IMBIBITIONAL AND TEXTURAL CHARACTERISTICS OF AGED BLACK BEANS (P. VULGARIS) AS RELATED TO COOKING FUNCTIONALITY

by

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TABLE OF CONTENTS

Introduction. 1

Review of Literature. 2

Seed coat factors. 2

Cotyledonary factors 6

Materials and Methods 12

Black bean samples 12

Accelerated aging. 12

Moisture analysis. 12

Cooking method 13

Imbibition 16

Autoradiography. 17

Electrolyte leakage. 18

Cellular structure 18

Texture measurements 18

Results and Discussion. 20

Effect of storage on cooking time. 20

Variability in water absorption among seeds. 25

Water absorption and solids loss 28

Mode of water penetration into beans 40

Effect of moisture contnent on cooking time. 50

Effects of storage on texture. 55

Summary and Conclusions 64

Acknowledgements. 66

References. 67

INTRODUCTION

The natural aging processes which occur during post-harvest storage of all seeds are especially detrimental to utilization of seed legumes. Cooking quality decreases rapidly in response to poor storage conditions resulting in functional and nutritive losses.

A widely accepted theory on cooking quality losses in stored legumes centers on water impermeability ("hardshell") which is thought to be a manifestation of hilar dysfunction (Quilivan, 1966 and Hyde, 1954). However, the work of Parrish and Leopold (1978) would tend to disprove age related impermeability in cotyledons. They showed that after four hours of soaking, all aged samples of soybean cotyledon, which had been stored at 41°C and 100% relative humidity for up to 7 days, achieved identical levels of absorptivity, (1.30 g. H_2O per g. dry cotyledon). Therefore, the imbibitional characteristics of stored legumes may not be related to their cooking quality. Hardshell and "hard-to-cook" phenomena must be differentiated if, indeed, they are separate problems.

Cotyledon cellular structure and testa layer play a role in water penetration and cooking time. Unfortunately, few reports have recognized the individual contributions of these structural entities to the cooking quality of legumes. The following study was undertaken to clearly delineate the roles of the testa and cotyledon in hardshell and to investigate the cellular mechanisms involved in creating losses in cooking quality of aged beans.

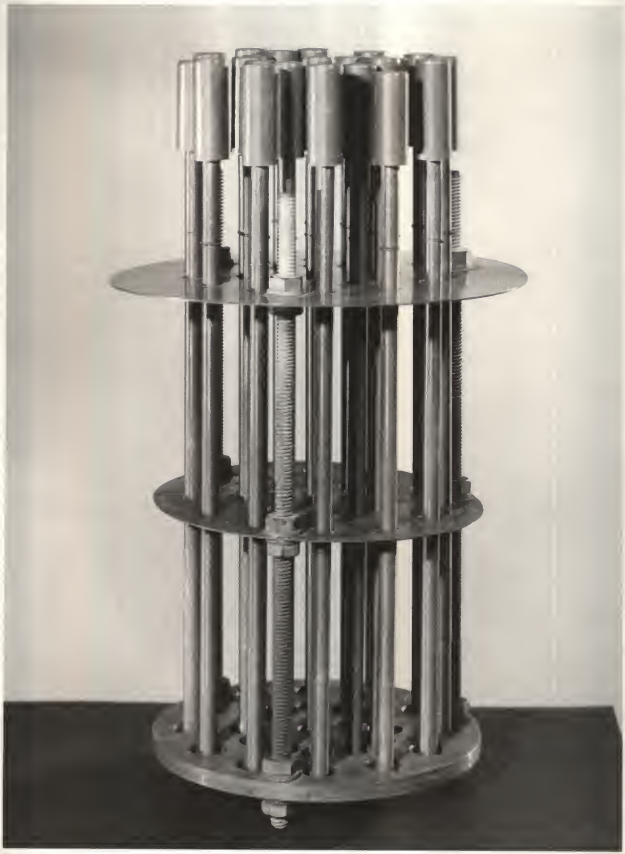
REVIEW OF LITERATURE

The hardshell problems characteristic of all Leguminosae have plagued their efficient utilization since the ancients. Hence, a great number of observations have been made on two utilization problems often encountered: germinability losses and decreased cooking quality. In some cases they seem to be related. The following review will concentrate on possible seed coat and cotyledonary factors responsible for hardshell and/or the "hard-to-cook" phenomenon in legumes.

Seed Coat Factors

Effects on seed vigor. The relationship between seed coat structure and seed vigor has centered on discussions of hilar dysfunction. Hamly (1932), working with osmic acid stains, was the first to show that the strophiolar fissure was responsible for regulating flow of moisture to and from the cotyledon and embryo. Sulfuric acid treatment removed the palisade cap and exposed the underlying lumen area on the outer testa but the treatment did not reduce impermeability. However, hard seeds could be softened by percussion, scarification of the testa, or moderate heating which ruptured the metastable double layer of palisade cells at the hilum. Hyde (1954) reported that the valve-like action of the hilum in regulating water flow was responsive to differences in environmental humidity and equilibrium humidity of the cotyledons. He postulated that the counter-palisade layer (double palisade layer) expanded in response to high humidity, closing the strophiolar fissure which restricts moisture flow to the cotyledons and embryo. At dessicating humidities, the palisade layer dries and contracts, opening the strophiole and allowing dessication of the cotyledons.

Figure 1. Modification of the device proposed by Mattson to generate continuous cooking curves for legumes.



Later work by Hagon and Ballard (1969) also showed that the strophiolar fissure was capable of re-sealing upon exposure to very low humidities. Scanning electron micrographs of the hilum and micropyle of hard and sound soybeans have confirmed this hypothesis, (Saio, 1976). Saio showed that hard seeds had closed hilar fissures and micropyle, while soft seed had large gaps at the same sites.

Some have suggested that the light line of the palisade layer is the anatomical feature which limits water movement into seeds (Esau, 1962). Recently, McKee et al., (1977) showed that impermeability could be eliminated by puncturing seeds to a depth of 99 μm. This falls into the range of the aleurone and epidermal cells for their Crownvetch seeds and its well below the seed coat osteo and macrosclereid cells.

Whether seed vigor is solely related to water impermeability due to the seed coat is doubtful. However, some types of dormancy do appear to be controlled by seed coat permeability, (Edwards, 1968). In other cases, the seed coat impermeability seems to result in a deteriorative effect on seed vigor. A report by Ballard (1973) indicates that the seed coat acts not only to restrict water via the strophiole, but also interferes with the passage of oxygen and carbon dioxide to the respiration cycle of the cotyledon. In 1961, Belderok indicated that loss of vigor appeared to be related to biochemical alterations in seeds. The relationship between the loss of vigor from hardshell and the loss of cookability from the "hard-to-cook" phenomena remains unclear.

Effects on cookability. Studies on the "hard-to-cook" phenomenon in legumes have also emphasized the role of the seed coat in preventing water penetration into the seed. Several authors have used blanching, steam or retort treatments to reduce cooking times for stored legumes (Morris, 1950;

Burr et al., 1968; Molina et al., 1976). Those authors provided no explanation for the success of their heat treatments. It is possible that alterations in the seed coat facilitated maximum moisture absorption rates so that cooking was not delayed by water absorption and equilibrium processes. On the other hand, alterations in the cotyledon, particularly at the middle lamella, may have shortened cooking time.

Morris (1963) examined the relative contributions of the seed coat and cotyledon to loss of cookability in pinto beans stored at high temperature and humidity. He established that there was only a minor seed coat contribution to the longer cooking times compared to that of the cotyledons.

Cotyledonary Factors

Studies on seed vigor. Burr et al., (1968) was the first to note that aged samples imbibed water at the same rate as did fresh samples when the hilum was fissured. Although this result was contradictory to hardshell theory, no conclusive work was done to corroborate Burr's work until recently when Parrish and Leopold (1978) found that aged and fresh samples of soybean cotyledons imbibed identical amounts of water on a dry basis when water absorption was corrected for solids lost during the soak. After a four hour period of soaking, they found that the apparent fresh weight gains decreased with increased accelerated aging treatment up to 7 days while the absolute amount of water imbibed by the cotyledon cells remained independant of aging. Specific conductivities of soak media indicated that solid loss during soaking increased with increased aging.

Ching and Schoolcraft (1968) confirmed that the conductivity of seed leachate was a good index of seed deterioration. Observed increases in leakage of metabolites in the seeds of weak, deteriorating and dead seeds was

thought to be related to degradation of cellular membranes with subsequent loss of permeability. Their study also showed that seed moisture was a major factor in membrane degradation.

A mechanism for plasmalemma hydration response was established by 1972 through a series of experiments by Simon and Harun (1972) in which pea embryos which had imbibed a small amount of water prior to soaking showed lower leakage rates during imbibition than those which had been initially drier. The same low rate of leakage was observed for fresh, succulent peas in which the initial extent of hydration was high. They concluded that the cell membranes lose their integrity with respect to permeability when the seed dries out and proposed that during imbibition there was a short period in which membrane integrity was being re-established when cellular leakage is very high followed by a rapid decline upon return of membrane function.

