ACCELERATED DEVELOPMENT OF THE HARD-TO-COOK STATE IN BEANS

by

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TO MY PARENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In developing countries, beans are an important source of protein, calories, and other nutrients (Deshpande et al, 1982: Jones and Boulter, 1983a). Unfortunately, if beans are stored improperly, they become hard-to-cook (Jones and Boulter, 1983a and 1983b; Varriano-Marston and Jackson, 1981; Mattson, 1946; Mattson et al., 1951). Hard-to-cook (HTC) or hardened beans require more fuel to cook, are less palatable, and are less nutritious.

Beans are known to resist cooking due mainly to two causes. One is "hardshell," which means the seed coat is impermeable to water. The other is hard-to-cook (HTC), which means the cotyledons do not soften during boiling, even though the seeds imbibe water (Morris et al., 1950; Bourne, 1967). Hardshell is promoted by low humidity and high temperature and can be reversed by hydrothermal treatment or scarification. On the other hand, the HTC condition is irreversible and is accelerated by high humidity and high temperature (Sgarbieri and Whitaker, 1982). Plant breeders have eliminated most "hardshell" varieties of beans, but all beans and other legumes remain susceptible to the HTC condition.

The mechanism causing the HTC phenomenon in stored beans has not been firmly established. Three mechanisms may be considered; (1) limited hydration of intracellular protein; (2) pectin insolubilization in the middle lamella by calcium and/or magnesium ion after the combined action of pectin methyl esterase and phytase (Jones and Boulter, 1983a), and (3) cross-linking of phenolics (lignification) and/or protein in the middle lamella (Varriano-Marston and Jackson, 1981). One objective of this work was to determine the most plausible hypothesis so that strategies might be developed to reduce the HTC condition. A second objective was to devise a

method to accelerate the hardening of bean cotyledons so that cultivars that rapidly harden can be identified.

MATERIALS AND METHODS

Materials

Beans

Pinto beans (Phaseolus vulgaris L.) were the Olathe variety grown in 1984 near Grand Junction, CO. The beans were kindly provided by Dr. Calvin H. Pearson, Fruita Research Center, Colorado State University, Grand Junction, CO. Four other varieties, a white "navy" bean (Sanilac), a brown bean (A-30), a red bean (15-R-148), and a black bean variety (Ica-Pijao No 12) were grown near Lansing, MI in 1982. These were gifts of Dr. George Hosfield, USDA/ARS Food Legume Program, Michigan State University, Lansing, MI. Medium-sized beans (0.3-0.4 g/bean) were hand-sorted and used in all experiments. Beans with broken hulls, shriveled kernels, or off-colored seed coats were discarded, as were small beans. The beans were stored in polyethylene bags at 5°C until used.

Methods

Moisture

The moisture content of whole beans (10-15 g) was determined by drying in an air-oven 72 h at 103°C (AACC Method 44-15A, 1968).

Bean Starch

Starch was isolated from pinto beans using the procedure of Schoch and Maywald (1968). Beans (100 g) were soaked overnight at 25°C in 250 ml of 0.2% sodium hydroxide. After cooling the mixture to ~ 10°C, the softened beans were ground in a Waring blender for 1.5 min, and the slurry sieved successively through 112 m and 63 m bolting cloths. The slurry was centrifuged, and the supernatant fluid and the dark-colored protein layer atop the starch were discarded. The starch was resuspended in 0.2% sodium hydroxide, centrifuged, and separated from the supernatant and

protein three more times. Finally, the starch cake was repeatedly washed with distilled water, and air dried.

Cookability Determined by the Tactile Method

The cooking method was a slight modification of the one described by Jones and Boulter (1983a). One-hundred pinto beans were soaked for 15 h in 200 ml of distilled water. The beans were drained, and the soaking water saved and combined with an additional 150 ml of distilled water in a 2-liter beaker covered with a watch glass. The water was heated to gentle boiling on a hot plate, then the beans were added. At 15, 30, 45, and 60 min, a sample of $\sim$ 20 beans was removed using a spoon. The beans were placed in single layer on a small plastic tray, covered with a paper towel, and cooled 10 min at room temperature. They were tested for softness by squeezing between the forefinger and thumb. Beans were classified as cooked when the cotyledons were soft and free of graininess. The graininess could also be detected between the teeth. Beans that were hard or contained grainy regions were classified as not cooked. Thin polyvinyl chloride gloves (Fisher Scientific) were used during testing when beans had been soaked in buffers containing chloramphenicol or fluoride ion. Cooking time (CT_{100}, Jones and Boulter, 1983a; Chhinnan, 1985) was calculated by multiplying the number of beans cooked at a given time by the cooking time, summing the products, and dividing by the initial number of beans. In this manner, beans cooked at short times were not counted again at long cooking times. Cooking times reported in this work are arithmetic means of duplicate or triplicate determinations. When all the beans were not cooked after 1 h in boiling water, then average cooking time was not used to assign cookability. Instead, cookability of beans was determined by boiling 1 h, then calculating the percentage of cooked beans.

To determine cookability of beans when the pH of the soaking water was to be held in a narrow range (± 0.2 pH units), the sample size of beans was 12 instead of 100. Duplicate or triplicate samples of 12 beans were rinsed with distilled water and placed in a 250 ml erlenmeyer flask containing 150 ml of water or buffer. After soaking the beans at 41°C for a given time period at a specific pH, the liquid phase was decanted and its pH recorded. The liquid was added to a graduated 600 ml beaker, made to 250 ml volume with distilled water, and heated to boiling. The 12 beans were added, cooked 60 min, cooled 10 min under ambient room conditions, and tested for softness as described above. Cookability was expressed as percentage of beans cooked.

Differential Scanning Calorimetry (DSC)

DSC was done using a Perkin-Elmer Instrument (Model DSC-2, Perkin-Elmer Corp., Norwalk, CT). Beans (25 g, aged or fresh) were soaked 15 hours in water (100 ml) and then cooked 15 min in boiling water (200 ml). The whole beans were freeze-dried and ground with dry-ice in a coffee grinder. Bean flour (3.0-4.5 mg) was weighed directly into a DSC aluminum sample pan using a Cahn 21 automatic electrobalance. Water (2 parts) was added to the bean powder (1 part) by syringe and the pan was weighed, hermetically sealed, then weighed again. The samples were run in the DSC under the following conditions: temperature scan, 280°-400°K (7-127°C); sensitivity (R), 0.2 mcal/sec; and scanning rate, 10°/min.

The same DSC procedure was followed for isolated bean starch, fresh and aged raw beans ground with dry-ice, cooked beans (fresh and aged) that were freeze-dried and ground, and cooked beans (fresh and aged) from which small pieces of cotyledons were examined directly after cooking.

Hardening of Beans by Holding at 45°C and 100% Relative Humidity

Four to nine desiccators (D=125mm, H=82mm) were washed and sanitized with water containing 10% Roccal II (Lehn and Fink Industrial Products Div., Sterling Drug Inc., Montvale, NJ), and distilled water was added to the wells of the desiccators. Four to nine lots of pinto, red, white, brown, and black beans (20-25 g each) were rinsed with distilled water and soaked in an aqueous solution of 0.01% chloramphenicol (Sigma Chemical Co., St. Louis, MO) for 5 min. After straining, the beans were placed in one layer in plastic petri dish lids that had been perforated extensively with a hot nail. One lid filled with beans was placed in each desiccator, and the desiccators were placed in a constant temperature cabinet at 45°C. One desiccator was removed daily for up to 7-9 days. Moisture was determined on ~10 g of the aged beans, while the remaining beans were dried at ambient room conditions for 2 days. Duplicate samples of 12 beans were soaked in 150 ml of distilled water for 17 h, and the pH of the soaking water recorded. The soaking water was diluted to 250 ml by adding distilled water and heated to boiling, then the beans were added, and the percent beans cooked in 60 min was determined by the tactile method.

