THE EFFECT OF SOAKING AND PECTIN COMPOSITION ON THE HARDNESS OF DRIED LEGUMES (*Phaseolus vulgaris*)

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ABSTRACT

Soaking time and pectin composition in accelerated stored versus control beans were investigated to determine their relationship to bean hardness. Beans were soaked for 0-44 hours following which hardness was determined and pectin fractionated into cold water, EDTA, and NaOH. Galacturonic acid content and percent methylation were determined for all pectin fractions. Aged beans showed dramatic increases in hardness with extended soak-times. A pattern suggesting a hardening mechanism primed by aging, with expression upon soaking was observed. Decreases in EDTA soluble pectin exhibited a temporal correlation to hardness development. Increases in NaOH soluble pectin with increased soak-times were observed. Pectin fractions from both aged and control beans were found to contain low-methoxy pectin.

INTRODUCTION

Legumes are a major food source world wide and the primary source of protein in many countries. When stored under adverse conditions of high temperatures and high humidities for extended periods of time, legumes often develop what is generally referred to as the "hard-to-cook" defect. When this problem is encountered, beans will hydrate, but fail to soften under normal processing conditions and require extended (up to 6 times longer than controls) cooking times to reach an acceptable texture (Dhahir, 1988). This results in decreased protein availability and economic losses due to increased fuel costs or product loss.

A commonly accepted mechanism which explains this problem is the middle lamella-cation-phytate-phytase theory. It is hypothesized that the high temperatures and high humidity conditions of storage potentiate phytase, deteriorate membranes, and demethylate pectin of the middle lamella. Membrane deterioration would allow contact between the otherwise compartmentalized phytase and its substrate phytate. Phytate, inositol hexaphosphate, is a powerful chelator and storage form of divalent cations. Once acted upon by phytase, phytate is broken down into inositol and phosphoric acid in a 1:6 molar ratio, and is no longer able to chelate calcium and magnesium. These freed divalent cations may now migrate through deteriorating membranes to the middle lamella and act as calcium and magnesium bridges to bind pectin, proteins, and

tannins to form intermolecular bonds. Prolonged heating would then be required for these bonds to be broken to allow molecular and cellular separation, which is required for the development of an acceptable texture in legumes (Hentges 1988; Jones and Boulter, 1983a; Moscoso et al., 1984; Rockland and Jones, 1974; Sefa-Dedeh et al., 1979; Stanley and Aguilera, 1985). There is a vast amount of research which has been done in an effort to substantiate and better understand the hard-to-cook defect of beans. Some studies support this theory and other research does not.

The role of phytate in the development of the hard-to-cook defect has been supported by the work of Stanley et al. (1990), Hentges (1988), Sievwright (1985), Hernandez-Unzon and Ortega-Delgado (1989), and Jones and Boulter (1983a), all of whom found a decreased phytate content accompanying increased cooking time.

This mechanism is also supported by changes in bean pectin observed by many researchers as the hard-to-cook defect develops. Hentges (1988) observed decreases in hot water soluble pectin and increases in EDTA soluble pectin for beans stored at high temperatures and humidities. A decrease in the content of water soluble pectin during storage at low temperature (4°C) and high relative humidity (65%) was demonstrated by Jones and Boulter (1983b). Jones and Boulter (1983a) showed a decrease in pectin solubility and esterification in hard-to-cook black beans along with an

overnight (Anonymous, 1982). Sometimes processing delays are encountered and beans may be soaked for extended time periods. Longer soak periods frequently yield bean products too hard to be acceptable to consumers.

This project was initiated to examine factors related to bean hardness. Length of soaking period in accelerated stored versus control beans, their pectin compositions, and relation of these factors to bean hardness were investigated.

MATERIALS AND METHODS

Legume Source

Great Northern beans (Phaseolus vulgaris), harvested in the fall of 1985, were used in the investigation. Since 1985 and until the investigation commenced, the beans were stored at 4-6°C. The moisture content of the beans was 12% when this study was initiated.

Accelerated Storage Treatment

For those beans subjected to accelerated storage treatment, beans were placed in a 300 millimeter (mm) desiccator-type glass container. The lower part of the container contained a saturated solution of sodium chloride to maintain the relative humidity near 75% (Greenspan, 1977). The top part of the container was lined with a double layer of

dried and ground to a flour for pectin analysis. These processes are described below.

Texture Analysis

Beans were placed in a #303 can and covered with a hot (80-85°C) 30-35% sucrose solution. The cans were then sealed and processed in a retort at 121°C for 25 minutes. Following processing, the samples were stored at room temperature for 27-28 days. At the end of this storage period, a subsample of 20 beans was taken from each can and hardness measured.

Hardness was measured on a Voland-Stevens LFRA Texture Analyzer with a T-shaped plunger (3.37 mm diameter, 25.5 mm total arm length) set to travel 6 mm at 0.5 mm/sec with 20 millivolt full scale sensitivity. Full scale deflection was equivalent to a 1 kilogram (Kg) load.

Drying and Grinding of Samples

Following soaking, the samples were drained and weighed, and half of the beans were placed in a VirTis Freezemobile 12 freeze dryer with a vacuum of 12 milliTorr and condenser temperature of -65°C for 44 hours or more. Plate temperature was 24°C.

Dried beans were ground to flour in a Grind-All mill set to its finest setting and bean flour was stored at 4-6°C until needed for further analyses.

Pectin fractionation

The method of Kon (1968) was used in the fractionation of pectic substances with the following modifications:

1) Starch gelatinization was accomplished by suspending bean flour in 0.1 N potassium phosphate buffer, Ph 4.5, and heating for 4-10 minutes at 75-80°C;

2) Starch was digested with 0.02 g amyloglucosidase (Sigma No. A-7255, Lot 56F-0647) for four hours at 36-38°C;

3) Collected supernatants were <u>not</u> brought to 0.05 N with NaOH.

This procedure results in three successively extracted pectin fractions: cold water soluble high methoxy pectin; 1% EDTA pH 6.0 soluble low methoxy pectin; and 0.05 N NaOH soluble protopectin.

Galacturonic acid assay

A. Sample preparation. An enzyme digestion, similar to the approach of Koseki et al. (1986), was used to prepare samples for the galacturonic acid assay. One ml aliquots of pectin fractions in distilled water, 1% EDTA at pH 6.0, or 0.05 N NaOH were added to 4 ml 0.1 N potassium phosphate buffer of pH 4.0, and the pH of each test tube was adjusted to 4.0 ± 0.2. One ml of polygalacturonase (Sigma No. P-5146, Lot

25f-0193), diluted to 2 units/ml in 0.1 N potassium phosphate buffer at pH 4.0, was then added, and the net weight of each test tube brought to near 7.00 g with distilled water. Samples were next incubated at room temperature for 4 hours. Following incubation, samples were centrifuged at 528 Relative Centrifugal Force (RCF) in a model UV IEAC International Centrifuge, and 1.00 ml of the supernatant was transferred to a test tube containing 10.00 ml 40% ethanol. This was mixed, sealed, and refrigerated until assayed for galacturonic acid. Samples were not further diluted prior to galacturonic acid assay.

B. Assay. Samples prepared as described above were analyzed for galacturonic acid content following the procedure used by Kinter and Van Buren (1982) with the following modifications:

1) Sample aliquots were not kept in an ice water bath prior to the addition of the sodium tetraborate in concentrated sulfuric acid reagent;
2) Following the addition of the sodium tetraborate in concentrated sulfuric acid reagent, samples were heated in a 95-97°C water bath for 7 minutes.

Total Soluble Pectin

Total soluble pectin was obtained as the sum of the galacturonic acid content of the three extracted pectin fractions.

Methanol Assay

The method of Klavons and Bennett (1986) was used, with some modifications, to determine the methyl ester content of the pectin fractions. Formation of turbidity was observed during the assay, a model UV IEAC International Centrifuge was used to remove the turbidity by centrifugation for 10 minutes at 528 RCF. Samples were chilled and centrifuged following potassium hydroxide (KOH) digestion and again prior to measuring absorbance. This reduced, but did not completely eliminate sample turbidity. Other modifications were as follows:

1) For incubation of samples in 0.5 N KOH, 1.00 ml aliquots of the pectin fractions were mixed directly with 1.00 ml 1 N KOH to get a final concentration of 0.5 N KOH;

2) Alcohol oxidase (Sigma No. A-2404, Lot 10H-590) was diluted to 1 unit/ml in 0.25 N potassium phosphate buffer (Ph 7.5), and frozen in 50 ml portions until needed for the assay.