Villiers (1972) proposed that membrane integrity cannot be re-established in aged seeds because of the extensive peroxidation of phospholipids which can occur even in the dry conditions of storage. However, biochemical observations of membrane integrity and function during hydration are confounded by a lack of clear structural evidence. Membrane structure itself is not well understood, but in addition, observations on membrane integrity are difficult to resolve experimentally due to inherent staining problems. Osmium vapor treatment of dry cotyledon sections are too poorly stained to resolve membrane structure (Simon, 1974). Aqueous stains do not allow observations of the dry state where changes presumably occur, (Swift and O'Brien, 1972).

Simon (1974) has suggested that the dry membrane may be in a porous, disconnected geometry, but at moistures greater than 20% transforms into the lamenar bi-layer necessary for permeability. Since this molecular change would

be difficult or impossible to resolve by transmission electron microscopy, the hypothesis remains unproven.

Studies related to cooking time. Gloyer (1921) was the first to suggest that changes in bean cotyledons during growth or storage resulted in decreased cookability. He indicated that some beans did not cook even after the seed coat was removed. Gloyer postulated that the changes responsible for this "hard-to-cook" phenomena were biochemical in nature.

Later, Mattson (1946, 1951) asserted that since cell walls and cellular contents remained intact in individual cells after cooking, the middle lamella and its breakdown processes were responsible for cookability. He suggested that an ion-exchange transport of magnesium and calcium from the pectin acidoids of the middle lamella to the phytate was the controlling factor in producing cookability. He further postulated the existance of a biochemical factor, phytase, as being responsible for inducing the "hard-to-cook" condition in stored peas through hydrolytic cleavage of the chelating phytate.

Ginzburg (1961) established the structure of the middle lamella as a gel containing an intermolecular chelate which is stabilized by various additional bonding. He proposed that the chelation was between specific protein sites in the middle lamella and an optimum ratio of mono and divalent cations. The primary chelate bonding and stability of the gel were conditional upon the presence of the metal cations and was enhanced by the hydrogen and disulfide bonding of the proteins.

No mechanism for the involvement of the pectins present in the middle lamella was offered, but later work (Letham, 1962) suggested that both the protein and pectins contribute to the chelation and stabilization of the gel. Recently, Varriano-Marston and de Omana (1979) confirmed that ion-exchange

mechanisms and chelation processes were operative in the dissolution of the intercellular cement and subsequent cell separation in black beans.

With the development of electron microscopy, changes in cellular structure during soaking, cooking and storage have drawn some experimental attention. Rockland and Jones (1974) used the scanning electron microscope to demonstrate a breakdown of the middle lamella of the cell walls during cooking. No differences in structure were observed between beans soaked in water and beans soaked in aqueous salt solutions. They attributed the cooking time differences to differential rates at which cell separation occurs.

Sefa-Dedeh (1978) also reported the breakdown of the middle lamella in beans heated at 100°C as the major change in cellular structure. Their microstructural evidence indicated that the middle lamella in cowpeas stored at 29°C and 85% relative humidity did not breakdown during cooking at 100°C. Sefa-Dedeh observed no changes in the microstructure of the dry aged cotyledons which would explain the textural differences observed in cooking; however, closer examination of the published scanning electron micrographs, especially at the cytoplasmic matrix, indicates that changes may, in fact, have occured.

The important question of how the middle lamella is affected by storage time and temperature remains unanswered, but the deteriorative effect of storage conditions on cooking quality is well documented. Morris and Wood (1956) related storage temperature and humidity to decreases in organoleptic quality and Muneta (1964) correlated increases in moisture content during storage. As cited above, Morris later demonstrated that it is the deteriorative effect of the cotyledon which controls cooking time in beans.

Of the many systems designed to deal with the "hard-to-cook" problem, none have addressed the basic causal factor but instead have circumvented the problem by introducing empirical quick-cooking treatments. Steinkraus

et al., (1945), developed a technique of producing a quick-cooking product by soaking in water, pre-steaming at 212°F for 15 minutes, pre-cooking in water for 90 minutes at 212°F, coating with sugar solutions and dehydrating. This method appears to pre-soften the cotyledons which reduces cooking times.

Rockland and Metzler (1967) proposed a quick-cooking treatment consisting of vacuum treatment, hydration in an aqueous solution of 2.5% NaCl, 1.0% Na tripolyphosphate, 0.25% $NaHCO_3$ and 0.25% Na_2CO_3, at pH 9.0 for 6 hours, rinsing and drying. The intended effect of the hydration media was to disperse or solubilize proteinaceous material as well as dissociating metal salt-protein complexes. Cooking times for lima beans were decreased from 65 to 25 min., but longer cooking times could be reproduced in processed beans by storage treatments at high temperature and moisture contents. The effect of the quick-cooking treatment on the cooking times of aged beans was not reported.

Recently, Bongirwar and Sreenivan (1977) combined the pre-cook method of Steinkraus and the salt treatment of Rockland to produce a product which hydrates in boiling water in 5 to 6 minutes. Storage stability of this product was good at low temperature and humidity, but again, longer cooking times are produced in quick-cooking beans by storage treatments at elevated temperatures and humidities.

Conclusion

Overall, seed coat contributions to impermeability are well defined. More work should be done to clearly define the relation of seed coat texture changes to changes in cookability. Further, an important relation between seed vigor and cooking quality has yet to be fully resolved.

No attempt has been made to answer the question of how the middle lamella stabilizing mechanisms are effected by storage conditions. In fact, a closer

examination of the biochemistry of the middle lamella is in order to further resolve the roles of the pectins and proteins in the three dimensional binding network.

Future research in this field should be directed toward the ultimate goal of understanding the textural hardening mechanisms of the middle lamella and cellular contents and their relative contributions to the "hard-to-cook" phenomenon in aged legumes.

MATERIALS AND METHODS

Black Bean Samples

Bean seed was purchased from the Vermont Bean Seed Company, Bomoseen, Vermont, and planted at the K.S.U. Ashland Research Farm on May 5th, 1978. Beans were harvested during August after drying on the plant. All beans were harvested, shelled and cleaned by hand to eliminate scarification artifacts and insure good quality. Cleaned seeds were stored in double-layer plastic at $4^{o}C$ and 13% moisture content.

Accelerated Aging

For the accelerated storage studies, the technique of Parrish and Leopold (1978) was used to age the beans. Refrigerated samples were placed in a single layer in petri dishes and floated in a water bath at $41^{o}C$ and 100% relative humidity for 7 and 14 days. Seeds were than removed and allowed to air-dry to approximately 8-9% moisture content.

Other samples were aged for 55 days by placing them over a wire mesh in a dessicator at $41^{o}C$ and 75% relative humidity. Humidity was maintained by an aqueous solution of 60% ethylene glycol. Samples were removed after 55 days and air-dried to 16% moisture content. Further dessication to the 9% value of the other samples was achieved by vacuum oven drying at $50^{o}C$ for 4 hr. followed by storaged in a dessicator over $CaSO_4$.

Fresh samples were removed from refrigerated storage, air-dried to 9% moisture and used as is.

Moisture Analysis

Moisture of samples was determined by the American Association of Cereal Chemists (AACC) Method 44-15 (AACC Approved Methods 1969) of oven drying at $130^{o}C$ for 1 hour.

Cooking Method

Cooking time was measured by the use of the Mattson cooking device, (Mattson 1946), shown in Figure 1. The 100 tube capacity device of Mattson and Burr was scaled down to hold a 25 bean sample and fit into a 2 liter beaker for convenience. Dimensions for the cylindrical cell are as in Figure 2.

To determine the cooking time, beans were positioned in each of the 25 cylindrical holes of the cooker so that the piercing tip of the 82.00 gram rod was in contact with the surface of the bean. A "cooked" condition was measured when the tip passed through the bean. The cooking time was taken as the time required to cook 50% of the sample.

Sample preparation prior to cooking

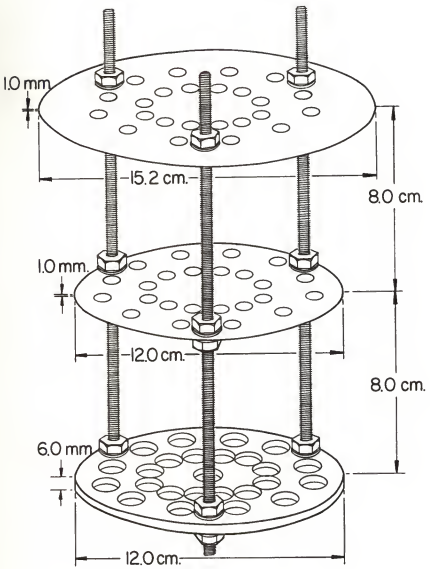
In all experiments, beans were sized to maintain uniformity and repeatability by selecting for weights between 200 to 300 mg.

