).

EXPERIMENTAL SECTION

Materials. Mature seeds of ten cultivars of mung bean with yellow and green seed coat color were obtained from the National Plant Genetic Resources Laboratory of the Institute of Plant Breeding, University of the Philippines at Los Baños. The dried seeds were ground in a UDY cyclone mill and passed through a 100-mesh sieve. All chemicals used were of analytical grade.

Analysis of Polyphenol Content. The polyphenol content of 10 mung bean cultivars was determined and compared by using three different assays: modified vanillin (Price et al., 1978), Prussian blue (Price and Butler, 1977), and protein precipitation (Hagerman and Butler, 1978). For the modified vanillin and the Prussian blue assays, 1% HCl in methanol was used as extractant. Plain methanol was used as extractant for the protein precipitation assay, since tannic acid in 1% HCl in methanol failed to precipitate due perhaps to its hydrolysis. Catechin and tannic acid were used as standards.

Localization. Polyphenol distribution in the mung bean was determined by the Prussian blue assay by using both raw and soaked seeds of two cultivars, Pag-asa 1 and

Biochemistry Laboratory, Institute of Plant Breeding, University of the Philippines at Los Baños, College, Laguna 3720, Philippines.

0021-8561/85/1433-1006$01.50/0

Table I. Analysis of Polyphenol Content of Selected Mung Bean Cultivars by Three Methods (Dry Weight Basis)

sample	seed coat color	polyphenol content: modified vanillin assay (mg of catechin/g)	polyphenol content: Prussian blue assay (mg of catechin/g)	polyphenol content: protein precipitation method (mg of tannic acid/g)
CES ID-21 (Pag-asa 1)	green	8.28 ab	4.04 b	0.41 bc
CES 3H-5	green	5.49 e	3.07 de	0.28 d
CES 2N-4	green	6.19 cd	3.21 d	0.19 e
V 2184	green	5.73 cde	3.54 c	0.17 e
CES 5G-1	green	4.49 f	3.21 d	0.10 f
CES 77-78-6	yellow	8.90 a	3.73 c	0.42 bc
CES 2F-1 (Pag-asa 2)	yellow	5.57 de	2.95 de	0.16 e
EGMY 161-1	yellow	6.34 c	3.09 de	0.43 b
CES 2G-4 (Pag-asa 3)	yellow	5.96 cde	2.83 e	0.29 d
VC 1469-4-3B	yellow	8.59 ab	4.50 a	0.61 a

[a] In a column, means followed by the same letter are not significantly different at the 5% level by Duncan's multiple test.

Table II. Distribution of Polyphenols in the Mung Bean Seed

component	polyphenol content (mg of catechin/g): raw seed, Pag-asa 1	raw seed, Pag-asa 3	soaked seed[b], Pag-asa 1	soaked seed[b], Pag-asa 3
seed coat	33.95 (9.9)[a]	32.82 (9.7)	22.36	20.38
cotyledon	0.67 (90.0)	0.78 (90.3)	1.23	0.94
whole seed	3.78	3.52	2.00	2.02

[a] Values in parenthesis refer to percent by weight of whole seed (average of 10 seeds). [b] Seeds were soaked in 25 mL of H_2O at 30 °C for 18 h and analyzed by following the Prussian blue assay (dry weight basis).

Pag-asa 3. The raw seeds were weighed, dehulled manually by a sharp scalpel, and ground while soaked seeds were dehulled, dried overnight, and ground.

Treatments. Pag-asa 1 seeds were soaked, cooked, and germinated and the polyphenol content was analyzed by the Prussian blue assay. Prior to analysis, treated grains were dried at 37 °C and then ground (100 mesh).

(a) Soaking. Ten grams of seeds were soaked in water (2–5 mL/g) for 18 h at 5, 10, and 15 °C. The whole seeds, cotyledons, seed coat, and the soak water were analyzed for polyphenols.

(b) Cooking. Twenty-five grams of seeds were boiled in 250 mL of H_2O for 120 min. Five-milliliter aliquots of broth and some seed samples were withdrawn at 15-min intervals. The broth, which contained fine solids, was centrifuged and 0.1 mL of the clear supernatant was used for analysis.

