Starch Alterations in Hard-To-Cook Beans (*Phaseolus vulgaris*)

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Dry common bean seeds (*Phaseolus vulgaris* cv. Carioca) were stored under conditions favorable for the development of hardness in order to investigate possible alterations in the starch component due to the hard-to-cook (HTC) phenomenon. Examination of isolated starch granules or seed fractures observed under scanning electron microscopy revealed that hard beans were more resistant to amyloglucosidase attack. Under polarized light microscopy, starch granules of HTC beans appeared more birefringent than those of the control beans. Differential scanning calorimeter thermograms showed an increase of the temperature of gelatinization of starch isolated from HTC-and-old beans (5 years of storage), whereas starch isolated from control (soft) bean seeds and HTC seeds stored for a short period of time (2 months) showed no such increase.

INTRODUCTION

Hard-to-cook (HTC) is a textural defect appearing in legume seeds stored under inappropriate conditions (high temperature and humidity). HTC leads to prolonged cooking times because cotyledonary cells fail to separate during cooking.

Different theories have been proposed for the hard-to-cook phenomenon in beans. The most widely cited explanation attributes an important role to the pectin in the cell wall/middle lamella and to phytin degradation (Mattson, 1946; Chang et al., 1977; Kon and Sanshuck, 1981; Jones and Boulter, 1983; Moscoso et al., 1984; Hentges et al., 1990). Another theory postulates the participation of phenolic compounds in the process of hardening (Hincks and Stanley, 1987; Srisuma et al., 1989). Also, ultrastructural data show that inadequate storage leads to membrane degradation (Varriano-Marston and Jackson, 1981), which seems to explain the leakage of electrolytes observed when HTC beans are soaked. It has been suggested that the HTC of legume seeds is the result of multiple mechanisms, with the participation of enzymatic and nonenzymatic reactions. However, the contribution of each mechanism to the overall process is not known (Stanley, 1992).

HTC causes an increase in cooking time, which leads to increased energy (fuel) consumption for cooking. It also reduces the nutritional value and changes the sensorial quality mostly because of poor properties of the cooking broth. A thick viscous broth is an important quality parameter for bean consumers in Latin America. Hard beans do not form a thick broth; this characteristic could be related to alterations of the starch properties induced by the inadequate storage conditions. Hard beans are therefore less acceptable to consumers and cause important postharvest losses.

Some authors have studied the alterations to starch and protein during the development of HTC. Hohlberg and Stanley suggested that a chemical or structural alteration occurs in bean starch with storage, but this change is independent of the environment. Paredes-Lopez et al. (1989) reported an increase in damaged starch content of

HTC beans and an increase in the viscosity of HTC bean flour pastes when compared to controls.

In this paper, starch was studied in soft (control) and hard beans in order to compare the changes to which it is submitted, probably as a consequence of hardening and/ or seed aging, rather than as a causative agent of HTC.

MATERIALS AND METHODS

Materials. Common bean (*P. vulgaris* cvs. Carioca and Carioca-80) were provided by the Instituto Agronômico de Campinas-SP. Carioca beans have a cream background with tan stripes.

Storage Conditions and Cooking Time. Control samples were kept at 5 °C/40% RH and accelerated hardened samples at 40 °C/75% RH for 60 days (Vindiola et al., 1986). A third lot of beans referred as HTC-and-old was maintained under local environmental conditions for 5 years: the seeds were kept in a cloth bag at an annual average RH of 75% and temperature range of 20–36 °C. A modified Mattson cooking device (Jackson and Varriano-Marston, 1981) was used to determine cooking time. The cooking times of Carioca beans were 89 min for the control sample, 159 min for the hard beans, and 520 min for the HTC-and-old seeds. For the assay of isolated starch digestion, samples of Carioca-80 were also used whose cooking times were 95 min (control) and 180 min (HTC).

Methods. *Isolation of Starch Granules.* The same procedure described by Hohlberg and Stanley (1987) was adopted with slight modifications at the end of the procedure. After the extraction with NaCl the residue was resuspended, treated with toluene-saturated water (Carson, 1981), and kept under agitation for 1 h. The toluene layer was removed through siphoning, and the starch obtained after vacuum filtration and acetone washing of the residue was lyophilized.

