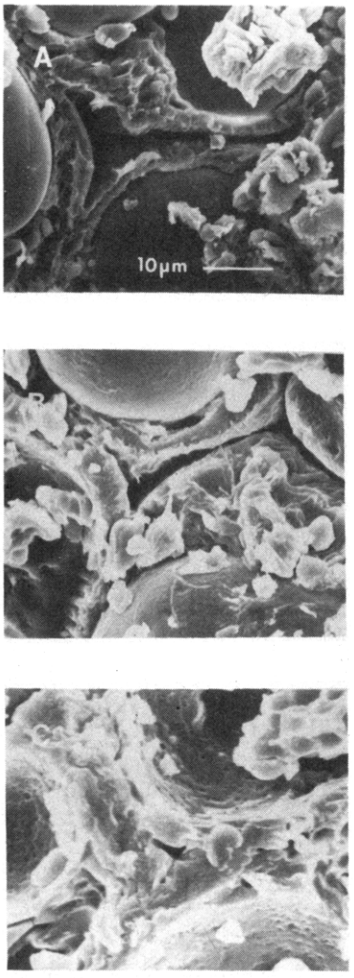

Figure 9. SEM micrographs of black beans after 8 months of storage: top, LTLH-stored beans; middle, MTMH-stored beans; bottom, HTHH-stored beans.

could certainly lead to a failure of the bean cells to separate upon cooking. Micrographs in Figure 9 depict the difference in intercellular material of raw beans after 8 months of storage. The presence of these deposits (lignin?) increased substantially from the LTLH to the HTHH storage. This observation supports the hypothesis that polymers are built up within the cell walls and in the middle lamella as the bean seeds age. Also, it is possible that water movement may be restricted within the cotyledons because of the deposited material.

ACKNOWLEDGMENT

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Registry No. Protease, 9001-92-7; polyphenolase, 9002-10-2; peroxidase, 9003-99-0; amylose, 9005-82-7; starch, 9005-25-8.

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Hard-to-Cook Defect in Black Beans. Soaking and Cooking Processes

Michael J. Hincks, Anne McCannel, and David W. Stanley*

Water absorption, solids loss, and electrolyte leakage were determined for soft and hard black beans during the soaking and cooking processes. All three parameters increased in hard beans during soaking. It is hypothesized that membrane damage or deterioration is responsible for these differences. During cooking, hard beans lost fewer solids and minerals and did not continue to hydrate to the same degree as the controls, probably as a result of restricted cell separation. Microscopic evidence indicated reduced starch gelatinization in hard beans. This is attributed to reduced water absorption.

The hard-to-cook defect that develops in dry beans stored under conditions of high temperature and high humidity has been investigated extensively. One often-repeated approach has been to establish a relationship among the imbibition characteristics of the beans and their rates of softening. Though initial work indicated no differences in the absorption patterns of hard and soft beans (Burr, 1968; Molina et al., 1976), no corrections were made for the loss of solids during soaking. More recently, Jackson and Varriano-Marston (1981) emphasized that the differences in cooking times for soft and hard beans could not be accounted for by absorption differences during

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soaking, even when corrected for lost solids.

The preparation of beans for consumption is bipartite; the beans are first soaked until saturation is achieved and then heated to induce softening. Although the soaking process has received much attention in reference to water absorption, electrolyte leakage, and loss of solids, little work has been reported on the cooking process in relation to these parameters. This is surprising since a full understanding of the cooking process would provide a more accurate overall picture of the chemical, physical, and biochemical changes affecting texture. For this reason, a series of experiments were conducted to follow these parameters over both the soaking and cooking processes.

MATERIALS AND METHODS

Materials. Black beans (*Phaseolus vulgaris*, var. orfeo) were provided by the Catholic University of Chile. The beans were field dried to a final moisture content of approximately 12% (dry-weight basis) and then shipped directly to Guelph for storage studies. Samples stored for 18 months from the 1984 harvest or for 6 months from the 1985 harvest from both storage conditions were used.

Storage Conditions. Beans were stored under two different environmental conditions: high temperature and high humidity (30 °C, 85% RH) to promote the hard-to-cook defect; low temperature and low humidity (15 °C, 35% RH) to provide controls. Lots (10 kg) of beans were put into jute bags and stored in environmental chambers (Conviron Controlled Environments, Winnipeg, Manitoba).

Water Absorption during Soaking/Cooking. Samples of approximately 10 g (weighed exactly) were placed in specially constructed nylon baskets (1000-μm mesh opening; B & SH Thompson and Co. Ltd., Scarborough, Ontario) and immersed in 50 mL of deionized water at 25 °C. After the appropriate soaking time beans were rinsed with deionized water to bring the volume back to the original 50 mL, drained, blotted dry, and weighed. The soak water was freeze-dried to determine solids lost. Absorption measurements were done for 6-, 12-, and 18-h soaking and were performed in triplicate. Water absorption was expressed as the percentage of water absorbed on a dry-weight basis either uncorrected or corrected for the loss of solids into the soak water for the time interval in question as recommended by Jackson and Varriano-Marston (1981). Calculations were as follows:

$$WA_u = \frac{\text{wt gain beans after soaking}}{\text{dry wt beans}} \times 100$$

$$WA_c = \frac{\text{wt gain beans after soaking + solids lost}}{\text{dry wt beans}} \times 100$$

WA_u = uncorrected water absorption, WA_c = corrected water absorption

Water absorption for the cooked product was determined in a manner similar to that for soaked beans. After 18-h soaking the beans were drained, placed in fresh deionized water, and cooked on a hot plate for 0.5, 1.0, 1.5 and 2.0 h, maintaining the volume of water. Cook time began when the water started boiling. The beans were rinsed and drained, and the bottom of the basket was blotted dry. In order to accurately assess water content the beans were allowed to dry for 45 s—the time required to lose any surface water—and immediately placed in a preweighed plastic bag and sealed so as to prevent evaporation during cooling. The beans were allowed to cool for 1 h and weighed. The cook water was freeze-dried, and water absorption was calculated as for soaked beans. For corrected water absorption solids lost included the initial loss from 18-h soaking plus the additional loss on cooking. Data for each soaking/cooking time were obtained from separate samples of beans. These and all subsequent studies discussed were preformed in triplicate.