Percent Methylation

The percent methylation was determined by dividing moles methanol by moles galacturonic acid and multiplying by one hundred.

RESULTS

Germination

As indicated in Figure 1, the germination rate of the aged beans decreased to zero at the end of 16 weeks of storage at 37°C and 75% humidity, while the germination rate of the control beans remained relatively constant. These findings are consistent with those of Sada (1980) who also found a reduced germination rate for beans stored at a high temperature and high relative humidity.

Hardness

Hardness of aged and control beans was measured on samples soaked for 0, 6, 20, 32, and 44 hours to determine when hardness occurred. Percent methylation and galacturonic acid content were then determined for each of the three pectin fractions to assess if changes were related to the onset of hardness.

As seen in Figure 2, mean hardness of aged beans was greater than mean hardness of control beans ($p < 0.05$). Aged beans which were unsoaked had a mean hardness 23% greater than control beans which were unsoaked. With soaking for 20 hours or more, aged beans had mean hardness values 2.4 - 3.1 fold greater than similarly soaked control beans.

Control beans increased slightly in hardness after 20 hours of soaking (hardness of control beans soaked 32 and 44

hours were significantly different than beans soaked 0 and 6 hours at $p < 0.05$); however, the control beans were not harder than 0.4 Kg - the borderline acceptability value of hardness established by He (1986) - even after 44 hours of soaking. Aged beans approached 0.4 Kg hardness with no soaking, exceeded 0.4 Kg hardness after only 6 hours of soaking, and showed dramatic increases in hardness by 20 hours of soaking. These results suggest that aging and soaking both contribute to the onset of bean hardness, with aging possibly priming the hardening mechanism and soaking allowing its full expression.

Percent Methylation

The fractionation method used was designed to successively separate the pectin into water soluble, 1% EDTA soluble, and 0.05 N NaOH soluble pectin (Kon, 1968). The mean percent methylation of these fractions as determined in this study were 24% for water soluble, 3-4% for EDTA soluble, and 26% for NaOH soluble pectin. (The 26% methylation found in the NaOH soluble fraction represents methanol remaining <u>after</u> extraction in NaOH which could cleave methanol esters and reduce the assay value.)

The only statistically significant difference in percent pectin methylation from aging was seen in the EDTA fraction. This involved an increase ($p < 0.05$) from a mean 3% for control to 4% for aged beans. Soaking did not significantly affect the percent methylation of the pectin fractions.

Galacturonic Acid Content.

The total soluble pectin decreased from 16.4 milligrams galacturonic acid per gram bean flour (mg/g) for control to 15.4 mg/g for aged beans. This decrease of 6% was statistically significant ($p < 0.05$).

The NaOH soluble pectin (protopectin) mean galacturonic acid content for aged and control beans plotted against soak-time is shown in Figure 3. This galacturonic acid content increased ($p < 0.05$) with soak-time in both aged and control beans.

The EDTA soluble pectin (very low methoxy pectin) mean galacturonic acid content for aged and control beans plotted against soak-time is shown in Figure 4. This galacturonic acid content was greater ($p < 0.05$) for control than aged beans.

Paralleling the pattern seen in the onset of hardness, the EDTA soluble pectin galacturonic acid content of aged beans soaked 0 and 6 hours was very close to those for control beans similarly soaked. Aged bean values decreased with soak-times of 20 hours or more, while control bean values remained relatively constant. A widening gap effect is seen which temporally correlates to the pattern for the development of hardness as shown in Figure 2. This aging-soaking interaction for the EDTA soluble pectin galacturonic acid content was statistically significant ($p < 0.05$).

The values for the aged and control bean EDTA soluble pectin galacturonic acid content were not statistically different ($p > 0.05$) at 0, 6 or 20 hours, but were statistically different ($p < 0.05$) at 32 and 44 hours. Although statistical differences were not detected until 32 hours of soaking, it can be seen from Figure 4 that the EDTA soluble pectin galacturonic acid content for aged beans soaked 20 hours follows the widening gap trend which parallels hardness development.

DISCUSSION

The effect of soaking and pectin composition on bean hardness were investigated in this study. This was done by comparing hardness and pectin composition of aged and control beans at different soak-times. Pectin composition was examined through successive fractionation into cold water, 1% EDTA, and 0.05 N NaOH, and determining the galacturonic acid content and percent methylation of each of these fractions.

Hardness

The hardness of the aged beans appeared to stabilize between 0.9 and 1.0 Kg after a soak-time of 20 hours (Figure 2). However, as previously mentioned, full scale deflection was set at 1.0 Kg resistance. Thus, it is possible that the mean hardness for aged beans soaked 20, 32 and 44 hours

continues to increase and could be detected if the instrument were not so limited. There was wide variation among the individual values that make up the means shown in Figure 1. Although many values for aged beans soaked 20 or more hours reached the maximum value of 1.0 Kg (see appendix B), some did not and consequently neither did their means.

Although not specifically determined in this study, calcium availability, as discussed previously, may play an important role in development of bean hardness. However, calcium released from internal phytate during prolonged soaking was not addressed in this work. Further, the calcium content of the tap water was small (40-65 ppm) and unlikely to have had a significant effect.

Percent Methylation

All values of percent methylation for the various pectin fractions as determined in this study were less than 30%. These are lower than normally found in ordinary "high methoxy pectin" or protopectin. The low value of methylation for protopectin may not be unusual when extracted with NaOH prior to the methanol assay, which could have cleaved methanol groups and reduced the assay value. Jones and Boulter (1983a) reported pectin methylation of black bean at 51% for soft and 15% for hard beans. Although they found a large methylation difference between hard and soft beans which was not found in

this study, the methylation values were those of low-methoxy pectin for both studies.

As previously mentioned, the only statistically significant difference in the percent methylation was seen in the EDTA fraction: a mean 3% for control beans and 4% for aged beans. However, this 1% difference does not seem to be of any practical importance.

Galacturonic Acid

Total soluble pectin. The decrease in total soluble pectin is consistent with the findings of Jones and Boulter (1983a), who also observed decreased pectin solubility in hard-to-cook black beans.

NaOH soluble pectin. Both aged and control beans showed significant increases in NaOH soluble pectin with increased soaking time. Although increases were slight for control beans, both aged and control beans also increased in hardness with increased soaking time. Increases in hardness from soaking <u>may</u> be affected by increases in the NaOH soluble pectin.

In a pattern similar to that seen in hardness development, aged beans which were soaked 0 and 6 hours had galacturonic acid contents very close to those for control beans similarly soaked; however, with soak-times of 20 or more hours, the increase of protopectin galacturonic acid in the aged bean NaOH fractions appeared greater (not statistically

significant) than the increase of protopectin galacturonic acid in the control bean NaOH fractions. Though this aging-soaking interaction is not statistically significant ($p > 0.05$), the widening gap effect is similar to that seen in the development of bean hardness (Figure 2), and may be of some importance.

The increase in the NaOH soluble pectin of aged and control beans might be explained by increased pectin polymerization on soaking. It is possible, that as the seed prepares for growth, more pectic substances are needed for use as structural support and pectin polymerization increases. Substrate sources for polymerization might include very low methoxy pectin from the EDTA soluble fraction (which decrease on soaking of aged beans) or galacturonic acid synthesized through metabolic activity which increases as the beans rehydrate and prepare for germination. It is also possible, that with rehydration and soaking, intermolecular association increases as pectic free carboxylic acid groups are allowed to align.

As stated by Whistler and Daniel (1985), "high gel strength correlates positively with high-molecular-weight pectin molecules and extensive intermolecular association." Increased pectic gel strength in the middle lamella, resulting from the increased protopectin (high molecular weight) fraction and increased intermolecular association upon soaking, could necessitate increased cooking time in order to

break down the gel and allow the cell separation which is required for development of acceptable bean texture.

EDTA soluble pectin. The temporal correlation between hardness development and decreased very low methoxy galacturonic acid in the EDTA fraction at first appears to contradict the generally accepted model of hardening involving demethylation of pectin and gel formation through calcium bridges. This is not necessarily the case.