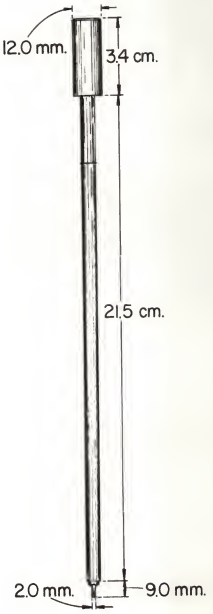
Seed coat intact. Samples of 25 beans were placed in a 150 ml. beaker with 100 ml. of deionized water. The beans were allowed to soak for 18 hours in a water bath maintained at 25°C. At the end of the soaking period the beans were removed and placed into the cooker which was, in turn, placed into 1400 ml. of boiling water in a two liter beaker. Time was measured from the point of contact between cooker and boiling water.

Decorticated beans. Decortication was achieved manually by removing the testa layer with a razor blade followed by scraping to remove the parenchymous layer adhering to the cotyledon surface. Decorticated samples were cooked in an identical manner. Cotyledon halves were held together by the pressure of the piercing rod until they were cooked.

Variable initial moisture. Beans were prepared by first fissuring the hilum to allow for uniform and homogenous moisture penetration and equilibrium

Figure 2. Dimensions for rack and plungers of Mattson device. Bottom plate contains depressions to hold bean stable. Holes to accomodate plunger tip are at the bottom of each depression, (not shown).


1.0 mm.
15.2 cm.
8.0 cm.
1.0 mm.
12.0 cm.
8.0 cm.
6.0 mm.
12.0 cm.


12.0 mm.
3.4 cm.
21.5 cm.
2.0 mm.
9.0 mm.

throughout the entire 25 bean sample lot. Fissured seeds were placed in deionized water at 25°C for variable time periods to produce a series of samples with moisture contents ranging from the initial 9% of air-dried seeds to the final free water equilibrium value of approximately 120% (dry basis). After soaking, the samples were removed from the water, sealed in a small beaker with parafilm, and allowed to equilibrate internal moisture gradients for 18 hours at 4°C. After equilibrium, samples were cooked as described above.

Imbibition

Weight uptake. Two experiments on gravimetric uptake were performed. Samples with and without seed coats were evaluated for fresh weight uptake verses time over various aging periods.

Fresh weight uptake. Fresh weight uptake patterns were measured by soaking 20 beans in deionized water at 25°C. Samples were removed and blotted dry then weighed. Samples with seed coats intact were measured for uptake at hourly intervals over a 24 hour soaking period. Water uptake by decorticated samples was measured in one and two minute intervals for the first ten minutes and then every hour for a total soaking period of 6 hours. In each case uptake was expressed as percent of fresh weight gained; two replicates were done.

Absolute uptake. Initial moisture content of seed coat and non-seed coat samples was determined as described above. Duplicate 10 bean samples were soaked in deionized water at 25°C for 4 hours. Seeds were then removed from soak water, washed with deionized water, blotted dry and weighed. Solids in the leachate plus wash water were determined by drying over a steam table followed by vacuum dessication at 60°C for 4 hours. Absolute uptake was calculated as follows:

$$\frac{\text{Weight after soaking - (dry weight at 0\% m.c. - solids)}}{\text{(dry weight at 0\% m.c. - solids)}}$$

The absolute uptake is the grams of water that the cotyledon cells are capable of binding after soaking, (Parrish and Leopold, 1978).

Autoradiography

A modification of the methods of Butcher and Stenvert (1973) and Rogers (1973) was used in the autoradiographic studies. Samples for autoradiography were maintained at 9% moisture content. Subsamples of ten beans (with or without seed coats) were placed in 10.0 ml. of tritiated water (ICN Chemical and Radioisotope Division, Irvine, California) at a concentration of 0.33 mCi./ml. Beans with seed coats intact were soaked for 1, 2, 4, 8, and 12 hours; decorticated beans were soaked for 1 or 2 hours. After the soaking period, beans were removed from the tritiated water, washed with deionized water, blotted dry, and then cut with a razor blade at the hilum and perpendicular to the plane of the intercotyledon face. The cut surface was again washed with deionized water, blotted dry, quick-frozen in liquid mitrogen cooled isopentane, and stored in liquid nitrogen. The frozen bean halves were then transferred to a dark room where the cotyledons were removed from the liquid nitrogen and cut surfaces placed in contact with precooled (-78^{o}C) 1" x 2" strips of Kodak NMB film (Eastman Kodak, Rochester, New York) which had been mounted on a glass slide with rubber bands. Beans were firmly anchored to the slide mount by wrapping with tape. The taped samples were then quickly transferred to a light tight insulated box filled with dry ice and exposed for 48 hours. Rapid transfer of bean halves from liquid nitrogen to film surfaces and exposure box was essential in order to avoid thawing or condensation artifacts on the final autoradiograms.

The film was removed from the mount after the exposure period and developed as follows: 5 minutes in Kodak D-19 developer, 2 minutes in 1% acetic acid stop bath, 10 minutes in Kodak hardening-fixer, and 20 minutes washing in tap water. Film strips were treated with Photo-flow 200 and dried. Autoradiograms were selected from an average of 30 replicate treatments.

Electrolyte Leakage

Leakage of metabolic electrolytes was measured on 10 whole beans for samples with seed coats or an equivalent 20 cotyledons for decorticated samples. Beans or cotyledons were placed in a florentine flask with 20 ml. of water and held at 25°C with gentle mechanical stirring. Specific conductivity was determined using a Beckman conductivity bridge, model RC16B2 (Beckman Instruments Inc., Cedar Grove, New Jersey). The micromho values were multiplied by a 0.1 cell constant; units of specific conductivity were reported as micromho x $cm.^{-1}$ verses soaking time.

Cellular Structure

Scanning electron microscopy. Decorticated beans were soaked for 0, 1, and 2 hour intervals at 25°C, quick-frozen, freeze-dried in an Edwards freeze-drier at -60°C for 48 hours, fractured with a razor blade, mounted on stubs, and coated with gold-palladium alloy. Duplicate samples were examined at each time interval. Scanning electron micorgraphs of the cotyledon cellular structure were taken on an Etec U-1 Autoscan Electron Microscope at an accelerating voltage of 10 to 20 kv.

Texture Measurements

Texture of aged and fresh dry beans (with and without seed coats) was measured on a Lee-Kramer shear press model SP-12 IMP., (Lee-Kramer,

Washington, D.C.) with a 500 pound load cell using an eleven bar extrusion grid. Twenty measurements were made on each sample. Individual beans were placed perpendicular to the slots of the extrusion grid, one per test, so that the cutting plates sheared a radial slice from the middle of the seed. Measurements of shearing force were divided by the weight of each seed to give a texture value in units of force per gram of seed tissue. Data produced was statistically analyzed for significant differences at the $P < 0.01$ level of probability.

RESULTS AND DISCUSSION

Effect of Storage on Cooking Time

Accelerated storage conditions had not previously been employed to study the "hard-to-cook" phenomenon in beans. Therefore, a test of the effects of high temperature and humidity on cooking time was run using the modified Mattson bean cooker.

The cooking rates of fresh and 14 day aged samples (seed coat on) are shown in Figure 3. Cooking times for fresh beans varied from approximately 17 minutes to 37 minutes, with a cooking half-time (Morris, 1950) of 31 minutes. The relationship between number of fresh beans cooked and cooking time was fairly linear. Conversely, the range of cooking times for 14 day stored samples was 24 minutes to 63 minutes, with a cooking half-time of 45 minutes. Much larger deviations from linearity were observed with aged beans probably because individual beans age at different rates.

The data indicate that the effects of accelerated storage on cooking time parallel the "hard-to-cook" phenomenon reported for long term storage of seed legumes. Therefore, accelerated storage conditions are applicable to studies on hardshell and bean cookability.

One critical question that must be addressed deals with the relative contribution of the testa and cotyledon to cooking time. A significant reduction in cooking time was observed for decorticated samples (Figure 4). The cooking half-time shifted downward to 12 minutes for the fresh samples and 17 minutes for 14 day aged samples. The 5 minute difference between fresh and aged samples was maintained throughout the course of the experiment. The uncookable 55 day intact beans were altered by decortication to a half-time cooking value of over 120 minutes, however an exact value was difficult to obtain due to variability.

Figure 3. Plot of the number of beans cooked vs. time for fresh and 14 day aged intact samples of black beans. Half cooked line indicated at 12.5 beans.