Accelerated Hardening of Beans by Soaking in Warm Aqueous Buffers

Nine buffers were prepared as described by Gomori (1955). The concentrations of the buffers ranged from 0.06-0.15M; all contained 0.002% chloramphenicol as an antimicrobial agent. The buffers at pH 1-2 were prepared by mixing hydrochloric acid and potassium chloride, pH 2.5-3.4 by mixing potassium acid phthalate and hydrochloric acid, and pH 6.8-8.6 by mixing *tris*-(hydroxymethyl)-aminomethane and maleic acid.

Duplicate and triplicate samples of 12 beans (pinto, red, white, and brown) were soaked at 32°C for 17 h or 41°C for 8 h in 150 ml of water

or buffer at different pH's in stoppered erlenmeyer flasks. The pH change after soaking usually did not exceed 0.2 pH units from the initial pH. Each sample of beans was cooked 60 min in boiling buffer in a 600 ml beaker covered with a watch glass. Prior to boiling, the buffer was adjusted to 250 ml volume by adding 100 ml of water. Cookability was reported as percentage of beans cooked.

In another experiment, pinto beans were soaked up to 7 days in *tris*-aminomethane-maleate buffers at pH 5.5, 6.0, 6.5, 7.6, and 8.6. The pH of the buffers and their concentrations were pH 5.5, 63.2 mM; pH 6.0, 76.0 mM; pH 6.5, 90.0 mM; pH 7.6, 108.0 mM; and pH 8.6, 137.0 mM. Samples of 12 pinto beans (in duplicate) were rinsed, strained, and placed in erlenmeyer flasks containing 150 ml buffer. The flasks were stoppered and placed in a constant temperature bath at 41°C for 0.5-7 days. The beans were strained from the soaking solution, and the pH of the soaking water was recorded. After adding 100 ml of distilled water to the soaking water, the mixture was heated to boiling, the beans were added, and cookability was determined as percent cooked in 1 h of boiling.

Effect of Blanching on Accelerated Hardening of Pinto Beans in Acetate Buffer at pH 4.4

Twelve pinto beans were soaked in water (150 ml) at 25°. The beans were removed from the water, the water was heated to boiling, and the beans were added to the boiling water for 15 min. The blanched beans were cooled under ambient conditions 10 min, and then soaked at 41°C in 150 ml of 0.2M acetate buffer (pH 4.4) containing 0.002% chloramphenicol. After soaking 8, 16 and 24 h, the pH of the soaking water and the cookability of the soaked beans were compared to those of unblanched beans.

Cookability of Fresh Pinto Beans Soaked in Acetate (pH 4.0) and Metaphosphate Buffer (pH 4.1)

Metaphosphate buffer (0.2M) and acetate buffer (0.1M) were prepared at pH 4.0-4.1 by mixing 0.2M metaphosphoric acid with 0.2M sodium trimetaphosphate, and 0.1M acetic acid with 0.1M sodium acetate, respectively. Duplicate samples of 12 beans were soaked 8-18 hours at 41°C in 150 ml of each buffer. After soaking 18 h, the pH of the metaphosphate buffer increased to 4.4-4.6, while that of the acetate buffer increased only slightly to 4.0-4.1. The cookability of the beans was determined as % beans cooked after 60 min boiling in a mixture of the soaking buffer (150 ml) and distilled water (100 ml).

Effect of Soaking in Metaphosphoric Acid on Cookability of Hard-to-Cook Beans

Pinto beans were aged in a desiccator at 100% relative humidity and 45°C for 2 weeks, at which time the beans were uncookable in boiling water for 60 min. Several lots of 12 uncookable beans were rinsed with distilled water and placed in erlenmeyer flasks containing either 150 ml of 0.002% chloramphenicol in distilled water or 150 ml of 0.002% chloramphenicol in 0.1M metaphosphate buffer at pH 4.0. The flasks were stoppered and held 3 and 7 days at 25°C. The cookability of the beans was determined after boiling 60 min as previously described.

Cookability of Pinto Beans After Soaking in Aqueous Calcium Chloride, Magnesium Chloride, and Sodium Chloride

Samples of pinto beans (20) were soaked in an erlenmeyer flask in 150 ml of 1 to 64 mM aqueous $CaCl_2$ for 18 h at 41°C, while 12 beans were soaked in 1 to 128 mM magnesium and sodium chloride at the same time and temperature. After soaking, the pH of the cooking water and the cookability of the beans were determined as previously described.

Effect of Sodium Fluoride on Hardening of Pinto Beans in Acetate Buffer at pH 4.8

Twelve pinto beans were soaked at pH 4.8 for 12 and 16 h in 0.1M acetate buffer containing 0.05M sodium fluoride. A control sample contained no fluoride. The pH of the soaking water and the cookability of beans were determined.

RESULTS AND DISCUSSION

Cookability of Beans

When investigating the hard-to-cook (HTC) problem in beans, the cookability of beans must be measured. Instrumental methods, such as those using the Mattson bean cooker (Mattson, 1946; Chhinnan, 1985), the Instron (Sefa-Dedeh, et al, 1978), and the Lee-Kramer shear press (Quast and da Silva, 1977), while objective, are slow or they do not measure directly whether individual beans are cooked as defined by humans. In addition, instruments are often unavailable or costly in developing countries where legumes are important foods. Even the Mattson bean cooker, though modest in cost, may not be available to a field investigator.

We prefer the tactile method used by Jones and Boulter (1983a). In this method, beans are squeezed between the forefinger and thumb. If a bean yields to slight pressure and is free of graininess, the bean is classified as cooked. The only other classification is not cooked. As beans develop the HTC state, the cotyledons pass through a grainy state to a hard solid.

When comparing the cookability of two bean samples, the beans can be boiled for a given period and the percentage of beans cooked can be compared. Alternatively, the times needed to cook all the beans in the samples (CT_{100}) can be compared (Jones and Boulter, 1983a; Chhinnan, 1985). We prefer the first approach, since it requires less time. We found that cookability as measured by the tactile method was reproducible when starting with as few as 24 and 12 beans (Table I).

Differential Scanning Calorimetry (DSC) of Fresh and Hard-to-Cook Beans

Cooking of beans is a hydrothermal process involving gelatinization and swelling of starch, denaturation of protein, solubilization of some

Table I. Cookability of Pinto Beans Soaked in Acetate Buffer at pH 4.7 and 41°C for Various Lengths of Time

Soaking Time, h.	pH After Soaking	Beans Cooked[a] after Boiling 60 min, %			
		Total Beans = 12		Total Beans = 24	
		Test	Mean + S.D.	Test	Mean + S.D.
8	4.8	92 92 83	88.9 ± 5.2	83 75	79.2 ± 5.6
10	4.8	75 75 67	72.2 ± 4.6	74 78	76.1 ± 2.8
12	4.8	50 33 36	39.9 ± 9.1	44 33	38.4 ± 7.9
13	4.8	25 17 33	25.0 ± 8.0	23 33	28.0 ± 7.1
14	4.8	0 0 0	0	0	0
16	4.8	0 0 0	0	0	0

[a]Beans (12 or 24) were soaked, respectively, in 150 ml or 300 ml of buffer. After soaking, the beans were removed, water (100 ml or 200 ml) added, and cookability determined by the tactile method.

polysaccharides, softening of structure, creation of flavor, and other physical and chemical changes (Sefa-Dedeh and Stanley, 1979). Differential scanning calorimetry (DSC) can be used to assess starch gelatinization and protein denaturation in a food system. The temperature at which the endotherms occur for gelatinization and denaturation depend, first of all, on the biological source (Arntfield and Murray, 1981; Donovan and Mapes, 1980). Also, gelatinization is influenced by water availability and solutes (Eliasson, 1980; and Ghiasi, et al, 1983). During hydrothermal cooking of beans, gelatinization and denaturation initiate the changes in starch and protein inside the cell. If water becomes limiting inside the cell, gelatinization and denaturation will be limited.

