

# Textural and microstructural changes associated with post-harvest hardening of trifoliate yam (*Dioscorea dumetorum*) pax tubers

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Received 22 May 2001; received in revised form 3 September 2001; accepted 3 September 2001

## Abstract

The textural and microstructural changes associated with the post-harvest hardening phenomenon of *Dioscorea dumetorum* tubers were studied to determine how texture relates to microstructure during the hardening process. A 2×2×4 factorial experiment with sample treatment, storage conditions and storage time as variables was performed. The tubers were harvested, matured and stored under prevailing tropical ambient conditions (28 °C) and cold room conditions (4 °C) for the study. All the samples were evaluated for their hardness (peak force) and adhesiveness (curve areas). Starch microscopy was done on the freshly harvested tubers, hardened tubers and tubers stored under low temperature conditions (4 °C) during the study. TA.XT2 analyses on the textural properties revealed consistent increases in the hardness and adhesiveness levels, measured during the hardening process of the tubers. Hardness levels increased markedly to significant levels ( $P < 0.05$ ) in the tubers (from 1208.4 g to 7801.6 g). Similarly, adhesiveness levels increased from 12.5462 to 30.2218 g m in the tubers within 72 h after harvest. Starch microscopy revealed variations in the cell microstructure of the hardened and unhardened tubers. Hardening of *D. dumetorum* was characterized by thickened cell walls and middle lamella in the cells of the tuber. The hardening varied according tuber treatment and temperature of storage. Several treatments and storage methods were tested and those that limited the contact of the tubers with the external environment were found to slow down the hardening process. © 2002 Elsevier Science Ltd. All rights reserved.

**Keywords:** *Dioscorea dumetorum*; Textural changes; Microstructural changes; Post-harvest hardening

## 1. Introduction

The textural characteristics of yams are very important because texture is one of the three main acceptability factors used by consumers to evaluate the quality of the yam tuber. The other two factors are appearance and flavour (Bourne, 1990). Food processors place a lot of weight on developing products that have the textural properties desired by consumers. Texture is important because the human masticatory apparatus has evolved to cope with particular types of mechanical properties and certain traditional foods are recognized by their mechanical properties (Lillford, 1991). Yams can therefore be accepted or rejected on the basis of their texture.

Food texture can be perceived as a direct consequence of its microstructure, which in turn is determined by the constituent chemical composition and the physical

forces binding it. Consequently, a knowledge of microstructure can be an antecedent to any plan aimed at either manipulation or regulation of texture (van Marle, Stolle-Smits, Donkers, van Dijk, Voragen, & Recourt, 1997). Microstructural observations and changes associated with its perturbations of composition and physical forces are reportedly directly related to texture (Stanley & Tung, 1976).

*Dioscorea dumetorum* originated from Africa and its cultivation is restricted to West and East Africa. It is an important source of starch for the energy requirements of the indigenous populations and it possesses a relatively high protein content and a favourable nitrogen/energy balance (Afoakwa & Sefa-Dedeh, 2001; Coursey, 1983). The storage ability of this yam is, however, restricted by a severe post-harvest hardening phenomenon of the tubers, which begins within 24 h after harvest and renders them unsuitable for human consumption. Therefore, only freshly harvested tubers can be consumed locally and technological transformation of

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*D. dumetorum* must be carried out promptly after harvest. Due to this handicap, the tubers are cooked after harvesting and sold to consumers. Initial studies (Afoakwa, 1999; Afoakwa & Sefa-Dedeh, 2002) revealed that the hardening of the tubers is characterized by lignification of the yam tissues and a consequential increase in fibre content, which consists of lignin, cellulose and hemicellulose.

The purpose of this work was to study how the microstructure of *D. dumetorum* starch granules relates to texture during the post-harvest hardening phenomenon of the tubers.

## 2. Material and methods

### 2.1. Materials

The white cultivars of trifoliolate yam (*D. dumetorum*) tubers were randomly harvested (matured) from a farm at Obomeng in the Eastern Region of Ghana, washed thoroughly with water, kept under ice-packed containers and transported immediately (within 3 h after harvest) to the laboratory.

At the laboratory, the tubers were divided into two main groups. One group of tubers was chopped into pieces of 5 cm thick and 5 cm diameter whilst the second group remained as whole tubers. Each of these two groups obtained were further sub-divided into two, one part of which was stored under prevailing tropical ambient conditions (28 °C), whilst the second was stored under cold room conditions (4 °C), for a period of 0, 24, 48 and 72 h. No growth of mould was observed on either the treated or untreated tubers during the storage period. During storage, the hardening of *D. dumetorum* was characterized, not only by a visible hardness, but by a rough and fluffy tuber surface as opposed to the smooth and moist surface of the freshly harvested tubers.