Ten grams of seeds were roasted for 10 min in an ordinary frying pan under low heat, ground, and analyzed for polyphenol.

(c) Germination. Fifty whole seeds were placed in petri dishes containing moist filter papers. These were placed in the dark at 25 °C, and the filter papers were moistened at 12-h intervals. Germinated seeds were collected after 1, 2, 3, 4, and 5 days. Dried germinated seeds were ground to pass a 60-mesh sieve and analyzed for polyphenols.

RESULTS AND DISCUSSION

Determination of Polyphenols. The three assays used differed in their capacity to detect various types of polyphenols and were selected to provide some specific information on the polyphenol characteristics of mung bean. Table I indicates that mung bean seeds contain a high level of flavanol groups as measured by the vanillin test which may be monomers or oligomers that are too short to precipitate proteins. The values obtained in the protein precipitation assay, ranging from 0.1 to 0.6 mg of tannic acid/g of sample (TAE), are very much lower than the values reported for sorghum (1.03–5.66 TAE) (Bullard et al., 1981), cowpea (1.44–4.77 TAE) (Laurena et al., 1984a), and common beans (1.61–5.2 TAE) (Bressani et al., 1983). When acidic methanol was used as extractant for some of the accessions tested, lower values of protein-precipitable phenols were obtained. This could be due to the presence of a large amount of hydrolyzable tannins which are unstable under acidic conditions. The high values of the modified vanillin assay (which measures only a fraction of the total phenols) over the Prussian blue assay (which measures total phenols) could mean that the components which reacted with vanillin produced at least twice as much color as does the catechin standard.

A significant correlation was obtained between the different methods: $r = 0.789^{**}$ for modified vanillin assay and Prussian blue assay, $r = 0.838^{**}$ for modified vanillin assay and protein precipitation method, and $r = 0.689^{*}$ for Prussian blue assay and protein precipitation method, similar to results reported earlier (Price et al, 1979; Price et al., 1980b; Earp et al., 1981; Bressani et al., 1983). No correlation was obtained between seed coat color (yellow and green) and polyphenol/tannin content ($r = 0.503^{ns}$, 0.364^{ns}, and 0.006^{ns}) for protein precipitation method, modified vanillin assay, and Prussian blue assay, respectively. For other legumes, Laurena et al. (1984a) reported a significant correlation between the level of condensed tannins in cowpea with the seed coat color (+0.64**), which varied from white to light brown, dark red, and black. Others reported also that dark-seeded beans contain higher amounts of tannins than the light seeded ones (Elias et al., 1979; Phillips et al., 1981; Fernandez et al., 1982). This was also similarly observed in sorghums and cereals (Ramachandra et al., 1977; Eggum et al., 1981).

Price et al. (1980a) did not detect tannins in 10 cultivars of mung bean with both vanillin and protein precipitation methods. Narasinga Rao and Prabhavathi (1982) and Laurena et al. (1984b) reported tannin values measured by the modified vanillin assay similar to those obtained in our study.

Localization. Analysis of the mung bean seed showed that 81–85% of the tannins (as polyphenols measured by the Prussian blue assay) were located in the seed coat while only 15–18% were in the cotyledon. The seed coat was only 10% by weight of the whole seed (Table II). Only about 55% of the polyphenols were recovered in the soaked seeds. About 34–38% of the lost polyphenols were from

Table III. Effect of Soaking on Polyphenol Content of Whole Mung Bean Seeds, Cotyledon, and Seed Coat at Different Temperatures

sample/part	polyphenol content[a] (mg of catechin/g) 5 C	15 C	30 C
Pag-asa 1			
whole seeds	2.04 (46.0)[b]	2.23 (41.0)	2.00 (47.1)
cotyledon	1.43	1.10	1.23
seed coat	25.62 (24.5)	21.69 (36.1)	22.36 (34.1)
Pag-asa 3			
whole seeds	1.75 (50.0)	2.02 (42.61)	1.91 (45.7)
cotyledon	1.05	1.01	0.94
seed coat	21.29 (35.1)	20.66 (37.0)	20.38 (37.9)

[a] Analyzed by Prussian blue assay of unsoaked seeds (in mg of catechin/g): 3.78 for Pag-asa 1; 3.52 for Pag-asa 3. [b] Values in parentheses refer to percent polyphenol reduction. Ten grams of seeds were soaked in 20–35 mL of H_2O for 18 h.

the seed coat. Analysis of the soak water revealed the presence of polyphenols and implies their leaching out from the seeds to the soak water.