In our work, fresh pinto beans were soaked 15 h in four parts of water, freeze-dried, ground, and heated in the DSC (see left side of Fig. 1). The thermogram of fresh pinto beans (curve a, Fig. 2) showed two endotherms at 80.5°C and 100°C. The peak at 80.5°C was attributed to starch gelatinization, since isolated pinto bean starch gave an endotherm at 74° (Fig. 3). The peak at 100°C was assigned to protein denaturation. The same two endotherms were observed starting with aged beans (curve a, Fig. 4).

When fresh or aged beans were soaked in water, cooked 15 min, freeze-dried, ground, mixed with two parts water, and heated in the DSC (right side of Fig. 1), the two major endotherms disappeared (curve b in Figs 2 and 4). The cooking times (CT_{100}) for the fresh and aged beans were 41 min and $\sim$ 120 min, respectively. Our results indicate that water is available for gelatinization and denaturation inside the bean cells within 15 min of boiling, a time well ahead of optimum cooking. Thus, the grainy texture of hard-to-cook beans is not due to ungelatinized starch or

Figure 1. Preparation of beans for DSC.

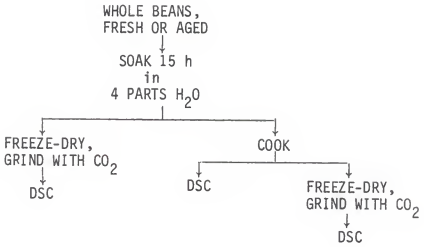

WHOLE BEANS,
FRESH OR AGED
SOAK 15 h
in
4 PARTS H_2O
FREEZE-DRY,
GRIND WITH CO_2
DSC
COOK
DSC
FREEZE-DRY,
GRIND WITH CO_2
DSC

Figure 2. Thermograms of (a) fresh raw beans that had been freeze-dried, and (b) fresh beans cooked 15 min and then freeze-dried.

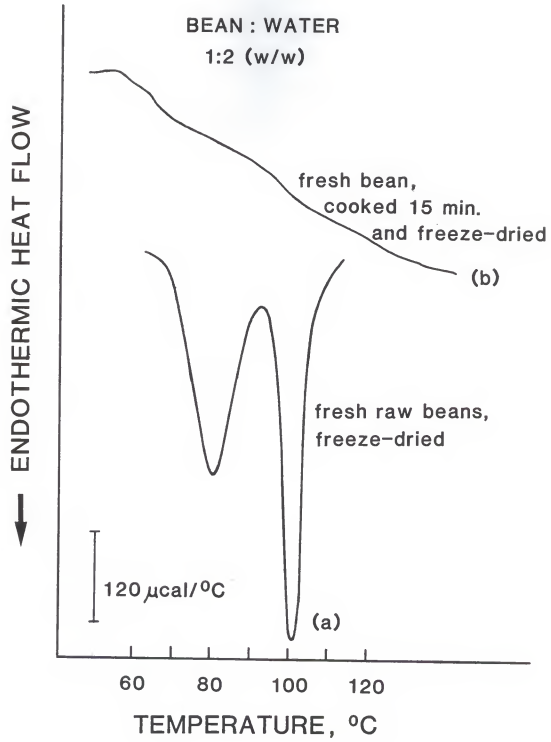

BEAN : WATER
1:2 (w/w)
fresh bean,
cooked 15 min.
and freeze-dried
(b)
fresh raw beans,
freeze-dried
120 μcal/°C
(a)
ENDOTHERMIC HEAT FLOW
60
80
100
120
TEMPERATURE, °C

Figure 3. Thermogram of isolated starch from pinto beans.

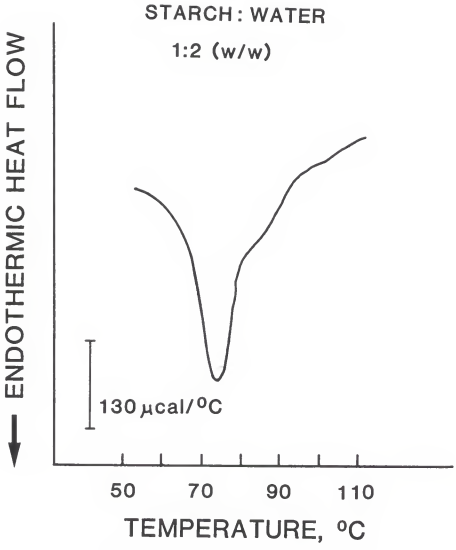
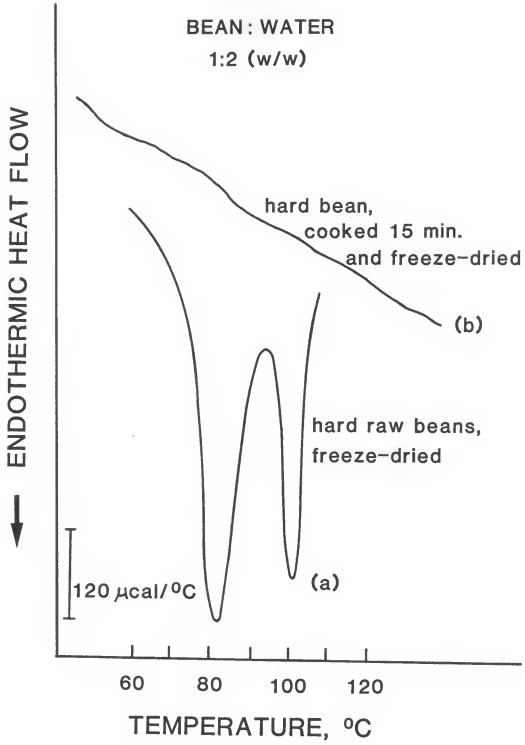

STARCH : WATER
1:2 (w/w)
ENDOTHERMIC HEAT FLOW
130 µcal/°C
50
70
90
110
TEMPERATURE, °C

Figure 4. Thermograms of (a) hard raw beans that had been freeze-dried, and (b) hard beans cooked 15 min and freeze-dried.


BEAN : WATER
1:2 (w/w)
hard bean,
cooked 15 min.
and freeze-dried
(b)
hard raw beans,
freeze-dried
(a)
120 µcal/°C
ENDOTHERMIC HEAT FLOW
60
80
100
120
TEMPERATURE, °C

raw protein, and water migration does not limit the cooking time of beans, as previously concluded by Varriano-Marston and Jackson (1981).

