Mechanism of Pectin Changes during Soaking and Heating As Related to Hard-To-Cook Defect in Cowpeas

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Cowpea (*Vigna unguiculata*) seeds were aged at 30 °C and 64% relative humidity for 6, 12, and 18 months. Pectin loss in control seeds (-18 °C and ambient humidity storage) was very low after 6 h of soaking and very high after 1 h of cooking. As aging progressed, pectin loss increased slightly after soaking but decreased significantly after cooking. The pH of soaking or cooking liquors also decreased, presumably resulting from decreased tissue pH reported earlier. With heating temperature, pectin loss was lower below 60 °C, higher at 85 °C, and maximum at 100 °C. These changes in pectin loss from cowpeas were similar to those in viscosity of pectin solution as affected by medium pH and temperature in a model study reported earlier, suggesting that pectin β-eliminative reaction occurs during heating of cowpeas. With aging time and heating temperature there was a negative correlation ($r = -0.926$) between seed texture and pectin loss during soaking or heating. Soaking in $CaCl_2$ or an acidic buffer also caused increased hardness and decreased pectin loss. However, with cooking time, no apparent relationship existed between the two. Overall results suggest that the hard-to-cook defect is caused in part by reduced pectin β-degradation during cooking, which apparently results from decreased tissue pH during aging.

INTRODUCTION

Legumes have been a primary source of dietary protein since ancient times, but there are still several constraints limiting their consumption. One of the major constraints is the storage-induced textural defect, commonly known as the hard-to-cook (HTC) phenomenon. Seeds with the HTC defect are resistant to softening during normal cooking. Therefore, the HTC phenomenon causes increased fuel consumption and decreased nutrition.

To investigate the mechanism of the HTC phenomenon, many investigators have focused on pectin changes during soaking or cooking of legume seeds and their relationship to the HTC defect (Kon, 1968; Jones and Boulter, 1983; Moscoso et al., 1984; El-Tabey Shehata et al., 1985; Hentges et al., 1991). Although conflicting results were reported among these studies because of inherent errors and variations in pectin extraction and analysis, there is little disagreement regarding the role of cell wall pectin in legume hardening. The explanation of pectin involvement has been based solely on a mechanism postulated by Mattson (1946). It is held that during storage, soaking, or cooking, pectin exchanges monovalent with divalent cations released from phytates, leading to insolubilization of pectin and thus strengthening of the cell wall.

However, pectin is known to be extremely sensitive to elevated temperature in a near-neutral medium, and under such conditions, the pectin degrades to lower molecular weight products via breakage of glycosidic bonds adjacent to carboxymethyl groups. This process is commonly known as the β-elimination reaction (Albersheim, 1959; Albersheim et al., 1960). Although this reaction has been associated with heat-related softening of many fruits and vegetables (Doesburg, 1961; Hughes et al., 1975a,b; Van Buren et al., 1990; Van Buren and Pitifer, 1992; Walter et al., 1992), its effect on legume hardening has not been reported. Therefore, the objective of this study was to investigate the mechanism of pectin changes in cowpeas during soaking/heating and the relationship of the changes to the HTC defect. Attempts were made to overcome some common problems associated with the study of pectin; pectin was monitored via loss into soaking or heating liquors instead of direct extraction from seed tissues and was analyzed according to a method which minimizes interference of neutral sugars (McFeeters and Armstrong, 1984).

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MATERIALS AND METHODS

Chemicals. D-Galacturonic acid was a product of Sigma Chemical Co. 3,5-dimethylphenol was purchased from Eastman Kodak Co. Deionized water was used throughout the study.

Cowpea Storage. Cowpeas (*Vigna unguiculata* cv. California Blackeye No. 5, 11.4% moisture) were obtained from Kerman Warehouse (Kerman, CA) and aged at 30 °C/64% relative humidity (RH) for 6, 12, and 18 months. Seeds stored at -18 °C and ambient humidity served as the control. Detailed procedures were reported elsewhere (Liu et al., 1992), and duplicate samples were prepared.

Soaking and Heating. Dry seeds (20 g) were wrapped in cheesecloth and soaked in 100 mL of water at room temperature for 6 h. Seeds were drained and soaking liquors saved. Samples were then heated on a hot plate in a 250-mL beaker with 100 mL of fresh water preheated to a selected temperature (60, 85, or 100 °C) for 60 min. For 100 °C, heating time was varied (30, 60, or 90 min). Heating was terminated by cooling the beaker in an ice bath. Additional soaking at room temperature served as a control against heating. Heated seeds were drained and cooled heating liquors saved. The volume of soaking or heating liquors was brought to 100 mL with water before measurements of pH and pectin content.