Several authors have confirmed the presence of tannins or polyphenols in the seed coat of sorghum and several legumes (Blessin et al., 1963; Jambunathan and Mertz, 1973; Ramachandra et al., 1977; Reichert et al., 1980; Earp et al., 1981; Phillips et al., 1981). A 90–98% polyphenol removal has been reported upon dehulling sorghum and cowpea (Chibber et al., 1978; Ramachandra et al., 1977; Griffiths, 1981; Laurena et al., 1984b).

Effect of Soaking and Heating on Polyphenol Content. Loss in polyphenol content could be due to (a) reduced extractability, (b) actual removal, or (c) change in chemical reactivity. Soaking of mung bean seeds in water reduced the assayable polyphenol content from 25 to 50%. [Reichert et al. (1980) reported a 40–75% tannin removal on high tannin sorghum on imbibition of H_2O and storage under anaerobic condition at 25 °C.] Most of the lost polyphenols were from the seed coat, while polyphenol content of the endosperm apparently increased, though to a small extent. Polyphenols (about 4–13 mg of catechin) were detected also in the soakwater of Pag-asa 1 and Pag-asa 3 which were colored green and golden brown, respectively. The soak water had two to three times greater polyphenols than the whole seed. This large difference could be due to the greater reactivity of polyphenols when not in contact with the endosperm constituents. Laurena et al. (1984a) reported 7–10 times greater values of condensed tannins in the seed coat and cooking broth than in the whole cowpea seed with the protein precipitation method. With modified vanillin and Prussian blue assays, similar results were obtained (Laurena et al., 1983). These results suggest that polyphenols/tannins which are not in contact with endosperm constituents are more reactive and give higher values. Though there was no apparent effect of temperature on the amount of polyphenols in the soaked grains, higher temperature evidently enhanced the leaching out process (Table III).

As shown in Table IV, a decrease in polyphenol content as boiling progressed was accompanied by a similar decrease in protein precipitable phenols. Laurena et al. (1984c) showed a +0.92** correlation between the tannin content obtained by the Prussian blue and protein precipitation assays. Boiling reduced polyphenols up to 73% and the protein precipitable phenols up to 91% (Table IV). The decrease in polyphenols was significantly positively correlated with the decrease in protein-precipitable phenols (+0.95**). An apparent loss of 20–39% from raw to cooked seeds when expressed as tannic acid and of 61–98% when

Table IV. Effects of Boiling on Tannin Content of Mung Bean Seeds[a]

boiling time (min)	polyphenol content (mg of catechin/g of seeds)	protein-precipitable phenols (mg of tanninc acid/g of seeds)
0	3.70	0.86
15	1.38 (62.7)[b]	0.35 (59.1)
20	1.08 (70.8)	0.27 (68.2)
30	1.00 (73.0)	0.17 (80.3)
45	1.22 (67.0)	0.20 (77.3)
60	1.25 (66.2)	0.16 (81.8)
90	1.28 (65.4)	0.08 (90.9)

[a] Twenty-five grams of Pag-asa 1 seeds were placed in a 500-mL Erlenmeyer flask containing 250 mL of H_2O and cooked for 15–90 min. Sample seeds were withdrawn at 15- and 30-min intervals. [b] Values in parentheses refer to percent polyphenol reduction.