Specific Conductivity of the Soak/Cook Water. Electrolyte leakage was determined by measuring the specific conductivity of the soak/cook water after beans had been removed and drained and the volume brought up to the original 50 mL. A YSI conductivity bridge was used (Model 31, Yellow Springs Instrument Co., Yellow Springs, Md), and the specific conductivity was expressed as micromhos/centimeter.

Cooked Hardness. Beans that had been previously soaked (18 h at 25 °C in 5 vol of deionized water) were placed in fresh deionized water and cooked for 2 h. The cooked beans were allowed to cool for 1 h prior to weighing for subsequent analysis. The hardness was measured using a 10-cm^2 Ottawa Texture Measurement System (OTMS) cell (Canada Machinery Ltd., Simcoe, Ontario) with an eight-bar wire extrusion grid (Sefa-Dedeh et al., 1978). Maximum force to compress and extrude a 30-g sample of cooked beans was measured, and three replicates were performed.

Mineral Analysis. Magnesium, calcium, and potassium contents of the leachates were obtained by atomic absorption spectrophotometry. Aliquots (5.0 mL) of the soak water/cook water were digested in 36 mL of nitric acid and 5 mL of perchloric acid, brought to a standard volume of 25 mL with water, and analyzed on a Varian AA4 atomic absorption spectrophotometer. Results were expressed as micrograms of mineral/gram of dry bean.

Isolation of Starch from Cooked Beans. Beans were removed from the cook water at the end of 2 h, dehulled, and freeze-dried. Samples (10 g) were homogenized in a precooled blender with 50 mL of deionized water at 4 °C for 5 min. The slurry was allowed to cool for 15 min and homogenized a second time for 5 min. The resultant slurry was washed through succesive screens (190 and 44 μm) as described by Hincks and Stanley (1987) for the isolation of cell wall material except that the screening procedure was performed only once. The crude starch preparation was taken as the material that had passed through both screens and settled to the bottom of the beaker. Excess water was decanted, and the samples were frozen at −40 °C and freeze-dried.

Microscopy. Cooked beans were prepared as described by Hincks and Stanley (1986). Thin sections (800–900 Å) were picked up on 200-mesh copper grids and stained with uranyl acetate and lead citrate (Venable and Coggeshell, 1965). These were viewed and photographed on a Phillips EM-300 transmission electron microscope operated at 60 kV.

Both whole bean samples and the crude starch isolate from cooked beans were mounted on aluminum stubs and coated with 300 Å of gold/palladium for examination on an Hitachi S-570 scanning electron microscope at an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

Texture. Cooked hardness of beans stored under the two different storage conditions for the time intervals of 6 and 18 months are given in Table I. As expected, high temperature and humidity induced hardening that was most pronounced at the longer storage period.

Water Absorption, Solids Loss, and Electrolyte Leakage. *Soaking.* Initial rates of water absorption (0–6 h) during soaking varied according to both time of storage and storage conditions (Figure 1). The amount of water absorbed by 6 h was significantly different for all four

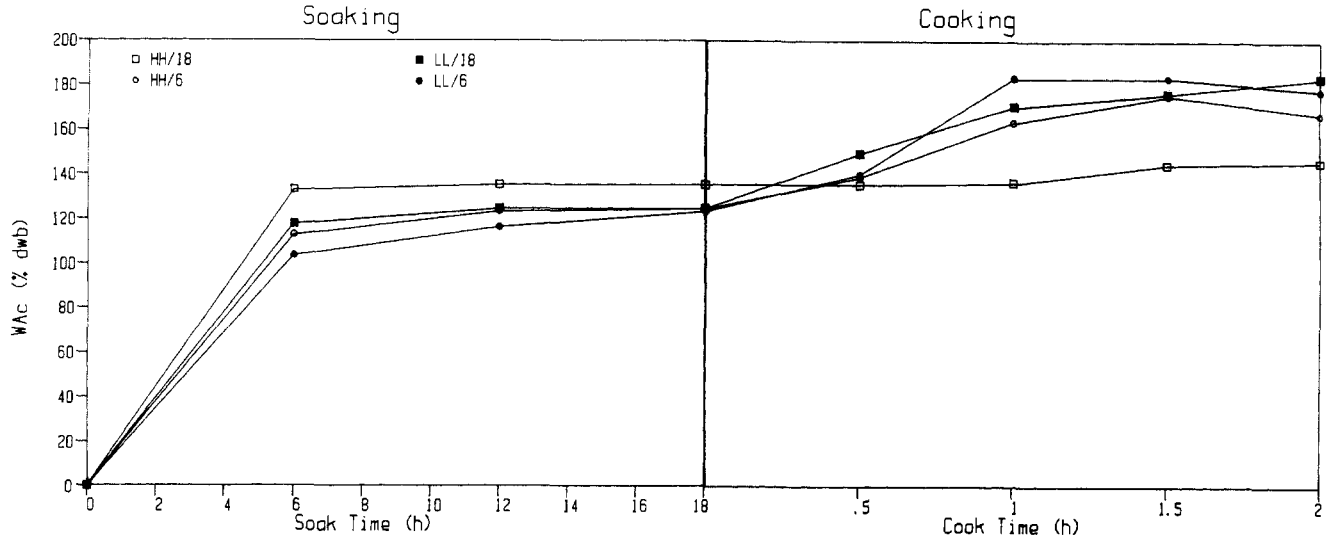

Figure 1. Corrected water absorption for black beans during soaking and cooking.