Soaking in $CaCl_2$ Solution. Seeds were soaked first in water for 3 h and then in 50 mM $CaCl_2$ solution for 3 h at room temperature. Samples were washed with water before cooking.

Seed Texture. Following soaking and heating, seeds were drained and measured for texture with an Instron universal testing instrument (Model 1122, Instron, Inc., Canton, MA). Hardness

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was expressed as newton force per gram of seeds (N/g). Details are given in a previous study (Liu et al., 1993).

Pectin Loss. Soaking or heating liquors were centrifuged at 20000*g* for 15 min to remove any cell debris. A portion of the supernatant (2–8 mL) was pipetted into another 50-mL centrifuge tube. One hundred percent ethyl alcohol was added to give a final concentration of 80% alcohol. The solvent was stored at 4 °C for 48 h before centrifugation at 20000*g* for 15 min. The precipitate was dissolved in 1 mL of water for 2 h with the aid of 2 drops of 1 N NaOH and occasional stirring.

This partially purified fraction was analyzed for pectin content using a modified method based on that of McFeeters and Armstrong (1984) with D-galacturonic acid as a standard. The detailed procedure is as follows. To the dissolved fraction was added 9 mL of cold 78% sulfuric acid, 4.5 mL at a time. The tube was vortexed and chilled after each addition, and then sonicated for 3 min, heated at 50 °C for 10 min, and cooled in an ice bath. A 1-mL aliquot was mixed with 2 mL of cold 6 N NaOH.

For color reaction, a 0.5-mL aliquot was added to a test tube and mixed with 0.5 mL of 2% NaCl solution. This was followed by addition of 4.0 mL of cold concentrated sulfuric acid. The tube was vortexed, heated in a 70 °C water bath for 10 min, and cooled in tap water for 10 min. 3,5-Dimethylphenol (0.1% in glacial acetic acid; 0.1 mL) was added and vortexed. After 15 min (no longer than 60 min) at room temperature, the absorption was read at 400 and 450 nm. The absorbance difference between the two wavelengths was used to calculate the galacturonic acid content. Pectin loss was expressed as milligrams of galacturonic acid per gram of dry seeds.

Statistics. Data were first subjected to analysis of variance (ANOVA). If significant differences existed among means of treatments, multiple confidence intervals were then determined.

RESULTS AND DISCUSSION

1. Method of Pectin Purification and Analysis. Using the slightly modified procedure of McFeeters and Armstrong (1984) for assaying galacturonic acid, neutral sugar interference was found to be minimal. This conclusion was based on two observations: (1) Addition of amylase into soaking or cooking liquors before alcohol precipitation did not significantly affect the final content of galacturonic acid. (2) Addition of glucose during the assay resulted in little change in the color difference between A_{450} and A_{400} as long as the concentration of glucose did not exceed about 1 M.

Pectin loss from kidney beans during soaking and cooking has been studied previously via measurement of the residual pectin in the bean tissue (Moscoso et al., 1984). Compared with this traditional practice, our new approach not only reduced sugar interference but also bypassed tedious extractions and thereby eliminated large variations associated with the extractions. The coefficient of variation for total pectin estimates in the soaking or cooking liquors was less than 5%.

2. Effect of Aging. *Texture.* Aging, that is, storage at 30 °C/64% RH, exerted little effect on the texture of raw soaked seeds but a significant effect ($p \leq 0.01$) on that of cooked seeds (Figure 1A). After 60 min of cooking, control seeds (−18 °C storage), which had hardness similar to that of freshly harvested seeds (data not shown), became softest. As aging progressed, the textural difference between cooked control and cooked aged seeds enlarged. Therefore, the HTC defect should refer to seed cooking quality rather than a physical state of raw seeds.

Pectin Loss. As shown in Figure 1B, after 6 h of soaking, little pectin was lost from control seeds (0 time aging). As aging progressed, there was a slight yet significant increase in pectin loss, although the rate of the increase was far lower than that of electrolyte leakage reported earlier (Jones and Boulter, 1983). In contrast to 6 h of soaking,

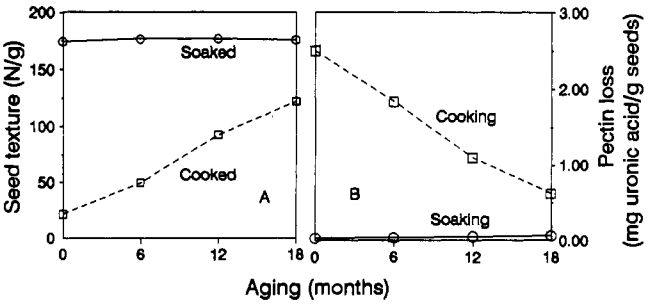

Figure 1. Effect of aging (storage at 30 °C and 64% relative humidity) on cowpea texture (A) and pectin loss (B) after soaking or cooking. Soaking time was 6 h, and cooking time was 1 h.

