Registry No. 1, 2274-58-0; 1·DCHA, 119853-36-0; 3, 107-95-9; 3 (benzyl ester), 14529-00-1; 3 (methyl ester), 4138-35-6; 4, 122663-58-5; 4 (methyl ester), 123486-25-9; 5, 94588-27-9; 6, 57133-29-6; 7, 123486-20-4; 8, 123486-19-1; 9, 123486-18-0; 10, 123486-23-7; 11, 123486-24-8; 12, 123486-26-0; HODSP, 45797-54-4; Boc-Orn(Cbz)-OH, 2480-93-5; Boc-Orn(Cbz)-β-Ala-OBzl, 123486-21-5; Ac-Orn(Cbz)-β-Ala-O-Bzl, 123486-22-6; Cbz-Orn(Boc)-OH, 7733-29-1; Cbz-Orn(Boc)-β-Ala-OBzl, 123486-27-1; H-Orn(Cbz)-β-Ala-OBzl·HCl, 123486-28-2; Cbz-Orn-β-Ala-OBzl·HCl, 123486-29-3; Cbz-Orn(Ac)-β-Ala-OBzl, 123505-62-4; OBA·1.3HCl, 123486-30-6; OBA·0.65HCl, 123486-31-7; OBA·1.3AcOH, 123486-32-8; OBA·1.3Ac-Tau-OH, 123486-33-9; OBA·0.65mal, 123486-34-0; Ac-Orn-β-Ala-OH·0.3HCl, 123486-35-1; H-Orn(Ac)-β-Ala-OH·0.3HCl, 123486-36-2; Ac-Orn(Ac)-β-Ala-OH, 123486-37-3; H-Orn-β-Ala-OMe·0.5HCl, 123486-38-4; H-Orn-β-Ala-OMe·1.0HCl, 123486-26-0; NaCl, 7647-14-5.

LITERATURE CITED

Huynh-ba, T.; Philippossian, G. Alleged Salty Taste of L-Ornithyltaurine Monohydrochloride. *J. Agric. Food Chem.* **1987**, *35*, 165.

Ishibashi, N.; Arita, Y.; Kanehisa, H.; Kouge, K.; Okai, H.; Fukui, S. Studies on Flavored Peptide. I. Bitterness of Leucine-containing Peptides. *Agric. Biol. Chem.* **1987**, *51*, 2389.

Kouge, K.; Koizumi, T.; Okai, H.; Kato, T. Peptide Synthesis in Aqueous Solution. I. Application of *p*-Dialkylsulfoniophenols as a Water Soluble Coupling Reagents. *Bull.Chem. Soc. Jpn.* **1987**, *60*, 2409.

Tada, M.; Shinoda, I.; Okai, H. L-Ornithyltaurine, a New Salty Peptide. *J. Agric. Food Chem.* **1984**, *32*, 992.

Received for review January 23, 1989. Accepted June 12, 1989.

Physicochemical Basis for Hardseededness in Mung Bean (*Vigna radiata* (L.) Wilczek)

Felicito M. Rodriguez and Evelyn Mae T. Mendoza*

Biochemistry Laboratory, Institute of Plant Breeding, College of Agriculture University of The Philippines at Los Baños College, Laguna, The Philippines 4031

Hard seeds ranged from 0 to 3.8% in four varieties and four seed lots of mung bean from commercial sources. Uncooked normal and hard seeds were indistinguishable although, after boiling for 30 min, the hard seeds remained uncooked and were hard, brownish, and wrinkled. The hardness and seed coat thickness of hard seeds were twice those of normal seeds. Hard and normal seeds had similar chemical proximate composition except for fiber content, which was 9–25% greater in the hard seeds. The seed coats of hard seeds had 12% higher fiber content, 7 times more lignin, and 23% higher silica than the normal. The amino acid composition and pectic substances content of the two types of mung bean were similar. Histochemical analysis and scanning electron microscopy revealed a more rigid and highly structured palisade layer in the hard seed than in the normal seeds.