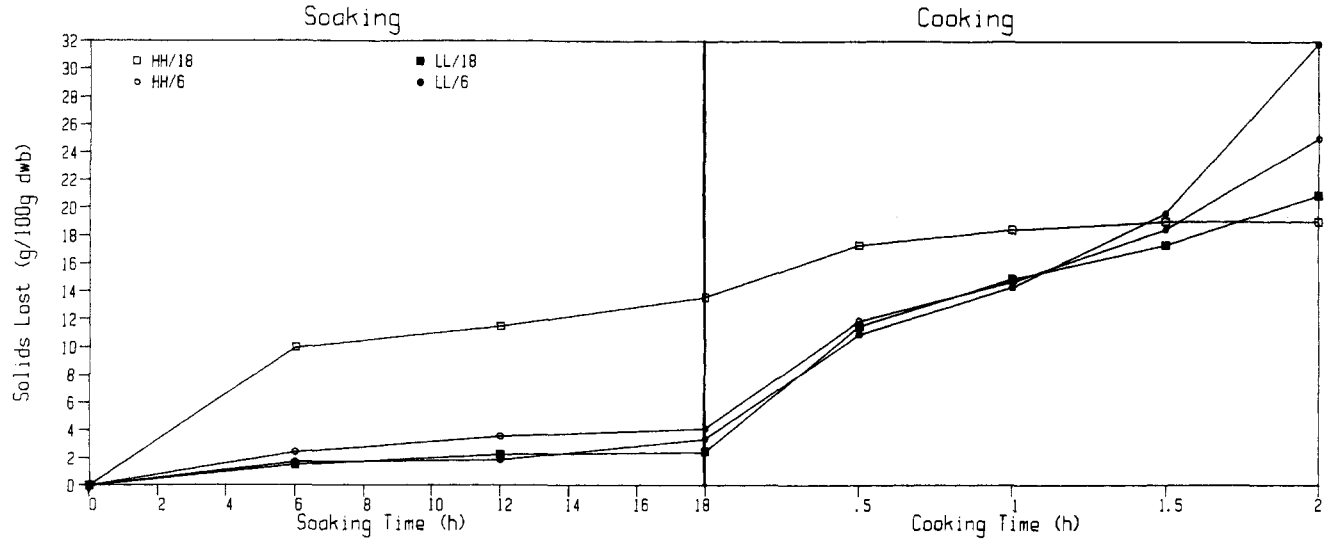

Figure 2. Solids lost during soaking and cooking of black beans.

Table I. Cooked Texture of Black Beans

storage condition/ time, months	cooked texture,[a] kg/30 g	storage condition/ time, months	cooked texture,[a] kg/30 g
HH/18	75.6[a]	HH/6	31.2[c]
LL/18	22.3[b]	LL/6	21.4[b]

[a] Means with different superscripts are significantly different ($P \leq 0.05$).

treatments ($P \leq 0.05$) regardless of whether or not the absorption values were corrected for lost solids. Bean hydration was most rapid for the longer storage period and adverse conditions. These data suggest that initial water absorption is affected by the higher moisture levels associated with storage at elevated humidity [as shown by Sefa-Dedeh et al. (1979)] as well as some aspect related to the physiological changes resulting from seed aging.

Final absorption figures obtained after 18-h soaking depended on whether values were corrected for loss of solids (Table II). The problems of interpretation due to this discrepancy have been discussed by Parrish and Leopold (1978), and corrections for this loss of material from the seeds have been recommended by these authors and others (Jackson and Varriano-Marston, 1981). When lost solids were not accounted for, the final absorption values

Table II. Water Absorbed for Soaking and Cooking Processes

process	storage conditions/ time, months	WA_c,[a] % dwb	WA_u,[a] % dwb
soaking (18 h)	HH/18	134.76[a]	121.21[a]
	LL/18	125.20[b]	122.80[a]
	HH/6	124.61[b]	120.44[a]
	LL/6	123.49[b]	120.19[a]
cooking (2 h)	HH/18	145.50[a]	126.57[a]
	LL/18	183.04[b]	162.12[b]
	HH/6	167.53[b]	142.55[ac]
	LL/6	178.22[b]	146.43[bc]

[a] Means with different superscripts are significantly different ($P \leq 0.05$). WA_c = water absorption corrected. WA_u = water absorption uncorrected.

were not significantly different; upon correction HH/18 beans had imbibed significantly more water ($p \leq 0.05$) than the other treatments. This trend was noted by Burr et al. (1968) and Jackson and Varriano-Marston (1981).

These differences in the WA_c can be attributed to the relatively large amounts of solids lost over the 18-h period of soaking by the HH/18 beans. Figure 2 illustrates the progression of leached solids over soaking time. By 18 h there was a significant difference ($P \leq 0.05$) between all four treatments, with the high temperature and humidity storage showing higher losses. The solids lost during

