Changes in Physicochemical Properties of Dry Beans *(Phaseolus vulgaris* L.) during Long-Term Storage

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Five different bean varieties (*Phaseolus vulgaris* L.) from Kenya, which were obtained either freshly collected or after having been stored for 5 years in tropical conditions (30−40 °C, >75% RH), were compared for their cooking characteristics. Beans under storage were susceptible to the hard-to-cook (HTC) defect, and the bean cooking time was up to 12 times that of the fresh beans, limiting their acceptability. A study was undertaken to investigate changes in nutritionally relevant physicochemical properties of beans during storage. Chemical analysis showed that storage resulted in a decrease in pH and an increase in titratable acidity in all varieties. Total polyphenol, non-tannin polyphenol, tannin, and lignin contents were determined; storage-induced HTC beans contained lower levels of total polyphenols (especially, non-tannin polyphenols) than the fresh beans but higher levels of tannins in all cultivars. Significant increases in lignin and lignified protein were also detected and accompanied by increases in cooking time. A reduction in phytic acid was also observed. These results are discussed in relation to the mechanisms which may underlie the development of the HTC defect.

Keywords: *Beans; hard-to-cook; phenolic compounds; storage*

INTRODUCTION

Dry beans of *Phaseolus vulgaris* are important food sources, especially in developing countries where they are a major source of dietary protein. Phaseolus beans are a good source of proteins, vitamins, and certain minerals (Ca, Fe, Cu, Zn, P, K, and Mg). They have beneficial effects on human health, being an excellent source of complex carbohydrates and polyunsaturated fatty acids. However, in tropical countries, storage of common beans under adverse conditions of high temperature and high humidity renders them susceptible to a hardening phenomenon, also known as the hard-to-cook (HTC) defect. Beans with this defect are characterized by extended cooking times (Jones and Boulter, 1983; Vindiola et al., 1986), are less acceptable to the consumer (Burr et al., 1968), and are of lower nutritive value (Sievwright and Shipe, 1986). In addition, in the raw state, common beans contain antinutritional and toxic substances such as polyphenols, phytates, enzymatic inhibitors, and lectins (Gupta, 1987; Liener, 1989) which limit their acceptability and should be removed or eliminated for effective utilization (Pusztai and Palmer, 1977).

At present, most studies carried out in extended storage at high temperature and high humidity have been directed to highlight the postharvest physiological changes in several physical and chemical components in relation to cooking times of legume seeds (Stanley and Aguilera, 1985; Plhak et al., 1989; Hentges et al., 1991). However, little work has been done to determine the changes in antinutrient compounds in beans in relation to the development of the HTC phenomenon. It has been reported (Martin-Cabrejas et al., 1995) that fresh and HTC beans contained nutritionally significant amounts of lectins and trypsin and α-amylase inhibitors; HTC samples had higher levels of lectin and lower levels α-amylase inhibitor, while the amounts of trypsin and chymotrypsin inhibitors were the same.

In recent years, concern over nutritionally harmful effects of certain biological compounds such as phytate and phenolics has increased (Bjorck and Nyman, 1987). These compounds occur naturally in the seeds of cereals and legumes and, if present in appreciable quantities, can lower the nutritional value and biological availability of dietary proteins and minerals. Phytic acid reduces the availability of minerals by forming complexes (Hallberg and Sölvell, 1967; Nävert et al., 1985). Polyphenols are known to inhibit the activity of proteolytic enzymes, hence reducing protein digestibility; recently attention has been paid to the role of these antinutrients in affecting the availability of carbohydrates for enzymic digestion (Björck and Nyman, 1987). However, there is little information regarding the effect of long-term storage on the changes in the different types of phenolics (total polyphenols, non-tannin polyphenols, tannins, and lignin).

The main objective of the present work was to determine the effects of the HTC phenomenon on several physicochemical parameters and, especially, phenolic constituents and phytic acid of seed bean varieties typically grown and stored in Kenya, under ambient environmental conditions.