Our results agree with the classical conclusion of Sosnin (1927) that "...the contents inside the cell, such as protein and starch, can have nothing to do with the cooking process because the cell wall is not ruptured; the cells merely fall apart, singly or in groups, when the peas are cooked". Many other authors since have shown that intact cells separate at the optimum cooking time of fresh beans (Mattson, 1946; Rockland and Jones, 1974; Bourne, 1976; Sefa-Dedeh et al, 1978; Varriano-Marston and Jackson, 1981; and Jones and Boulter, 1983a). In other words, the grainy texture of hard-to-cook beans is due to clumps of cells that cling together after cooking.

Accelerated Hardening of Beans by Soaking and Cooking in Buffers

Mattson (1946) was probably the first to demonstrate that the pH of the soaking water affected the cookability of peas. In our work, we modified the procedure of Mattson to examine the effect of soak-water pH on cookability of pinto beans. In order to maintain constant pH, we used buffer solutions instead of hydrochloric acid or sodium hydroxide, and we used a much lower bean to liquid ratio (12 beans or $\sim$ 6 g/150 ml) than Mattson (50 g beans/200 ml H_2O). In addition, we added 0.002% chloramphenicol to prevent microbial activity. After soaking, we diluted the soaking buffer with 0.67 volumes of distilled water, and then cooked the beans in the diluted medium. Dilution gave only a slight change in pH. Finally, we increased the soaking temperature from room temperature to 41° to accelerate the rate of development of the HTC state or hardening.

When soaking pinto beans at 41°C for 8 h, the pH range over which

cookability disappeared was rather narrow (pH 4 to 4.5), whereas soaking for 24 h gave a broader range (pH 3 to 5) over which none of the beans cooked in boiling buffer for 60 min (Fig. 5). The sharp decrease in bean cookability as pH was decreased from 5.5 to 4.2 may be due to the dissociation of a ternary complex between phytate, magnesium, and protein (Maga, 1982; Prattley and Stanley, 1982). Plant phytase normally has optimum activity near pH 5 (Irving, 1980), thus, it appears that the increase in bean hardening at pH 4.2 corresponds to increased accessibility of phytate to phytase. Sefa-Dedeh et al (1979) demonstrated loss of protein bodies during soaking of cowpeas previously stored 12 months at 29°C and 85% RH. The same peas without soaking contained the protein bodies. Varriano-Marston and Jackson (1981) observed autolysis of protein bodies in black beans stored at 75% RH and 41°C for 55 days.

Pinto beans remained 100% cookable during 60 min boiling when soaked 8 to 24 h at pH 1.1, 1.6 and above pH 5.7 (Fig. 6). The gradual increase in cookability of beans with soaking time at pH 1.2 or 5.8 is shown in Fig. 7. Cookability increased linearly to 100% after 5 h soaking at pH 1.2 or 5.8, and remained at 100% for 5 to 16 h soaking. Sefa-Dedeh and Stanley (1979) found that five different legumes softened steadily up to 6-hours soaking, at which point softening reached a plateau. Eventually, beans soaked between pH 5.5 and 8.6 still hardened and would not cook in 60 min. The time to develop the HTC state increased with pH as follows; pH 5.5, 3 days; pH 6.0, 4 days; pH 6.5, 6 days; and pH 7.6, 7 days (Fig. 8). Thus, for each soaking pH, there was a "window" of soaking time during which the beans could be fully cooked in 60 min. The "window" was wide (4 to 7 days) between pH 6-7.6, but narrow (1/3 day) at pH 2.8 and

Figure 5. Cookability of pinto beans soaked at different pH's in 150 ml buffer for 8 h (●) or 24 h (◇), then cooked 60 min by boiling in the soaking liquid that had been diluted with 100 ml of distilled water.

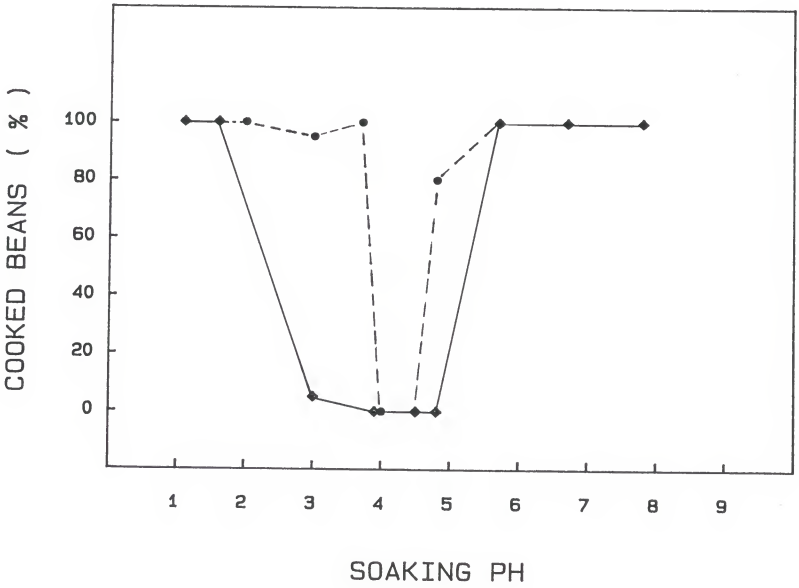

COOKED BEANS (%)
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
SOAKING PH

Figure 6. Cookability of pinto beans after soaking at 41°C and different pH's. pH 1.1, 1.6, 5.7, 6.7, 7.8 and 8.6 (■), pH 4.8 (△), pH 2.9 (●) and pH 3.9 (◇).

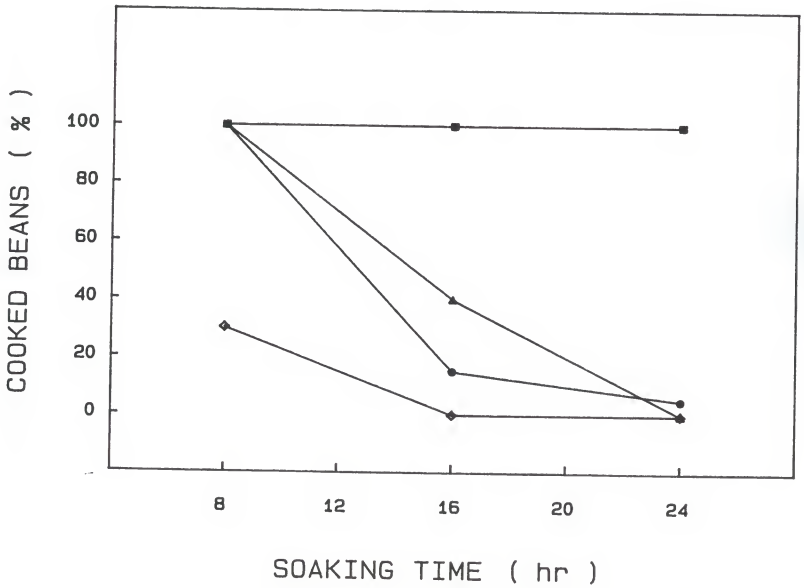

COOKED BEANS (%)
100
80
60
40
20
0
8
12
16
20
24
SOAKING TIME (hr)

Figure 7. Cookability of pinto beans soaked at 41°C in buffers at pH 1.2 (□) and pH 5.8 (●). Beans were treated as described in the caption to Fig. 5.