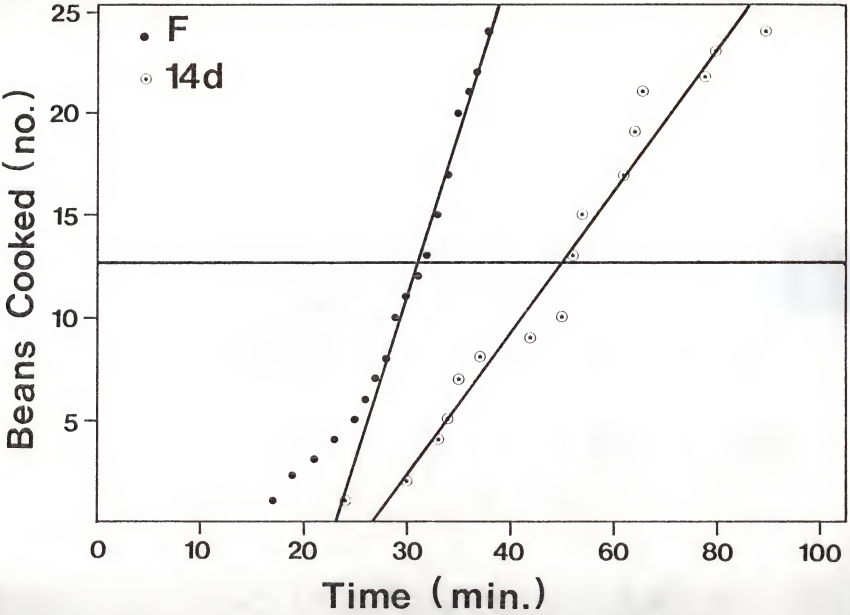
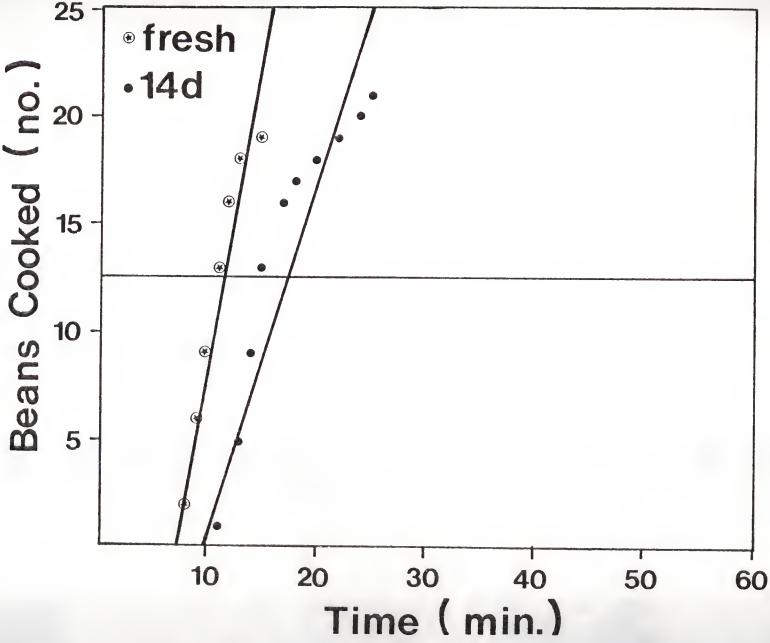

F
14d
Beans Cooked (no.)
25
20
15
10
5
0
10
20
30
40
60
80
100
Time (min.)

Figure 4. Plot of the number of beans cooked vs. time for decorticated fresh and 14 day aged black beans.


fresh
14d
Beans Cooked (no.)
25
20
15
10
5
10
20
30
40
50
60
Time (min.)

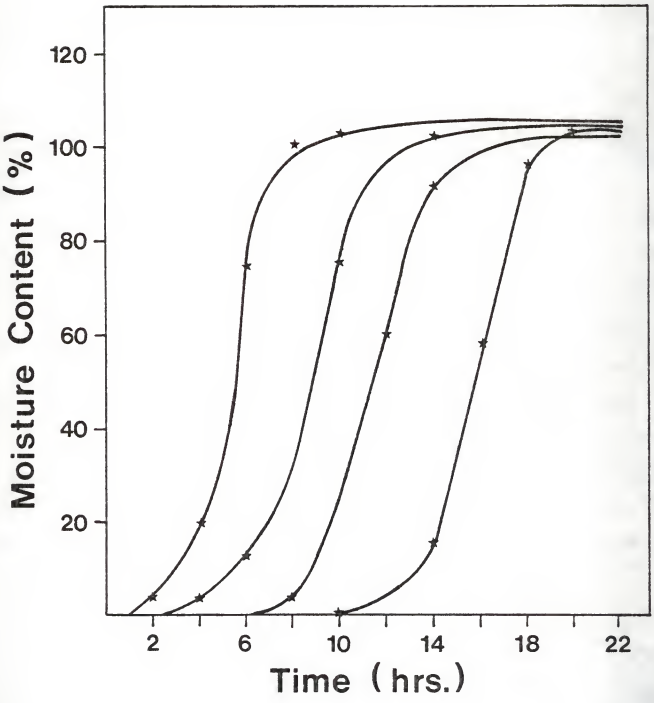
These data indicate that biophysical and/or biochemical changes occured in both seed coat and cotyledon during storage. In addition, a comparison of cooking times for intact beans and decorticated samples suggests that the seed coat is the major barrier to the softening of beans during cooking. However, changes in the cotyledon during storage cannot be considered insignificant since about 39% of the total cooking time for intact aged beans was associated with the softening of the cotyledon.

The cooking process for beans involves two steps: water absorption to an equilibrium condition with free water followed by softening of the texture by heat. From the cooking data presented in Figures 1 and 2, it is impossible to distinguish whether the cooking problem is solely due to impaired water absorption rates or a manifestation of physicochemical changes in the bean components; both factors could increase cooking times. In order to shed some light on factors contributing to the reduction in bean cookability with storage, a series of experiments were conducted to determine: (1) the variability in water absorption among seeds, (2) the relationship between water uptake and solids lost, (3) mode of water penetration over time, and (4) the effect of seed moisture content (after soaking) on cooking time.

Variability in Water Absorption Among Seeds

Water imbibition patterns for some individual seeds are shown in Figure 5; each curve represents a single fresh bean with intact testa. The sigmoidal curves indicate that for every sample there is an initial lag time prior to rapid water uptake and eventual equilibrium at 100% fresh weight. Lag periods varied from a few minutes for quickly imbibing beans to 24 hours for more impermeable samples, indicating increasing levels of hilar dysfunction. Apparently, the counter-palisade layer in individual seeds, as described by

Figure 5. Water uptake patterns of individual black (intact) beans. Moisture content (% fresh weight) vs. time.


Moisture Content (%)
120
100
80
60
40
20
2
6
10
14
18
22
Time (hrs.)

Hyde (1954), is in various stages of sensitivity with respect to the surrounding free water.

Not only does the level of water imbibed vary among seeds, but the rate of uptake also varies. The rate of water imbibition of fresh beans as affected by soaking time is plotted in Figure 6. High maximum rates of imbibition were observed for beans with short lag periods. Conversely, low maximum imbibition rates were observed for beans with longer lag periods, suggesting, again, that hilar dysfunction was greatest for these latter seeds. However, each individual bean, regardless of rate of uptake, eventually attains the 100% fresh weight equilibrium value.

Water Absorption and Solids Loss

Recognizing that the seed coat limits water uptake and causes a wide variability in the rate of water imbibition, future studies were directed at evaluating hydration processes of decorticated seeds only.

The water absorption patterns of fresh and aged decorticated beans (as % dry basis vs. time) are presented in Figure 7. Values recorded in this figure were corrected for solids lost during soaking. The results indicate that if corrections for solids are considered, fresh and aged water absorption patterns were essentially identical. In all cases, water imbibition was rapid within the first 1.5 hour, followed by an equilibrium value of 100% to 110% in 2.0 to 2.5 hours.

Most of the literature reports that stored legumes imbibe less water than fresh samples. However, data misinterpretation has apparently resulted because those authors recorded water absorption as a percentage of the fresh weight. To further illustrate this point, and to compare our data to that reported by other authors, water absorbed by decorticated beans after 4 hours of soaking

Figure 6. Rate of water imbibition of individual fresh intact seeds.

