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Effect of the Hardening Phenomenon on some Physico-chemical Properties of Common Bean

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ABSTRACT

*Several aspects of the hard-to-cook phenomenon in beans (*Phaseolus vulgaris*) were investigated. Two varieties of black and light yellow seeds were stored for 135 days at 40°C and 80% rh. Texture measurements produced force–time deformation curves of two peaks for the fresh samples and of three peaks plus a substantial increase in Instron hardness for the aged beans. The damaged starch content of the stored seeds tended to increase. The paste viscosity of flour dispersions from fresh seeds increased outstandingly after storage. Scanning electron microscopy studies showed that longitudinal sections of the cotyledons from hard beans had very densely packed cells, whereas they were loosely arranged in fresh samples. No changes were observed in the gelatinisation temperature of bean flour and isolated starch of hard-to-cook samples, as assessed by differential scanning calorimetry.*

INTRODUCTION

In Latin America, common beans are, after cereals, the most important source of protein, calories and other nutrients (Paredes-López *et al.*, 1985). It is now established that, after prolonged storage at high temperatures and humidities, common beans and other legume seeds develop the hard-to-cook (HTC) phenomenon (Mattson *et al.*, 1950; Jones & Boulter, 1983*a*, *b*). HTC or hard beans require more time to cook, are less acceptable to the consumer and of lower nutritive value (Bressani *et al.*, 1963). Therefore, this

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textural defect yields poor quality products and also inflicts important postharvest losses.

The mechanisms involved in the HTC phenomenon have not been satisfactorily elucidated. One of the favoured hypotheses ascribes prolonged cooking times in stored legumes to a failure of phytate to chelate divalent cations in the pectates of the middle lamella, rendering this structure unsusceptible to heat-softening. The phytate is degraded enzymatically due to the action of phytase becoming thus unable to fulfil its role; this enzyme is probably activated by elevated storage conditions (Rockland & Jones, 1974; Jones & Boulter, 1983*a*).

The objective of this study was to investigate some of the physico-chemical changes taking place in hard beans in an attempt to understand better the HTC defect, so that strategies might be developed to reduce it.

MATERIALS AND METHODS

Materials and storage conditions

Two important bean cultivars (*Phaseolus vulgaris*) in the commercial market were seeded in plots during the spring of 1986. Negro Qro and Canario, with black and light yellow seeds, respectively, were harvested, shelled and cleaned. Fresh samples were kept in plastic bags inside tightly covered containers at 4°C until used. Another portion of bean samples was stored at 40°C, 80% relative humidity for 135 days. An attempt was made to simulate storage conditions prevalent in bean production areas of the tropical regions.

Methods

Moisture and water acitivity (Aw)

The mosture content of whole beans (10–15 g) was determined by drying them in an air-oven at 103°C for 72 h (AACC, 1984).

Water activity of whole beans was measured with a Rotronic Hygroscop DT apparatus at 25°C.

Seed weight and test weight

Weights of 100 randomly selected beans were determined, replicating the measurement three times. Test weight (kg/Hl) determinations were carried out in triplicate.

Water absorption and cooking test

The procedure for water absorption described previously (Paredes-López *et al.*, 1986) was followed. Intact and decorticated bean samples were used. Results were reported as percentage of water absorbed on a dry weight basis corrected for loss of solids.

A Mattson cooking device with some modifications was built (Mattson *et al.*, 1950; Jackson & Varriano-Marston, 1981) for the cooking test. After 16 h of soaking, 25 seeds were positioned into each of the cylindrical holes of the cooker so that the piercing tip of the rod was in contact with the bean surface. The cooker was placed into a 4 litre beaker containing 3 litres of boiling water. Beans were judged as cooked when the rod tip penetrated 68% of the sample. The cooking time was recorded and used to prepare samples for texture determination.

Texture determination

The texture determination, reported here as Instron hardness, was performed in an Instron Universal Testing Instrument, model 1000 (Moscoso *et al.*, 1984). A flat faced 1/8 in diameter circular steel punch was used. 0·5 and 5 kg load cells were mounted on the moving crosshead with a speed of 30 cm/min for fresh and hard samples, respectively. The maximum puncture force was taken as an index of bean hardness. A total of 100 individual beans, previously soaked for 16 h and cooked for 60 min, were punched for each treatment and the mean peak force calculated. The cooking time for all samples was chosen using the treatment time estimated for the fresh samples.

