Induced Hard-to-Cook State in Cowpeas by Freeze-Thawing and Calcium Chloride Soaking[1]

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ABSTRACT

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Cowpeas stored at −18°C and ambient humidity (control) or 30°C and 64% rh (aged) for 12 months were hydrated before being subjected to freeze-thawing (FT) cycles, $CaCl_2$ soaking, or a combination of these. The cowpeas were monitored for electrolyte leakage, a possible indicator of cell membrane damage, and for hard-to-cook (HTC) state. Results showed that electrolyte leakage depended on storage conditions and the number of FT cycles. HTC state depended on storage conditions, the number of FT cycles, and the application sequence of FT and Ca^{2+} soaking. For control cowpeas, FT slightly softened seed texture but greatly enhanced the hardening effect of Ca^{2+} soaking applied subsequently. For aged cowpeas exhibiting a certain degree of HTC state, the greatest inducement of hardness was observed with Ca^{2+} soaking alone. Other divalent cations exerted effects similar to those of Ca^{2+} but to a lesser extent. With respect to the number of FT cycles, there was a parallel relationship in control cowpeas between electrolyte leakage after FT and HTC state induced by subsequent Ca^{2+} soaking. This relationship was not observed in aged seeds because of initial high electrolyte leakage during hydration. Overall results suggest that one part of HTC inducement during cation soaking occurs through loss of cell membrane integrity that allows cations to bind intracellular components.

For most developing countries, legumes are second only to cereals as a source of dietary protein. However, for certain legume seeds such as cowpeas, consumption is curtailed due to a tendency of seeds to develop the hard-to-cook (HTC) defect during prolonged storage at high temperature and humidity.

Various studies have sought to explain the causes of the HTC defect (Mattson 1946, Burr et al 1968, Moscoso et al 1984, Garcia-Vela et al 1991). One postulated mechanism points to cell membrane deterioration as a primary event leading to the textural defect (Jones and Boulter 1983, Richardson and Stanley 1991, Stanley 1991). Further investigation of how the membrane deterioration eventually leads to the textural defect is necessary.

More recently, Liu et al (1992a) reported a model study using both accelerated storage and treatments to induce HTC state in cowpeas. They found that, for control cowpeas, a significant increase in the HTC state resulted from incubation in water at temperatures between 60–85°C with subsequent soaking in a $CaCl_2$ solution. However, for aged seeds exhibiting a certain degree of HTC defect due to 12-month storage at 30°C and 64% rh, a further increase in HTC state was readily achieved by Ca^{2+} soaking alone. Because increased HTC state in cowpeas resulted from Ca^{2+} uptake by cotyledons, the different responses of control and aged seeds toward incubation and Ca^{2+} soaking was attributed to the difference in initial Ca-uptake capacity of cotyledons. A subsequent study (Liu et al 1992b) showed a parallel relationship between electrolyte leakage during incubation and hardness induced by subsequent $CaCl_2$ soaking with respect to incubation temperature. This result suggested that increased cation-uptake capacity in aged or incubated control cowpeas results from loss of cell membrane integrity that allows cations to migrate to the sites where they cause the HTC defect. Overall results suggested a possible role of cell membrane at an initial hardening stage and roles of intracellular components, such as proteins and starch, at subsequent stages of bean hardening.

Freezing-thawing (FT) of flaccid plant tissue is known to disrupt plasma membrane integrity (Lyons et al 1979). However, unlike the temperatures of heat incubation, the temperatures encountered during freezing would minimize chemical and biochemical deteriorative reactions. Consequently, the role of cell membranes in HTC development could be more clearly elucidated in cowpeas subjected to FT than in those subjected to heat incubation. Therefore, to continue our efforts to understand development of HTC beans, we conducted the following experiment to induce HTC state in cowpeas. This involved hydration, FT cycles, and soaking in various chloride salts.

MATERIALS AND METHODS

Aged and Control Cowpeas

Cowpea seeds (*Vigna unguiculata*, cv. California Blackeye peas No. 5) were purchased from Kerman Warehouse (Kerman, CA). Aged cowpeas were obtained by storing seeds in a tightly covered polyethylene container at 30°C and 64% rh. The humidity was maintained by placing a glass jar of saturated sodium nitrite solution inside the container. After 12 months, samples were taken out of the container and kept at ambient temperature and humidity for one week and then stored at 4°C until used. Cowpeas stored at −18°C and ambient humidity for the entire period were used as a storage control. For each storage condition, two separate containers provided duplication.

Hydration

Cowpeas (30 g, 11.4% moisture) were wrapped in cheesecloth and hydrated in 150 ml of water (deionized) at 25°C for 4 hr before being subjected to FT, cation soaking, or a combination of these.