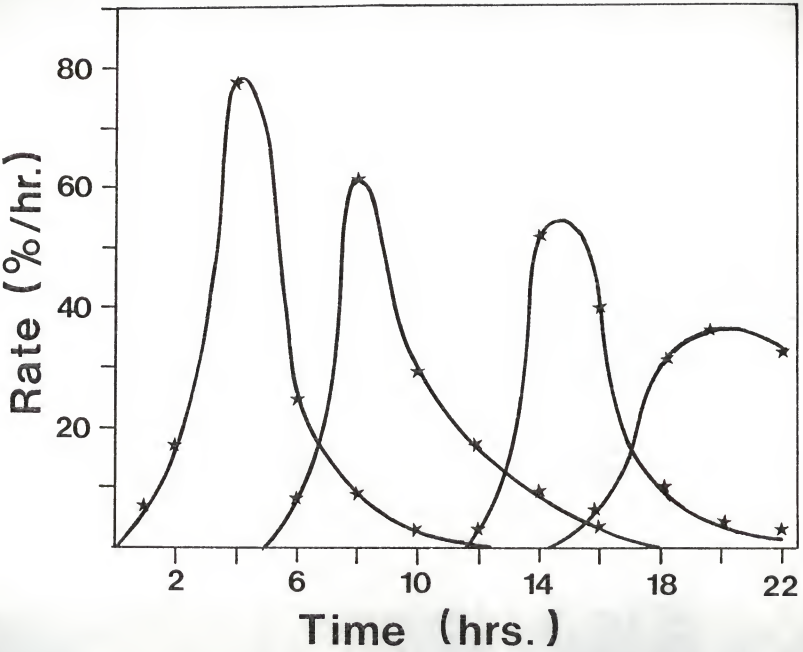
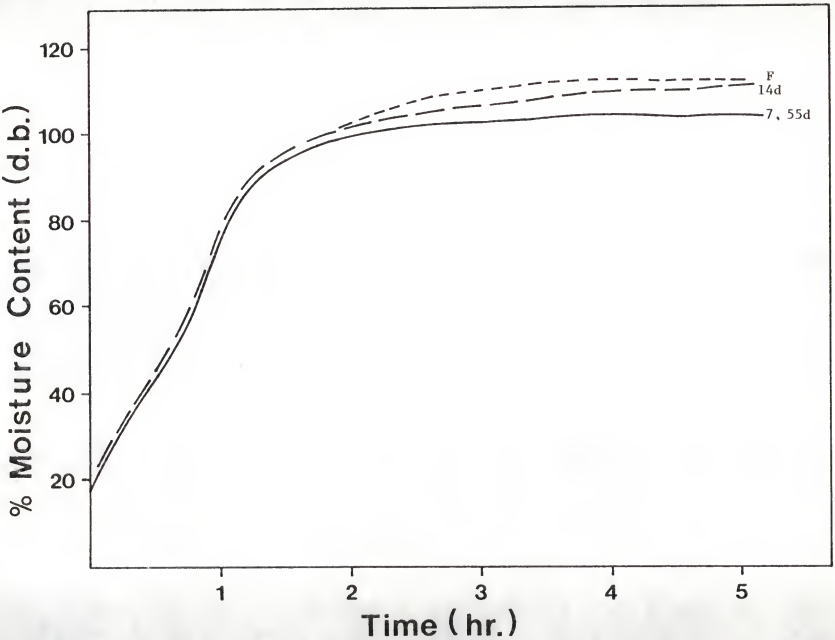

Rate (%/hr.)
80
60
40
20
2
6
10
14
18
22
Time (hrs.)

Figure 7. Water absorption patterns for fresh, 7, 14, and 55 day aged decorticated black beans.


120
100
80
60
40
20
% Moisture Content (d.b.)
1
2
3
4
5
Time (hr.)
F
14d
7, 55d

was calculated on a fresh weight basis and then corrected for solids lost. These data are presented in Table 1. The amount of water absorbed, when calculated as a percentage of the fresh weight, decreased with increasing storage time; 90.05% for fresh samples and 60.87% for 55 day aged beans. On the other hand, when water absorption was calculated as grams of water bound to 100 gram dry matter after soaking, the decline was from 119.23% for fresh beans to 116.86% for day samples. The difference in values for fresh and aged samples falls within experimental error. Therefore, our water absorption data corraborates Burr's (1968) work in concluding that there are no differences in imbibition characteristics of fresh and stored beans.

It should be noted that, although moisture imbibition rates and capacity were not affected by aging, the 55 day samples demonstrated a higher affinity for water when air dried. When 0 to 14 day aged samples were air dried, they attained an equilibrium moisture content of 8 to 9%. Conversely, 55 day samples that were vacuum dried to 8 to 9% moisture followed by re-equilibration in air, attained a moisture content of 16%. It is possible that aging affects certain ultrastructural characteristics of the bean which in turn, changes the effective surface area and increases the sorption sites for water vapor without changing the maximum hydration capacity of the cells. Water activity studies as well as structural studies would be useful to confirm this hypothesis.

It is apparent from the studies above that aged black beans lost more solids during soaking than their fresh counterparts. We also wanted to determine if the rate of leakage was affected by storage. Therefore, conductance measurements were made on the leachate from fresh and aged samples.

The specific conductance of intact black beans during soaking is shown in Figure 8. The rate of leakage from intact 55 day aged beans was very rapid in the first 4 hours of soaking. In fact, after 4 hours the specific conductance

Table 1. Absolute water absorption by black beans soaked 4 hours.

Storage Time (days)	Fresh Weight %	Water Absorption (gH_2O/g Dry Seed)*X100
0	90.05	119.23
7	91.83	121.02
14	86.42	118.27
55	60.67	116.86

*Absorbed Weight - (CALC'D DRY WEIGHT - DRY SOLIDS LOSS)

(CALD'D DRY WEIGHT - DRY SOLIDS LOSS)

Figure 8. Specific conductance of the leachate from intact fresh, 7, 14, and 55 day aged black beans.

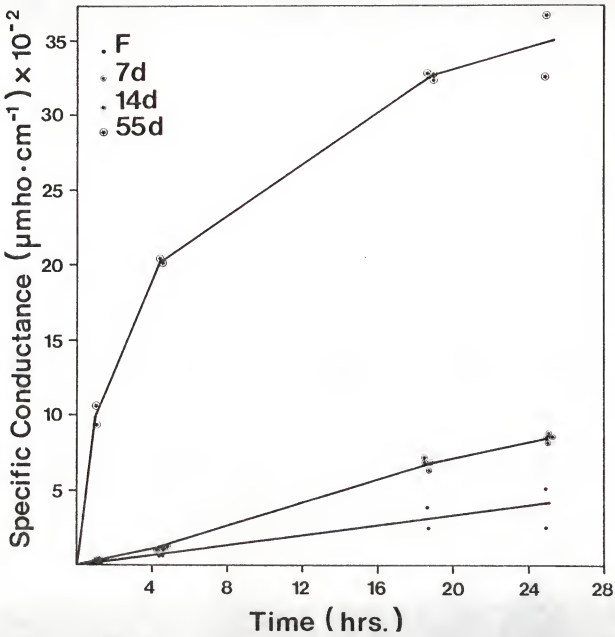
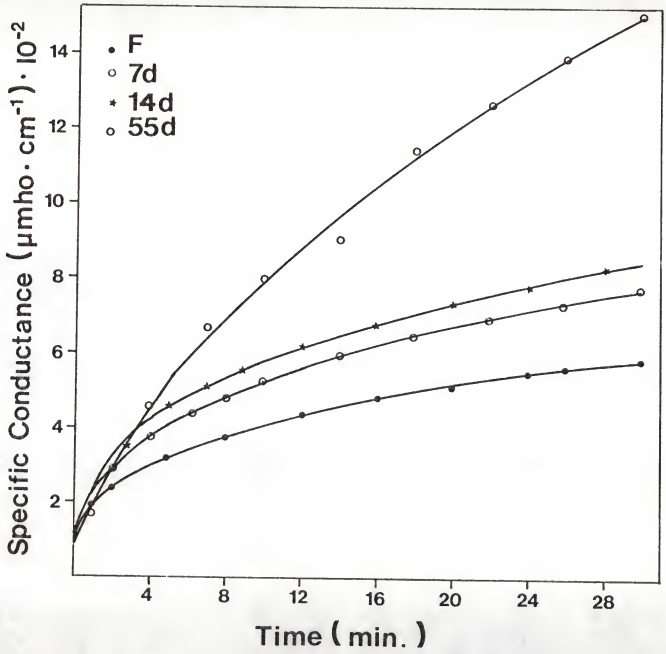

. F
. 7d
. 14d
. 55d
Specific Conductance (μmho·cm⁻¹) x 10⁻²
35
30
25
20
15
10
5
4
8
12
16
20
24
28
Time (hrs.)

Figure 9. Specific conductance of the leachate from decorticated fresh, 7, 14, and 55 day aged black beans.


• F
○ 7d
* 14d
○ 55d
Specific Conductance (μmho · cm⁻¹) · 10⁻²
14
12
10
8
6
4
2
4
8
12
16
20
24
28
Time (min.)

of the leachate from 55 day samples was more than 2x greater than that of the 24 hour leachate from fresh or 14 day beans.

Conductance studies were also done on the leachate from decorticated beans, but only the first 30 minutes of soaking was monitored. As can be seen in Figure 9, the shapes of the specific conductance curves for aged and fresh samples are nearly identical. The rate of electrolyte leakage, however, was considerably greater for the 55 day samples than for the fresh or 14 day beans. After a 30 minute soaking period, electrolyte leakage from 55 day beans was 2.5x greater than that from the fresh samples. Furthermore, the specific conductance of decorticated samples was higher than that observed for intact beans after 30 minutes of soaking. The testa and structures at the hilum can, therefore, be considered as restrictive barriers to electrolyte leakage.

Leakage of electrolytes from black beans during soaking appears to be related to the accelerated storage conditions employed in this study. Others have also shown that specific conductance is related to aging phenomenon in seeds (Parrish and Leopold, 1977; Ching and Schoolcraft, 1968). If hilar permeability was decreased during bean storage, it was not manifested by reduced electrolyte leakage during soaking.