#### 2.1.1. Experimental design

A 2×2×4 factorial experimental design was used and the principal factors were:

1. sample treatment: whole tubers (Whole) and chopped tubers (Chopped);
2. storage condition: tropical ambient (28 °C) and cold room (4 °C) temperatures; and
3. storage period: 0, 24, 48 and 72 h.

### 2.2. Textural analysis

The tuber samples of sizes 15 cm diameter and 50 cm length, were cooked in boiling water (100 °C) for 1 h on a hot plate and made to cool completely at room temperature (28 °C). The cooked tubers were cut into slices

of 1 cm thick and 5 cm diameter, and evaluated using Warner-Blatzler test cell in a TA.XT2 Texture Analyzer (Stable Micro Systems, Halmere, Surrey, England). The following test parameters were used: pre-test speed, 10 mm/s; test speed, 5.0 mm/s; post-test speed, 10 mm/s; distance, 20 mm. The peak force required to cut completely through the slices as well as the area under the curve were recorded. Determinations were done in triplicate.

### 2.3. Microstructural studies

A formol-saline fixative was prepared according to the procedure described by Mahoney (1973). Cut tissues of dimensions 7.5×5×5 mm were sectioned, using a dissection blade from the middle portion of hardened and unhardened *D. dumetorum* tubers. Fixation was carried out by placing the cut tissues in the formol-saline solution for 24 h, washed with distilled water and dehydrated through a graded series of aqueous ethanol. The dehydrated tissues were cleared in antemedial (toluene) for 2 h and embedded in a paraffin medium. Specimens of a thickness around 7 µm were sectioned using a laboratory scale sledge microtome (Erma Inc. Tokyo, Japan) and made to fix in an albumen solution on a slide.

The fixed tissues on the slide were immersed in xylene to remove the wax and then passed through series of aqueous ethanol. Staining was done following the procedure of Fowell (1962). The samples were stained in safranin for 10 min, washed again in the series of ethanol and counter stained in fast green for 1 min. Clearing was done using clove oil and the specimens were mounted in Euparal (Flatters and Garvett Ltd., England). Examination of starch granules were done using TMS-F Light Microscope (Nikon Co., Tokyo, Japan) and micrographs taken.

### 2.4. Statistical analyses

The data obtained from the textural analyses were statistically analyzed using Statgraphics (Graphics Software System, STCC, Inc. USA). Comparisons between sample treatments and the indices were done using analysis of variance (ANOVA) and significance of difference was defined at  $P \leq 0.05$ .

## 3. Results and discussion

### 3.1. Changes in texture

The TA.XT2 Texture Analyzer was used for the measurement of the textural properties of the *D. dumetorum* tubers after harvesting. Measurements were made from the cooked samples of the freshly harvested tubers and after every 24 h in storage for a period of 72 h, to

find out the changes occurring in the textural properties of the tubers after harvesting. Peak forces of the curves generated by the Texture Analyzer were recorded as the hardness of the samples and the area under the curves was also recorded as a measure of the adhesiveness.

Hardness and adhesiveness levels of the *D. dumetorum* tubers, as determined by the Warner-Bratzler blade, increased significantly in the samples stored under both storage conditions during the storage period (Figs. 1 and 2). Both the whole tubers and tubers chopped prior to storage increased consistently with storage time. However, the chopped tubers showed comparatively higher levels of hardness and adhesiveness during the period of storage. Storage caused increases in the hardness levels of the chopped tubers from 1208.4 to 7801.6 g within 72 h after harvest while the whole tubers increased from 1208.4 to 5921.9 g during the 72 h of storage under ambient conditions. Similarly, the adhesiveness levels of the tubers increased significantly ( $P < 0.05$ ) from 12.5462 to 30.2218 g m in the chopped tubers and from 12.5462 to 25.6583 g m for the whole tubers within 72 h after harvest when stored under ambient conditions. These high levels of hardness and

adhesiveness, observed in the tubers chopped into pieces after harvest, are probably due to the increased surface area exposed to the external environment, which might facilitate chemical reactions, as well as physical forces, binding the tuber cells together and leading to the hardening phenomenon in the tubers after harvest. The consistent increases in the hardness and adhesiveness of the tubers during storage results from the rapid hardening of *D. dumetorum* tubers after harvesting which might be due to increases in cell wall stiffness and subsequent strengthening of cell wall bondings during storage of the tubers.