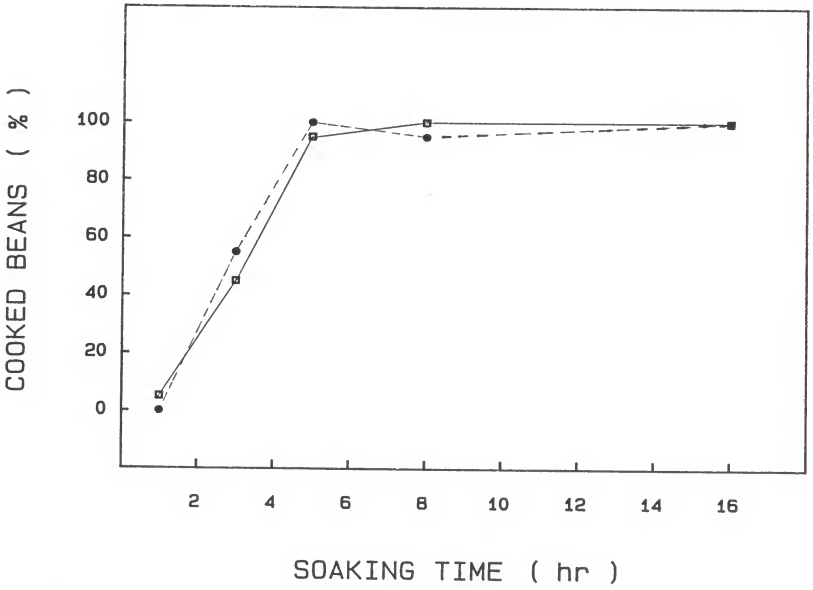
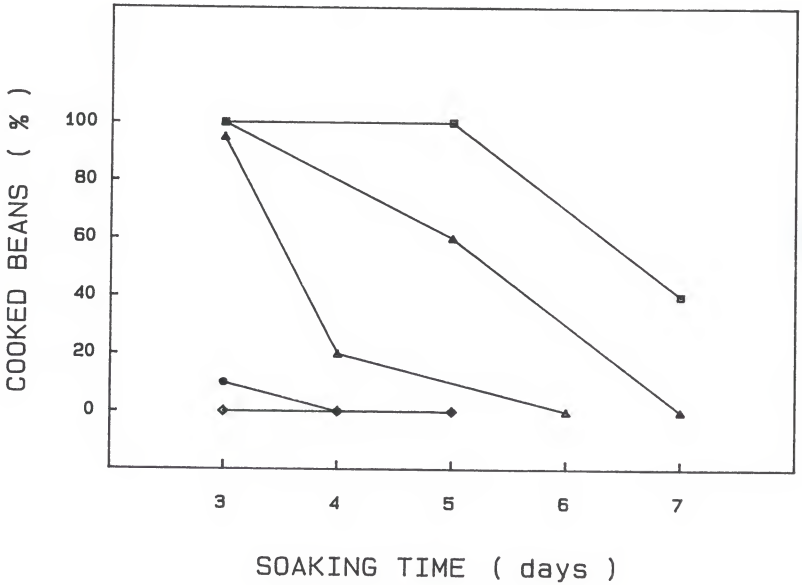

COOKED BEANS (%)
100
80
60
40
20
0
2
4
6
8
10
12
14
16
SOAKING TIME (hr)

Figure 8. Cookability of beans soaked in buffers of various pH's at 41°C. Beans were treated as described in the caption to Fig. 5. Soaking pH's were pH 5.5 (◇), pH 6.0 (●), pH 6.5 (△), pH 7.6 (▲) and pH 8.6 (□).


COOKED BEANS (%)
100
80
60
40
20
0
3
4
5
6
7
SOAKING TIME (days)

4.8 (Fig. 9). Soaking pinto beans at pH 3.8 gave no "window" for 100% cookability (Fig. 9) in 60 min of boiling.

It should be noted that during soaking of beans, the pH of the soaking buffers increased by 0.1-0.2 pH units in most experiments, except when soaking 7 days at pH's 7.4 and 8.3, where the pH decreased by 0.7 pH units. The pH changes were probably due to leaching of minerals and protein from the bean, and to the insoluble protein.

Our results show that the HTC state developed at all pH's above 4, but most rapidly at pH 4-5 and at temperatures of 40-45°C. The data on the accelerating effect of temperature on hardening are given in another part of this thesis; we did not test temperatures above 45°C. To obtain good cookability, it appears beans should be soaked in cold, soft water 14-18 h at pH 7-8.

Mechanism of Cotyledon Hardening in Beans

Jones and Boulter (1983a) proposed a dual-enzyme mechanism to explain the development of the HTC condition during storage of beans at elevated temperature and humidity (Fig. 10). Inside the cotyledon cells, phytase hydrolyzes phytic acid to release inorganic phosphate and magnesium, while outside the cells in the middle lamella, pectin methyl esterase hydrolyzes pectin to pectinic acid and methanol. The magnesium is thought to migrate from inside the cell to the middle lamella, producing insoluble magnesium pectinate that cements the cells together. Calcium also may migrate to the middle lamella to give insoluble calcium pectinate. It is known (McNeil et al, 1979) that pectin and xyloglucan are the major non-cellulosic polysaccharides in the cell walls of dicotyledons, and that magnesium and potassium are the major cations in plants (Moscoso et al, 1984;

Figure 9. Cookability of pinto beans soaked at 41°C and pH 2.8 (△), pH 3.8 (□) and pH 4.8 (●). The beans were treated as described in the caption to Fig. 5.

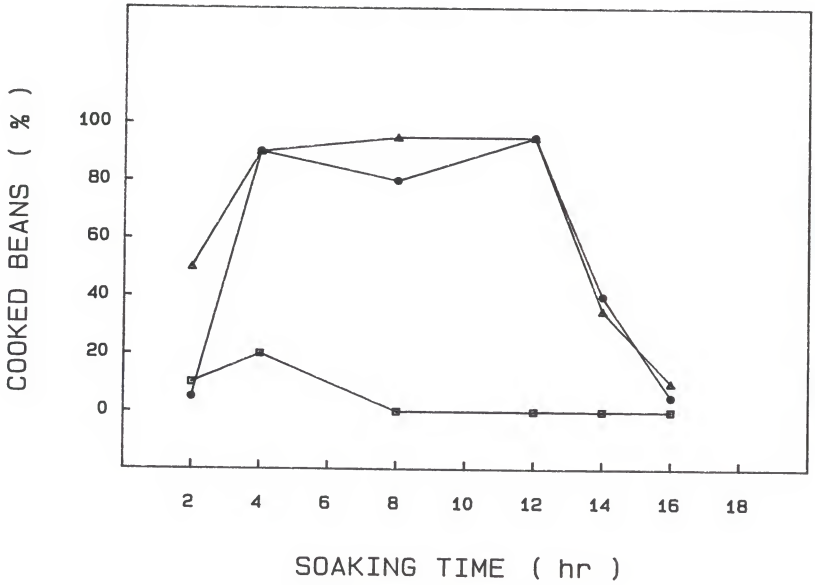
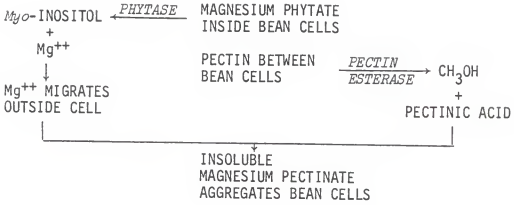

COOKED BEANS (%)
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
SOAKING TIME (hr)

Figure 10. Schematic of mechanism proposed by Jones and Boulter (1983a) to explain the development of the hard-to-cook condition in stored beans.