MATERIALS AND METHODS

Bean Samples. Dry beans (*P. vulgaris)* of five different cultivars, namely, Mwitemania, Canadian Wonder, Mwezi Moja, Rose Coco, and Red Haricot, were supplied by the

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S0021-8561(97)00069-1 CCC: $14.00

Table 1. Physical Properties, Moisture Content, and Cooking Characteristics of Fresh and Stored Beans[a]

cultivar	type	100-seed wt (g)	seed vol (mL/seed)	seed density (g mL^{-1})	moisture (%)	cooking time (min)
Mwitemania	fresh	44.4 ± 0.8^a	0.34 ± 0.03^a	1.24 ± 0.06^a	5.0	15 ± 1^a
	HTC	35.6 ± 1.2^a	0.28 ± 0.04^b	1.21 ± 0.05^a	5.2	75 ± 5^b
Canadian Wonder	fresh	34.0 ± 0.9^a	0.33 ± 0.03^a	1.23 ± 0.04^a	5.7	25 ± 3^a
	HTC	39.2 ± 1.0^a	0.26 ± 0.02^b	1.35 ± 0.02^b	4.8	$>300 \pm 15^b$
Mwezi Moja	fresh	50.0 ± 0.5^a	0.37 ± 0.05^a	1.30 ± 0.03^a	5.0	10 ± 1^a
	HTC	36.0 ± 1.2^b	0.30 ± 0.04^b	1.34 ± 0.01^a	4.2	120 ± 9^b
Rose Coco	fresh	47.6 ± 0.8^a	0.43 ± 0.03^a	1.28 ± 0.02^a	6.2	20 ± 2^a
	HTC	46.4 ± 1.1^a	0.37 ± 0.02^b	1.30 ± 0.03^a	4.7	135 ± 10^b
Red Haricot	fresh	20.0 ± 0.2^a	0.26 ± 0.03^a	1.34 ± 0.01^a	4.9	20 ± 2^a
	HTC	31.2 ± 0.6^b	0.14 ± 0.02^b	1.31 ± 0.02^b	5.0	75 ± 6^b

[a] Different superscript letters within a column indicate statistically significant differences ($p \leq 0.05$) for each variety.

Kenyan Agricultural Research Institute (Nairobi, Kenya). There were two batches from each cultivar: one consisted of fresh beans just harvested in 1994, and the other was obtained from the National Cereal Board Stores (Kenya), harvested 5 years ago. The latter batches of seeds identified as exhibiting the HTC defect by having increased cooking times or not cooking at all were stored in tropical conditions (30−40 °C, 75% humidity). These varieties are very common in Kenya.

Cooking Time. Prior to cooking, seed samples ($n = 50$) were soaked for 16 h in ultrahigh purity water. A Mattson-type cooker (Downie et al., 1996) was used to determine cooking time of the seeds. Cooking time was reported as time taken to cook 50% of the seeds (median cooking time).

Physical Analysis. One hundred randomly selected seeds of each cultivar of beans were weighed separately and recorded as 100-seed weight. In a test cylinder containing water, 20 previously weighed seeds were immersed, and the amount of water displaced was recorded as volume of seeds (mL). Seed density was calculated from the values obtained for weight (g) and volume (g/mL) (Giami and Okwechime, 1993).

The water absorption was determined by soaking 10 g of whole seed beans at room temperature (25 °C) in a distilled water ratio of 1:5. After 12 h the beans were removed from the soaking water, drained, surface-dried with filter paper, and reweighed. Gain in weight was taken as the amount of water absorbed and expressed as a percentage of initial dry weight of the beans.

Chemical Analysis. Proximate composition was determined by AOAC (1984) methods in triplicate on raw material and expressed on a dry weight basis. Moisture (method 14003) was determined by vacuum drying the sample at 70 °C, crude protein (method 2055) by the micro-Kjeldahl method (N × 6.25), and ash (method 14006) by combustion for 5 h at 525 °C. Carbohydrate content was determined by difference.

pH and titratable acidity were measured using AOAC methods 14022 and 22058 (AOAC, 1984), respectively. pH was measured on a slurry prepared with 10 g of bean flour in 40 mL of boiled, deionized water. Acidity was measured on the same slurry by titration to pH 8.1 with standardized titrant and calculated as g kg^{-1} acetic acid.