First, all pectin fractions of Great Northern beans showed a low percentage of methylation, and could feasibly participate in the formation of calcium bridges and intermolecular bonds. Secondly, as previously mentioned, other researchers (Jones and Boulter, 1983a) have found a decrease in the extractability of pectin from hard beans. Lignification may be a factor adversely affecting the extractability of these very low methoxy pectin.

Another possibility is that some of the EDTA soluble, low methoxy pectin, are polymerizing with the protopectin and associating with that fraction.

It is important to note that Hentges (1988) reported an increase in the EDTA soluble pectin in beans stored at high temperatures and high humidities; however, soaking beans for extended periods of time prior to extracting the pectin was not part of this study. Only after soaking for extended periods of time were significant differences in the EDTA soluble pectin of aged and control beans observed. Further,

Kon (1968) and Molina et al. (1976) found no significant changes in the pectic fractions hard-to-cook beans.

Conclusions

The following conclusions and observations were noted:

1) A pattern suggesting a hardening mechanism primed by aging, with expression upon soaking was observed.

2) Decreases in EDTA soluble pectin exhibited a temporal correlation to hardness development.

3) Increases in NaOH soluble pectin with increased soak-times were observed.

4) Pectin fractions from both aged and control beans were found to contain low-methoxy pectin.

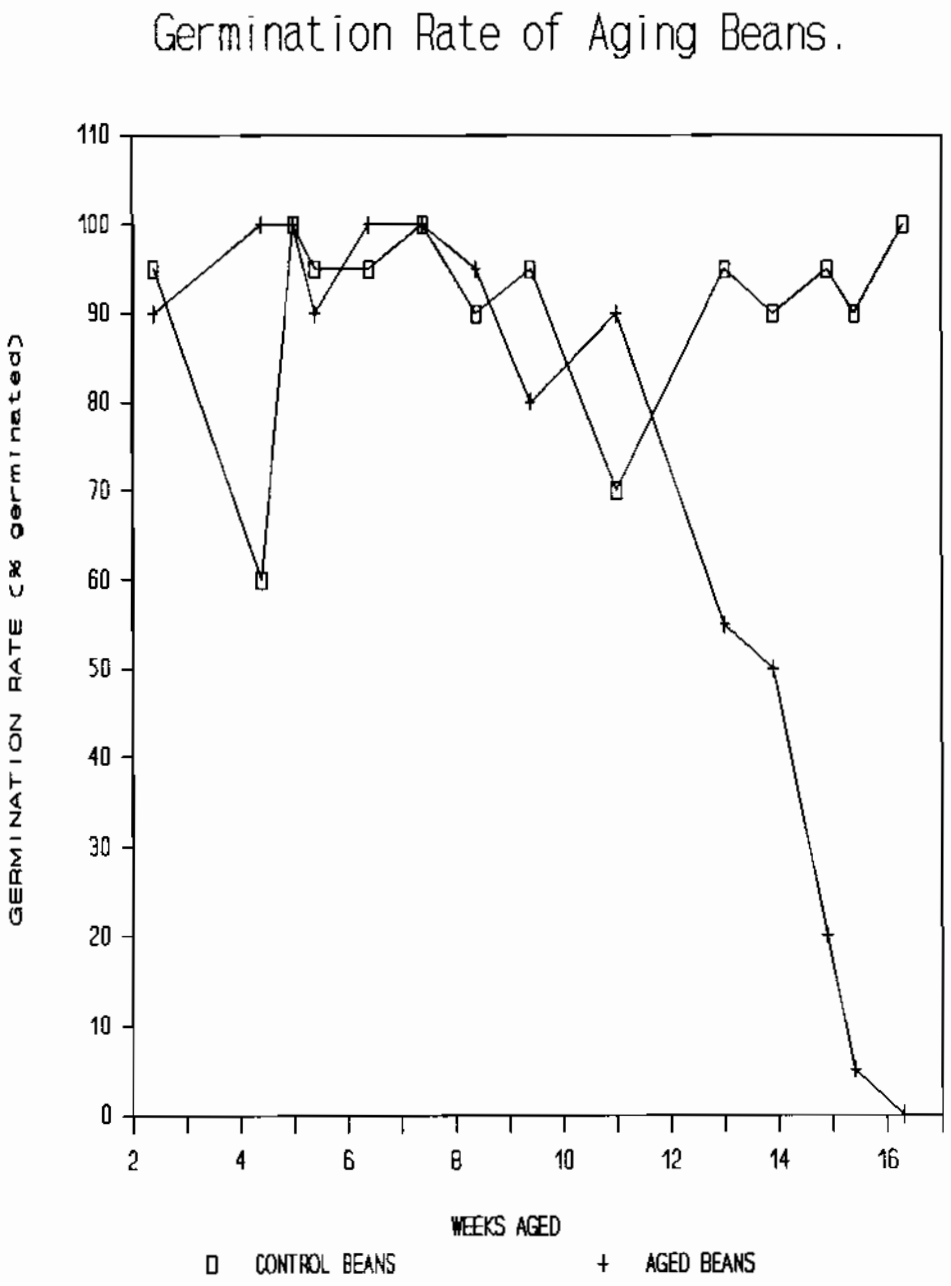

Figure 1. Germination rate of aged and control beans over the 16 weeks during which aged beans were stored at 36-38°C and 75% humidity.

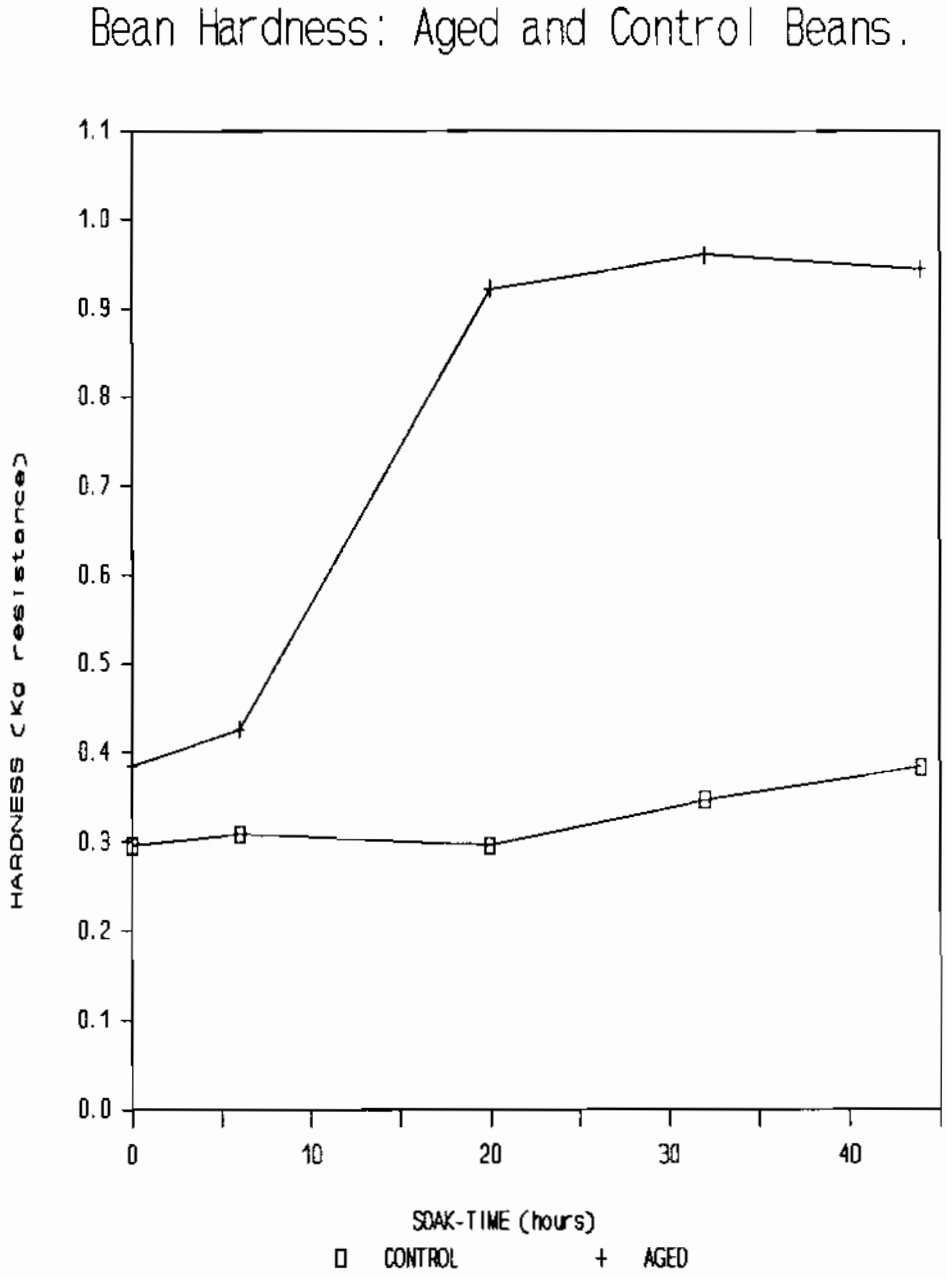

Figure 2. Hardness of aged and control beans which were soaked for 0, 6, 20, 32, and 44 hours.