Simon (1974) and others have suggested that loss of plasmallema integrity is responsible for the increased leaching observed in aged seeds. All low molecular weight soluble metabolites would leach from the seeds in direct proportion to their cytoplasmic concentrations (Simon and Harun, 1972). However, we observed that the leachate from fresh beans was more turbid than that from 55 day stored samples. This could suggest that a decrease in protein or carbohydrate leakage occurred with aging. If selective leaching did occur in the 55 day samples, this would cast doubt on the theory of plasmallema

malfunction. Quantification of the chemical components of the leachate from fresh and aged samples could shed light on this controversy.

Mode of Water Penetration into Beans

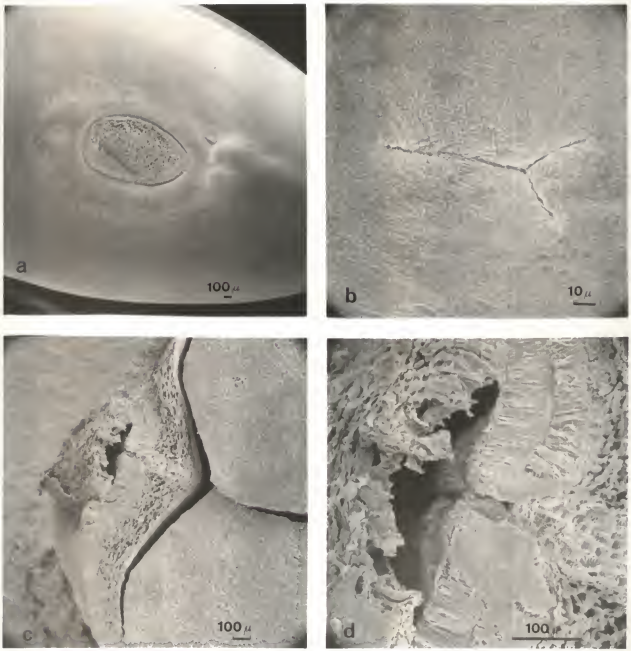
Although gravimetric studies on water absorption furnish information on the amount of water absorbed, they do not provide data concerning the structural regulators of imbibition or possible water concentration gradients in soaked beans. Such information would be important to obtain if, as part of a quick-cooking regiment, recommendations are made to reduce soaking time. Therefore, two physical methods were employed to study the mode of water penetration in black beans: autoradiography and scanning electron microscopy.

Autoradiography. Micrographs in Figure 10 are presented to familiarize the reader with the structural features that will be discussed in this section.

When fresh intact seeds are analyzed via autoradiography, the images in Figure 11 were obtained. A darkening appears at the hilum area after 2 hours of soaking, indicating penetration at the strophiole. After 4 hours, the entire periphery of the bean becomes darkened. Apparently, water which has entered the strophiole redistributes itself in the layer of parenchyma cells under the testa. The darkening of the cotyledon near the hilum is no greater than that at the periphery. Therefore, no water concentration gradient exists between the place of entry and the imbibed areas.

Water penetration increased uniformly from the periphery to the center of each cotyledon after 8 and 12 hours of soaking. Water entering the strophiole, therefore, is continuously transported to the sub-testa parenchyma throughout the entire imbibition period. Water does not penetrate the cotyledon at the hilar area any more rapidly than at the cotyledonary cells adjacent to the strophiole. After 18 hours, the seed is fully penetrated although the maximum

Figure 10. Structure of hilar area in black beans, a. Micropyle, b, and cross section of metastable double-palisade layer, c and d.


a
100 μ
b
10 μ
c
100 μ
d
100 μ

level of water imbibition has not been attained (see Figure 11). Water absorption continues after 18 hours, producing progressively darker autoadiograms. It should be emphasized that at no point in the water imbibition process do water concentration gradients exist across the cotyledon.

Water uptake by beans appears to be a continuous process which is regulated by structural features at the hilum. The regulatory mechanism, however, remains unclear. It is possible that the hilum opens when the external free water concentration is high relative to the water concentration in the interior of the bean. Conversely, the hilum would close when the water concentration in the seed interior was too high. However, this type of metering action has never been reported. Alternately, the transport of water across the hilum may be slower than the rate of diffusion through the seeds in which case this would be the rate limiting factor in hydration. The latter hypothesis is supported by a comparison of the rates of hydration when seed coats are intact versus when they are removed (Figures 5 and 7). The conclusion is that the hilum is a passive anatomical bottle-neck which is the rate limiting factor in imbibition.

Autoradiograms were also done on decorticated beans in order to observe their responses to water uptake without the metering action of the hilum (Figure 12). A uniform darkening around the entire cotyledon periphery resulted after soaking fresh or aged beans for 1 hour. After 2 hours of soaking, the water had penetrated the center of the cotyledons; fresh and aged samples showed identical responses. These data corraborate the equilibrium condition that was reported earlier for gravimetric studies on water absorption (see Figure 7). However, the autoradiographic technique has one advantage over gravimetric analysis: it is completely free of artifacts due

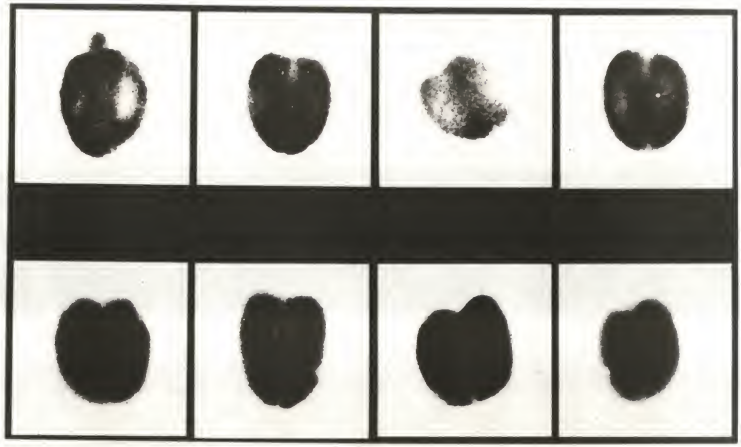
Figure 11. Autoradiograms of fresh and 7 day aged intact beans soaked 2, 8, and 14 hr.

F

7d

14 hr. 7 hr. 2 hr.

Figure 12. Autoradiograms of fresh, 7, 14, and 55 day decorticated beans soaked 1 and 2 hr.


1 hr.
2 hr.
F
7
14
55 d

to solids loss. Again, an important conclusion that can be drawn from this study is that fresh and aged beans show identical water imbibition rates which confirm the earlier work of Burr et al.(1968) and Parrish and Leapold (1978).

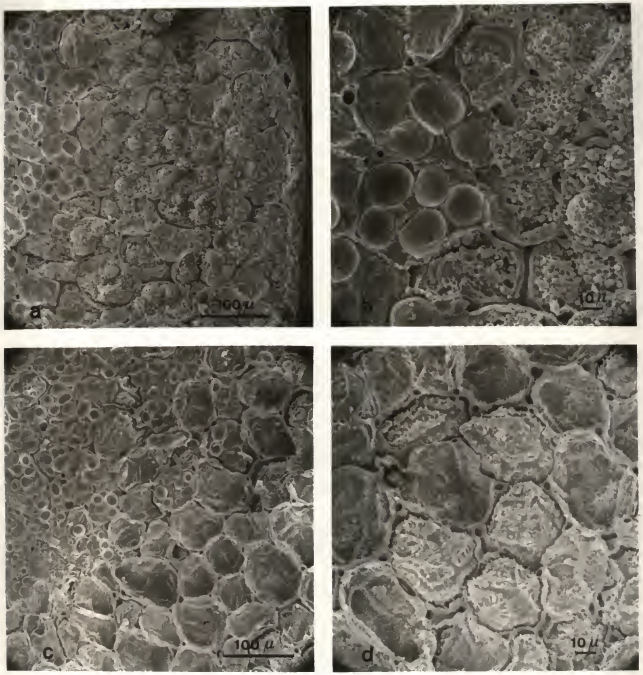
Scanning electron microscopy (SEM). To confirm the results obtained with the autoradiographic technique, water penetration in decorticated beans was also studied using the SEM.

Water penetration fronts were observed in fresh and aged cotyledons after 1 hour of soaking at 25°C (Figure 13). A clear demarcation between hydrated and dry cells is evident. Cytoplasmic structures of dry cells of fresh beans (Figure 13a and b) are densely packed and protein bodies are barely discernable. Conversely, spherical protein bodies were clearly visible in hydrated cells (Figure 13b). On fracturing, the shear stress planes in the dry cells were generally at the interface between the starch granules and the protein matrix. After hydration and freeze-drying, the shear plane occurred between the protein bodies and the cytoplasmic matrix. A softening of the intercellular contents is implied by these observations on fresh hydrated beans.