Hunter colour and starch damage

Surface colour of whole beans was measured with the HunterLab model D25-2 reflectance colourmeter (Paredes-López *et al.*, 1986). The Hunter parameters L (lightness) and ΔE (total colour difference) were determined.

The colourimetric procedure 76-30A of AACC (1984) was used to measure damaged starch content. It was expressed as g of damaged starch/100 g sample at 14% moisture content.

Pasting properties

The Brabender viscograph model E was used to develop viscosity curves of the samples at a concentration of 80 g of flour (14% moisture content) in 400 ml of a phosphate buffer, pH 5·3 (Shuey & Tipples, 1980) using the

1000-cmg sensitivity range. The mixture was transferred to the bowl of the viscograph, which rotated at 75 rpm, and heated from 30 to 90°C at a rate of 1·5°C/min. It was maintained at that temperature for 15 min, then cooled at the same rate to 50°C. Consistency and temperature profiles were simultaneously recorded (Colonna & Mercier, 1985).

Scanning electron microscopy (SEM)

Halves of raw beans without soaking and cooking were separated along the longitudinal axis. These samples for SEM were critical point-dried, mounted on aluminium stubs and coated with 60 nm of gold. These were viewed and photographed on a JEOL 35CX scanning electron microscope at an accelerating voltage of 15 kV.

Bean starch isolation

The method for starch isolation was based on that described by Sathe & Salunkhe (1981) with minor modifications. Raw beans (400 g) were soaked in 1 litre of deionised water containing 0·01% sodium metabisulphite for 12 h at room temperature. The swollen seeds were rinsed with water and as much hull as possible was manually removed during this process. The beans were blended with 2 litres of deionised water in a Waring blender at medium speed for 5 min. The homogenate was shaken for 24 h at 4°C and then centrifuged at 10 000 × *g* for 30 min. The resulting residue was mixed with 2 litres of 2% sodium chloride, stirred for 24 h at 4°C and centrifuged as above. After discarding the supernatant, the residue was washed with 1 litre of deionised water, suspended in 2 litres of 0·1N sodium hydroxide, mixed in a blender for 1 min and shaken for 48 h at 4°C. After centrifuging, the extraction with alkali was repeated. The mucilagenous overlayer of sediment was discarded, the residue resuspended in 80% aqueous ethanol, blended for 1 min and heated in a water bath for 1 h at 50°C. This sample was allowed to sediment for 4 h at 4°C and the supernatant discarded. The isolated starch was freeze-dried and stored at 4°C until use.

Differential scanning calorimetry (DSC)

DSC was done using a DuPont calorimeter, model 9000. The whole beans were ground in a Udy Cyclone Sample mill. Bean flour (3·0 mg) was weighed directly into a DSC aluminium sample pan and deionised water (12 μl) added by microsyringe. After sealing, the pan was left to equilibrate for 1 h (Ghiasi *et al.*, 1982). The samples were scanned at a rate of 10°C/min with a sensitivity of 0·005 mcal/s. The same DSC procedure was followed for

isolated bean starch. The endotherm peak temperature obtained by DSC was recorded and it is referred to here as the gelatinisation temperature of starch (Donovan, 1979; Biliaderis, 1983).

RESULTS AND DISCUSSION

The stored beans showed an increase in moisture content, *Aw* and weight of 100 seeds as compared to the fresh samples (Table 1). However, after storage a slight decrease was observed in the test weight of Canario. It means that the gain in moisture content was not enough to compensate for the volume expansion of the aged beans. The influence of high moisture content in bean hardening has been studied by several workers (Burr *et al.*, 1968; Jones & Boulter, 1983*a*, *b*). The increase in *Aw*, as observed here, might be one of the key factors leading to previously restricted enzymatic activities important in the hard-to-cook phenomenon (Hincks & Stanley, 1986).