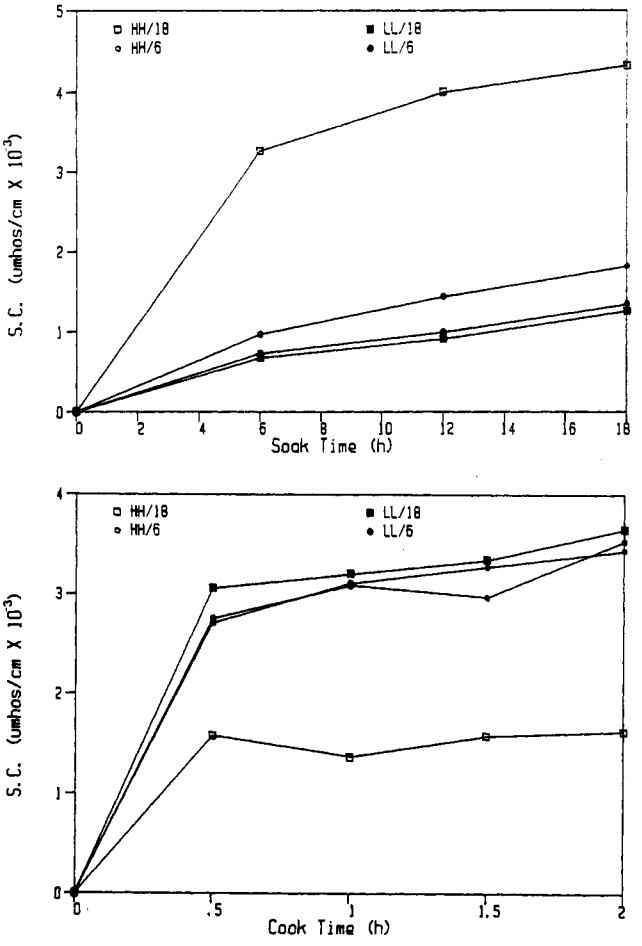

Figure 3. Specific conductance of the leachates during soaking (top) and cooking (bottom).

soaking were positively correlated to the final cooked texture ($r = 0.995$, $P \leq 0.01$) as was the WA_c ($r = 0.982$, $P \leq 0.05$).

These two trends—increased loss of solids and increased water absorption—may be explained by the aging process of the quiescent seed. Several authors have indicated that aging involves loss of membrane integrity leading to increased permeability and a resultant leakage of sugars, amino acids, and inorganic salts (Harman and Granett, 1972; Bewley and Black, 1985). This disruption of membranes may also allow more uncontrolled hydration and subsequent higher water absorption in seeds that are most severely affected (HH/18).

It should be noted that there is a question as to the validity of the ultrastructural studies on dry seeds with present fixation techniques and, thus, the interpretation of membrane changes (Abdul-Baki and Baker, 1973). There is also some discussion as to whether observed changes such as plasmalemma abberations (Jackson and Varriano-Marston, 1981) are the cause of, or an effect due to, seed aging.

Electrolyte leakage during soaking, as determined by the specific conductance of the leachate, paralleled the loss of solids and 18-h conductance values were correlated with the final cooked texture ($r = 0.999$, $P \leq 0.01$) (Figure 3). It has been shown that several of the parameters measured during soaking are related to the final cooked texture. In order to fully explain the series of events that lead to hardening, or are secondary effects of these events, it is imperative to investigate the cooking process as well.

Cooking. During the cooking process defective beans (HH/18) absorbed little additional water while the softer controls and somewhat harder HH/6 beans continued to hydrate (Figure 1). After 2 h the HH/18 beans had significantly lower WA_c ($P \leq 0.05$). Water absorption at this time was negatively correlated to cooked texture ($r = -0.970$, $P \leq 0.05$).

The amout of soluble solids lost increased only slightly during the first 30 min in hard beans, after which no further losses occurred (Figure 2). The other three treatments showed a progressive loss of solute over the cooking time, having lost amounts equal to the HH/18 beans by 1.5 h and surpassing this by 2.0 h. After cooking, beans previously stored for shorter intervals lost significantly more solids than their 18-month counterparts, though in both cases unfavorable storage reduced the solids lost. This reduction was only significant for 6-month storage.

The trend seen with lost solids indicates that fresher seeds are more susceptible to solute leakage during cooking and that restriction of this leakage due to high temperature and humidity is significant over short storage periods. Specific conductance of the leachate from cooked beans did not parallel the loss of solids as closely as in the soaking procedure (Figure 3). The major increase in specific conductance occurred in the first 30 min, leveling off thereafter. Although there was a further loss of solute after this time, it did not contribute greatly to the specific conductance of the leachate.

An important result is the restriction of further hydration in the hard-to-cook beans upon cooking. This should not be misinterpreted as a cause of the defect but is rather a consequence of its development. Reduced absorption over the cooking process may, in turn, have secondary effects that could contribute to cooked texture. The main consideration here would be starch gelatinization as will be discussed.

As previously mentioned, it is thought that reduced hydration in the cooked product is a consequence of the mechanisms causing the hard-to-cook defect. Previous studies have indicated that bean hardening is a multistaged process, involving at least two mechanisms: phytate loss as a minor mechanism during initial storage and lignin deposition as a major mechanism during later storage times (Hincks and Stanley, 1986, 1987). The lower water absorption pattern seen is thought to result from the lack of cell separation brought about by these two mechanisms, especially the incorporation of lignin into the cell wall matrix. In the controls, cooking leads to a breakdown of the middle lamella, unimpeded by either Ca/Mg pectate cross-linking or lignin, and the individual cells are allowed to separate from one another. As this progresses, absorption is enhanced due to the exposure of greater surface areas of the cells. In the defective beans, cell separation is prevented, precluding further hydration.

The results show that defective beans do not continue to hydrate upon cooking to the same degree as the controls. This difference in water uptake would be expected to affect starch gelatinization and subsequently the final cooked texture. Under the assumption that all components of bean tissue hydrate equally, the volume fractions of water (v_1) for the HH/18 and LL/18 beans at the end of the cooking process were 0.69 and 0.74 (density of water 1.00; density of starch 1.55; Donovan, 1979). These values have been found critical to the gelatinization process. Previous work using differential scanning calorimetry (DSC) has established the dependance of gelatinization on moisture content (Lund, 1984). Although oversimplified, the following applies. When $v_1 > 0.70$, a single endotherm is observed in the thermal curve (G endotherm). This is explained as a water-mediated cooperative melting of