The hard seed (hard coat, hard shell) phenomenon has been reported for several leguminous species including soybean (Saio, 1976), cowpea (Sefa-Dedeh et al., 1979; Sefa-Dedeh and Stanley, 1979), and black beans (Molina et al., 1976; Varriano-Marston and Jackson, 1981; Jackson and Varriano-Marston, 1981) as well as for several species of Papilionaceae, like yellow peas (Werker et al., 1979). Various studies have sought to explain the causes of this phenomenon. The impermeability to water of *Pisum elatius*, *Pisum fulvum*, and *Pisum humile* is said to be due to the continuous, very hard pectinaceous layer of the caps of the palisade cells as well as the presence of quinones in a continuous layer of cells around the seed both in the lumen and the cell wall (Werker et al., 1979). Hard soybeans have higher amounts of crude fiber and calcium than normal soybeans (Saio, 1976). Lignification and presence of pectate could be possible causes of the hard-coat phenomenon resulting in descreased cookability (Bourne, 1967; Molina et al., 1976). The cross-linking of hydroxyprolyl residues in proteins to lignin has been suggested as an initial step in lignification of the cell wall (Whitmore, 1978). This phenomenon has also been established to occur after prolonged storage under unfavorable conditions of high temperature and high humidity (Molina et al., 1976).

Hard seeds, locally termed "patol", have also been observed in mung bean, a popular legume in the Philippines and in Southeast Asia. These hard seeds remain raw even after the normal seeds are fully cooked. To avoid the presence of these hard seeds in viands of mung bean, some crush the seeds before cooking. Others preboil the mung bean and then remove the uncooked seeds before fully cooking the rest of the seeds.

The mung bean hard seeds occur from 2 to 5% in newly harvested seeds during the dry season and occur to a greater extent among the yellow variety (Mendoza et al., 1988). This study is aimed at providing some basic information on the physicochemical characteristics of the mung bean hard seeds, which could explain this phenomenon and aid in the search for methods to preserve its cooking quality and acceptability.

EXPERIMENTAL SECTION

Materials. Samples of known varieties of mung bean seeds were obtained from the Legume Division of the Institute of Plant Breeding through the courtesy of Rudy S. Navarro. Samples

0021-8561/90/1438-0029$02.50/0 © 1990 American Chemical Society

Table I. Proximate Composition of Raw Mature Seeds of Several Mung Bean Varieties

variety[a]	moisture,[b] %	fat, %	protein, % N × 6.25	ash, %	fiber, %	NFE, %
CES 87 (N)	7.22 c	1.46 a	21.21 d	4.12 a	5.07 c	60.92
Pag-asa 1 (N)	7.29 b	1.19 b	22.84 c	3.98 b	4.51 e	60.19
Pag-asa 2(N)	7.24 c	1.06 d	24.96 a	3.56 d	4.15 f	59.03
Pag-asa 3 (N)	7.12 d	1.12 c	23.42 b	3.64 c	4.56 d	60.14
Pag-asa 2 (H)	7.14 d	1.12 c	24.78 a	3.44 e	5.48 b	58.04
Mixed variety (H)	7.56 a	1.21 b	20.46 e	3.56 d	5.61 a	61.60

[a] Key: N, normal; H, hard; NFE, nitrogen-free extract. [b] Means followed by a common letter are not significantly different at the 5% level (Duncan's multiple-range test, DMRT).

Table II. Proximate Analysis of Mung Bean Seed Coats of Pag-asa 2 (Dry Weight Basis)

seed coat	protein,[a] % N × 6.25	fat, %	fiber, %	ash, %	NFE, %
hard seeds	8.00 b	2.37 a	35.11 a	4.11 a	50.41
normal seeds (boiled)	8.64 a	2.40 a	31.12 b	4.02 a	53.82

[a] Means followed by a common letter are not significantly different at the 5% level by DMRT.

of unknown varieties were obtained from the market and stores in Los Baños, Laguna.

Preparation of Samples. (*a*) *Separation of Hard Seeds.* A 500-g portion of mature mung bean seeds was boiled in water for 30 min. Uncooked intact seeds were sorted from the boiled seeds. These seeds were considered as hard seeds and were dried in a forced-draft oven at 70 °C for 12 h.

(*b*) *Removal of Seed Coat.* Seed coats were removed by hand from cooked normal seeds and from hard seeds. The seed coats were ground to 60 mesh on an intermediate Wiley mill grinder.