Figure 1 shows the water absorption results with the Negro Qro cultivar; the patterns followed by Canario were similar and are not shown here. It was observed that up to about 18-h soaking, the water absorption of intact beans was higher for the stored seeds than for the fresh samples. The higher rate of the intact aged seeds agrees with data reported by Burr *et al.* (1968) and Hincks & Stanley (1986). However, in this study the absorbed water was mostly retained between the seed coat and cotyledons as reported by Elías (1982) and Jones & Boulter (1983*a*). Then, decorticated samples were

TABLE 1
Changes in Some of the Physico-chemical Properties of Fresh and Hard-to-Cook Beans[a]

Determinations	*Bean cultivars*				*Least significant difference (95%)*
	Negro Qro		*Canario*		
	Fresh	*Hard*	*Fresh*	*Hard*	
Moisture (%)	10·2 ± 0·3	17·2 ± 0·1	13·8 ± 0·2	17·3 ± 0·2	0·5
Aw	0·46 ± 0·02	0·79 ± 0·01	0·65 ± 0·01	0·80 ± 0·02	0·01
Weight of 100 seeds (g)	22·5 ± 0·4	24·8 ± 0·4	42·5 ± 0·8	46·5 ± 0·7	1·2
Test weight (kg/Hl)	73·9 ± 0·6	73·1 ± 0·6	75·2 ± 0·7	72·7 ± 0·5	1·2
Cooking time (min)	60 ± 2	360	60 ± 3	360	—
Instron hardness (kg/bean)	0·44 ± 0·10	2·26 ± 0·62	0·41 ± 0·13	2·69 ± 0·50	0·2
Hunter colour					
L	14·4 ± 0·1	15·1 ± 0·1	47·8 ± 0·4	22·8 ± 0·2	0·3
ΔE	76·8 ± 0·1	76·1 ± 0·1	46·7 ± 1·5	70·6 ± 0·8	1·3
Starch damage (%)	13·1 ± 0·1	14·3 ± 0·2	5·1 ± 0·2	8·1 ± 0·2	0·4

[a] Mean values ± standard deviations.

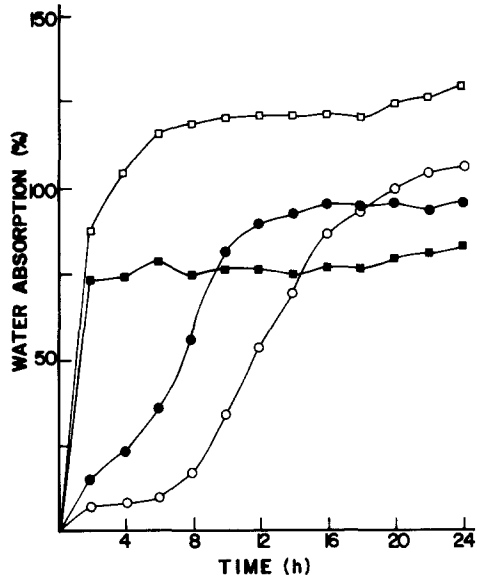


Fig. 1. The water absorption patterns of intact and decorticated seeds of fresh and hard beans from Negro Qro cultivar. ○, intact/fresh; ●, intact/hard; □, decorticated/fresh; ■, decorticated/hard. Least significant difference at 0·05%, 4·1.

tested and showed that the water absorption of the aged seed remained almost unchanged after a sudden enhancement in 2-h soaking time, whereas the fresh sample exhibited a higher rate of absorption up to about 8 h. The decorticated stored seeds had significantly lower absorption values than the corresponding fresh sample (Fig. 1). The patterns of water uptake restrictions and loss of solids during soaking of cotyledons of aged seeds have also been studied by several authors (Parrish & Leopold, 1978; Jackson & Varriano-Marston, 1981; Elías, 1982).

The cooking time for both cultivars in the fresh state was 60 min as assessed by the Mattson cooker (Table 1). As expected, after storage this time changed to more than 6 h even with the use of 16 h soaking. This means that the weather conditions of the tropics produce, after bean harvesting, outstanding enhancement of the cooking time, which is accompanied by other undesirable changes (Bressani *et al.*, 1963; Molina *et al.*, 1975). The storage changed the Instron hardness of the black beans from 0·44 to 2·26 kg/bean (Table 1); a similar effect was observed for the Canario bean. These results demonstrate that, even after a soaking period of 16 h and 60-min cooking time, the texture of HTC beans was much more resistant to the puncture force than that of fresh samples. Typical force–time deformation curves for fresh and stored beans, which were soaked and cooked, are illustrated in Fig. 2. Fresh seeds (Figs 2A & 2C) of the two cultivars showed