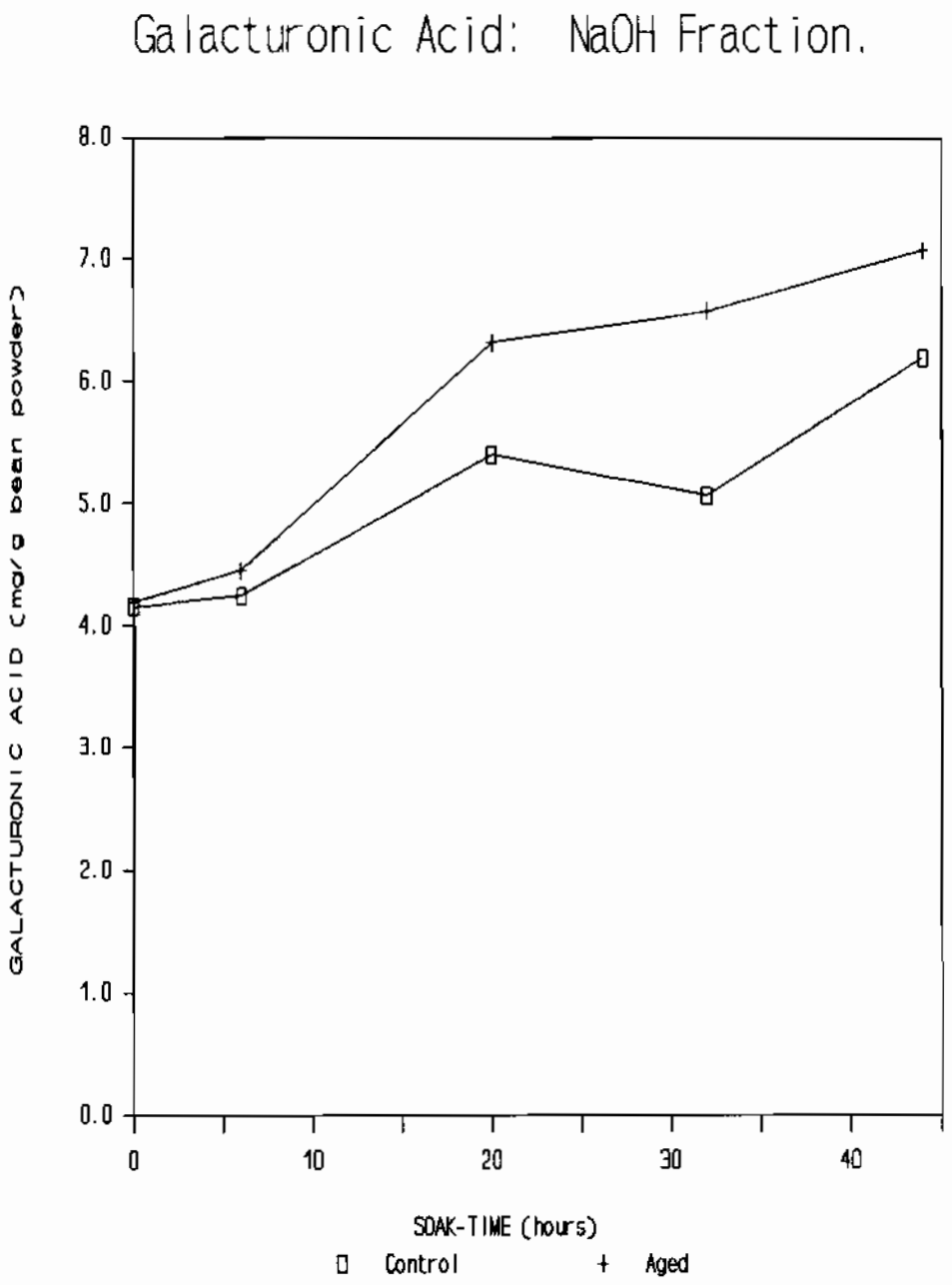

Figure 3. NaOH fraction galacturonic acid content of aged and control beans which were soaked 0, 6, 20, 32, and 44 hours.

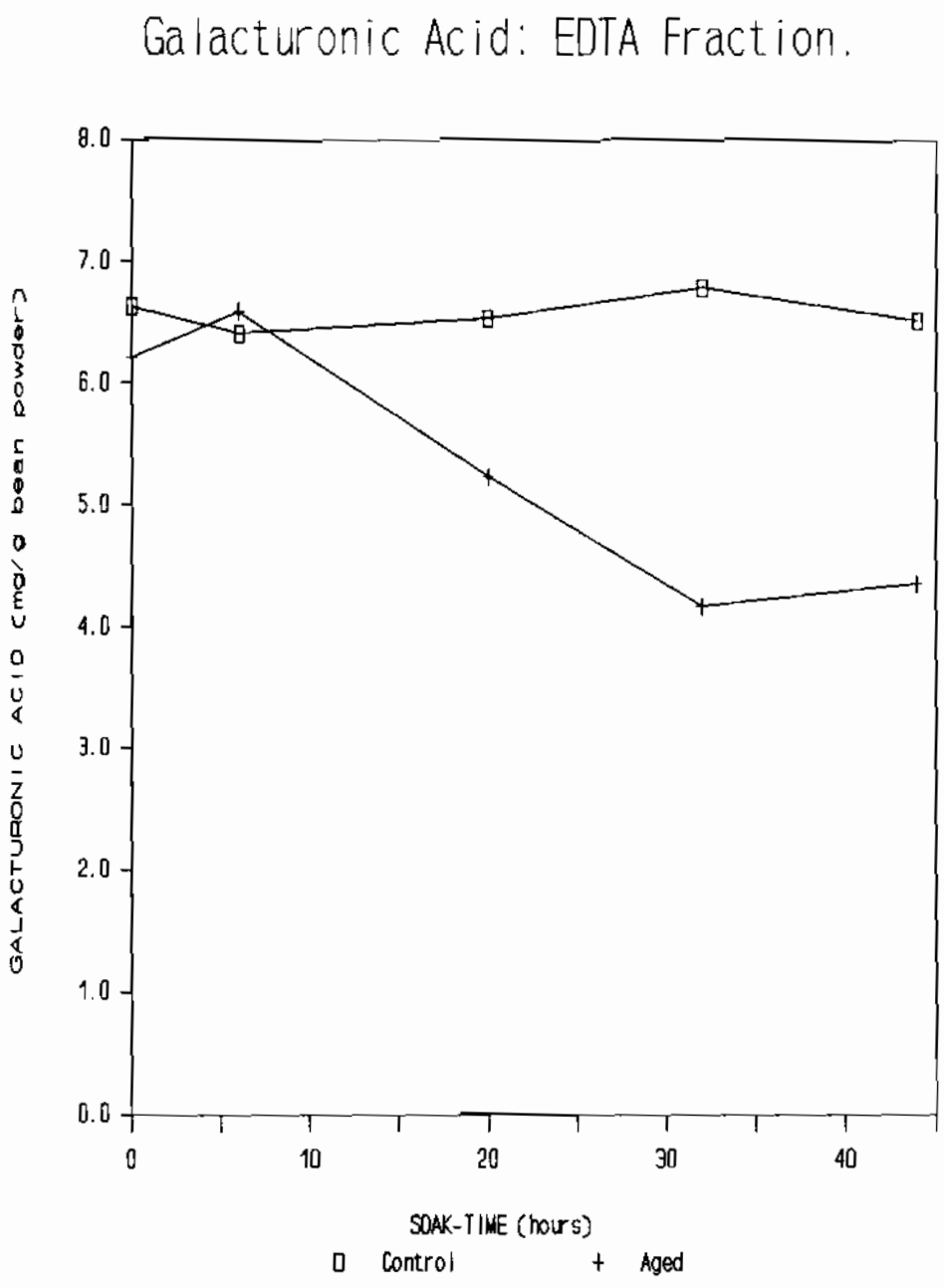

Figure 4. EDTA fraction galacturonic acid content of aged and control beans which were soaked 0, 6, 20, 32, and 44 hours.