Unlike fresh beans, the cytoplasmic structures of hydrated 55 day beans were not visible (Figure 13c and d). Instead, all cells pulled away from the intercellular interface when beans were fractured. A weakening of the attachments between cell walls and intercellular cement must have occurred as a result of storage conditions. However, the intercellular cement still formed a rigid, three-dimensional honeycomb network that was not distrubed by the soaking treatment.

In general, the SEM data is in agreement with autoradiographic studies on the extent of water penetration in decorticated beans after soaking for 1 hour. However, more samples must be examined in order to obtain the same

Figure 13. Water penetration front associated with fresh beans, a and b, and 55d beans, c. Micrograph d shows the degenerated cell wall-middle lamella binding in 55d beans.


a
100 μ
b
10 μ
c
100 μ
d
10 μ

degree of confidence as that obtained with autoradiography. More than 60 seeds per treatment were studied using the latter technique. Conversely, the small capacity of the tissue freeze-drier employed for preparation of samples for SEM did not facilitate studies on large numbers of seeds.

Effect of Moisture Content on Cooking Time

Although the data above indicates that bean storage did not appreciably affect water imbibition rates, information concerning the effect of bean moisture content (after soaking) on cooking time was lacking. A series of experiments were, therefore, conducted to clarify the role of seed moisture content in bean cookability. In these experiments, the hilum was fissured to facilitate water uptake but the seed coat remained attached to the beans.

Cooking experiments were done using seeds at moisture contents ranging from about 8% (air dry seeds) to over 110% (fully equilibrated samples). The results presented in Figure 14 demonstrate the linear relationship that was observed between cooking time and initial moisture content of intact fresh and 14 day samples. The 55 day aged beans were not softened even after cooking periods of over 6 hours.

The cooking time of intact beans was shown to be inversely proportional to moisture content, producing a series of parallel lines for each storage period. These plots demonstrate that there is no moisture content that will give identical cooking times for fresh and aged samples. Therefore, differences in cooking times for intact beans are not a result of the impairment of imbibition by the hilum.

Parallel patterns for cooking times were also observed for decorticated samples (Figure 15). However, there appears to be an approach to an asymptotic cooking time at the higher moisture contents for both fresh and aged samples.

Figure 14. Cooking times of fresh and 14 day aged intact black beans as affected by moisture content.

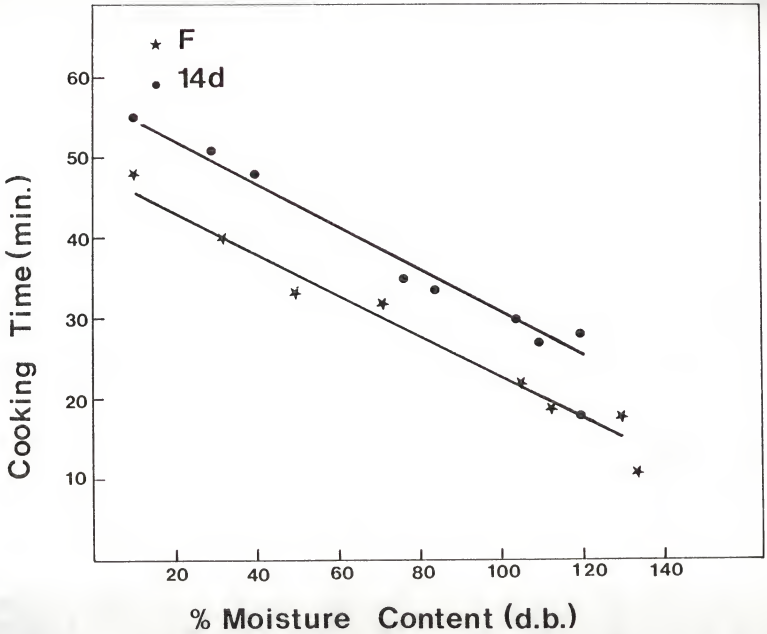
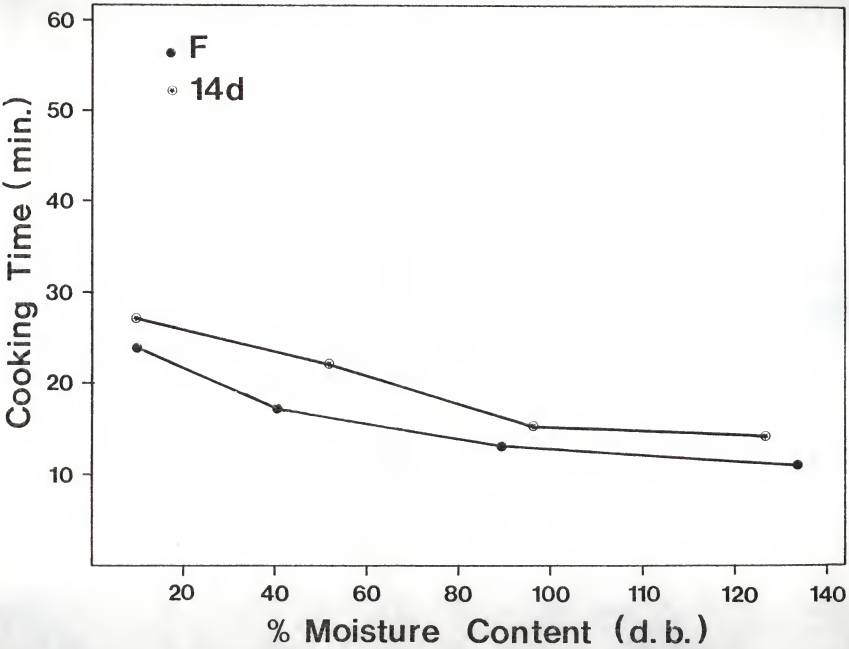

★ F
• 14d
Cooking Time (min.)
60
50
40
30
20
10
20
40
60
80
100
120
140
% Moisture Content (d.b.)

Figure 15. Cooking times of fresh and 14 day aged decorticated black beans as affected by moisture content.


F
14d
Cooking Time (min.)
60
50
40
30
20
10
20
40
60
80
100
110
120
140
% Moisture Content (d.b.)

The 14 day beans approach a 13 minute cooking value at moisture contents above 130% dry weight. Fresh seeds approach a value of 9 to 10 minutes for the same moisture content.

The asymptotic behavior exhibited in the cooking experiments with decorticated beans may emphasize that a relationship exists between the energy requirements necessary to breakdown cellular structures and the level of free water present in the beans. At some optimum cotyledonary moisture content, the energy requirement is at its minimum.

A minimum energy requirement may also exist for intact seeds, but it is probably at a considerably higher moisture content. The seed coat apparently has a separate cooking time requirement. Nevertheless, the data in Figure 15 indicates that at equal moisture contents, real differences in cooking times exist between fresh and aged samples, and that aged beans require more energy to break down cellular structures. These data were confirmed by SEM's of fresh and 55 day aged beans cooked for some time period.

Effects of Storage on Texture

It seems readily apparent from the water absorption data presented above that the "hard-to-cook" phenomenon observed for stored beans has very little to do with imbibition rates or capacity. The causal factor responsible for the increased cooking time, therefore, must be related to textural changes in aged beans. If the aging process results in increased binding forces in the testa and cotyledon, these changes should be obvious in physical measurements of the force required to shear through fresh and aged samples. The Kramer Shear Press was, therefore, used to determine the hardness of dry beans.

The texture measurements presented in Table 2 were obtained by dividing the weight of the seed which was tested into the force required to shear

Table 2. Hardness of fresh and aged dry black beans as determined by the Kramer shear press.

Storage Time at 41°C (days)	Relative Humidity During Storage	Crushing Force (Kg/g) Seed Coat Intact	Testa	Decorticated Cotyledons
0	—	74.3	24.2	50.1
7	100 %	60.1	10.3	49.8
14	100 %	70.2	25.5	44.7
55	75 %	101.9	41.5	60.4

LSD = 4.0 at $P < 0.01$

through that seed. The use of the extrusion grid for textural measurements results in values for crushing force as opposed to the cutting force measurement used by Sefa-Dedeh (1978). Statistical analysis of the data from 20 replicates showed that the 55-day intact seeds required a significantly ($P < 0.01$) greater crushing force than any of the other samples. It can be concluded that, in this instance, storage conditions did cause a physical hardening of the dry beans.

The relationship between hardness of dry beans and cookability, however, may only pertain to samples stored for long periods of time. Hardness measurements were not related to the cookability of beans stored for short periods of time at 41°C, 100% R.H. Intact fresh and 14 day aged beans required essentially the same crushing force while 7 day aged samples required less force to crush than did the fresh samples. However, these aged beans required longer cooking times than the fresh counterparts (Figure 3).

Crushing force measurements on the decorticated beans showed the same trends as described above. The 55 day samples required a significantly greater crushing force than all other treatments.

Subtracting the decorticated value from the total force gives the hardness contribution due to the testa, which show an increase in the 55 day samples compared to that for 0, 7, and 14 days (Table 2). Thus, the seed coat not only adds significantly to the force required to crush the bean, but this contribution increases with storage time. Similar increases were noted for the cotyledon.