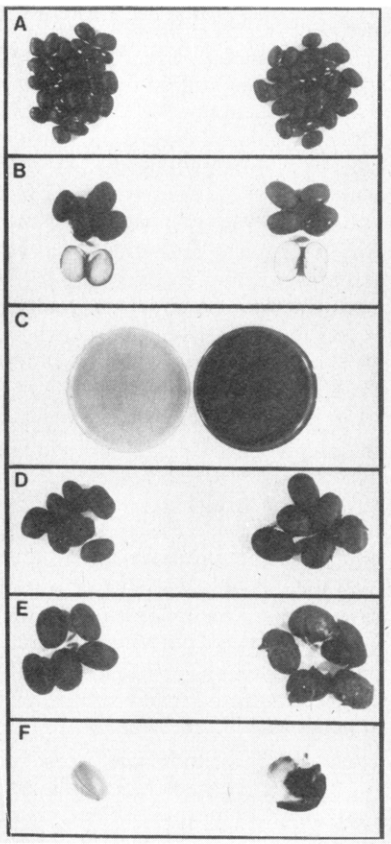

Figure 4. Visual appearances of black beans during soaking/cooking: A, raw seeds (left side HH/18, right side LL/18); B, seeds soaked 18 h; C, leachate from 18-h soaked beans; D, seeds cooked 0.5 h; E, seeds cooked 2.0 h; F, cooked bean after pressing between fingers. Note whole discolored cotyledon that separated easily from seed coat in the HH beans; LL beans were soft and extruded from the fractures under pressure.

starch crystallites (Burt and Russel, 1983). Biliaderis et al. (1980) equate this event to the swelling of the amorphous regions that strips chains from starch crystallites. When $v_1 < 0.70$, two endotherms are observed: a G endotherm at the same temperature as when excess water is available but of less intensity (reduced enthalpy) and an M_1 endotherm at a higher temperature relating to the melting of remaining crystallites. In this case, the gelatinization temperature range is broadened.

Recent work on starch isolated from black beans found that at $v_1 = 0.69$ an M_1 endotherm was present while at $V_1 = 0.78$ only a G endotherm was observed (Hohlberg and Stanley, 1987). This emphasizes the importance of the critical value ($v_1 = 0.70$). That the volume fractions of water for the defective and control beans in this study fall below and above the 0.70 value supports the contention that the extent of gelatinization would be expected to differ between the two storage conditions.

Visual Changes in Beans over Soaking/Cooking. Visual differences between HH/18 and LL/18 beans were observed over the soaking and cooking processes (Figure 4). There were no discernible distinctions between the raw product in either testae color or size distribution, but the cotyledons of the HH beans were darker with a brown/yellow discoloration compared to the white cotyledons of the controls. Several differences were apparent as a result of soaking. These included a greater volume expansion as well as a loss of testae color in the LL beans; LL seeds were purplish/gray while HH seeds retained their original black color. The leachate from the controls was a transparent purple; the HH leachate was muddy brown and solids were visible. It is likely that the color changes of the testae and cotyledons are associated with polyphenols since they are more difficult to extract from defective seeds (Hincks and Stanley, 1986), perhaps as a result of polymerization under high temperature and humidity conditions. Discoloration of raw bean cotyledons may indicate that this process occurs over storage, although some mobilization from seed coat to cotyledons is apt to occur during soaking.

A further feature of soaked hard beans was that cotyledon surfaces were wet when separated, with water seemingly held within the space between the cotyledons as well as between the seed coat/cotyledon interface. In contrast, cotyledons from the controls were dry. Jackson and Varriano-Marston (1981) reported that WA_c for decorticated fresh and aged beans was not significantly different. As our results confirm, however, intact HH beans absorbed more water than controls. Water associated with the testa and cotyledon space may account for this difference, indicating that when the defect is present, there is a maximum hydration limit and any additional water is retained outside the cotyledons.

When cooked, the control beans continued to increase in volume and lose color from the testa. Seed surfaces were smooth, while that of the HH beans developed an uneven and wrinkled surface. The size and color of HH beans was unchanged. Breakdown of the LL beans over cooking time seemed to follow a set pattern. By 0.5 h a characteristic fracture occurs below the hilum at the micropyle end. As time progressed, the beans often split, at times severing in half. By the end of 2-h cooking, control beans are soft and their cotyledons yield to finger pressure and flow from the fractures as a smooth paste. When HH seeds are pressed, whole cotyledons are expelled from the testa, their surfaces badly discolored.

Mineral Contents of the Leachates. Mineral contents of the leachates for all treatments as a function of soaking and cooking times are shown in Figure 5 and Figure 6. During soaking, significantly greater quantities of magnesium, calcium, and potassium were leached at each soaking interval from the HH/18 beans than from the three other treatments ($P \leq 0.05$). Leachate mineral contents decreased from 12 to 18 h for the HH/18 beans. This is thought to be a result of sampling error due to the higher solids content of this leachate though resorption by the beans from the soak water cannot be discounted. In the three remaining treatments a general trend was seen where HH/6 beans showed a greater loss of minerals than the controls; however, this was only significant in the case of potassium after 12-h soaking.

Leakage has been attributed to lack of membrane integrity, either as a result of the rapid influx of water into dry seeds that displaces the membranes or of the time lag required for molecular reorganization of the membranes upon hydration (Simon, 1984). Another source of leakage is that of membrane degradation over storage catalyzed by lipoxygenase (Mazliak, 1983) or phospholipase (Nakayama et al., 1981) activity. In the case of the HH/18 beans, leakage is probably a result of a combination of these mechanisms. Beans stored under high-temperature and high-humidity conditions have a much more rapid rate of hydration than controls. Though this is not illustrated well in Figure 1, this trend is striking when shorter soak times are examined (Hincks and Stanley, 1986).