Isolated Starch Digestion with Amyloglucosidase. Twenty-five milligrams of isolated starch granules were incubated with 5 mL of amyloglucosidase solution (Sigma A-7255: 14 units/mL in 0.1 M acetate buffer pH 4.8 at 37 °C) for 30 min, 1 h, 2 h, 4 h, and 6 h, respectively. Five hundred microliters was taken after each incubation period, 50 μL of 0.6 N perchloric acid was added, and the amount of released glucose was determined according to Bergmeyer (1974). Complete assays were carried out in triplicate.

Scanning Electron Microscopy. Cotyledons of dehulled beans were previously fixed in glutaraldehyde/formaldehyde (3%/3%) in 0.05 M phosphate buffer pH 6.8 for 24 h. The seeds were transversally fractured with a razor blade and incubated with amyloglucosidase (as described for isolated starch digestion) for 24 h under constant low agitation. The fractures were then washed with phosphate buffer, dehydrated using a graded ethanol series, and finally critical-point dried (Humphreys, 1974). The
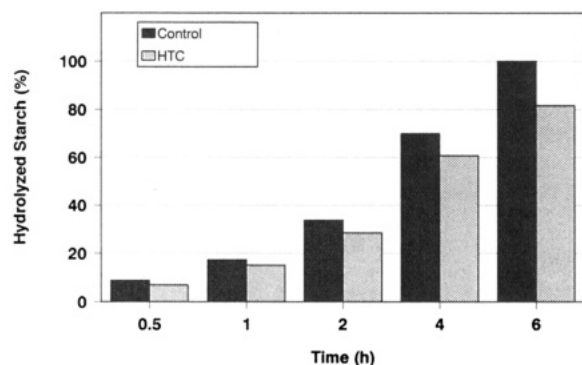
Figure 1. Digestion of isolated bean starch during incubation with amyloglucosidase. Starch was from control beans (solid bars) and HTC bean seeds (shaded bars).

samples were mounted on aluminum stubs using colloidal graphite cement and sputter coated with gold (300 μm). The examination of the samples was carried out on a Hitachi S-570 scanning electron microscope at an accelerated voltage of 20 kV. The pictures were taken on Polaroid type 55 (P/N) film.

Polarizing Microscopy. Dehulled beans were fixed in ethanol/formaldehyde/acetic acid/water (30:10:10:10) for 7 days, with three substitutions of the fixative solution. Then the samples were embedded on paraffin and sectioned. The deparaffinized sections were mounted with immersion oil and observed under polarized light with a Carl Zeis Model Jenaval microscope. The pictures were taken with Kodacolor Gold 200 ASA film.

Differential Scanning Calorimetry. Samples weighing between 2 and 6 mg of isolated starch were accurately weighed into sample pans, and water was added to keep a 40–45% proportion of starch to water. Sample pans were sealed, reweighed, and equilibrated for 1 h. DSC thermograms were obtained with a Perkin-Elmer DSC-7; the temperature range for scanning was from 25 to 110 °C, at a 10 °C/min rate, and water was used in the reference pan. Analyses were run in triplicate.

RESULTS AND DISCUSSION

In order to compare possible alterations of starch susceptibility to enzymatic attack, isolated starch granules of control (soft) and HTC beans were incubated with amyloglucosidase (AMG). Susceptibility was measured by the amount of glucose released by the enzymatic digestion. Figure 1 shows the results of the digestion expressed in percentage of digested starch. HTC bean starch was less susceptible to AMG action than control starch. After 6 h of incubation, about 80% of HTC bean starch was digested compared to 100% hydrolysis of the control (soft) sample. Least-squares regression lines were calculated for both the control and HTC beans. The equations for these lines were $y = 16.76x + 0.7055$ for the control ($r = 0.9994$) and $y = 13.82x + 1.24$ for the HTC beans ($r = 0.9968$). A t test applied for comparison of the two regression lines (Brownlee, 1965) showed significant differences at the $P = 0.01$ level between the two regression coefficients ($t_{obs} = 4.03 > t_{0.995,6df} = 3.71$). This result was observed either in accelerated hardened samples or in long-term stored beans (5 years old). This observation may have nutritional implications, specifically with regard to impaired caloric utilization. Compared to other carbohydrate foods, the ingestion of cooked dried bean seeds causes a low blood glucose level (Jenkins et al., 1980). One could infer that ingestion of HTC beans should lead to even lower starch utilization. In a preliminary assay conducted in our laboratory (results not presented here), a comparison was made between the blood glycemic curves of rats 4 h after the ingestion of meals composed of cooked rice (control) or beans. Compared to the utilization of rice starch, the soft and HTC bean meals had a utilization of 89.3% and 74.7%, respectively.