Examination of Physical Properties. Appearance and color were noted. Hardness of the seeds was measured with a seed hardness tester (Kiva Seisakustus). Seed coat thickness was measured with a micrometer caliper.

Chemical Analysis. (*a*) *Proximate Analysis.* Proximate analysis of the seed coats was done following methods of the Association of Official Analytical Chemists (1980) with some modifications.

(*b*) *Amino Acid Determination.* The amino acid profile of the mung bean seed coats was determined on the acid hydrolysates in a LKB Model Alpha amino acid analyzer. Acid hydrolysates were prepared under nitrogen, filtered, evaporated, and taken up in citrate buffer (pH 4).

(*c*) *Fiber Component Determination.* Acid detergent fiber, lignin, and silica contents were determined by the method of Van-Soest (1967).

(*d*) *Determination of Pectic Substances.* Pectic substances of the seed coats were analyzed for anhydrouronic acid (AUA) by the modified procedure of McComb and McCready (1952).

Localization of Lignin in the Seed Coat. Cross sections (15 μm thick) of normal and hard seeds were fixed on glass slides with Mayer's adhesive and were stained with ammoniacal basic fuchsin, a dye specific for lignin.

Stained tissues were then viewed in a stereo microscope under high-power objective (100×). Normal and hard seed coats were compared by the degree of reaction with the dye (color intensity) of the coat as an index.

Electron Microscopy of the Seed Coats. Seed coats of both normal and hard mung bean seeds were viewed in a Hitachi Model HEM 101 scanning electron microscope for differences in seed coat structures. Vertical and horizontal views were taken and examined for physical and structural differences.

RESULTS AND DISCUSSION

Occurrence, Hardness, and Seed Coat Thickness. Among the four mung bean varieties tested, the yellow-seeded variety, Pag-asa 2, contained the highest level of hard seeds (3.8%) whereas both green varieties, Pag-asa 1 and CES 87, had no hard seeds. Four samples of unknown variety of genotype obtained from the market had hard seeds ranging from 0.58 to 3.24%. With hand-harvested seeds of crownvetch (*Coronilla varia* L.), 65%

Table III. Fiber Components and Pectic Substances Content of Mung Bean Seed Coats of Pag-asa 2 (Dry Weight Basis)

sample	ADF,[a,b] %	lignin, %	silica, %	anhydrouronic acid, mg/100 g
hard seeds	43.11 a	10.50 a	40.63 a	0.40 a
normal (boiled)	40.36 b	1.24 b	31.38 b	0.42 a

[a] ADF, acid detergent fiber. [b] Means followed by a common letter are not significantly different at the 5% level by DMRT.

or even more can be hard (McKee et al., 1977).

Uncooked normal and hard seeds are indistinguishable from one another. After boiling, hard seeds could be sorted from the cooked soft normal seeds. The boiled hard seeds of Pag-asa 2 were brownish and wrinkled and were found to have higher hardness than normal (6.91 ± 1.21 vs 3.42 ± 0.66 kg). Their seed coats were thicker (0.290 ± 0.062 mm) than normal (0.166 ± 0.018 mm). Saio (1976) observed thicker seed coat for the hard soybean than normal.

Chemical Analysis. The proximate composition of the whole hard seeds of mungbean did not differ from those of cooked normal seeds except in crude fiber content (Table I). Hard seeds had 9–25% higher fiber content than the normal. The seed coats of hard seeds had significantly higher crude fiber content than normal (Table II). Further analysis of the fiber components showed the hard seeds to have 7 times more lignin than normal (Table III). Hard seeds also had 23% higher silica than normal. A high correlation of 0.91 was earlier found between the hardness value of stored cooked black beans and the lignified protein content of the cotyledon but not of the seed coat (Molina et al., 1976). Saio (1976) showed that high contents of crude fiber and calcium and the solidness of the seed coat structure are responsible for the hardness of and impermeability to water by hard soybeans.

The amino acid compositions of the hard and normal mung bean seeds were similar, and no hydroxyproline was seen in their amino acid profiles (data not shown).

Pectic substances content (Table III) of the normal and hard seed coat did not exhibit any statistically significant difference. Anhydrouronic acid contents of the normal and hard seed coat ranged from 0.36 to 0.40, and the small differences are insignificant. In contrast, other workers have correlated the hard seed coat phenomenon with high concentrations of pectic substances (Bourne, 1967; Molina et al., 1976; Werker et al., 1979).