Freeze-Thawing

Hydrated or Ca-soaked seeds were frozen at −18°C overnight and thawed at room temperature for 4 hr.

Soaking in Chloride Salt Solutions

The hydrated or hydrated-FT cowpeas were soaked in 150 ml of $0.1N$ solution of various chloride salts (Na^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, La^{3+}, or Ce^{3+}) at room temperature for 3 hr. Deionized water was used as a control solution. Samples were washed with water several times before cooking.

Cooking and HTC State Measurement

Before textural measurement, samples, still wrapped in cheesecloth, were cooked on a hot plate in water preheated to 100°C in a beaker covered with a watch glass for 90 min. Cooking was terminated by transferring the samples to an ice bath. HTC state measurement was made using an Instron universal testing machine (model 1122, Instron, Canton, MA) with a Kramer shear compression test cell (model 2830-018, 500-kg load, Instron) filled with all of the cooked seeds removed from one cheesecloth wrapping. Compression was performed at a crosshead speed of 50 mm/min. Maximum force (N) was determined from the peak height of the force-deformation curve. HTC state was expressed as Newton force per gram of dry sample.

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Electrolyte Leakage

Leakage of electrolytes during hydration was determined by measuring conductance of hydration liquors after samples had been removed and the volume brought up to the original 150 ml with water. The electrolyte leakage after FT was determined by soaking the treated samples in 150 ml of water for 1.5 hr and then measuring conductance of the soaking liquor. A digital conductivity meter (Fisher Scientific, Pittsburg, PA) was used. The conductance was expressed as micromhos per 30 g of seeds in 150 ml of water.

RESULTS AND DISCUSSION

Leakage Due to Storage

A nonlinear increase in electrolyte leakage with hydration time, as indicated by conductance, was observed in both control and aged cowpeas (Fig. 1). However, aged seeds, as a result of 12-month storage at 30°C and 64% rh, exhibited a much higher level of electrolyte leakage during the entire hydration period.

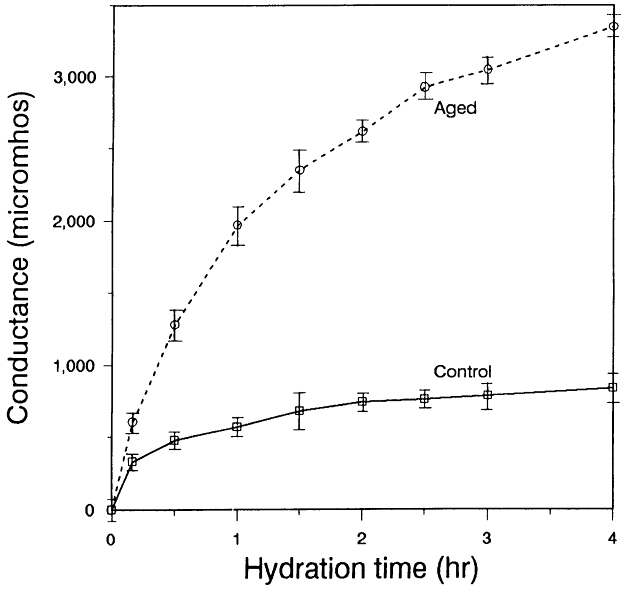

Fig. 1. Electrolyte leakage from control and aged cowpeas as a function of hydration time, as assessed by conductance of hydration solutions.

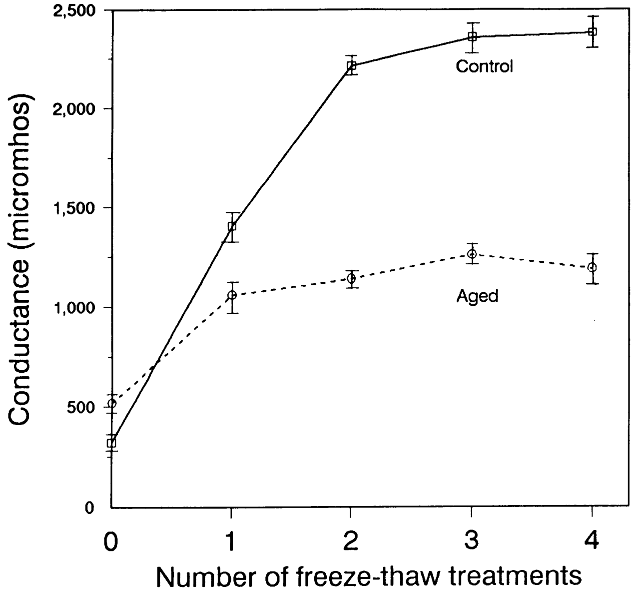

Fig. 2. Electrolyte leakage from control and aged cowpeas as a function of the number of freeze-thaw treatments, as assessed by conductance of soaking solutions after freeze-thaw treatments.