Mineral losses during cooking are opposite to those during soaking, with softer beans losing large amounts of magnesium, calcium, and potassium in the first 30 min, after which the rate of leakage slowed. HH/18 seeds lose

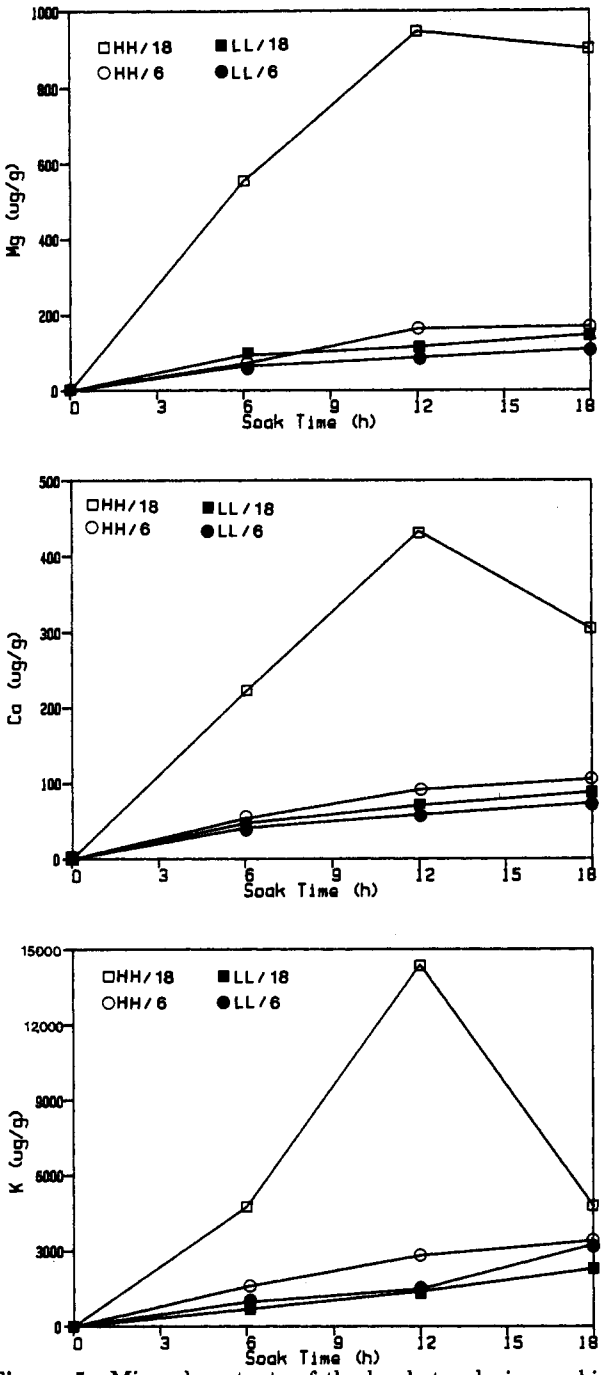

Figure 5. Mineral contents of the leachates during soaking.

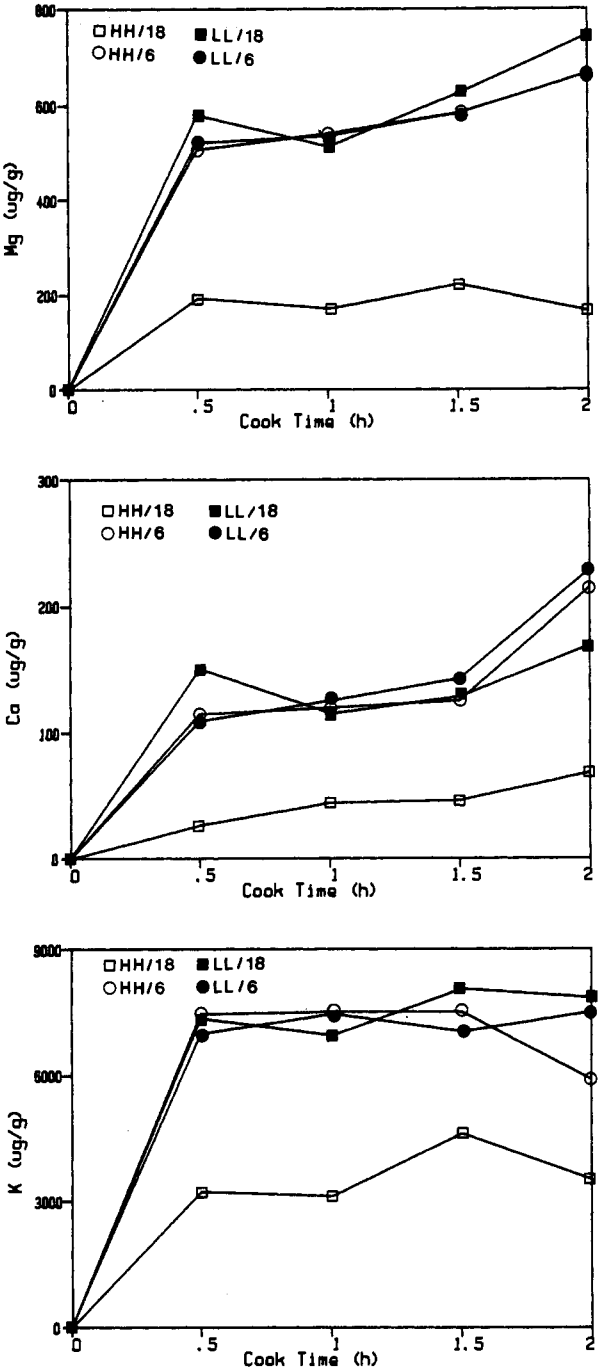

Figure 6. Mineral contents of the leachates during cooking.

additional minerals upon cooking, but this loss was significantly less than the other treatments ($P \leq 0.05$). The rapid initial efflux over the first 30 min may be related to the first fracture noted in the LL beans at this time (Figure 4).