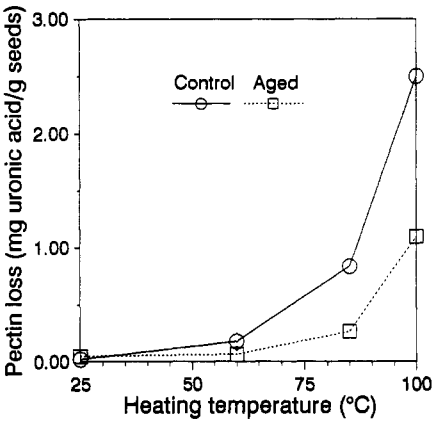

Figure 2. Effect of heating temperature on pectin loss from control and aged cowpea seeds. Heating time was 1 h. Control: cowpeas were stored at −18 °C and ambient humidity. Aged: cowpeas were stored at 30 °C and 64% relative humidity for 12 months.

60 min of cooking caused the greatest loss of pectin from control seeds, and this loss decreased sharply with aging time.

3. Effect of Heating Temperature. The effect of heating temperature on texture and pectin loss at a fixed heating time (60 min) was also studied. Comparison was made between control seeds and those stored at 30 °C/64% RH for 12 months (aged).

Texture. Results of textural change in cowpeas with heating temperature were reported elsewhere (Liu et al., 1993). Briefly, when heating temperature was below 60 °C, there was a slight change in texture of both control and aged seeds. At 85 °C, control seeds softened significantly, while aged seeds firmed slightly. The most dramatic softening of both control and aged seeds was with 100 °C cooking. At any temperature, control seeds were softer than aged ones.

Pectin Loss. From 25 to 60 °C, there was a slight increase of pectin loss into heating liquors (Figure 2). A dramatic increase in pectin loss was observed at 85 °C, but the most dramatic loss occurred at 100 °C. Control and aged seeds showed similar temperature-dependent patterns, but the former exhibited higher loss. As heating temperature increased, the difference between the two enlarged. Literature dealing quantitatively with the effect of heating temperature on pectin loss from legume seeds is lacking, but there is a study of the effect of temperature on pectin extraction from citrus peel (El-Nawawi and Shehata, 1988).

When the data of Figures 1 and 2 and of Liu et al. (1993) were replotted as shown in Figure 3, we found that there was a negative correlation ($r = -0.926$) between texture and pectin loss during soaking/heating with respect to aging and heating temperature. This finding clearly

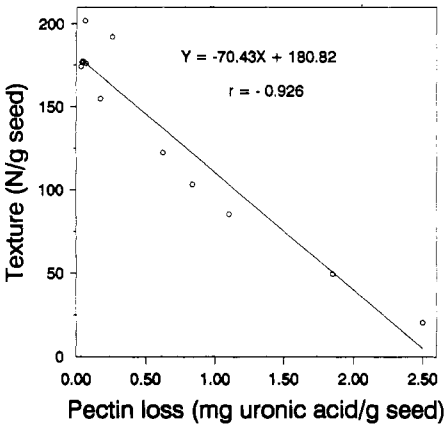

Figure 3. Correlation between seed texture and pectin loss from cowpeas after soaking or heating. Data are from Figures 1 and 2 and Liu et al. (1993).

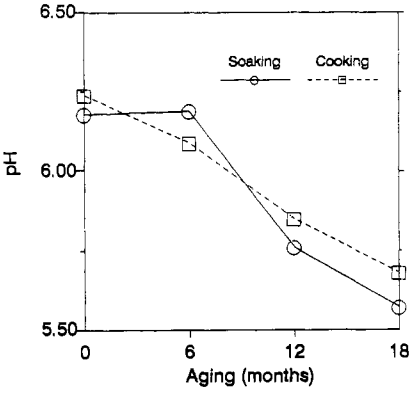

Figure 4. Effect of aging (storage at 30 °C and 64% relative humidity) on pH of soaking or cooking liquors of cowpeas. Soaking time was 6 h, and cooking time was 1 h.

indicates an involvement of pectin in the HTC defect of legume seeds and is consistent with those of previous studies. Our question is whether loss of pectin during-soaking/heating results from both β-eliminative degradation and solubilization rather than from solubilization alone. If pectin loss comes solely from solubilization, as explained by previous investigators (Kon, 1968; Jones and Boulter, 1983; Moscoso et al., 1984; El-Tabey Shehata et al., 1985; Hentges et al., 1991), we would expect a less dramatic effect of temperature. We would also expect more pectin loss from control seeds than from aged seeds at the soaking stage. The present study showed results opposite to our expectations.