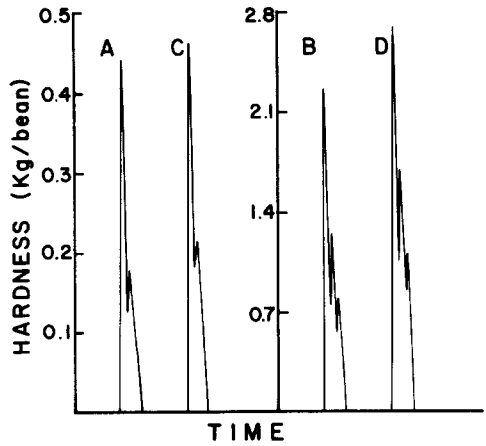


Fig. 2. Force–time deformation curves for soaked and cooked beans of both cultivars. Negro Qro: A, fresh; B, hard; Canario: C, fresh; D, hard.

two well-defined peaks while the hard samples (Fig. 2B & 2D) had three peaks. In all cases, the magnitude of the individual peaks decreased consecutively. The yield point (point at which the punch begins to penetrate the bean seed) and maximum force of secondary peaks increased substantially for stored samples. The puncture force of these peaks might well be used to follow changes in seed texture during storage. The first and second peaks were ascribed by Hincks & Stanley (1986) to the force needed to cut through the seed coat + first coyledon and to the second cotyledon, respectively. They found profiles of two major peaks and reported that differences in force–time deformation curves of fresh and stored seeds were no longer evident after soaking. The present results showed outstanding differences in the profiles of these curves even after soaking and cooking. The third peak might be due to the resistance to puncture force of the seed coat of hard beans.

The Hunter colour of the stored black bean remained practically unchanged whereas remarkable modifications were observed for Canario (Table 1). This cultivar had a reduction in the L value, meaning a loss of lightness in the colour of the stored seed. Also, the ΔE parameter related to the yellow standard used was larger for the aged seed than that for the fresh sample.

The damaged starch content increased for both stored seeds. The method used for this determination is based on the content of total reducing sugars. Thus, the observed increase might be ascribed to a higher amylolytic activity during storage and/or to a higher starch breakdown during milling of the hard beans as compared to the fresh samples. Jones & Boulter (1983*b*) found a reduction in total sugar content along the storage of bean samples at 34°C

and 70% rh, and suggested that, under these conditions, the available moisture content was perhaps not sufficient to activate enzyme synthesis.

Viscograms of dispersions with the HTC flours from both cultivars had an overall consistency remarkably higher than those of the corresponding fresh samples (Fig. 3). All flours displayed a net increase in paste consistency upon cooling from 90 to 50°C; however, this retrogradation did not have a defined trend for both samples. The Canario flour pastes were less viscous than those of Negro Qro. The pasting profiles of fresh flours were somewhat different from those reported by Agbo *et al.* (1986). The increase in paste consistency of the hard beans is related to the intracellular components, of which starch is the primary constituent, comprising at least 45% of the cotyledons (Reddy *et al.*, 1984). Maza-Calviño (1987) found that the isolated starch from hard beans exhibited a higher peak viscosity than that of fresh samples. However, the starch from defective seeds had a lower retrogradation capacity than the controls.

SEM studies demonstrated that differences in cotyledon cell separations were evident between fresh and HTC Canario seeds (Fig. 4(a) & 4(b), respectively). Also, the appearance of middle lamella for both samples was outstandingly different. These characteristics were similar in Negro Qro samples (not shown). SEM of stored seeds exhibited a dense packing of cotyledon cells with no separation between them as shown by the control