Low temperature storage of the samples was observed to decrease the rate of hardening of the tubers during storage compared to the tubers stored under tropical ambient conditions (Figs. 1 and 2). This suggests that the hardening of *D. dumetorum* tubers after harvest is temperature dependent and that low temperature storage can be used to effectively prolong the hardening process after harvesting.

ANOVA on the data indicated that the sample treatment, storage condition and storage time significantly affected ( $P < 0.05$ ) the hardness (peak forces) and

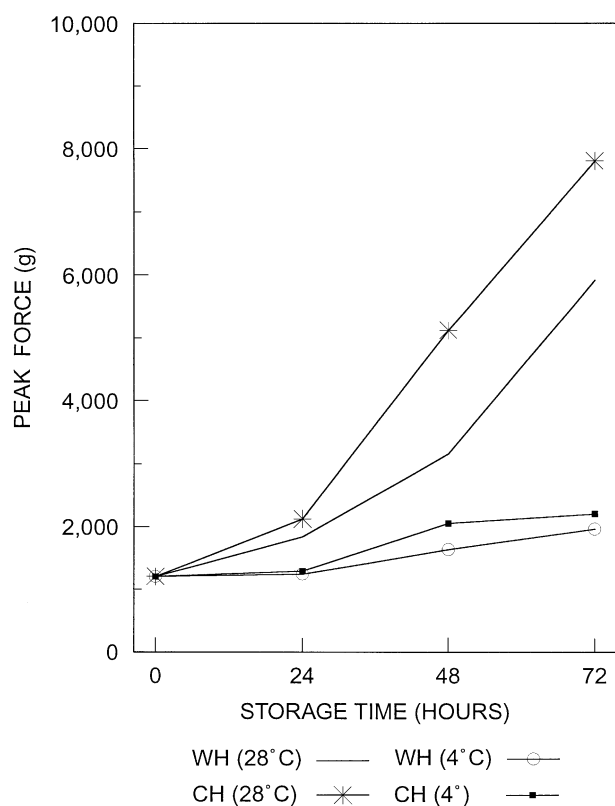


Fig. 1. Changes in hardness (peak force) of *Dioscorea dumetorum* tubers during hardening. WH (28 °C), whole tubers kept under tropical ambient conditions; CH (28 °C), tubers chopped into pieces and kept under tropical ambient conditions; WH (4 °C), whole tubers kept under cold room conditions; CH (4 °C), tubers chopped into pieces and kept under cold room conditions.

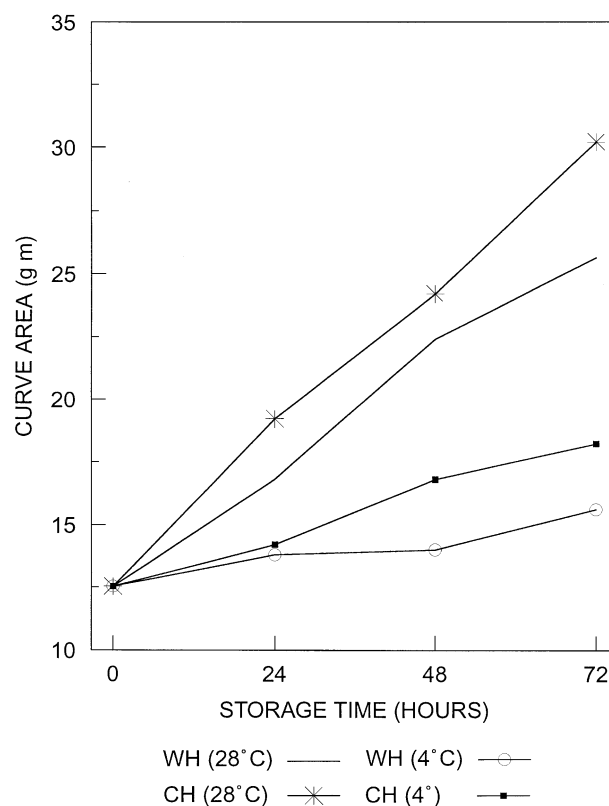


Fig. 2. Changes in adhesiveness (curve areas) of *Disocorea dumetorum* tubers during hardening. WH (28 °C), whole tubers kept under tropical ambient conditions; CH (28 °C), tubers chopped into pieces and kept under tropical ambient conditions; WH (4 °C), whole tubers kept under cold room conditions; CH (4 °C), tubers chopped into pieces and kept under cold room conditions.

adhesiveness (curve areas) levels of the tuber during storage (Table 1).