Cotyledons of soft and HTC beans were fractured, treated with AMG, and examined under SEM (Figure 2). The peripheral cotyledonary cells of HTC bean seeds showed more residual starch than control samples, confirming the lower susceptibility of HTC bean starch to enzymatic digestion. Probably the starch alterations associated with HTC begin in the outer cell layers of the cotyledon and extend toward the center of the cotyledon.

In histological sections of bean seeds under polarized light, starch granules of both control and HTC bean seeds showed the characteristic "maltese cross" (Figure 3). However, a much stronger birefringence was observed in starch granules from HTC bean cells, suggesting that starch isolated from HTC beans had a higher degree of crystallinity. Also, starch from HTC-and-old bean starch showed stronger birefringence than the control.

To investigate any possible alteration of the gelatinization temperature, DSC thermograms of the starches were prepared. Figure 4 presents the results obtained with starch from control (soft bean), HTC, and HTC-and-old samples. As expected for water concentrations lower than 60%, the thermal profiles revealed multiple endotherms (Biliaderis, 1992). The gelatinization temperature of the control sample was 64.2 °C; for the HTC bean starch, gelatinization occurred at 65.7 °C, and for the starch from HTC-and-old bean, it occurred at 71.1 °C. The difference of 1.5 °C between soft and HTC bean starches was not large, but the difference of 6.9 °C between soft and HTC-and-old bean starches represented an increase of the gelatinization temperature of more than 10%. The second endothermic transition was detected at 78.8, 78.5, and 86.1 °C, respectively, for the soft, HTC, and HTC-and-old bean starches. Paredes-Lopez et al. (1989) did not detect an alteration of the gelatinization temperature with the hardening process. Hohlberg and Stanley (1987) noticed a change in the gelatinization temperature but no modification of the fusion temperature (second endotherm). Our results showed that starch isolated from HTC beans stored for short periods revealed an atypical DSC pattern with a third transition at 92.7 °C. HTC beans bind less water than normal beans (Plhak et al., 1989), and water can be a limiting factor of starch gelatinization. The gelatinization temperature of starch inside cells is higher than that of starch outside cells (Rockland et al., 1977; Hahn et al., 1977). If the gelatinization temperature of isolated starch from HTC-and-old beans is already higher than for the soft beans, one can infer that to attain the gelatinization of the starch *in situ* a more intense heating is required to obtain an appropriate nutritional utilization of the starch.

Although it may not be among the factors responsible for hardening, starch is being altered during this process. Our data, however, do not allow us to conclude that starch alterations are a direct consequence of the hardening. Those changes could also result from the seed aging process. It is well-known that accelerated aging of seeds is achieved through storage under high temperature and humidity (Parrish and Leopold, 1978). These are the same conditions used to obtain HTC samples. During aging, membrane systems are exposed to deterioration, and membrane degradation problems have been reported in HTC beans (Varriano-Marston and Jackson, 1981). Because of that we conclude that the investigations on starch changes related to HTC should be conducted in a well-controlled situation where hardening and aging do not occur concurrently.