Determination of Phenolic Constituents. Total polyphenols and non-tannin polyphenols were determined with the Prussian blue method (Price and Butler, 1977); tannin contents were determined according to the modified vanillin assay proposed by Price et al. (1978) using a Sephadex LH-20 resin which allows to recover 90% of the original material. Phenolic compounds were extracted with 0.3 N HCl in methanol as described by Carmona et al. (1991). All reagents were of analytical grade except where otherwise stated.

The residue obtained after the phenolic compound extraction was sequentially hydrolyzed with 12 M H_2SO_4 for 3 h at 20 °C, being afterwards diluted to 1 M for 2.5 h at 100 °C, according to Selvendran et al. (1979). The insoluble material from hydrolysis was recovered quantitatively on a glass filter (Pyrex no. 2), washed through with hot water, dried for 18 h at 105 °C, and determined gravimetrically as Klason lignin.

Determination of Phytic Acid. Phytic acid was determined by the method of Latta and Eskin (1980) with modifications Reyes-Moreno et al. (1994). The samples were extracted by placing 2 g of bean flour in a 50 × 125 mm screwtop tube to which 40 mL of 0.67 N HCl was added. Tubes were capped tightly and shaken for 3 h at 20 °C. After filtration (GF-A, Whatman), an extra filtration through Sep-Pak filters (c-18, Waters) was carried out. The supernatant was diluted with MilliQ water in a 1:25 ratio; 10 mL of supernatant was added onto a prepared ion exchange column containing 0.5 g of 200−400 mesh AG1-X8 chloride anion exhange resin (Biorad). Inorganic phosphorus and impurities were eluted with 30 mL of 0.05 M NaCl. Phytate was eluted with 30 mL of 0.7 M NaCl. To each tube containing 3 mL of eluant was added 1 mL of Wade reagent. The tubes were covered with parafilm and mixed. After centrifugation (5000*g*), the absorbance was measured at 499.7 nm.

Statistical Analysis. All analyses were performed in triplicate. Results were analyzed by the Statistical Analysis System program (SAS, 1985). When effects of storage conditions were shown, the results for each parameter of each variety were evaluated further using Duncan's test. Differences were considered significant at ≤0.05.

RESULTS AND DISCUSSION

The physical data for the bean cultivars are presented in Table 1. The weight of 100 seeds ranges from 20.0 to 50.0 g, similar to other African bean cultivars, although far higher compared to other African grain seeds (Giami and Okwechime, 1993). Seed volume and density are lower compared to other legumes (Giami and Okwechime, 1993). All the HTC beans exhibit a significantly lower volume compared to fresh ones.

The extent of the effect of storage temperature and humidity on cooking time varied with the cultivar, but all stored beans required prolonged cooking times compared to the freshly harvested beans. Storage resulted in more than a 12-fold increase in cooking time for Canadian Wonder and Mwezi Moja in the worst cases, demonstrating the negative effect of the HTC phenomenon (Hentges et al., 1991).

The differences in chemical compounds of fresh and stored beans are shown in Table 2. The crude protein content of beans ranged from 18.2% to 23.3% of the seed meal. Stored beans generally had a slightly higher protein content. The pH and acidity of beans were also affected; all beans exhibited a neutral or slight acidic pH (5.8−6.4), and stored beans demonstrated a lower pH in all the cultivars studied. It is believed that the low pH of aged beans is a cause of both reduced pectin loss and increased seed hardness (Liu et al., 1992). Interestingly, the greatest pH decrease (8.9%) was observed for Canadian Wonder variety which showed the most accentuated HTC defect. This observation is consistent with the possibility that acidification during storage leads to textural defects and might also suggest that tissue pH can be a convenient indicator of seed HTC defect induced by adverse storage. Hydrolysis of lipids into fatty acids, oxidation of these acids into organic acids, and other biological processes might also contribute to a pH decrease during adverse storage (Saio