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APPENDIX A: MICROSTRUCTURE STUDY

TITLE

THE EFFECT OF SOAKING ON MICROSTRUCTURE AND COOKABILITY OF GREAT NORTHERN BEANS (Phaseolus vulgaris).

ABSTRACT

The possible relationship between microstructure and increased bean hardness is investigated. Great Northern beans (Phaseolus vulgaris), were soaked in water for 0 to 44 hours and their hardness measured. Samples of beans soaked 0, 20, and 44 hours were used in a microscopy study to determine the effect of soaking on legume microstructure. In soaked beans, reductions in starch granule population and protein bodies were observed, as well as disintegration of endoplasmic reticulum cellular matrix. These factors may all affect bean softening by reducing mechanical stress on the cells from starch gelatinization, protein denaturation, and osmotic pressure.

INTRODUCTION

Dried legumes are used as a food source for many of the world's population, and are a major source of protein in some countries. Dried beans are generally prepared for consumption by soaking in water for 16-24 hours and cooked in fresh water with or without salt and other condiments (Anonymous, 1982). Processing not only improves the texture and flavor of the legumes, but is necessary to make the bean protein nutritionally available (Rockland and Jones, 1974).

One problem frequently encountered in preparing beans is the "hard-to-cook" phenomenon in which the legumes do not develop a desirable texture after normal processing. This defect is especially pronounced in beans stored at high temperatures and humidities or moisture contents. This phenomenon has been investigated quite extensively, but is still not well understood. Microscopy has been used to study this phenomenon. It has been used to determine relationships between texture and structure in the following: black beans during storage and water imbibition (Varriano-Marston and Jackson, 1981), lima beans during cooking (Rockland and Jones, 1974), cowpeas under different soaking and cooking conditions (Sefa-Dedeh et al., 1978), and several other varieties during water absorption and cooking (Sefa-Dedeh and Stanley, 1979). More recently, He et al. (1988) have developed a method for opening cells of heat processed legumes for microscope studies.

Jones and Boulter (1983) and Shomer et al. (1990) observed cell separation in soft beans, which did not occur in hard beans, and concluded that cell separation is necessary for beans to soften to an acceptable texture. These authors, and others (Rockland and Jones, 1974; Sefa-Dedeh and Stanley, 1979), have suggested that starch granules and protein matrix may play a role in softening of bean cotyledons, possibly through mechanical stress from starch gelatinization, protein denaturation, and cotyledon swelling. However, Vindola et al. (1986) hypothesized that cellular contents such as protein and starch could not participate in the hard-to-cook phenomenon because cells separate, rather than rupture, as beans soften.

There is evidence of changes in the protein and starch fractions of beans which develop the hard-to-cook defect. Protein bodies of beans stored at high temperatures and high moisture contents were unrecognizable in raw bean cotyledons observed with scanning electron microscopy (Dhahir, 1988). Sefa-Dedeh et al. (1979) observed the loss of some protein bodies from soaking in water. Hussain et al. (1989) found additional protein bands on electrophoregrams of hard beans as compared to soft beans, suggesting there may be some association and/or denaturation of proteins during hardening. Hohlberg and Stanley (1987) noted an increase in low molecular weight proteins which accompanied a decrease in high molecular weight proteins, suggesting protein breakdown during storage. Decreased starch and protein solubility was observed by

Hentges (1988). Paredes-Lopez et al. (1989) reported an increase in the damaged starch content and paste viscosity of hard-to-cook beans.

The objective of this study was to determine the effect of soaking on microstructure and hardness of dried legumes. Microstructural components observed included: starch granules, protein bodies, and the cellular endoplasmic reticulum matrix.

MATERIALS & METHODS

Legume Source

Great Northern beans (Phaseolus vulgaris), harvested in the fall of 1985, were used in the investigation. Since 1985 and until the time of this study, the beans were stored at 4-6°C.

Soaking Treatments

One hundred gram samples of beans were soaked for 0, 6, 20, 32, and 44 hours in a continuous flow of cold (8-12°C) tap water (40-65 ppm calcium). After soaking, beans were processed for subsequent texture analysis. Prior to processing, samples were taken and dried for microscopy studies.

Texture Analysis

Beans were placed in a 303 can and covered with a hot (80-85°C) 30-35% sucrose solution. The cans were then sealed and processed in a retort at 121°C for 25 minutes. Following processing, the samples were stored at ambient temperature for 27-28 days. At the end of this storage period, a subsample of 20-40 beans was taken from each can and hardness measured. Hardness was measured on a Voland-Stevens LFRA Texture Analyzer with a T-shaped plunger (3 mm diameter, arms 1 cm each) set to travel 6 mm at 0.5 mm/sec with 20 millivolt full scale sensitivity. Full scale deflection was equivalent to a 1 kilogram (Kg) load.

SEM Sample Preparation

Samples from the 0, 20 and 44 hour soak-times were used in the microscopy study. Samples were placed in a VirTis Freezemobile 12 freeze dryer with a vacuum of 12 milliTorr and condenser temperature of -65°C for a minimum of 44 hours. Plate temperature was 24°C. Once dried, beans were fractured, mounted on gummed stubs, sputter coated with gold, and micrographs were taken with a JEOL 840A Scanning Electron Microscope.

RESULTS

Hardness.

Figure 5 contains a plot of mean hardness for each soak-time and the best fit line. Each mean represents 5-7 trials with a subsample of 20-40 beans from each trial for a total of 140-200 beans for each mean. A regression performed on the means showed a positive linear relationship between soak-time and hardness.

Micrographs were taken on beans from trials in which a subsample of 20 beans was used for hardness determination. The mean hardness values for these trials were 296, 295, and 383 grams resistance for beans soaked 0, 20, and 44 hours respectively. These hardness values were not statistically different ($p > 0.05$) for 0 and 20 hour soak-times; however, a soak-time of 44 hours had a hardness value significantly ($p < 0.05$) greater than 0 and 20 hours.

Microstructure.

The microstructure of beans with different soak-times are seen in Figure 6. Differences seen between cells soaked for different times included: 1) decreased starch granule population (not shown) with fissure formation in existing starch granules of beans soaked 44 hours (Figure 6c); 2) decreased protein body population (Figure 6a, 6b); and 3) disintegration of the endoplasmic reticulum of the cellular matrix (Figure 6b, 6c).

DISCUSSION

As beans get harder with increased soaking time (Figure 5), starch granules and protein bodies decrease in number and cellular membrane material breaks down, as evidenced by the disintegration of the cellular endoplasmic reticulum (Figure 6).

Starch granules may have been absorbed, and fissures formed, due to starch utilization by the beans during soaking. Small sprouts, indicating germination, were seen in soaked beans. Future studies are needed to quantitate the number of the starch granules for an empirical comparison.

The endoplasmic reticulum of the matrix surrounding the starch granules was markedly different for each treatment. The matrix of the unsoaked beans (Figure 6a) resembled a full honeycomb, the 20 hour sample (Figure 6b) resembled an empty honeycomb, and the 44 hour (Figure 6c) sample matrix resembled flaked mica. It is probable that the "full honeycomb" compartments seen in the control beans (Figure 6a) were protein bodies. These were absent in the soaked samples. These changes indicated a general breakdown of the cellular structure, and may also decrease the nutritional quality of the bean.