In summary, an increase in the starch gelatinization temperature was observed as a consequence of prolonged periods of storage (HTC-and-old beans). In HTC beans
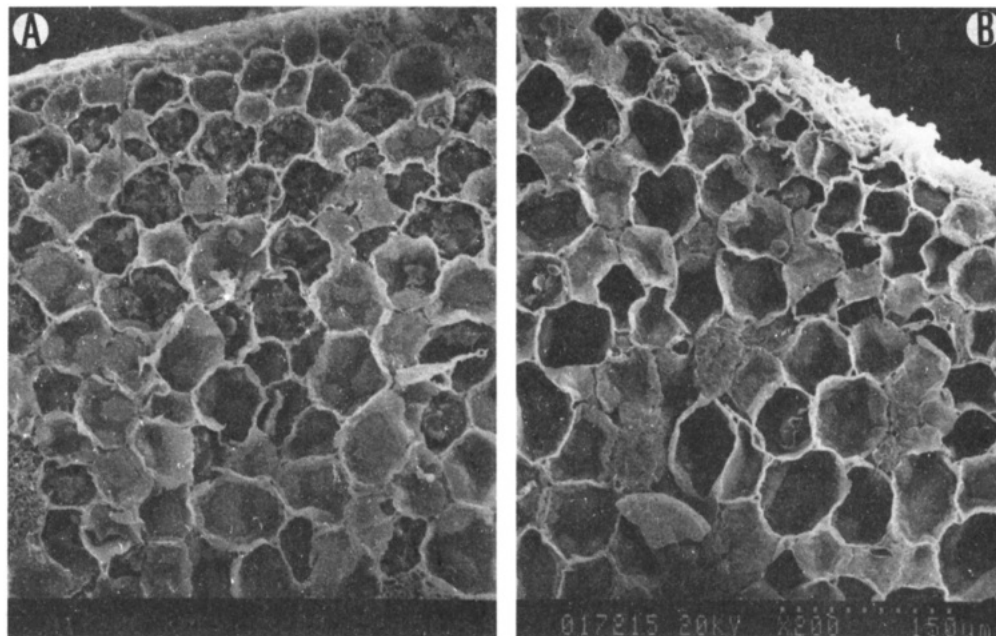
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Figure 2. SEM micrographs of bean seed fractures after incubation with amyloglucosidase: A, control; B, HTC bean seed.
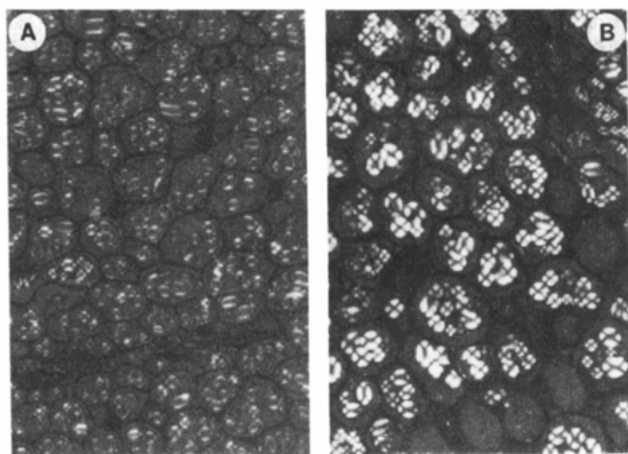

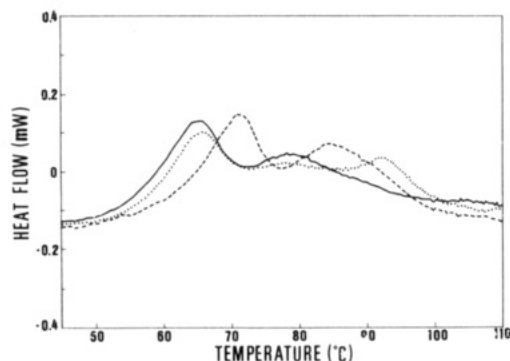**Figure 3.** Polarizing micrographs of bean seed sections: A, control; B, HTC bean.

Figure 4. DSC thermograms of isolated bean starch: (—) control; (···) HTC; (- - -) HTC-and-old bean starch.

the starch is more resistant to the attack of amyloglucosidase, as evidenced by SEM of bean fractures and after incubation of isolated starch with AMG. Also, the stronger birefringence revealed by polarizing microscopy suggests alteration of starch crystallinity in the granules.

Probably the changes in HTC beans involving the starch are only a consequence of that process, but they represent one of the mechanisms which lower the cooking quality of common beans and affect its acceptance by consumers.

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