### 3.2. Changes in starch microstructure

Starch microstructural studies were carried out, using the light microscope, to determine changes occurring in the starch granules of *D. dumetorum* tubers during the hardening process. The light microscope provides a useful and rapid means of determining microstructural properties of native starch. A micrograph, showing the cells of the freshly harvested *D. dumetorum* tuber (Fig. 3), revealed very thin and flexible polygonal cells. These microscopic examinations showed very pronounced variations in the cells of the tubers with storage time and temperature. Comparison of the micrographs

Table 1

Table of *F*-values for hardness (peak force) and adhesiveness (curve areas) of *Disocorea dumetorum* tubers

Process variables	Hardness (peak force)	Adhesiveness (curve area)
Cultivar	3.828*	5.608*
Tuber treatment	9.874*	9.532*
Storage condition	10.259*	5.098*
Storage time	7.625*	11.997*

\* Significant at  $P < 0.05$ .

between the hardened (Fig. 4) and unhardened *D. dumetorum* tubers (Fig. 3) showed increases in the thickness of the cell walls of the hardened tubers as compared to the very thin cell wall of the unhardened tubers. This may be due to changes in cell wall constituents and middle lamella of the *D. dumetorum* as the tuber hardens. In the case of the unhardened tubers, the cell walls were very thin and reacted with the safranin and thus stained light green (Fig. 3). However, on hardening, the thickened cell walls reacted strongly to both the safranin and fast green stains used during the staining process, and thus stained dark pink, showing lignification and thickening of the cell walls which is one of the characteristic features of the hardening process (Fig. 4). Fig. 5 shows a higher magnification ( $\times 350$ ) of the hardened *D. dumetorum* which clearly shows pronounced increases in the thickness of the middle lamella membranes of the cell wall in the tuber as it hardens. Therefore, the hardening of *D. dumetorum* tubers during storage is brought about by the deposition of additional cell wall constituents, which results in the thickening and lignification of the middle lamella membranes of the cell walls in the tubers, thus bringing about increases in texture during storage. This explains that changes in the microstructure of *D. dumetorum* tubers is directly linked with texture, leading to the hardening of the tubers after harvesting. Work done by Thybo, Martens,

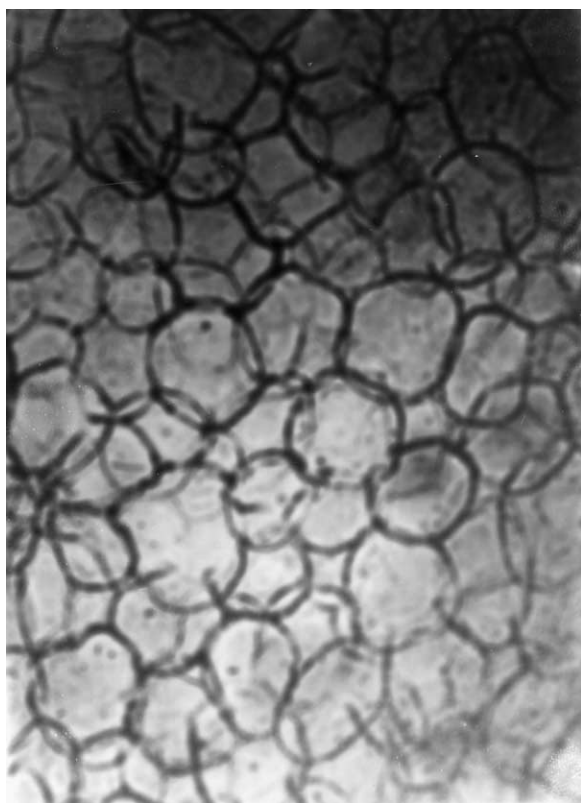


Fig. 3. Micrograph showing starch microstructure of freshly harvested *Disocorea dumetorum* tuber ( $\times 150$ ).