Myo-INOSITOL
PHYTASE
MAGNESIUM PHYTATE
INSIDE BEAN CELLS
+
Mg++
PECTIN BETWEEN
BEAN CELLS
PECTIN
ESTERASE
CH3OH
+
PECTINIC ACID
Mg++ MIGRATES
OUTSIDE CELL
INSOLUBLE
MAGNESIUM PECTINATE
AGGREGATES BEAN CELLS

Varriano-Marston and Omana, 1979). Calcium is also present (Jones and Boulter, 1983a; Moscoso et al, 1984).

In support of their hypothesis, Jones and Boulter (1983a) found that pectin solubility (sum of cold water, hot water, and oxalate extracts) was 31.4% in fresh beans and 17.2% in hardened beans. At the same time, the degree of pectin esterification decreased from 51% to 15%, and phytic acid from 29 mg/g to 19 mg/g. Furthermore, incubating fresh beans in 0.03 M calcium ions with and without pectin methyl esterase increased cooking time $\sim$ 60%. Other workers (Moscoso et al, 1984 and reference therein) have shown that high phytate levels in beans favors good cookability.

We found that blanching (15 min in boiling water) of pinto beans retarded their rate of hardening during soaking in acetate buffer at pH 4.4 (Table II). The blanched beans, however, still became uncookable after soaking 24 h, whereas the unblanched control beans were uncookable after soaking $<$ 8 h. Either the blanched beans retained some residual enzyme activity or a slow chemical change eventually caused hardening. To answer that question, the enzyme activities in blanched beans must be determined. Mattson (1946) soaked peas 4 h, boiled the suspension 10 min, then allowed it to cool and stand 8 days at 37^{o}C. He found that the blanched peas were as easy to cook as fresh peas, whereas unblanched peas were uncookable. Molina et al (1976) found that steaming black beans 2 min at 120^{o}C or 10 min at 98^{o}C decreased the development of the hard-to-cook phenomenon during storage for 9 months at 90% RH and 25^{o}C.

Excessive heat treatment of beans may cause chemical hydrolysis of pectin and extra mobility of mineral ions. Such a mechanism would explain why excessive steaming of beans reduced cookability (Molina et al 1976). It would also explain why Jones and Boulter (1983a) found that soaking

Table II. Blanching[a] of Pinto Beans and Subsequent Hardening of the Beans in Acetate Buffer (pH 4.4) at 41°C.

Bean	Soaking Time, h	pH After Soaking	Cookability after 60 min Boiling, %
Blanched	8	4.5	55
Unblanched	8	4.5	0
Blanched	16	4.5	15
Unblanched	16	4.5	0
Blanched	24	4.6	0
Unblanched	24	4.6	0

[a]Beans (12) were soaked 4 h at 25°C in 150 ml of distilled water, boiled 15 min, then drained and placed in 150 ml of acetate buffer pH = 4.4.

beans in hot 80% ethanol for 18 h, followed by treatment with calcium ion and pectin methyl esterase, rendered them uncookable. Unfortunately, the cooking time of beans soaked 18 h in hot 80% ethanol without subsequent treatment with calcium and enzyme was not reported.

Fluoride ion, an inhibitor of phytase (Irving, 1980), prevented the hardening of pinto beans during soaking at pH 4.7 and 41°C (Table III). After 16 h soaking in 0.05M fluoride, the beans were 96% cooked after 60 min boiling, whereas with no fluoride the beans were uncookable. The correlation of cookability with phytate content, the reduced rate of hardening of blanched beans, and the inhibition of hardening by fluoride ion indicate that a phosphatase enzyme is involved.

The interaction of calcium and magnesium ions with pectin in the middle lamella appears to be the second step in the hardening reaction. We found that soaking pinto beans in metaphosphate buffer at pH 4.1-4.6 and 41°C for 8-18 h caused no hardening, while in acetate buffer under practically the same conditions the beans were completely hardened (Fig. 11). Soaking HTC beans in metaphosphate buffer could not reverse the HTC state (data not given), which verifies the irreversible change in the hard cotyledons.

Varriano-Marston and Omana (1979) found that soaking black beans in aqueous sodium triphosphate (pH 8.7-9.9) at 25°C for 24 h gave improved separation of cells upon cooking. Oxalate, phosphate, carbonate, and phytate also improved cookability of beans, probably by chelating or precipitating magnesium and calcium ions (Mattson, 1946; Kon and Sanshuk, 1981). On the other hand, adding low concentrations of calcium or magnesium chloride to the soaking waters decreased the cookability of pinto beans, but sodium chloride had no effect (Fig. 12). Pinto beans

Table III. Cookability of Pinto Beans Soaked at 41°C in Acetate Buffer pH = 4.7 With and Without Sodium Fluoride.

Fluoride Concentration, M	Soaking Time, h	pH After Soaking	Cookability[a], %
0.05	12	4.85	95.5
none	12	4.80	39.0
0.05	16	4.85	90.0
none	16	4.80	0

[a]Pinto beans (12) were soaked in buffer (150 ml) at 41°C. Cookability was determined by diluting the soaking water with distilled water (100), bringing the liquid to boil, cooking the beans 60 min, and using the tactile method.

Figure 11. Cookability of pinto beans after soaking in 0.2M metaphosphate (□) and 0.1M acetate (●) buffers at 41°C. The initial and final pH of the soaking solutions were 4.1 and 4.5-4.6 for metaphosphate buffer, respectively, and pH 4.0 and 4.0-4.1 for acetate buffers. The beans were treated as described in the caption to Fig. 5.

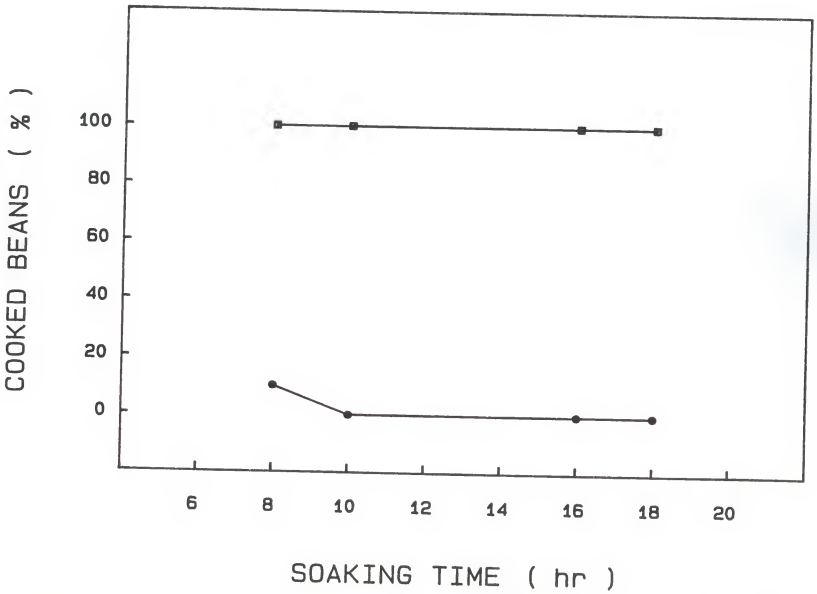
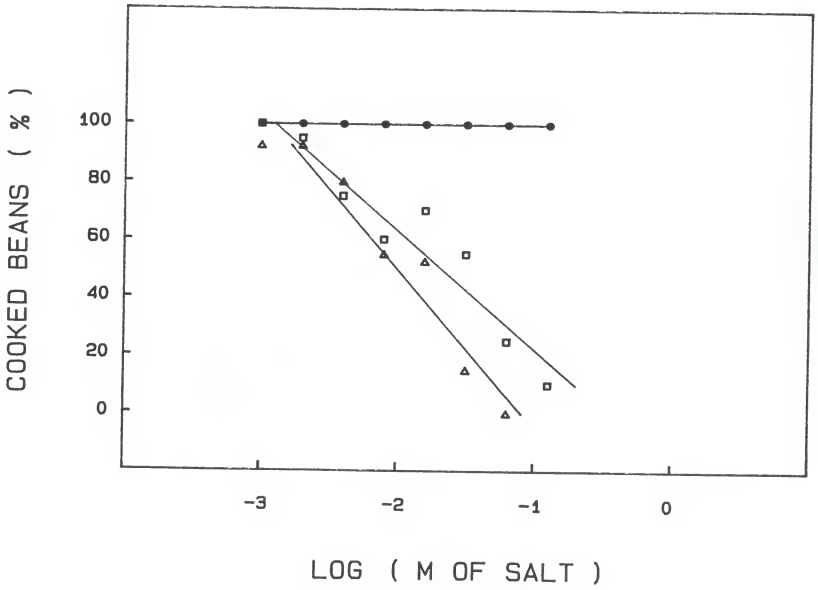