Breakdown of cellular membranes in hard beans has been demonstrated by the observations of many researchers. Varriano-Marston and Jackson (1981) reported disintegration of organelles in beans stored at high temperatures and relative

humidities. Other investigators have observed evidence of cellular membrane breakdown in hard-to-cook beans through increased electrolyte leakage (Duxbury, 1988; Jones and Boulter, 1983; Hentges, 1988; Varriano-Marston and deOmana, 1979; Jackson and Varriano-Marston, 1981; Hincks, 1987; Parrish and Leopold, 1978).

Bean softening has been associated with the break down of the middle lamella and the subsequent separation of individual cells (Jones and Boulter, 1983; Shomer et al., 1990). Other reports suggest that cell separation may be aided by mechanical stress imparted from the swelling and gelatinization of the starch granules, along with protein denaturation during cooking (Rockland and Jones, 1974; Sefa-Dedeh and Stanley, 1979; Narasimha et al., 1989, Sefa-Dedeh et al., 1978; Jones and Boulter, 1983). The ability of beans to absorb water has also been correlated with softening (Sefa-Dedeh and Stanley, 1979). Osmotic pressure present when cellular membranes are intact may affect the beans ability to absorb water and also exert mechanical pressure to encourage cellular separation.

Findings in this study suggest two possible explanation for increase in bean hardness with increased soaking time. First, the decrease in protein body and starch granule population could lead to a decrease in mechanical stress and water absorption from starch gelatinization and protein denaturation. Secondly, disintegration of the endoplasmic

reticulum, could signify a general breakdown of the cellular membrane, resulting in a loss of osmotic pressure on the cell walls and decreased uptake of water due to the expansion of the cell wall.

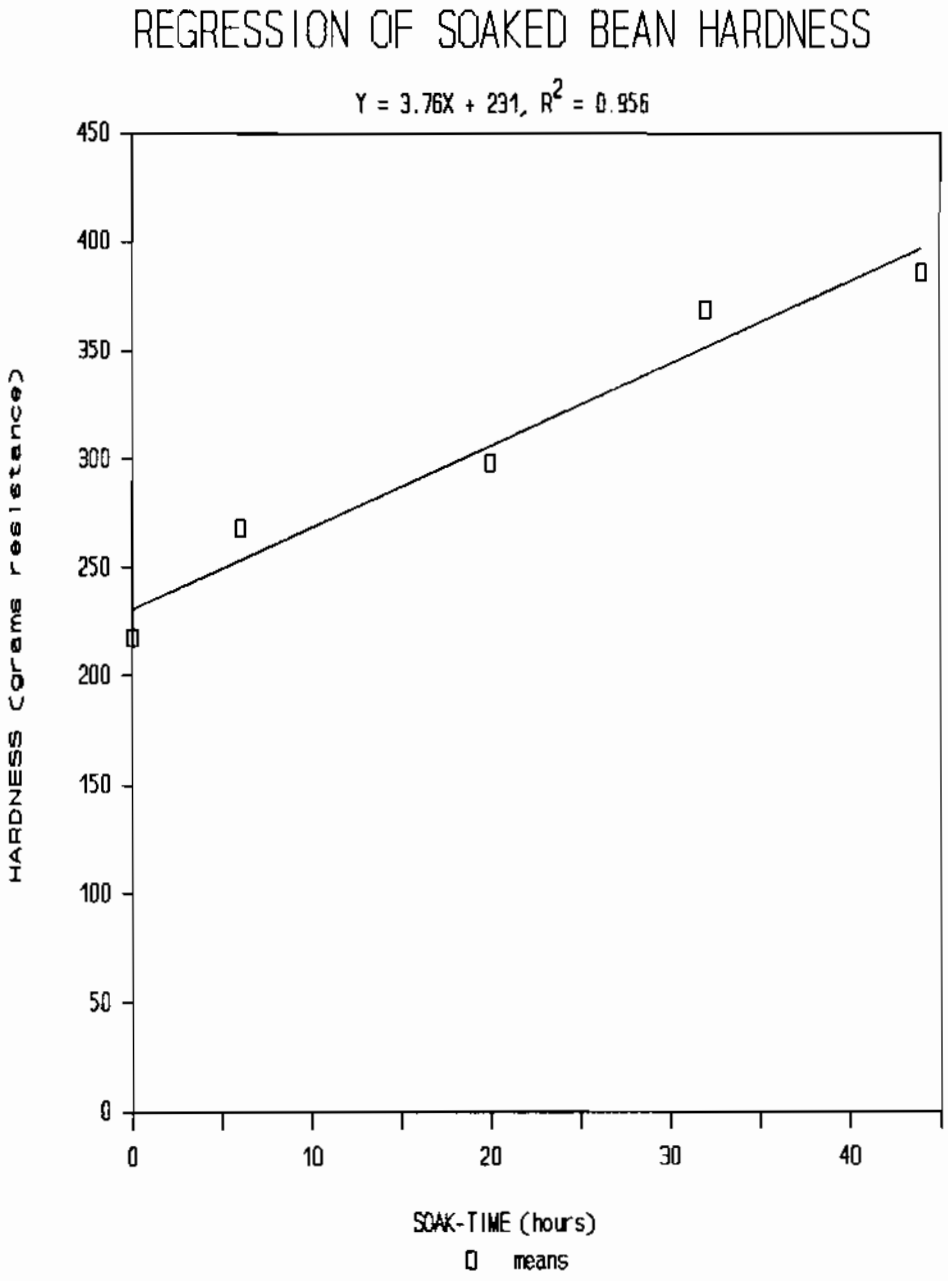

Figure 5. Mean bean hardness of control beans versus soak-time and best fit line.

Figure 6. Scanning electron micrograph of bean cotyledon cells (bar=10 μm, S-starch granule, P-protein body, arrow - fissure): (A) bean soaked 0 hours; (B) bean soaked 20 hours; (C) bean soaked 44 hours.

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APPENDIX B: HARDNESS RAW DATA

T = Treatment (1 = Control, 2 = Aged)
R = Replication Number
S = Soak-time (hours)
HARD = Hardness (grams)

T	R	S	HARD	T	R	S	HARD	T	R	S	HARD	T	R	S	HARD
1	1	0	170	1	1	0	230	1	1	0	110	1	1	0	130
1	1	0	300	1	1	0	310	1	1	0	110	1	1	0	570
1	1	0	280	1	1	0	300	1	1	0	370	1	1	0	280
1	1	0	230	1	1	0	370	1	1	0	250	1	1	0	420
1	1	0	410	1	1	0	250	1	1	0	230	1	1	0	230
1	1	6	300	1	1	6	320	1	1	6	330	1	1	6	620
1	1	6	240	1	1	6	450	1	1	6	180	1	1	6	330
1	1	6	240	1	1	6	320	1	1	6	540	1	1	6	300
1	1	6	180	1	1	6	310	1	1	6	160	1	1	6	120
1	1	6	250	1	1	6	230	1	1	6	250	1	1	6	240
1	1	20	460	1	1	20	280	1	1	20	220	1	1	20	390
1	1	20	430	1	1	20	310	1	1	20	340	1	1	20	250
1	1	20	260	1	1	20	110	1	1	20	290	1	1	20	250
1	1	20	330	1	1	20	350	1	1	20	350	1	1	20	250
1	1	20	420	1	1	20	230	1	1	20	240	1	1	20	480
1	1	32	380	1	1	32	230	1	1	32	530	1	1	32	420
1	1	32	330	1	1	32	650	1	1	32	290	1	1	32	90
1	1	32	340	1	1	32	180	1	1	32	530	1	1	32	310
1	1	32	310	1	1	32	370	1	1	32	220	1	1	32	460
1	1	32	320	1	1	32	430	1	1	32	200	1	1	32	340
1	1	44	530	1	1	44	250	1	1	44	340	1	1	44	470
1	1	44	410	1	1	44	320	1	1	44	390	1	1	44	300
1	1	44	520	1	1	44	210	1	1	44	540	1	1	44	300
1	1	44	490	1	1	44	530	1	1	44	490	1	1	44	330
1	1	44	500	1	1	44	1000	1	1	44	290	1	1	44	440
1	1	20	320	1	1	20	330	1	1	20	510	1	1	20	300
1	1	20	250	1	1	20	410	1	1	20	210	1	1	20	280
1	1	20	230	1	1	20	210	1	1	20	280	1	1	20	150
1	1	20	390	1	1	20	240	1	1	20	360	1	1	20	230
1	1	20	250	1	1	20	140	1	1	20	270	1	1	20	370