Physiologically, leakage of dry seeds during soaking (imbibition) has been attributed to lack of cell membrane integrity allowing free release of cytoplasmic solutes into solution (Simon 1974). The increased electrolyte leakage in aged seeds (Fig. 1) suggests that cowpeas may undergo membrane deterioration during prolonged adverse storage. Previous investigators reported similar findings with other seeds (Ching and Schoolcraft 1968, Stewart and Bewley 1980, Jones and Boulter 1983).

Electrolyte Leakage Due to Freeze-Thawing

For hydrated control cowpeas, FT caused a pronounced increase in electrolyte leakage, and this increase was doubled in seeds that had two FT cycles (Fig. 2). Further increase in the number of FT cycles did not significantly increase electrolyte leakage. For hydrated aged cowpeas, a singular treatment increased the electrolyte leakage, but additional FT cycles resulted in no further increase.

The increased electrolyte leakage in hydrated-FT control cowpeas (Fig. 2) suggests that FT leads to loss of cell membrane integrity. The lower levels of electrolyte leakage from hydrated aged seeds after FT are attributed to the fact that the cell membrane of aged seeds had already been disrupted during adverse storage. As a result, most solutes had already leaked out from aged seeds during the initial hydration stage (Fig. 1).

Hard-to-Cook State

HTC state in cowpeas depended on storage condition, application sequence of FT and Ca soaking, and the number of FT cycles (Fig. 3). Aged seeds showed HTC state of 69.6 N/g as compared with 16.1 N/g of control cowpeas (Fig. 3, water label). This increase in HTC state was due to 12 months of storage at 30°C and 64% rh. For control cowpeas (Fig. 3, shaded bars), soaking in 0.1 *N* $CaCl_2$ solution caused an 80% increase in HTC state compared to that caused by soaking in water, but FT slightly reduced seed hardness. Ca^{2+} soaking followed by FT caused only a 32% increase, but FT followed by Ca^{2+} soaking resulted in a 270% increase in HTC state. This increase was doubled if seeds were hydrated and then underwent two FT cycles. Increasing the number of FT treatments before Ca^{2+} soaking did not lead to a further increase in HTC state of control cowpeas.

For aged seeds (Fig. 3, white bars), the response was different. Combining FT with Ca^{2+} soaking induced less HTC than did Ca soaking alone, regardless of application sequence. Furthermore, as the number of FT cycles increased, the decrease in HTC state continued.

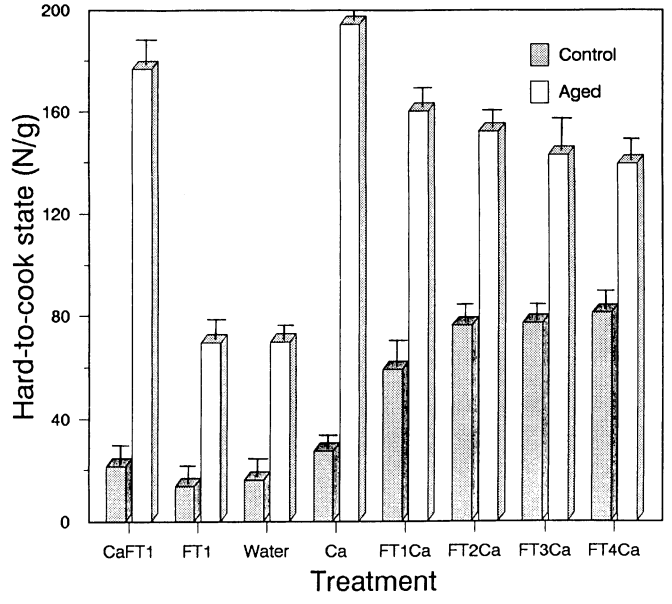

Fig. 3. Hard-to-cook state in control and aged cowpeas induced by freeze-thaw, $CaCl_2$ soaking, or combinations of these. Water = water soaking; Ca = $CaCl_2$ soaking; FT = freeze-thawing (cycle number following FT). The treatment labels reading from left to right give the order.