Increased binding forces in the cytoplasm or middle lamella and in seed coat structures may explain the increases in shear force observed for 55 day aged samples. Further work must be done on the correlation between these

textural properties and the biochemical and biophysical modifications in both testa and cotyledon during aging.

Ultrastructural studies. Ideally, to differentiate between the hardening contributions to texture due to cytoplasmic and cell wall contributions, a method should be found which could evaluate the textural strength of each component independently. Alternately, information on the relative binding strength of cellular components can be gathered indirectly from observations on structural responses to shearing forces under various conditions (Hoseney and Seib, 1976).

Scanning electron micrographs of fresh, 14 day, and 55 day aged beans are displayed in Figures 16 and 17. The structure of fresh samples (Fig. 16a) is very similar to the structure of 14 day beans (Fig. 16d) at low magnifications. However, 55 day beans (Fig. 17g) showed evidence of alterations in their response to the shearing action of the blade during fracturing (See upper right corner of Fig. 17g). The shear planes that were produced suggest that a weakening of the attachments between the cell wall and the middle lamella occurred during storage.

Changes in the interior of the cells during storage were also observed. Higher magnifications of the cytoplasmic matrix of fresh, 14 day, and 55 day samples (Figs. 16b and f; and 17h and i, respectively) revealed that the granular structure observed in fresh beans decreased during storage. It should be noted, however, that the granularity of the matrix of 55 day seeds is somewhat greater than for 14 day seeds. Differences may be a result of the much higher relative humidity used in storing the latter samples (100% vs. 75%, respectively).

A reduction in adhesion between cell walls and middle lamella was observed when 55 day samples were soaked (Fig. 13d). However, the middle

Figure 16. Scanning electron micrographs of dry sections of fresh, a, b, c, and 14 day, d, e, f, aged beans. Note lack of granularity at higher magnifications in 14d beans.

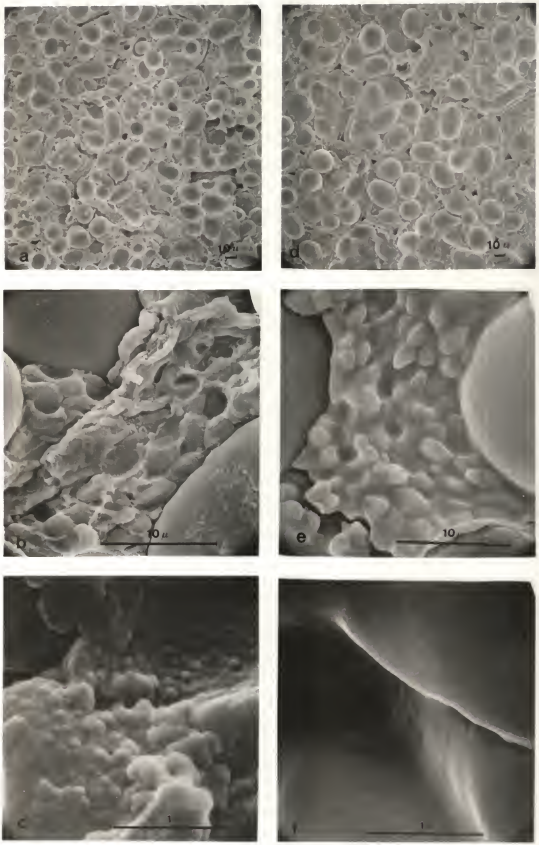
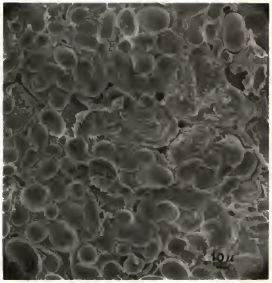
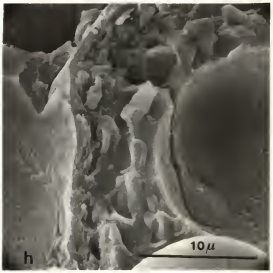
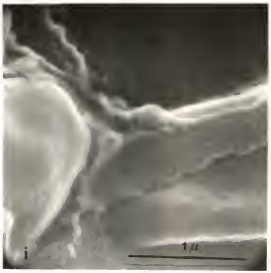

a
10 μ
d
10 μ
b
10 μ
e
10 μ
c
1
f
1

Figure 17. Scanning electron micrographs of dry section of 55d bean. Granularity of h intermediate to fresh and 14d, (16b and c). Greater shearing at cell wall-middle lamella interface, g, than fresh and 14d, (16e and d).


10 μ


h
10 μ


i
1 μ

lamella remained as a rigid, three-dimensional, honeycomb structure, with free-floating cells positioned within the matrix. These structural observations indicate that the major contributor to cotyledonary hardness in aged beans, as measured by the shear press, was due to the middle lamella.

Since 55 day beans take 2 to 3 times longer to cook than the 14 day samples, the changes in intracellular order of the cotyledonary cells may be irrelevant to the increased hardness observed between these two samples. On the other hand, alterations in the chelating components of the cellular interior (e.g. phytate) may have an indirect effect on intercellular binding. Mattson (1946) suggested that phytate in peas played a key role in preventing "hard-to-cook" phenomena by chelating the ions responsible for crosslinking of pectic substances.

SUMMARY AND CONCLUSIONS

Cooking studies on black beans stored at 41°C and 100% or 75% relative humidity indicated that the "hard-to-cook" phenomenon involved age-related changes in both seed coat and cotyledon. Data on intact and decorticated beans showed that the seed coat contribution to cooking time was greater than that of the cotyledon.

Shear press data indicated that hardening occurred in both testa and cotyledon of 55 day aged samples. However, the relative contribution of the testa to the total hardness was greater than that of the cotyledon.

Gravimetric and autoradiographic studies showed that there were no differences between fresh and aged samples with respect to water binding capacity (when values were corrected for solids loss) and water penetration rates. Increased electrolyte leakage from aged samples was, however, correlated to storage time.

Adjusting fresh and aged beans to identical moisture contents (from 8% to 120%) prior to cooking, did not alleviate the effects of storage on cooking time. In other words, differences in cooking times between fresh and aged samples persisted regardless of bean moisture content.

These data, along with that from water absorption studies, suggest that biophysical or biochemical changes, which are unrelated to water absorption, must be responsible for the increased cooking time requirements for aged beans. Therefore, the deteriorative changes occurring in "hard-to-cook" beans are not necessarily related to "hardshell". These two processes must be clearly differentiated in any future studies on bean cookability.

Possible biophysical alterations in stored beans were investigated by scanning electron microscopy. Ultrastructural changes in 55 day beans occurred

at the middle lamella - cell wall interface creating a rigid 3-dimensional network of intercellular cement. This network may be the major anatomical structure influencing hardness.

Future studies should concentrate on determining biophysical and biochemical changes in seed coat and middle lamella during storage. For example quantitation of lignin formation, browning reaction polymers, protein polymerization and denaturation reactions during storage may prove useful in evaluating the causes of the increased textural binding in old seeds. A better understanding of the structure of the middle lamella and its relationship to cooking functionality is needed before observations on changes are meaningful. The physicochemical changes in the seed coat have never been studied, but are certainly important in a comprehensive analysis of deterioration of cookability.

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IMBIBITIONAL AND TEXTURAL CHARACTERISTICS OF AGED BLACK BEANS (P. VULGARIS) AS RELATED TO COOKING FUNCTIONALITY

by

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Cooking studies on black beans stored at 41°C and 100% or 75% relative humidity indicated that the "hard-to-cook" phenomenon involved age-related changes in both seed coat and cotyledon. Data on intact and decorticated beans showed that the seed coat contribution to cooking time was greater than that of the cotyledon.

Shear press data indicated that hardening occurred in both testa and cotyledon of 55 day aged samples. However, the relative contribution of the testa to the total hardness was greater than that of the cotyledon.

Gravimetric and autoradiographic studies showed that there were no differences between fresh and aged samples with respect to water binding capacity (when values were corrected for solids loss) and water penetration rates. Increased electrolyte leakage and aged samples was, however, correlated to storage time.

Adjusting fresh and aged beans to identical moisture contents (from 8% to 120%) prior to cooking, did not alleviate the effects of storage on cooking time. In other words, differences in cooking times between fresh and aged samples persisted regardless of bean moisture content.

These data, along with that from water absorption studies, suggest that biophysical or biochemical changes, which are unrelated to water absorption, must be responsible for the increased cooking time requirements for aged beans. Therefore, the deteriorative changes occurring in "hard-to-cook" beans are not necessarily related to "hardshell". These two processes must be clearly differentiated in any future studies on bean cookability.

Possible biophysical alterations in stored beans were investigated by scanning electron microscopy. Ultrastructural changes in 55 day beans occurred at the middle lamella - cell wall interface creating a rigid

3-dimensional network of intercellular cement. This network may be the major anatomical structure influencing hardness.