Table 2. Effect of Storage on Proximate Composition, pH, and Acidity of Fresh and Stored Beans

cultivar	type	crude protein (%N × 6.25)	ash (%)	pH	acidity (g kg^{-1} of acetic acid)	carbohydrate[a]
Mwitemania	fresh	18.2 ± 0.1	4.2 ± 0.01	6.30 ± 0.02	8.56 ± 0.08	70.6
	HTC	18.5 ± 0.8	4.4 ± 0.02	5.86 ± 0.01	11.68 ± 0.04	68.4
Canadian Wonder	fresh	19.8 ± 0.1	4.4 ± 0.01	6.42 ± 0.03	8.20 ± 0.08	69.3
	HTC	21.4 ± 0.4	4.8 ± 0.01	5.85 ± 0.01	13.64 ± 0.04	62.9
Mwezi Moja	fresh	18.2 ± 0.1	4.6 ± 0.01	6.41 ± 0.01	9.63 ± 0.12	70.7
	HTC	19.9 ± 0.1	4.0 ± 0.01	6.02 ± 0.02	11.64 ± 0.08	66.5
Rose Coco	fresh	20.2 ± 0.1	4.3 ± 0.01	6.36 ± 0.01	8.20 ± 0.04	69.1
	HTC	23.3 ± 0.1	4.4 ± 0.01	5.93 ± 0.01	12.64 ± 0.08	58.6
Red Haricot	fresh	18.5 ± 0.1	4.7 ± 0.01	6.30 ± 0.02	9.36 ± 0.04	68.2
	HTC	22.5 ± 0.6	5.1 ± 0.02	6.16 ± 0.01	12.64 ± 0.08	59.6

[a] Determined by difference: 100 − protein − ash − lignin − total polyphenols.

Table 3. Influence of Storage on Phenolic Compounds in Beans[a]

cultivar	type	total polyphenols (mg of gallic g^{-1})	non-tannin polyphenols (mg of gallic g^{-1})	tannins (mg of catechin g^{-1})
Mwitemania	fresh	3.20 ± 0.20^{a}	1.46 ± 0.01^{a}	nd
	HTC	2.55 ± 0.04^{b}	1.24 ± 0.02^{b}	1.28 ± 0.02
Canadian Wonder	fresh	3.73 ± 0.03^{a}	1.90 ± 0.02^{a}	0.03 ± 0.01^{a}
	HTC	2.95 ± 0.03^{b}	1.33 ± 0.02^{b}	0.49 ± 0.03^{b}
Mwezi Moja	fresh	2.66 ± 0.02^{a}	1.49 ± 0.08^{a}	0.07 ± 0.01^{a}
	HTC	2.34 ± 0.01^{b}	0.85 ± 0.04^{b}	0.64 ± 0.07^{b}
Rose Coco	fresh	2.90 ± 0.04^{a}	2.09 ± 0.04^{a}	0.48 ± 0.08^{a}
	HTC	2.92 ± 0.04^{a}	1.88 ± 0.05^{b}	1.42 ± 0.02^{b}
Red Haricot	fresh	4.14 ± 0.06^{a}	2.33 ± 0.06^{a}	0.42 ± 0.07^{a}
	HTC	3.66 ± 0.06^{b}	1.27 ± 0.01^{b}	1.56 ± 0.04^{b}

[a] Different superscript letters within a column indicate statistically significant differences ($p \leq 0.05$) for each variety.

et al., 1980; Thomas et al., 1989). As pH decreased, titratable acidity increased for all stored samples (Table 2). Values ranged from 8.2 to 13.6 g of acetic acid/kg, the highest value being for HTC Canadian Wonder beans as compared to fresh beans. A similar increase in acidity during storage was also reported in soy beans (Thomas et al., 1989). Carbohydrate content was significantly less in the HTC beans, and this results in changes to the dietary fiber levels (Martin-Cabrejas et al., 1997, in preparation).