Table V. Changes in Polyphenol Content of Mung Bean Seeds during Germination

germination (h)	polyphenol content[a] (mg of catechin/g)	polyphenol reduction (%)
0	3.16	
24	2.44	22.8
48	2.01	36.4
72	2.20	30.4
96	2.25	28.8
120	2.96	6.4

[a] Average of three replicates analyzed by Prussian blue assay. Fifty seeds of Pag-asa 1 were placed in filter-lined petri dishes and allowed to germinate in the dark at 25 °C.

expressed as catechin equivalent was reported by Bressani et al. (1983). Elias et al. (1979) reported that cooking decreased tannin content in whole seeds while a high concentration was found in the cooking broth.

Roasting lowered the polyphenol content of mung bean seeds by 16.67% (roasted seeds contained 3.15 mg of catechin/g of sample compared to 3.78 mg of catechin/g of sample of the raw sample). The amount of polyphenols reduced by roasting might represent the polyphenols changed chemically and not assayable by the method used.

The lower amounts of assayable polyphenols in the soaked and cooked seeds may be due to the physical leaching out of the polyphenols into the water, the process occurring at a faster rate at higher temperature. It is also likely that part of the polyphenols may have entered the endosperm with the imbibed water (Chavan et al., 1981), as was noted by the slightly higher polyphenol content of the soaked cotyledon compared to the raw cotyledon. Satwadhar et al. (1981) claimed that the apparent decrease in polyphenols was most likely not due to the actual decrease of tannins but to a change in their solubility and chemical reactivity. Soaking in water may provide a suitable environment for tannin–protein interactions, promoting the formation of insoluble complexes and unreactive tannins. The high temperature and dry heat may have brought about change in chemical structure and hence, reactivity. Laurena et al. (1984c) noted that cooking cowpea by boiling, roasting, and autoclaving was accompanied by a removal of polyphenols and an increase in in vitro protein digestibility. They further noted that a reduction in assayable polyphenols could be brought about by removal of polyphenols (by leaching out into the soak water) or change in the chemical reactivity of the polyphenols. Further, although a large reduction in assayable polyphenol content was brought about by roasting the increase in in vitro protein digestibility was not as much as in boiling or germination.

Effect of Germination. Germination reduced polyphenol content only by 23–36%, with maximum poly-

phenol removal occurring after 48 h. Continued germination up to 120 h resulted in a decrease in polyphenol removal or an increase in polyphenol content (Table V). Narasinga Rao and Prabhavathi (1982) noted a 20–30% loss of tannin content on germination of green gram dhal and bengal gram.

In this experiment, the significant browning of the filter paper indicated leaching of the polyphenols. Formation of tannin–protein insoluble complexes and unreactive tannins may also account for the decrease in polyphenol content. However, a significant increase in polyphenol content of germinated seeds after 120 h may be due to a fresh synthesis of polyphenols or the polymerization of existing phenolic compounds or degradation of high molecular weight insoluble polymers into smaller molecular weight soluble polymers that give color reaction to the reagent (Satwadhar et al., 1981).

Soaking, cooking, and germination are ordinary methods employed in the preparation of food using mung bean. As such, they provide a convenient, inexpensive, easy, and practical means of deactivating or removing polyphenols from this legume.

ACKNOWLEDGMENT

We thank Fernando Santiago for his help in the statistical analysis of the data and Cleotilde M. Vicencio for her technical assistance.

LITERATURE CITED

Barroga, C. F.; Laurena, A. C.; Mendoza, E. M. T. *J. Agric. Food Chem.*, in press.

Blessin, C. W.; VanEtten, C. H.; Dimler, R. J. *Cereal Chem.* **1983**, *40*, 421.

Bressani, R.; Elias, L. G.; Wolzak, A.; Hagerman, A. E.; Butler, L. G. *J. Food Sci.* **1983**, *48*, 1000.

Bressani, R.; Flores, M.; Elias L. G. In "Potentials of Field Beans and Other Food Legumes in Latin America"; Wall, D. Ed.; Centro International de Agricultura Tropical: Cali, Colombia, 1973; p 17.

Bullard, R. W.; York, J. O.; Kilburn, S. R. *J. Agric. Food Chem.* **1981**, *29*, 973.

Chavan, J. K.; Kadam, S. S.; Salunkhe, D. K. *J. Food Sci.* **1981**, *46*, 638.