An alternative explanation of pectin involvement would be its β-eliminative reaction during heating. This is evidenced by the similar changing pattern between pectin loss from cowpeas as affected by aging and heating temperature (Figures 1B and 2) and viscosity of pectin solution as affected by buffer pH and temperature in the model study of Albersheim (1959). Furthermore, in the previous study (Liu et al., 1992), we showed that control cowpea seeds have a tissue pH of 6.64. As aging (storage at 30 °C/64% RH) progresses to 18 months, the tissue pH decreases to 5.57. In the present study, we also observed that the pHs of both soaking and cooking liquors decreased significantly ($p \leq 0.01$) with aging (Figure 4). Apparently, these decreases result from the decreased tissue pH observed earlier. The low value of soaking liquor pH at 0 aging time might be due to the slight acidity of the deionized water and low leakage of the control seeds.

We believe that the low pH of aged seeds is a cause of both reduced pectin loss and increased seed hardness (HTC

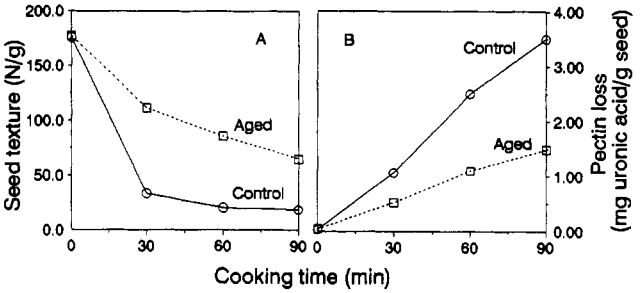

Figure 5. Effect of heating time at 100 °C (cooking) on texture (A) and pectin loss (B) of control and aged cowpea seeds. Control: cowpeas were stored at −18 °C and ambient humidity. Aged: cowpeas were stored at 30 °C and 64% relative humidity for 12 months.

defect). During soaking, the temperature was ambient, which is unfavorable for β-elimination regardless of medium pH. Thus, little pectin was lost and seeds were very hard. However, during heating, particularly at a high temperature, pectin β-eliminative reaction mainly depends on medium pH. Since their tissue pH is nearest to neutral, control seeds exhibited the highest pectin loss and the softest texture among seeds aged for four different times. To confirm this reasoning, we soaked control cowpeas in 0.1 N sodium acetate buffer, pH 4, for 20 h at room temperature prior to cooking and found that the texture of these buffer-soaked and then cooked seeds (62.3 ± 3.4 N/g) was much harder than that of those soaked in water and then cooked (20.5 ± 0.9 N/g) and that there was also a decrease in pectin loss after cooking (1.62 ± 0.07 mg of uronic acid/g of seeds from the buffer-soaked seeds vs 2.51 ± 0.12 mg/g from water-soaked seeds). However, we failed to show that control seeds, when cooked directly in the acidic buffer instead of soaking, had a similar increase in hardness. Apparently, the seed tissue pH has a greater effect on seed cookability than the cooking medium pH, and a period of time is required for the two to reach equilibrium.

As early as 1946, Mattson soaked yellow peas for 18 h in water to which HCl or NaOH was added to adjust the pH. He found that the cookability of yellow peas was a function of pH, with a minimum in the region of 4 and maxima beyond neutral and in very acidic pHs. Later, Vindiola et al. (1986) observed similar pH-dependent U-shaped curves in cookability when pinto beans and black beans were soaked in buffers with various pH values for a prolonged time. Evidently, all of these observations are consistent with the theory connecting pectin β-eliminative degradation and the HTC defect in legume seeds.

Pectin degradation and solubilization during cooking have been linked with heat-related textural changes of many other plant tissues, including horticultural products (Doesburg, 1961), potatoes (Hughes et al., 1975a,b), snap beans (Van Buren et al., 1990; Van Buren and Pitifer, 1992), and sweet potatoes (Walter et al., 1992). Not surprisingly, all of these previous investigators have emphasized the important role of pH on softening of plant tissues. Thus, the present study points out a similarity between legume seed hardening and plant tissue softening in terms of the mechanism of pectin changes during heating.