COOKED BEANS (%)
100
80
60
40
20
0
6
8
10
12
14
16
18
20
SOAKING TIME (hr)

Figure 12. Cookability of beans after soaking in calcium (△), magnesium (□) or sodium chloride (●) for 18 h at 41°C. The beans were treated as described in the caption to Fig. 5. M is the molarity of salt in the soaking water.


COOKED BEANS (%)
100
80
60
40
20
0
-3
-2
-1
0
LOG (M OF SALT)

were 50% cookable during 60 min of boiling when the beans were soaked in ∿ 0.008M calcium ion and ∿ 0.03M magnesium ion.

The retardation of bean hardening by metaphosphate at pH ∿ 4 is difficult to rationalize by the cross-linking or lignification mechanism, especially since hardening is so rapid in acetate buffer at the same pH. However, the effect of metaphosphate as a chelating agent does support the enzyme mechanism involving phytate and pectin. The minimum cookability of beans at pH ∿ 4.2 (Fig. 5) also supports the phytate-pectin hypothesis. As pH for soaking beans is increased from 4 to 7, phytate inside the cell is thought to chelate more and more Ca^{++} and Mg^{++} ions (Tangkongchitr et al 1982), which keeps the pectinic acid in the middle lamella in a soluble form and allows the beans to cook normally. As soaking pH is decreased from 4 to 1, the precipitated calcium and magnesium pectinate dissolve as pectinic acid, and cookability again improves (Mattson, 1946).

The Jones-Boulter hypothesis (Fig. 10) differs from that of Mattson (1946) in that pectin methyl esterase has been added. More data are needed to determine whether pectin methyl esterase is involved. Jones and Boulter (1983a) did not expose fresh beans to pectin methyl esterase alone. Furthermore, when they did soak fresh beans in a mixture of 0.03M calcium chloride and pectin methyl esterase, the mean cooking time increased from 24 min for the control sample (soaked in 0.03M calcium chloride) to 28 min. The increase of 4 min in cooking time was small. On the other hand, the methyl ester content of pectin did decrease from 51% to 15% upon storage of the black beans for 6 months at 34°C and 70-75% RH (Jones and Boulter, 1983a), but the authors did not report the cooking times of the beans.

Accelerated Hardening of Beans

It is well known (Sgarbieri and Whitaker, 1982) that holding beans at high humidity and high temperature accelerates the development of the HTC condition. Agronomists could use accelerated hardening of beans at 100% R.H. in warm air to select varieties that harden at a slow rate during drying, storage, *and*, as our results show here, possibly during soaking.

To develop a reproducible method of accelerated hardening of beans, two variables must be controlled, (i) the ratio of beans to the volume of humid air in the container, and (ii) mold growth on the beans. The volume of air to beans was 40 cm^3/g, which we speculate was sufficiently large to ensure that the air was saturated with water vapor throughout the storage period for the beans. We sanitized the desiccators used to hold beans, and pretreated the beans (5 min) with water containing 0.02% chloramphenicol. Those precautions prevented microbial attack on all but 3 of 45 samples of beans for at least 9 days at 45°C. Otherwise, beans held at 100% R.H. and 45°C became visually moldy in 3-7 days. Moscoso et al (1984) used tetramethylthiuram disulfide to prevent molding in kidney beans stored at 17.9% moisture and 32°C.

Table IV shows results that were typical of the humid-air accelerated-hardening of pinto and black beans. For both varieties, moisture increased from $\sim$ 11% up to 32-36% in 5 days aging at 100% R.H. and 45°C, and 34-39% in 7 days. Simultaneously, the cookability of the beans decreased from 100% at 0 days to 5-20% in 5 days, and fell further to 0-5% in 7 days. Surprisingly, the pH of the soaking water increased from 5.7 at 0 days to 6.4-6.6 in 5-7 days of humid aging.

Other properties of the beans changed with increased storage in humid air. The color of the seed coat darkened during humid storage, as

Table IV. Accelerated Hardening[a] of Pinto[b] and Black[c] Beans by Storing in a Desiccator at 45°C and 100% R.H.

Storage Time, d	Moisture, % (w.b.)		pH After Soaking 17 h in Water		Beans Cooked in 60 min, %	
	Pinto	Black	Pinto	Black	Pinto	Black
0	10.9	11.6	5.7	5.7	100	100
1	19.6	24.8	5.8	5.7	95	95
2	25.8	29.4	6.0	6.2	90	95
3	25.1	32.0	6.0	6.2	95	60
4	31.9	33.5	6.3	6.5	50[d]	35
5	32.4	35.5	6.4	6.4	5	20
6	33.1	35.6	6.4	6.6	10	10
7	33.5	38.6	6.4	6.3	0	5

[a]Each measurement is the average of two replications.

[b]Pinto beans were Olathe variety grown in 1984 in Colorado, and stored 12 months at 5°C.

[c]Black beans were Ica-Pijao variety grown in Michigan and stored 12 months at 5°C.

[d]The same percentage (50%) of cooked beans was found when the beans were not dried after storing in a desiccator, but were soaked and cooked immediately. All other beans that had been stored in a desiccator were dried 2 days under ambient room conditions prior to soaking and cooking.

previously observed (Sartori, 1982). Darkening of beans, therefore, may be used as a crude indicator of cookability. Cracking of the seed coat of aged beans diminished during cooking; aged beans tended to remain intact during cooking. Finally, during humid storage, the clarity of the soaking water changed. When pinto beans were stored at 100% RH and 45°C for 1 to 2 days or 5 or more days, and then soaked 17 h at 25°C, the soaking water was clear. But humid aging for 3-4 days gave turbid soaking water. The significance of the precipitate during soaking of beans is unknown.

Comparison of Hardening of Three Varieties of Beans

Besides the pinto and black beans, three other varieties of beans were accelerated hardened at 100% R.H. and 45°C. The cooking data in Table V show the order in which the three varieties of beans developed the HTC state in the warm humid atmosphere. The white bean (Sanilac) became uncookable sometime between 0 and 3 days storage, while the red (15-R-148) and brown (A-30) beans were uncookable after 7-9 days storage. However, the plant breeder (Hosfield, 1984) reported to us that the red bean (15-R-148) tended to harden faster than the brown bean (A-30), which hardened faster than the white bean (Sanilac).