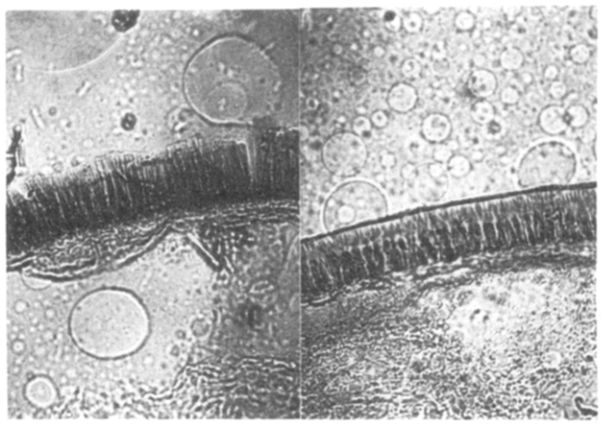

Figure 1. Cross section of seed coats of normal (left) and hard (right) seeds of mung bean stained with basic fuchsin (10×).

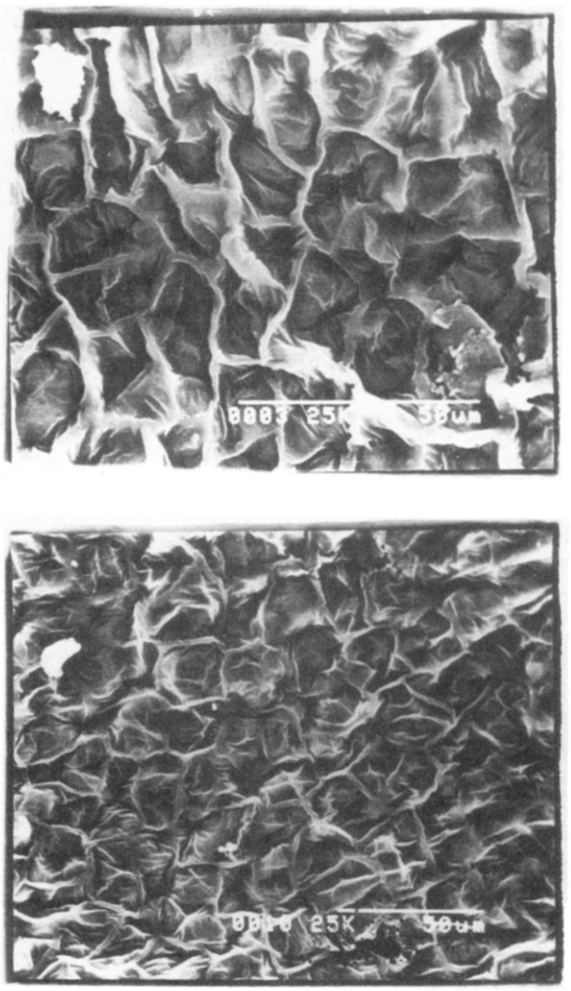

Figure 2. Scanning electron micrographs of the top view of seed coats of normal (top) and hard (bottom) seeds of mung bean.

Histochemical Analysis. The cross section of the seed coat of normal seeds stained more intensely with basic fuchsin than that of the hard seed (Figure 1). The normal seed coat but not the hard seed coat showed fractures in the cuticle membrane and palisade layer. These indicate that the hard seed coat had palisade cells that were tough and highly dense perhaps due to their high lignin and silica contents, which could prevent penetration by the basic fuchsin.

Electron Microscopy of the Seed Coat. The seed coats of the hard and normal seeds were observed to have