T	R	S	HARD	T	R	S	HARD	T	R	S	HARD	T	R	S	HARD
1	1	32	250	1	1	32	550	1	1	32	520	1	1	32	350
1	1	32	340	1	1	32	330	1	1	32	150	1	1	32	260
1	1	32	320	1	1	32	160	1	1	32	240	1	1	32	260
1	1	32	340	1	1	32	480	1	1	32	280	1	1	32	400
1	1	32	460	1	1	32	410	1	1	32	370	1	1	32	290
1	1	44	430	1	1	44	260	1	1	44	360	1	1	44	220
1	1	44	530	1	1	44	430	1	1	44	250	1	1	44	340
1	1	44	350	1	1	44	390	1	1	44	430	1	1	44	310
1	1	44	570	1	1	44	290	1	1	44	300	1	1	44	330
1	1	44	260	1	1	44	350	1	1	44	460	1	1	44	220
1	2	0	250	1	2	0	160	1	2	0	570	1	2	0	130
1	2	0	300	1	2	0	240	1	2	0	210	1	2	0	200
1	2	0	260	1	2	0	300	1	2	0	350	1	2	0	290
1	2	0	330	1	2	0	530	1	2	0	360	1	2	0	270
1	2	0	240	1	2	0	350	1	2	0	480	1	2	0	540
1	2	44	250	1	2	44	250	1	2	44	490	1	2	44	410
1	2	44	800	1	2	44	210	1	2	44	400	1	2	44	270
1	2	44	350	1	2	44	210	1	2	44	570	1	2	44	390
1	2	44	440	1	2	44	270	1	2	44	260	1	2	44	420
1	2	44	390	1	2	44	500	1	2	44	150	1	2	44	420
1	2	44	180												
1	2	6	240	1	2	6	340	1	2	6	190	1	2	6	310
1	2	6	180	1	2	6	310	1	2	6	180	1	2	6	200
1	2	6	310	1	2	6	400	1	2	6	280	1	2	6	250
1	2	6	290	1	2	6	550	1	2	6	180	1	2	6	420
1	2	6	270	1	2	6	340	1	2	6	390	1	2	6	170
1	2	20	300	1	2	20	230	1	2	20	300	1	2	20	340
1	2	20	340	1	2	20	320	1	2	20	220	1	2	20	390
1	2	20	380	1	2	20	160	1	2	20	360	1	2	20	280
1	2	20	340	1	2	20	190	1	2	20	420	1	2	20	410
1	2	20	120	1	2	20	310	1	2	20	50	1	2	20	310
1	2	44	310	1	2	44	290	1	2	44	650	1	2	44	160
1	2	44	230	1	2	44	360	1	2	44	450	1	2	44	320
1	2	44	390	1	2	44	480	1	2	44	330	1	2	44	440
1	2	44	530	1	2	44	430	1	2	44	510	1	2	44	390
1	2	44	330	1	2	44	220	1	2	44	400	1	2	44	540
1	2	32	430	1	2	32	160	1	2	32	300	1	2	32	260
1	2	32	500	1	2	32	370	1	2	32	430	1	2	32	450
1	2	32	260	1	2	32	230	1	2	32	340	1	2	32	380
1	2	32	410	1	2	32	310	1	2	32	330	1	2	32	210
1	2	32	430	1	2	32	430	1	2	32	670	1	2	32	200

T	R	S	HARD
1	2	0	440
1	2	0	170
1	2	0	230
1	2	0	330
1	2	0	260
1	2	6	270
1	2	6	240
1	2	6	260
1	2	6	170
1	2	6	560
2	1	0	330
2	1	0	470
2	1	0	510
2	1	0	390
2	1	0	670
2	1	44	1000
2	1	44	1000
2	1	44	1000
2	1	44	1000
2	1	44	1000
2	1	32	970
2	1	32	1000
2	1	32	1000
2	1	32	1000
2	1	32	1000
2	1	20	1000
2	1	20	1000
2	1	20	1000
2	1	20	1000
2	1	20	1000
2	1	0	420
2	1	0	260
2	1	0	190
2	1	0	400
2	1	0	290
2	1	20	800
2	1	20	1000
2	1	20	570
2	1	20	870
2	1	20	1000

T	R	S	HARD
1	2	0	210
1	2	0	250
1	2	0	370
1	2	0	300
1	2	0	500
1	2	6	170
1	2	6	420
1	2	6	270
1	2	6	680
1	2	6	410
2	1	0	410
2	1	0	350
2	1	0	660
2	1	0	460
2	1	0	340
2	1	44	1000
2	1	44	1000
2	1	44	1000
2	1	44	1000
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2	1	20	800
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2	1	20	1000
2	1	20	1000
2	1	20	900
2	1	0	590
2	1	0	260
2	1	0	230
2	1	0	400
2	1	0	290
2	1	20	1000
2	1	20	1000
2	1	20	1000
2	1	20	560
2	1	20	1000

T	R	S	HARD
1	2	0	190
1	2	0	300
1	2	0	310
1	2	0	180
1	2	0	310
1	2	6	380
1	2	6	330
1	2	6	430
1	2	6	220
1	2	6	280
2	1	0	540
2	1	0	440
2	1	0	340
2	1	0	270
2	1	0	630
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2	1	44	1000
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2	1	44	1000
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2	1	32	1000
2	1	32	1000
2	1	20	1000
2	1	20	1000
2	1	20	1000
2	1	20	1000
2	1	20	1000
2	1	0	410
2	1	0	280
2	1	0	380
2	1	0	420
2	1	0	280
2	1	20	1000
2	1	20	1000
2	1	20	1000
2	1	20	950
2	1	20	1000

T	R	S	HARD
1	2	0	260
1	2	0	340
1	2	0	340
1	2	0	180
1	2	0	380
1	2	6	440
1	2	6	170
1	2	6	340
1	2	6	320
1	2	6	450
2	1	0	500
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2	1	0	370
2	1	0	520
2	1	0	520
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2	1	32	1000
2	1	32	1000
2	1	20	1000
2	1	20	980
2	1	20	1000
2	1	20	1000
2	1	20	1000
2	1	0	450
2	1	0	400
2	1	0	590
2	1	0	400
2	1	0	320
2	1	20	1000
2	1	20	760
2	1	20	1000
2	1	20	1000
2	1	20	920

T	R	S	HARD	T	R	S	HARD	T	R	S	HARD	T	R	S	HARD
2	1	6	660	2	1	6	500	2	1	6	800	2	1	6	390
2	1	6	640	2	1	6	550	2	1	6	650	2	1	6	320
2	1	6	410	2	1	6	360	2	1	6	720	2	1	6	840
2	1	6	550	2	1	6	750	2	1	6	610	2	1	6	470
2	1	6	640	2	1	6	370	2	1	6	910	2	1	6	240
2	1	6	350	2	1	6	270	2	1	6	320	2	1	6	720
2	1	6	700	2	1	6	270	2	1	6	280	2	1	6	260
2	1	6	790	2	1	6	440	2	1	6	660	2	1	6	380
2	1	6	140	2	1	6	220	2	1	6	290	2	1	6	340
2	1	6	240	2	1	6	980	2	1	6	680	2	1	6	590
2	2	32	1000	2	2	32	430	2	2	32	1000	2	2	32	1000
2	2	32	1000	2	2	32	1000	2	2	32	1000	2	2	32	950
2	2	32	660	2	2	32	1000	2	2	32	980	2	2	32	1000
2	2	32	1000	2	2	32	1000	2	2	32	1000	2	2	32	900
2	2	32	1000	2	2	32	940	2	2	32	1000	2	2	32	1000
2	2	44	1000	2	2	44	1000	2	2	44	1000	2	2	44	1000
2	2	44	1000	2	2	44	960	2	2	44	1000	2	2	44	1000
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2	2	44	940	2	2	44	330	2	2	44	1000	2	2	44	1000
2	2	44	1000	2	2	44	1000	2	2	44	600	2	2	44	1000
2	2	44	840	2	2	44	1000	2	2	44	790	2	2	44	860
2	2	44	800	2	2	44	600	2	2	44	1000	2	2	44	1000
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2	2	44	1000	2	2	44	880	2	2	44	850	2	2	44	1000
2	2	44	1000	2	2	44	780	2	2	44	1000	2	2	44	670
2	2	6	330	2	2	6	290	2	2	6	180	2	2	6	230
2	2	6	200	2	2	6	100	2	2	6	320	2	2	6	250
2	2	6	310	2	2	6	590	2	2	6	460	2	2	6	260
2	2	6	240	2	2	6	360	2	2	6	100	2	2	6	300
2	2	6	150	2	2	6	370	2	2	6	120	2	2	6	120
2	2	20	780	2	2	20	310	2	2	20	640	2	2	20	790
2	2	20	830	2	2	20	990	2	2	20	730	2	2	20	970
2	2	20	960	2	2	20	1000	2	2	20	850	2	2	20	970
2	2	20	930	2	2	20	700	2	2	20	780	2	2	20	1000
2	2	20	1000	2	2	20	1000	2	2	20	600	2	2	20	1000
2	2	20	700	2	2	20	1000	2	2	20	1000	2	2	20	970
2	2	20	1000	2	2	20	730	2	2	20	1000	2	2	20	1000
2	2	20	1000	2	2	20	1000	2	2	20	1000	2	2	20	1000
2	2	20	1000	2	2	20	1000	2	2	20	610	2	2	20	800
2	2	20	1000	2	2	20	1000	2	2	20	1000	2	2	20	1000