The disagreement between the assigned rates of hardening for the three bean varieties may be explained by knowing that beans become HTC not only during humid storage, but also during the soaking period prior to cooking. Over 40 years ago, as previously pointed out, Mattson (1946) showed that peas hardened when soaked longer than 1 day at 25°C, especially between pH 4-5.

The three varieties of beans in our work were soaked 8 h in a large excess of aqueous buffer between pH 1 and 9 at 41°C, and their cookabilities determined after boiling in diluted soaking buffer for 1 h. The curves

Table V. Accelerated Hardening[a] of Three Varieties[b] of Beans Stored in Desiccators at 45°C and 100% R.H.

Storage Time, d	Moisture % (w.b.)			pH After Soaking 17 h in Water			Beans Cooked in 60 min, %		
	A	B	C	A	B	C	A	B	C
0	8.2	7.5	7.7	---	---	---	100	100	100
3	30.7	22.8	25.1	6.4	5.7	5.8	0	95	80
5	37.7	28.3	32.2	6.7	6.0	6.0	0	90	60
7	41.0	36.9	39.6	6.8	6.4	6.5	0	20	15
9	----	37.3	38.0	---	6.4	6.4	---	0	0

[a]Each measurement is the average of two replications.

[b]A=Sanilac variety (white); B=A-30 variety (brown); and C=15-R-148 variety (red).

in Fig. 13 show that during soaking, the red bean became hard-to-cook over a broad pH range (2-7), the white over a narrow pH range (4-5), and the brown bean intermediate between the two. The relative tendencies of the three bean varieties to harden during soaking 8 h at 41°C and pH 6-7 agreed with the judgement of the plant breeder, but disagreed with the relative tendencies to harden in a warm humid atmosphere.

When the temperature of soaking the beans was lowered from 41°C to 32°C and the soaking time increased from 8 to 17 h, the red bean again showed less cookability than the white bean or brown bean at ∿ pH 5.7 (Table VI). It was unfortunate that at this point in our investigation, we exhausted the three samples of beans and could not do additional soaking tests at 32° and 25°C for 17-24 h. Curves similar to those in Fig. 13, which were obtained by soaking beans at 41°C, are needed at temperatures near 25°C and 32°C in the soaking step.

The data given in Fig. 13 were obtained using a large ratio of buffer to beans, which is much different than the 2:1 proportion of water:beans normally used by consumers. When we soaked one part of Sanilac (white) or the red variety 15-R-148 in two parts distilled water, the pHs of the soaking waters after 17 h were 6.5 and 5.9, respectively. The curves in Fig. 13 show that the white bean would be expected to cook well after soaking under normal conditions, since the pH of the soaking water was above pH 6. On the other hand, the red bean would harden under normal soaking conditions, since the pH of its soaking water was 5.9.

It appears that new bean varieties may have to be screened by two different tests to discriminate hardening that might occur during soaking rather than during storage. The first test would involve soaking beans between pH 5-8 for 16-24 h at 32°C to determine how rapidly a sample

Figure 13. Cookability of three varieties of beans soaked 8 h at 41°C in various buffers. The beans were treated as described in the caption to Fig. 5. Sanilac (white bean) = (□); A-30 (brown bean) = (△); and 15-R-148 (red bean) = (●).

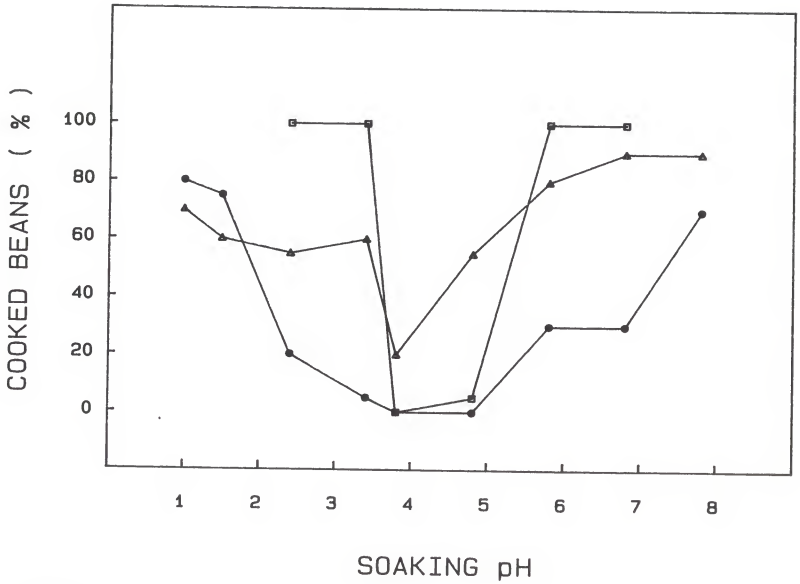

COOKED BEANS (%)
100
80
60
40
20
0
1
2
3
4
5
6
7
8
SOAKING pH

Table VI. Accelerated Hardening[a] of Three Varieties[b] of Beans by Soaking 17 h at 32°C in Buffers of pH 2.8 to 8.1.

Buffer pH	pH After Soaking 17 h in Buffer			Beans Cooked in 60 min, (%)		
	A	B	C	A	B	C
2.8	3.0	-	2.9	100	70	55
3.5	3.7	3.6	3.6	100	55	55
3.9	4.0	4.0	3.9	0	10	10
4.4	4.4	4.4	4.4	10	50	30
5.7	5.7	5.7	5.7	100	90	70
6.3	6.3	6.3	6.3	100	100	100
8.1	-	8.1	8.1	100	100	100

[a]Each measurement is the average of two replications.

[b]A=Sanilac variety (white); B=A-30 variety (brown); and C=15-R-148 variety (red).

hardened during soaking. If a bean variety cooked fully after soaking at a desired pH, that variety should then be exposed to a warm humid atmosphere to test its hardening under simulated storage conditions.

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To my friends in the Grain Science Department, to Amir, and to my beloved family, THANKS.

ACCELERATED DEVELOPMENT OF THE HARD-TO-COOK STATE IN BEANS

by

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B.S., University of Sonora, Mexico, 1972

AN ABSTRACT OF A MASTERS'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1986

ABSTRACT

Differential scanning calorimetry (DSC) of a 2:1 (w/w) mixture of water and fresh pinto beans (freeze-dried and ground) showed two endotherms at 80.5° and 100°C that were assigned to starch gelatinization and protein denaturation. After boiling 15 min in water, fresh (cooking time 41 min) and hard-to-cook (HTC) pinto beans (cooking time ∿ 120 min) did not show either thermal event. When soaked at 41°C in buffers containing 0.002% chloramphenicol, pinto beans developed the HTC state most rapidly at pH 4; the beans were essentially uncookable in boiling water for 60 min when soaked at pH 4 for 8 h. Pinto beans also became uncookable upon soaking at pH 5.5 for 3 days; pH 6.0, 4 days; pH 6.5, 6 days; and pH 7.6, 7 days. When fluoride ion (0.05M) or metaphosphate (0.2M) was added to the soaking medium at pH 4.7 and pH 4.1, respectively, the beans remained 90-100% cookable after soaking 16 h at 41°C, whereas control beans were uncookable. Red and white beans developed the HTC condition at opposite times when stored at 100% RH and 45°C or when soaked in acidic buffers at 41°C. Beans that are prone to the HTC state may harden during soaking and/or storage.