4. Effect of Cooking Time. The effect of heating time at 100 °C on texture and pectin loss of both control and aged seeds was also monitored.

Texture. When heated at 100 °C (cooking), control and aged seeds exhibited different time-dependent patterns of textural changes (Figure 5A). For control seeds, a dramatic softening occurred within the first 30 min of

Table I. Effect of $CaCl_2$ Soaking on Texture and Pectin Loss of Control and Aged Cowpeas after 60 min of Cooking and on pH of Soaking or Cooking Liquors[a]

treatment	cowpeas[b]	seed texture, N/g	pectin loss, mg of uronic acid/g of seeds	medium pH after soaking	medium pH after cooking
water soaking (6 h)	control	20.5 ± 0.9[c]	2.51 ± 0.12[c]	6.18 ± 0.03[c]	6.24 ± 0.02[c]
	aged	85.4 ± 3.3[d]	1.10 ± 0.07[d]	5.75 ± 0.02[e]	5.80 ± 0.02[e]
$CaCl_2$ soaking (50 mM, 3 h) plus water soaking (3 h)	control	47.6 ± 2.3[e]	1.47 ± 0.07[e]	6.02 ± 0.05[d]	6.04 ± 0.01[d]
	aged	208.7 ± 6.2[f]	0.09 ± 0.02[f]	4.93 ± 0.02[f]	5.28 ± 0.01[f]

[a] Means of duplicate measurements ± standard deviation. [b] Control: −18 °C storage. Aged: 12 months of storage at 30 °C and 64% relative humidity. [c-f] Column means with different superscripts differ significantly at $p \leq 0.01$.

cooking, while additional heating had little effect. For aged seeds, softening proceeded gradually during 90 min of cooking.

Pectin Loss. Pectin loss during cooking increased almost linearly with time for both control and aged seeds. Overall, control seeds lost more pectin than aged seeds, and this difference enlarged with cooking time (Figure 5B). As compared with textural change (Figure 5A), pectin loss with cooking time showed a different temperature-dependence pattern. Moscoso et al. (1984) studied the relationship between textural change of kidney beans and pectin dissolution during cooking and found that with cooking time both softening rate and pectin dissolution rate followed an apparent first-order kinetics and that the two apparent rate constants correlated highly with each other. The contrast of our findings to those of Moscoso et al. (1984) might be due to the differences in bean materials and the pectin analytical method used in the two studies.

The difference in changes with cooking time between texture and pectin loss (Figure 5) indicates that, in addition to cell wall pectin, other factors may also be involved in the HTC defect. Indeed, several alternative mechanisms have been proposed, including cell lignification via cross-linking of phenolic substances with cell wall protein (Hincks and Stanley, 1986) and denaturation of storage protein leading to restricted starch swelling during heating (Liu et al., 1992, 1993).

5. Effect of Ca^{2+} Treatment. The hardening effect of Ca^{2+} on legume seeds is well-known (Mattson, 1946; Jones and Boulter, 1983). To further study the mechanism of pectin involvement in cowpea cookability, the effects of $CaCl_2$ soaking on texture and pectin loss of cowpeas were also studied (Table I). As compared with water soaking, there was a significant increase in HTC state after Ca^{2+} treatment, and this was accompanied by a significant decrease in pectin loss from both control and aged seeds.

There might be several explanations for reduced pectin loss by Ca^{2+} treatment. These include the following: (1) The ability of this divalent cation to insolubilize pectin prevails over its ability to accelerate pectin degradation (Keijbets et al., 1976). (2) Ca^{2+} replaces such monovalent cations as Na^+ and K^+, which have been shown to increase pectin solubility (Kaneko et al., 1984). (3) Ca^{2+} caused a decrease in soaking/cooking pH (Table I), which in turn could lead to reduced pectin loss. Reduction in cooking medium pH after Ca treatment has also been reported with potatoes (Hughes et al., 1975a).

In summary, our previous studies (Liu et al., 1992, 1993) have shown that the hard-to-cook defect in cowpeas is caused directly by restricted starch gelatinization within the cell cytoplasm and lack of cell separation between cell walls during cooking and that restricted starch gelatinization results from decreased protein solubility and thermal stability. Results of this study suggest that lack of cell separation results from resistance of pectin to β-eliminative degradation in addition to solubilization.

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