The deleterious changes which occur during postharvest storage of legumes, particularly hardening, have been associated with reactions of polyphenolic substances (Bressani and Elias, 1980). Recently, polyphenolic compounds in many edible plant products have received increasing attention as a result of their influence on the nutritional and aesthetic quality of foods and biochemical and physiological functional properties (Salunkhe et al., 1981). Regarding total polyphenols, Table 3 shows significant levels of total polyphenols in all beans. HTC beans exhibit lower polyphenol contents compared with the freshly harvested beans in nearly all cultivars studied. The HTC-related decreases within cultivars range from 11.4% to 38.4% on a dry basis except Rose Coco cultivar in which no appreciable changes were evident. These results complement those found by Hincks and Stanley (1986).

The percentage of non-tannin polyphenols as a function of total polyphenols varies from 45.6% to 72.1%. Lower proportions were observed in all HTC beans. Such storage-related differences have also been found during fruit maturation (Goldstein and Swain, 1963) and seed development in sorghum (Chavan et al., 1979). The HTC-related reduction in total polyphenols and non-tannin polyphenols is probably due to polymerization of existing phenolic compounds, resulting in insoluble, high molecular weight polymers (Kadam et al., 1982); it is known that precipitation of polyphenol−protein complexes occurs more readily at elevated temperatures (Clarkson et al., 1992). However, the possibility of binding of polyphenols that renders them incapable of giving a chemical color reaction measured by the blue Prussian method used in the present investigation cannot be ruled out.

Table 4. Changes in Lignin and Lignin−Protein Complexes of Seed Beans during Adverse Storage[a]

cultivar	type	lignin (%)	crude protein in lignin (%N × 6.25)
Mwitemania	fresh	6.7 ± 0.01^{a}	3.0 ± 0.1^{a}
	HTC	8.4 ± 0.05^{b}	3.1 ± 0.1^{a}
Canadian Wonder	fresh	6.1 ± 0.03^{a}	2.4 ± 0.1^{a}
	HTC	10.6 ± 0.13^{b}	3.4 ± 0.3^{b}
Mwezi Moja	fresh	6.2 ± 0.01^{a}	3.1 ± 0.2^{a}
	HTC	9.2 ± 0.04^{b}	3.2 ± 0.3^{a}
Rose Coco	fresh	6.1 ± 0.01^{a}	2.3 ± 0.5^{a}
	HTC	13.4 ± 0.22^{b}	4.0 ± 0.1^{b}
Red Haricot	fresh	8.2 ± 0.02^{a}	2.7 ± 0.1^{a}
	HTC	12.1 ± 0.03^{b}	3.9 ± 0.1^{b}

[a] Different superscript letters within a column indicate statistically significant diffences ($p \leq 0.05$).

Tannin contents ranged from 0.03 to 1.56 mg of catechin/g (Table 3). This is similar to that observed by Desphande et al. (1982) and Barampama and Simard (1994). In general, long-term bean storage tended to substantially increase tannins by between 11.8% and 49.9% except for the Mwitemania variety which is even more. It is possible that these compounds are derived from small molecular weight non-tannin material, indicating postharvest biochemical activity during storage. Such activity might include that of peroxidase enzymes (Fry, 1986), which may be active in the moist tissues which result from storage in high humidity and high temperatures. Such activity would help to explain the decrease of total polyphenols and especially non-tannin polyphenols.

Klason lignin content was higher in stored beans than in fresh in all the varieties studied (Table 4). Values range from 8.4% to 13.4% on a dry matter basis for beans which showed HTC defect and between 6.1% and 8.2% on a dry matter basis for fresh beans. The increases are significant for all cultivars ($p \leq 0.05$), ranging from 26% for Mwitemania cultivar to 120% for