Chibber, B. A. K.; Mertz, E. T.; Axtell, J. D. *J. Agric. Food Chem.* **1978**, *26*, 679.

De Lumen, B. D.; Salamat, L. A. *J. Agric. Food Chem.* **1980**, *28*, 533.

Earp, C. F.; Akingbala, J. O.; Ring, S. H.; Rooney, L. W. *Cereal Chem.* **1981**, *58*, 234.

Eggum, B. O.; Alabata, E. P.; Juliano, B. O. *Qual. Plant. Plant Foods Hum. Nutr.*, **1981**, *31*, 175.

Elias, L. G.; Bressani, R. *Arch. Latinoam. Nutr.* **1974**, *24*, 365.

Elias L. G.; Colindres, R.; Bressani, R. *J. Food Sci.* **1964**, *29*, 118.

Elias, L. G.; De Fernandez. D. G.; Bressani, R. *J. Food Sci.* **1979**, *44*, 524.

Elkowicz, K.; Sosulski, F. W. *J. Food Sci.* **1982**, *47*, 1301.

Fernandez, R.; Elias, L. G.; Braham, J. E.; Bressani, R. *J. Agric. Food Chem.* **1982**, *30*, 734.

Griffiths, D. W. *J. Sci. Food Agric.* **1981**, *32*, 797.

Griffiths, D. W.; Moseley, G. *J. Sci. Food Agric.* **1980**, *31*, 255.

Hagerman, A. E.; Butler, L. G. *J. Agric. Food Chem.* **1978**, *26*, 809.

Jaffe, W. G. *Arch. Latinoam. Nutr.* **1968**, *18*, 205.

Jambunathan, R.; Mertz, E. T. *J. Agric. Food Chem.* **1973**, *21*, 692.

Laurena, A. C.; Garcia, V. V.; Mendoza, E. M. T. *Phil. Agric.* **1984b**, *67*, 3, 329.

Laurena, A. C.; Garcia, V. V.; Mendoza, E. M. T., unpublished results, 1984c.

Laurena, A. C.; Truong, V. D.; Mendoza, E. M. T., unpublished results, 1983.

Laurena, A. C.; Truong, V. D.; Mendoza, E. M. T. *J. Agric. Food Chem.* **1984a**, *32*, 1045.

Liener, I. E. "Toxic Constituents of Plant Foodstuffs"; Academic Press: New York, 1980.

Narasinga Rao, B. S.; Prabhavathi, T. *J. Sci. Food Agric.* **1982**, *33*, 89.

Phillips, D. E.; Eyre, M. D.; Thompson, A.; Boulter, O. *J. Sci. Food Agric.* **1981**, *32*, 423.

Price, M. L.; Butler, L. G. *J. Agric. Food Chem.* **1977**, *25*, 1268.

Price, M. L.; Butler, L. G.; Rogler, J. C.; Featherson, W. R. *J. Agric. Food Chem.* **1979**, *27*, 441.

Price, M. L.; Hagerman, A. E.; Butler, L. G. *J. Agric. Food Chem.* **1980a**, *28*, 459.

Price, M. L.; Hagerman, A. E.; Butler, L. G. Nutri. Rep. Int. **1980b**, *21*, 761.

Price, M. L.; Scoyoc, S. V.; Butler, L. G. *J. Agric. Food Chem.* **1978**, *26*, 1214.

Ramachandra, G.; Virupaksha, T. K.; Shadaksharaswany, M. *J. Agric. Food Chem.* **1977**, *25*, 1101.

Reichert, R. D.; Fleming, S. E.; Schwab, D. J. *J. Agric. Food Chem.* **1980**, *28*, 824.

Satwadhar, P. N.; Kadam, S. S.; Salunkhe, D. K. *Qual. Plant.—Plant Foods Hum. Nutr.* **1981**, *31*, 71.

Received for review November 27, 1984. Revised manuscript received April 23, 1985. Accepted July 22, 1985. This study was supported by the Institute of Plant Breeding under the Legumes Program. IPB Journal Paper 84–13.