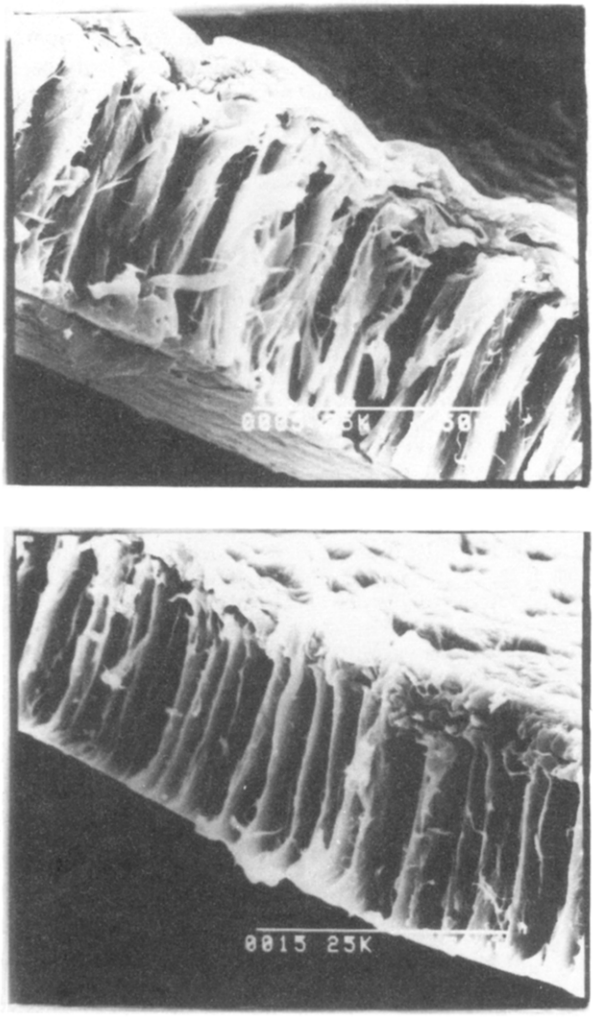

Figure 3. Scanning electron micrographs of the cross section of seed coats of normal (top) and hard (bottom) seeds of mung bean.

similar morphology from the top view (Figure 2). However, the cross section of the seed coat shows a more rigid formation of the palisade layer in the hard seed in contrast to that of the normal seed (Figure 3). The high structural integrity of the hard seed palisade layer could be due to their high lignin and silica contents.

The seed coats of the hard seeds of soybean were observed to have greater thickness and more solid ultrastructure, having tough palisade cells and hourglass cells (Saio, 1976). Moreover, the micropyle of the hard seed was closed and covered with outside palisade cells unlike the open micropyle of the normal seeds. Sefa-Dedeh et al. (1979) observed an incomplete breakdown of the middle lamellae in hard seeds of cowpea during cooking.

CONCLUDING REMARKS

The above results indicate that the high lignin and silica contents and their more solid structure may explain the hardness of the seed coat of mung bean hard seeds as well as their impermeability to water. Apparently, hydroxyprolines and pectins are not involved in the hard coat phenomenon of mung bean.

The hard seeds used in this study were those from newly harvested mung bean. No study has been done on the incidence of hard seeds during long-term storage of mung bean. Although it seems that hardseededness is a genetic character, the effect of environment on the seeds especially during the maturation stage cannot be discounted.

ACKNOWLEDGMENT

We thank Dr. Ricardo M. Lantican for his encouragement, Rudy S. Navarro for providing most of the seed samples, and Luiza Nazareno Villamael for preparation and staining of the cross section of the seed coats.

Registry No. Lignin, 9005-53-2.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 13th ed.; Association of Official Agricultural Chemists: Washington, DC, 1980.

Bourne, M. C. Size, Density and Hardshell in Dry Beans. *Food Technol.* **1967**, *21*, 335.

Jackson, G. M.; Varriano-Marston, E. Hard-to-Cook Phenomenon in Beans: Effects of Accelerated Storage on Water Absorption and Cooking Time. *J. Food Sci.* **1981**, *46*, 799.

McComb, E. A.; McCready, R. M. Colorimetric Determination of Pectic Substances. *Anal. Chem.* **1952**, *24*, 1630.

McKee, G. W.; Peiffer, R. A.; Mohsenin, N. N. Seed Coat Structure in *Coronilla varia* L. and its Relations to Hard Seed. *Agron. J.* **1977**, *69*, 53.

Mendoza, E. M. T.; Barroga, C. F.; Rodriguez, F. M.; Revilleza, M. J. R.; Laurena, A. C. Factors Affecting Nutritional Quality and Acceptability of Mung Bean (*Vigna Radiata* (L.) Wilczek). *Trans. Natl. Acad. Sci. Tech. (Phils.)* **1988**, in press.