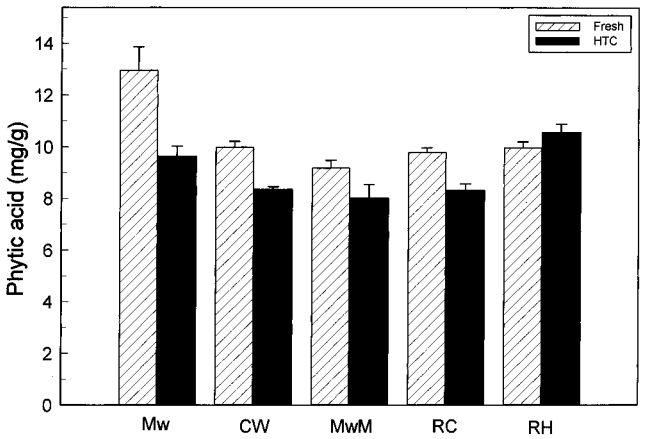

Figure 1. Effect of the HTC on phytic acid content in bean seeds. Beans cultivars: Mw, Mwitemania; CW, Canadian Wonder; MwM, Mwezi Moja; RC, Rose Coco; RH, Red Haricot.

Rose Coco cultivar. These results are in agreement with the lignification hypothesis suggested by Varriano-Marston and Jackson (1981) and the qualitative studies by Hincks and Stanley (1987) during the development of the HTC defect. It has been reported that storage conditions favoring development of the HTC defect produce significant increases in non-N-proteins and free aromatic amino acids in beans (Hohlberg and Stanley, 1987; Martin-Cabrejas et al., 1995). Aromatic amino acids such as phenylalanine and tyrosine are immediate precursors of hydroxycinnamic acids (C6−C3) which are the building blocks of lignin.

In addition, the stored beans exhibit higher insoluble N-protein associated with Klason lignin (3.0−4.0% on a dry matter basis) compared with the freshly harvest beans (2.4−3.1% on a dry matter basis) for all the cultivars studied. Both Klason lignin and lignified protein contents were generally correlated with increased cooking time ($r^2 = 0.50$ and 0.48, respectively), complementing the results of Molina et al. (1975) and Mafuleka et al. (1993). However, Srisuma et al. (1989) associated the development of the HTC defect with large increases in free hydroxycinnamic acids and did not detect significant changes in lignin content; this may be attributable to the differences in the methods employed for the extraction and determination of the lignin (thioglycolic method). More research needs to be conducted to develop better quantitative methods to define and determine lignin.

Adverse storage had a significant effect on the phytic acid (PA) content (Figure 1). Phytic acid contents of bean flours ranged from 12.9 to 8.0 mg/g of beans and were similar to the range of reported values for several legumes (Jones and Boulter, 1983; Bernal-Lugo et al., 1990; Reyes-Moreno et al., 1994; Bhatty, 1995). Comparing cultivars, Mwitemania beans showed 36% more phytic acid than the others. The levels of PA decreased significantly ($p < 0.05$) as the seeds stored for all cultivars, except in the case of Red Haricot variety. The differences in PA however do not appear to correlate with the extent of hardening, suggesting that changes in PA do not necessarily underlie the susceptibility of bean to hardening. This is consistent with the results of other works that have suggested that the decrease of PA content is not continuous during storage but may be important in the first stages of the hard-to-cook defect of beans (Hentges et al., 1991; Mafuleka et al., 1993).

In conclusion, the five varieties of common beans examined in this study with or without long periods of storage showed differences in the physical and chemical components, such as decreased tissue pH. The fractionation of the polyphenols has been investigated, and the results highlight a significant storage-related reduction in total polyphenol and non-tannin polyphenol contents. These changes may indicate postharvest physiological activity; formation of tannins and lignin (the oxidation and polymerization of polyphenolic compounds) within bean seeds may be the result of a stress response initiated by adverse conditions, could be responsible, at least in part, for the hard-to-cook condition that develops in common beans during extended storage, and may limit the availability of nutrients through their protein-precipitating action.

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Received for review January 27, 1997. Accepted May 28, 1997.[⊗] We thank the European Communities for their financial support (STD-3 Project TS3-CT92-0085). This work was also partly financed by the U.K. Office of Science and Technology to Dr. Waldron.

JF970069Z

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.