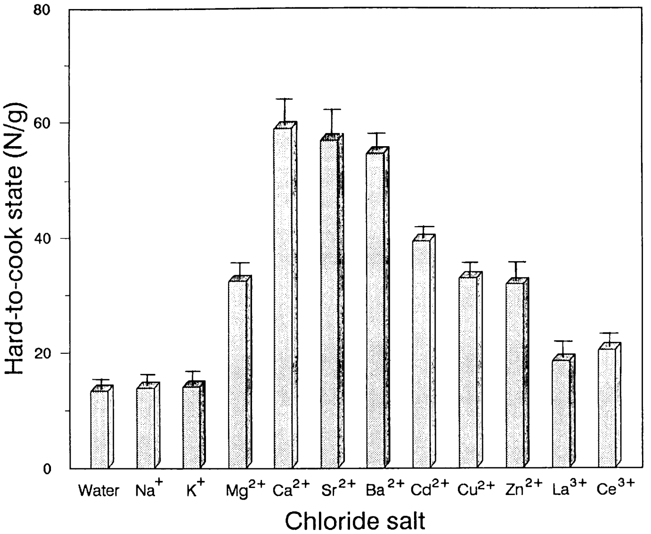

Fig. 4. Hard-to-cook state of control cowpeas induced by one-time freeze-thawing cycle followed by soaking in various solutions of $0.1N$ chloride salts or water.

Electrolyte Leakage and Hard-to-Cook State

Liu et al (1992b) reported a parallel relationship between electrolyte leakage from cowpeas during incubation and induced HTC state due to subsequent $CaCl_2$ soaking, with respect to incubation temperatures. In this study, we also observed a parallel relationship in hydrated control cowpeas between electrolyte leakage after FT (Fig. 2) and HTC state induced by subsequent Ca^{2+} soaking (Fig. 3 shaded bars), with respect to the number of FT cycles. The correlation coefficient between the two parameters was 0.964 ($P = 0.05$). This linear relationship was not observed with hydrated aged cowpeas, possibly because of the initial high electrolyte leakage during hydration.

The HTC state in cowpeas induced by heat incubation and $CaCl_2$ soaking has been shown to result from Ca uptake by seed cotyledon (Liu et al 1992a). The increase in Ca-uptake capacity after heat incubation is attributed to loss of cell membranes that lead to improved access of cations to existing binding sites (Liu et al 1992b). Likewise, in this study, the observed parallel relationship in control cowpeas between electrolyte leakage after FT and induced HTC with subsequent Ca soaking further supports a possible role of the cell membrane in cation-induced hardening of cowpeas. We reason that the importance of the cell membrane in this hardening process lies in its biological function as a cell boundary and as a barrier to molecules in the surrounding media. In an active cell, a passive influx of cations is restricted because a transmembrane action of a cation is carried out by a plasma-membrane-based, adenosine 5′-triphosphate (ATP) dependent pump called membrane-associated H^+-ATPase. However, when cell membrane integrity is lost due to aging (storage at 30°C and 64% rh), physical disruption (such as ice crystal formation during freezing), or thermal stress (such as incubation in water at 60–85°C employed in Liu et al [1992a]), denaturation of the ATPase occurs (Palta 1990). As a result, a passive influx of cations into the cell should occur upon soaking in a cation solution.

Accordingly, in aged cowpeas where membrane integrity had already undergone deterioration during prolonged adverse storage, as suggested by high electrolyte leakage during hydration (Fig. 1), Ca^{2+} soaking increased the HTC defect. The slight reduction in HTC state in aged cowpeas as a result of repeated FT cycles might be due to loss of cation-binding sites. In control seeds, the cell membrane remains intact and a passive influx of Ca^{2+} is restricted; therefore, Ca^{2+} soaking alone resulted in limited hardening. When cell membranes were disrupted by FT, as suggested by increased electrolyte leakage, a passive influx of Ca^{2+} would occur during Ca^{2+} soaking leading to enhanced hardening effect of Ca^{2+} treatment (Fig. 3, shaded bars). The sequence effect of FT and Ca^{2+} soaking can also be explained by the boundary function of cell membranes.

Effects of Other Chloride Salts

Other divalent cations were able to induce hardness in hydrated-FT control cowpeas but to a lesser extent than Ca^{2+} (Fig. 4). Trivalent cations had less hardening effect than divalent cations, whereas monovalent cations had little effect. In an earlier study, Liu et al (1992a) observed a similar finding with aged control or heat-incubated control cowpeas, although, for a particular cation, the HTC state varied among cowpea samples in the two studies. Garcia-Vela et al (1991) showed that softening black beans by soaking them in an aqueous salt solution was affected by cations as well as anions. These results suggest that change in bean texture is a complex process, possibly involving interaction with cations as well as anions.

In summary, this study showed effects of aging (storage at 30°C and 64% rh) and FT on cowpea electrolyte leakage and the effects of aging, FT, and cations on cowpea texture. We also found a significant inducement of HTC state in aged or hydrated-FT control cowpeas by soaking with a divalent cation. Overall results suggest that one part of HTC inducement occurs through loss of cell membrane integrity, which allows cations to migrate inside cells to bind intracellular components, causing HTC defect.

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