In conclusion, the greater leakage of minerals from defective beans during soaking appears to be associated with membrane changes that occur during storage or as a result of rapid water influx while the restriction of further losses upon cooking is probably a result of limited cell separation and maintenance of cotyledon integrity. Differences seen between the control vs. short-term storage at high temperature and high humidity were not as clearly defined.

Microscopy. Scanning electron micrographs of cooked whole bean are shown in Figure 7. LL/18 samples exhibited advanced cell separation, with individual cells showing raised patches believed to be cell wall/middle lamella (cw/ml) remnants from areas of prior cell to cell contact. Association between cells consisted predominantly of fine reticular-like strands; however, some larger bridges of cw/ml material were observed. In contrast, the HH/18 samples showed minimal cell separation, often so restricted that intercellular spaces were clearly distinguishable. Cell to cell association was extensive, the cw/ml bridging generally extending the entire length of the cell. These bridges were both thicker and rougher in texture that their LL counterparts. No cw/ml residual patches were evident.

The crude preparation of isolated starch from cooked beans showed some startling differences (Figure 8). The isolate from HH/18 beans was reasonably pure with many starch granules and only minimal contamination by cell wall material (CWM); the separation behavior resembled that of raw beans. The LL/18 isolate had few granules, and these were dispersed amid cell debris and whole, intact cells.

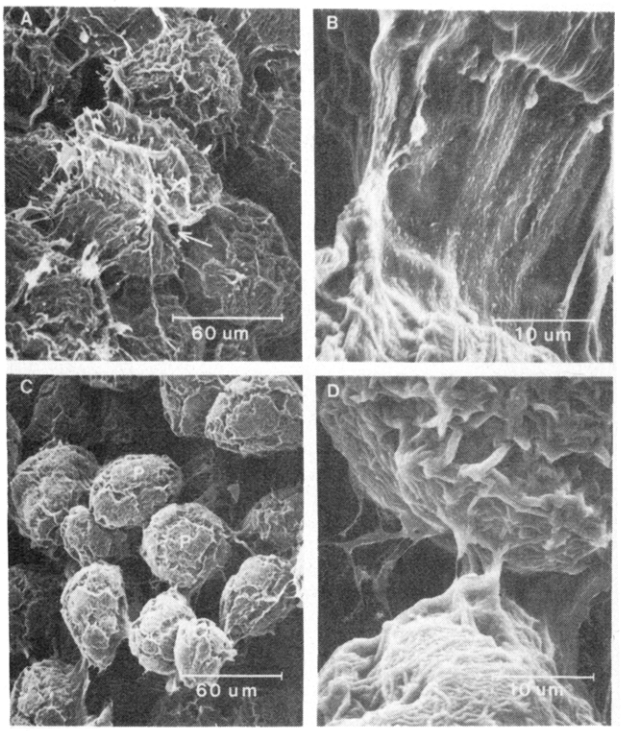

Figure 7. Cooked black beans: A, HH/18 sample showing minimal cell separation, intercellular spaces (arrow) still intact; B, increased magnification of cw/ml bridge between hard bean cells, note rough texture; C, LL/18 sample showing extensive cell separation and cw/ml patches (P) on individual cells; D, example of both the reticular-like strands and cw/ml bridge in soft beans.

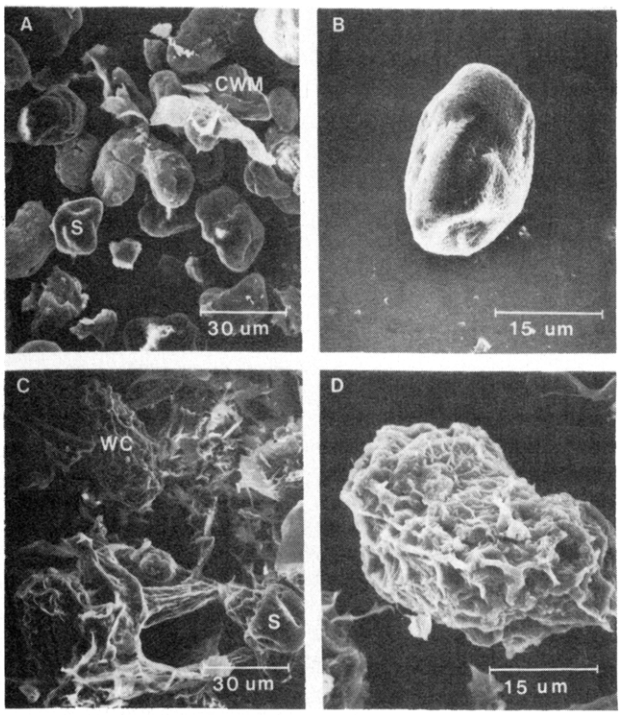

Figure 8. Crude starch isolate from cooked beans: A, isolate from HH/18 beans showing abundance of starch granules (S) and minimal cell wall material (CWM); B, individual starch granule from HH/18 isolate; C, isolate from LL/18 beans showing few starch granules amid cell debris and whole cells (WC); D, increased magnification of whole cell found in LL/18 isolate.

These micrographs indicate that the shearing action of the separation procedure fractured the cell walls of HH-cooked beans. These remained on the 44-μm screen and allowed the released intracellular contents to pass through.