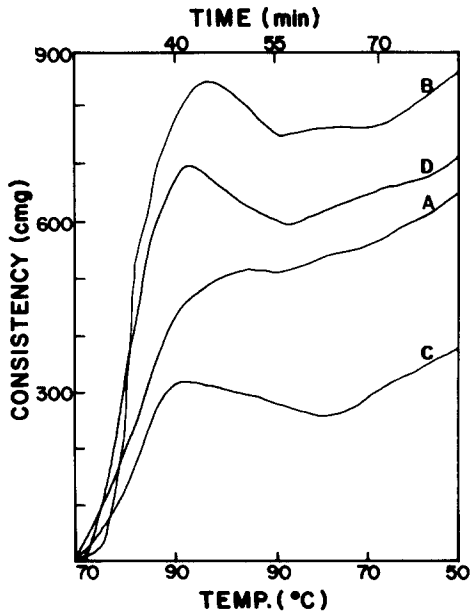
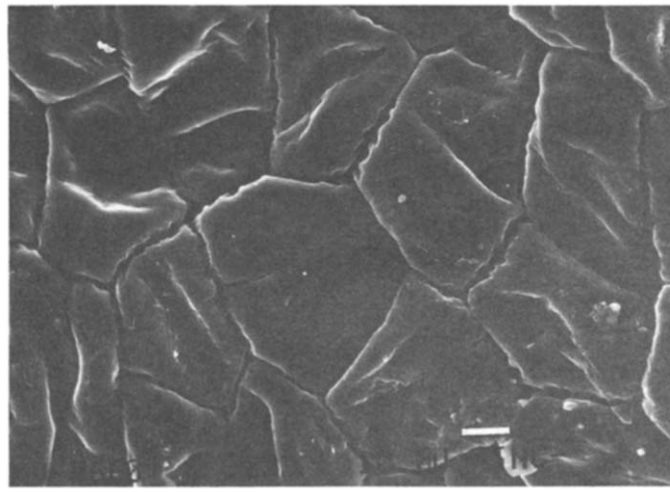
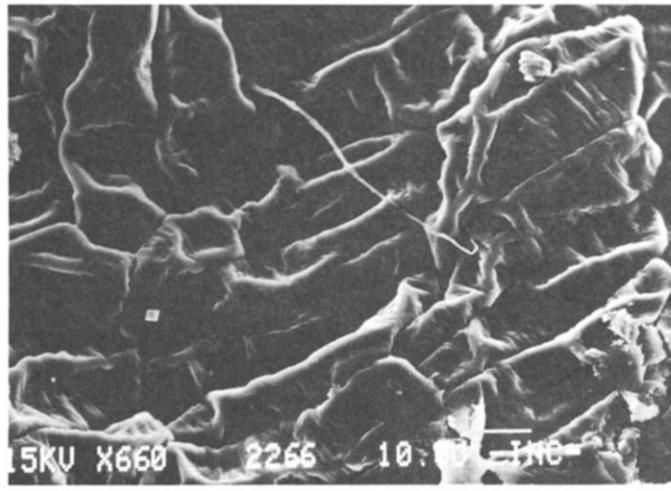


Fig. 3. Viscograms of flour dispersions from raw beans. Negro Qro: A, fresh; B, hard; Canario: C, fresh; D, hard.



(a)



(b)

Fig. 4. Scanning electron microscopy of cotyledons of raw beans from Canario cultivar: (a) fresh; (b) hard. (Scale bar = 10 μm).

beans. Other workers (Jackson & Varriano-Marston, 1981; Jones & Boulter, 1983*a*) found that cooking did not produce cell separation in the cytoplasm of aged beans as compared to the control samples. The strong adhesion between cells observed here for hard seeds might partially explain the reduced water uptake and consequently the lower rate of cooking of these samples (Molina *et al.*, 1975; Hincks & Stanley, 1987).

TABLE 2
Endotherm Peak Temperatures Obtained by Differential Scanning Calorimetry of Fresh and Hard-to-Cook Beans and Starch Isolated from Them[a]

Samples	*Peak temperatures (°C) of bean cultivars*			
	Negro Qro		*Canario*	
	Fresh	*Hard*	*Fresh*	*Hard*
Raw beans	75·2 ± 0·3	75·2 ± 0·2	75·6 ± 0·2	75·8 ± 0·3
Isolated starch	75·3 ± 0·4	75·6 ± 0·1	75·8 ± 0·1	76·4 ± 0·2

[a] Mean values ± standard deviation.

The DSC endotherm peak temperatures for fresh and stored seeds appear in Table 2. It was evident that the hardening phenomenon did not modify the gelatinisation temperature of the starch present in the flour. The isolated starch from the fresh and HTC beans of both cultivars had about the same gelatinisation temperature as compared to that of the flours. Refined starches from *Phaseolus* (Biliaderis *et al.*, 1980) and from *Phaseolus vulgaris* (Sosulski *et al.*, 1985) showed similar gelatinisation temperatures determined by DSC as those found in this study.

In summary, it was found that HTC beans showed an increase in water activity and in individual seed weights but a decrease in test weight and in water absorption capacity. The force–time deformation curves with two peaks for the fresh samples changed after storage to three peaks with a substantial increase in Instron hardness. A loss of Hunter lightness was observed in the surface of the light yellow bean but not in the black seed. The damaged starch content of the stored samples tended to increase. The hard beans showed a higher paste viscosity and a more compact packing of the cotyledon cells as compared with the fresh samples. DSC studies showed that the hardening phenomenon did not modify the gelatinisation temperature of the bean starch.

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