Molina, M. R.; Bates, M. A.; Gomez-Brenes, R. A.; King, K. W. Bressani, R. Interrelationships between Storage, Soaking Time, Cooking Time, Nutritive Value and Other Characteristics of the Black Bean. *J. Food Sci.* **1976**, *41*, 661.

Saio, K. Soybeans Resistant to Water Absorption. *Cereal Foods World* **1976**, *21*, 168.

Sefa-Dedeh, S.; Stanley, D. W. The Relationship of Microstructures of Cowpea to Water Absorption and Dehulling Properties. *Cereal Chem.* **1979**, *56*, 379.

Sefa-Dedeh, S.; Stanley, D. W.; Voisey, P. W. Effect of Storage Time and Conditions on the Hard-to-Cook Defect in Cowpea [*Vigna unguiculata*]. *J. Food Sci.* **1979**, *44*, 790.

Van-Soest, P. J. The Use of Detergents in the Analysis of Fibrous Feeds: Determination of Plant Cell Wall Constituents. *J. Assoc. Anal. Chem.* **1967**, *50*, 50.

Varriano-Marston, E.; Jackson, G. M. Hard-to-Cook Phenomenon in Beans: Structural Changes During Cooking and Storage. *J. Food Sci.* **1981**, *46*, 1379.

Werker, E.; Marback, I.; Mayer, A. M. Relation Between the Anatomy of the Testa, Water Permeability and the Presence of Phenolics in the genus *Pisum*. *Annu. Bot.* **1979**, *43*, 765.

Whitmore, F. W. Lignin-Protein complex Catalyzed by Peroxidase. *Plant Sci. Lett.* **1978**, *13*, 241.

Received for review March 1, 1988. Revised manuscript received November 11, 1988. Accepted June 20, 1989. This study was supported by grants from The Philippine government to the Institute of Plant Breeding.

Structural and Gelling Properties of Dry-Heating Egg White Proteins

Akio Kato,*,† Hisham Radwan Ibrahim,† Hiroyuki Watanabe,‡ Kazuo Honma,‡ and Kunihiko Kobayashi†

Department of Agricultural Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753, Japan, and Q.P. Corporation, Fuchu-shi, Tokyo 183, Japan

Gel strength of dried egg white greatly increased by heating in the dry state at 80 °C. The structural changes responsible for the enhancement of gel strength were studied. Heat-induced gelation of dry-heated egg white in the presence of denaturing reagents resulted in a decrease of gel strength in the order SDS > urea > 2-mercaptoethanol. CD spectra revealed only little changes in the secondary structure of some egg white proteins upon dry-heating, except ovotransferrin. Proteolytic digestibility of ovalbumin and ovotransferrin increased progressively with an increase of dry-heating time, indicating enhancement of protein flexibility by such heating in the dry state. DSC thermograms showed that enthalpy of denaturation (ΔH) of dried egg white was markedly decreased with an increase of dry-heating time. In parallel, the Gibbs free energy of unfolding in water (ΔG) was also found to decrease at approximately the same rate as ΔH. A good correlation was obtained between the decrease in ΔH and the increase in gel strength of egg white proteins heated in the dry state.

Dry-heating is required for the manufacture of processed foods and the reduction of their microbial population. These processes could cause substantial protein denaturation, which is critical to functionalities such as solubility, gelation, emulsification, and foaming (Kinsella, 1976). Our previous report (Kato et al., 1989) showed that when spray-dried egg white was heated in a controlled dry state at 80 °C for various periods of time, its functionalities (gelling, emulsifying, and foaming properties) improved significantly with an increase in heating time without loss in solubility. However, the molecular basis of structural changes causing the improvements of functional properties in dry-heating egg white has yet to be unraveled. Furthermore, there is little information about the dry denaturation of proteins, despite the importance to industrial applications.

The present paper describes the conformational changes in spray-dried egg white proteins by dry-heating treatment and the relationship between the structural and functional properties, especially gel formation.

MATERIALS AND METHODS

Dried egg white (DEW) was prepared by spray-drying at 70 °C after decarbohydrate treatment. Ovalbumin was prepared from fresh egg white by a crystallization method in sodium sul-

† Yamaguchi University.
‡ Q.P. Corp.

0021-8561/90/1438-0032$02.50/0 © 1990 American Chemical Society