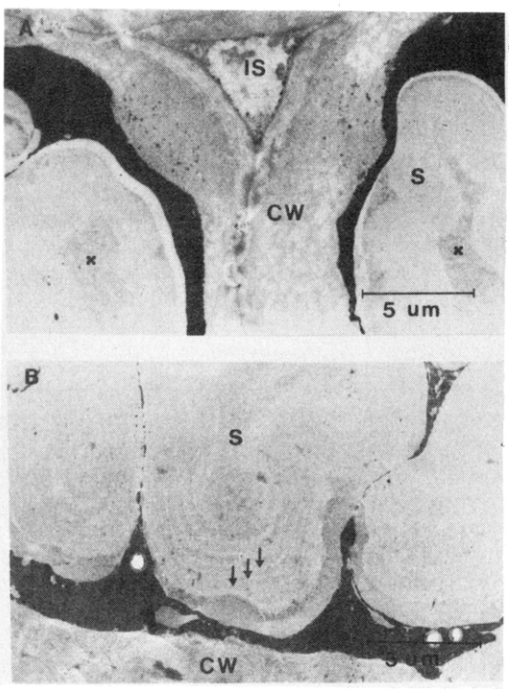

Figure 9. TEM micrographs of cooked bean: A, cross-section of hard bean at the junction of two cells, note central staining (*) in starch granules (S) and retention of intercellular space (IS) from restricted cell separation (CW = cell wall); B, cross-section of soft bean showing stain penetration into starch granules to form concentric shells (arrows).

In the case of the LL beans either cell walls were reduced in size to the point where they passed through the 44-μm screen or whole cells were malleable enough to be forced through. The lack of intact starch granules may be a result of containment in the cells or disintegration during the screening procedure. In either case starch gelatinization was more advanced in the control samples.

Transmission electron microscopy on thin sections of cooked beans also indicated a difference in the starch granules from soft and hard beans. In the control samples starch granules had concentric rings through the entire cross section where the stain had penetrated (Figure 9). Hard bean samples had granules that stained only slightly and irregularly, generally with the most intense staining in the center of the granule, where water appears first during soaking (Allen et al., 1976). It is suggested that as the soft beans have unrestricted cell separation (leading to increased water uptake), more complete in situ swelling of the granules is allowed, making the granule more susceptible to stain penetration. With hard beans gelatinization appears to be minimal and confined to central regions. The lack of detail and predominently central staining demonstrate a lack of water absorption.

That these differences are related to water absorption and not to starch structure or composition is supported by data of Hohlberg and Stanley (1987). They showed that the temperature of the G endotherm increased with aging, presumably due to an increase in crystallinity, but that this was not related to storage temperature or humidity.

This study has reported some of the events that occur during soaking and cooking of soft and hard beans and related these events to the physical and chemical changes resulting from the mechanisms responsible for the development of the hard-to-cook defect. During soaking, defective seed hydrates more rapidly and loses more solids and minerals than controls. These differences have been attributed to membrane changes resulting from high-temperature and high-humidity storage for long periods.

During cooking, hard beans absorb less water and lose fewer solids and minerals than controls. This results from severe restriction of cell separation caused by at least two mechanisms contributing to the hard-to-cook defect: phytate depletion leading to Ca/Mg pectate formation and lignin deposition. Lack of further hydration during cooking suggests that starch gelatinization may be hindered in hard beans.

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Registry No. H_2O, 7732-18-5; Mg, 7439-95-4; Ca, 7440-70-2; K, 7440-09-7; starch, 9005-25-8.

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Mineral and Phytate Content and Solubility of Soybean Protein Isolates

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Laboratory preparations of sodium proteinate, obtained by aqueous extraction of defatted soybean flakes, precipitation at pH 4.5, and neutralization to pH 8, were fractionated by centrifugation and filtration into an easily sedimented insoluble fraction (4–7%), a gellike material (1–3%), and a soluble filtrate. The pH 8 soluble filtrate was then acidified to pH 4.5 and refractionated at pH 8 into a second insoluble (1–6%), a second gellike (1–3%), and a final soluble filtrate fraction (80–89%). These fractions plus a trypsin inhibitor concentrate were analyzed for calcium, magnesium, potassium, sodium, iron, manganese, copper, zinc, phosphorus, and phytic acid and compared to values for commercial soy proteinates. Mineral–phytate–protein interactions were examined by dialysis and gel filtration. Differences in binding by the various minerals were noted, but phytate content of the proteins did not correlate with mineral binding or protein insolubility. The calcium level of the first insoluble fraction was 4 times that of the unfractionated isolate.

The complex relationships among minerals, phytic acid, and plant proteins and their association with reduced mineral bioavailability have been studied extensively (Smith and Rackis, 1956; Saio et al., 1968; Okubo et al., 1975, 1976; O'Dell and de Boland, 1976; Erdman, 1979; Cheryan, 1980; Reddy and Salunkhe, 1981; Prattley and Stanley, 1982; Turnlund et. al., 1984). Neutralization of soy protein products reportedly decreases zinc bioavailability compared to acid-precipitated protein (unneutralized), soy flour, or egg white when fed to rats (Erdman et al., 1980; Prattley et al., 1982; Ketelson et al., 1984). Rackis and Anderson (1977) suggested that formation of protein–phytate–mineral complexes during processing of soybean protein isolates, rather than phytic acid content per se, may be an important factor in reduced bioavailability of minerals such as zinc. They reported that when soy protein is fed as the sole protein source in the diet, the need for supplemental zinc may vary from 0 to 100 ppm, depending on the soybean protein product used and the conditions of manufacture.

In the preparation of soybean protein isolates, maximum yields occur when precipitation is carried out near pH 4.5, the isoelectric region for the major proteins. Phytic acid reacts with the proteins in water extracts of raw, defatted soybean flakes during acidification to pH 4.5, and the pH range of minimum solubility of the proteins is affected by both phytic acid and calcium concentrations (Saio et al., 1967). When the isoelectric protein isolate is subsequently neutralized (pH 6–8.5), soluble and insoluble protein–phytate complexes are formed (Smith and Rackis, 1956).

In a previous study, Honig et al. (1984) determined phytic acid contents of commercial and laboratory-prepared soybean protein isolates, including soluble and insoluble fractions of pH 8 sodium proteinates. In the present study we determined levels of major mineral elements as well as phytic acid levels in these same products before and after dialysis in order to determine relationships

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