T	R	S	HARD	T	R	S	HARD	T	R	S	HARD	T	R	S	HARD
2	2	0	580	2	2	0	200	2	2	0	420	2	2	0	510
2	2	0	360	2	2	0	260	2	2	0	400	2	2	0	300
2	2	0	220	2	2	0	500	2	2	0	200	2	2	0	330
2	2	0	390	2	2	0	380	2	2	0	470	2	2	0	100
2	2	0	270	2	2	0	190	2	2	0	380	2	2	0	480
2	2	32	1000	2	2	32	810	2	2	32	1000	2	2	32	870
2	2	32	1000	2	2	32	1000	2	2	32	1000	2	2	32	1000
2	2	32	1000	2	2	32	1000	2	2	32	1000	2	2	32	1000
2	2	32	1000	2	2	32	870	2	2	32	1000	2	2	32	1000
2	2	32	1000	2	2	32	1000	2	2	32	1000	2	2	32	600

APPENDIX C: GALACTURONIC ACID DATA

OBS Observation Number
C/A Control=1 Aged=2
R Replication Number (1 or 2)
ST Soak-time (hours)
H_2O Water Soluble Pectin or high methoxy pectin
EDTA EDTA Soluble Pectin or very low methoxy pectin
NaOH NaOH Soluble Pectin or Protopectin

Unless otherwise indicated, values are in μg galacturonic acid/ g bean flour.

OBS	C/A	R	ST	H_2O	EDTA	NaOH
1	1	1	0	6225	5976	3613
2	1	1	6	5189	6732	4316
3	1	1	20	6088	7082	4407
4	1	1	20	5974	*	5056
5	1	1	32	5469	6180	4679
6	1	1	32	4865	6809	5315
7	1	1	44	6030	6932	5930
8	1	1	44	6154	*	5275
9	1	2	0	4444	6834	4252
10	1	2	0	2857	7093	4577
11	1	2	6	4892	6541	4364
12	1	2	6	4919	5957	4029
13	1	2	20	3831	5991	6728
14	1	2	32	3324	7351	5188
15	1	2	44	4840	7680	6205
16	1	2	44	3424	4929	7347
17	2	1	0	4458	6111	4150
18	2	1	0	4696	5964	3396
19	2	1	6	5266	5611	*
20	2	1	6	4621	6495	3691
21	2	1	20	5259	4968	4826
22	2	1	20	4911	5334	5623
23	2	1	32	4193	4649	5707
24	2	1	44	4416	3313	5560
25	2	2	0	4258	6556	5005
26	2	2	6	3072	7680	5198
27	2	2	20	4947	5535	7547
28	2	2	20	3441	5089	7268
29	2	2	32	4865	4736	7913
30	2	2	32	4576	3116	6088
31	2	2	44	2960	4760	7911
32	2	2	44	4047	4989	7718

APPENDIX D: METHANOL DATA

OBS Observation Number
C/A Control=1 Aged=2
R Replication Number (1 or 2)
ST Soak-time (hours)
H_2O Water Soluble Pectin or high methoxy pectin
EDTA EDTA Soluble Pectin or very low methoxy pectin
NaOH NaOH Soluble Pectin or Protopectin

Unless otherwise indicated, values are in μg methanol/ g bean flour.

OBS	C/A	R	ST	H_2O	EDTA	NaOH
1	1	1	0	311	45	312
2	1	1	6	254	53	415
3	1	1	20	355	53	346
4	1	1	20	431	*	270
5	1	1	32	328	44	283
6	1	1	32	336	54	285
7	1	1	44	394	43	297
8	1	1	44	339	*	372
9	1	2	0	282	43	330
10	1	2	0	309	40	359
11	1	2	6	316	30	301
12	1	2	6	271	34	331
13	1	2	20	277	59	304
14	1	2	32	260	46	240
15	1	2	44	305	44	180
16	1	2	44	237	35	371
17	2	1	0	*	36	359
18	2	1	0	227	24	376
19	2	1	6	276	30	385
20	2	1	6	280	33	370
21	2	1	20	*	40	256
22	2	1	20	278	49	436
23	2	1	32	273	42	442
24	2	1	44	*	45	489
25	2	2	0	180	53	341
26	2	2	6	176	56	268
27	2	2	20	236	75	346
28	2	2	20	152	72	354
29	2	2	32	245	74	287
30	2	2	32	194	*	254
31	2	2	44	188	67	253
32	2	2	44	203	79	345

APPENDIX E: PERCENT METHYLATION DATA

OBS Observation Number
C/A Control=1 Aged=2
R Replication Number (1 or 2)
ST Soak-time (hours)
H_2O Water Soluble Pectin or high methoxy pectin
EDTA EDTA Soluble Pectin or very low methoxy pectin
NaOH NaOH Soluble Pectin or Protopectin

Unless otherwise indicated, values are the percent methylation, moles methanol/moles galacturonic acid X 100.

OBS	C/A	R	ST	H_2O	EDTA	NaOH
1	1	1	0	20	3	35
2	1	1	6	20	3	39
3	1	1	20	24	3	32
4	1	1	20	29	*	22
5	1	1	32	24	3	25
6	1	1	32	28	3	22
7	1	1	44	27	3	20
8	1	1	44	22	*	29
9	1	2	0	26	3	32
10	1	2	0	44	2	32
11	1	2	6	26	2	28
12	1	2	6	22	2	33
13	1	2	20	29	4	18
14	1	2	32	32	3	19
15	1	2	44	26	2	12
16	1	2	44	28	3	20
17	2	1	0	*	2	35
18	2	1	0	20	2	45
19	2	1	6	21	2	*
20	2	1	6	25	2	41
21	2	1	20	*	3	22
22	2	1	20	23	4	31
23	2	1	32	26	4	31
24	2	1	44	5	*	36
25	2	2	0	17	3	28
26	2	2	6	23	3	21
27	2	2	20	19	5	19
28	2	2	20	18	6	20
29	2	2	32	20	6	15
30	2	2	32	17	*	17
31	2	2	44	26	6	13
32	2	2	44	20	6	18

THE EFFECT OF SOAKING AND PECTIN COMPOSITION ON THE HARDNESS OF DRIED LEGUMES (*Phaseolus vulgaris*)

Holly Curtis

Department of Food Science and Nutrition

M. S. Degree, December 1991

ABSTRACT

Length of soaking period and its effect on pectin composition and hardness of "aged" and control beans was investigated. Effect of soaking on microstructure of control beans was also investigated. Aged beans exhibited hardness values greater than control beans, with dramatic increases in hardness for aged beans soaked 20 or more hours. This suggests that the hardening mechanism is primed by aging, but requires soaking for extended periods of time for its full expression. Decreases in EDTA soluble very low methoxy pectin exhibited a temporal correlation to hardness development. Pectin fractions from both aged and control beans were found to contain low-methoxy pectin. Observed microstructural changes of soaked control beans included: reductions in protein body and starch granule populations as well as disintegration of the endoplasmic reticulum cellular matrix.

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