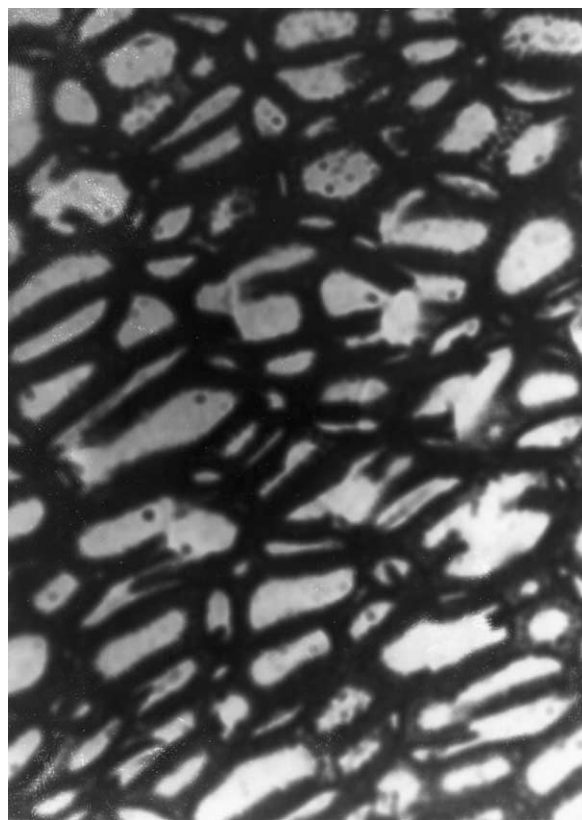


Fig. 4. Micrograph showing starch microstructure of hardened *Disocorea dumetorum* tuber ( $\times 150$ ).

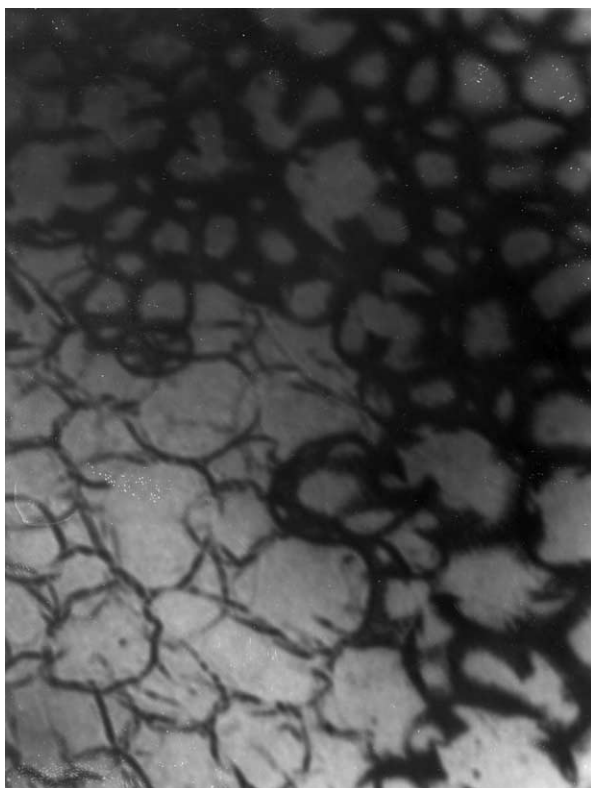


Fig. 5. Micrograph showing starch microstructure of hardened *Disocorea dumetorum* tuber ( $\times 350$ ).

and Lyshede (1998) reported that the textural properties and microstructure of potatoes are directly affected by processing conditions. The textural attributes declined with increasing cooking time while cell separation and disintegration of tissue structure made the cooked tubers less firm. Several studies have been carried out on potato tuber structure during processing (Huang, Weber, Purchell, & Huber, 1990; McComber, Horner, Chamberlin, & Cox, 1997; van Marle et al., 1997), using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). They suggested that differences in starch grains could be related to different textural attributes in foods.

Storage of the tubers at low temperatures ( $4\text{ }^{\circ}\text{C}$ ) leads to the slowing down of the hardening process. The samples stored under cold room conditions ( $4\text{ }^{\circ}\text{C}$ ) showed a section with normal cell walls and a section with thickened cell walls (Fig. 6). This suggests that the changes in starch microstructure of the tubers after harvesting are temperature-dependent. Low temperature storage of *D. dumetorum* tubers can therefore be used to effectively minimize the rate of thickening and lignification of the cell walls after harvesting.

The results therefore suggest that changes in microstructure of *D. dumetorum* tubers after harvest influence the post-harvest changes in textural properties (hardness and adhesiveness) of the tubers, leading to the hardening phenomenon. This explains that changes in the

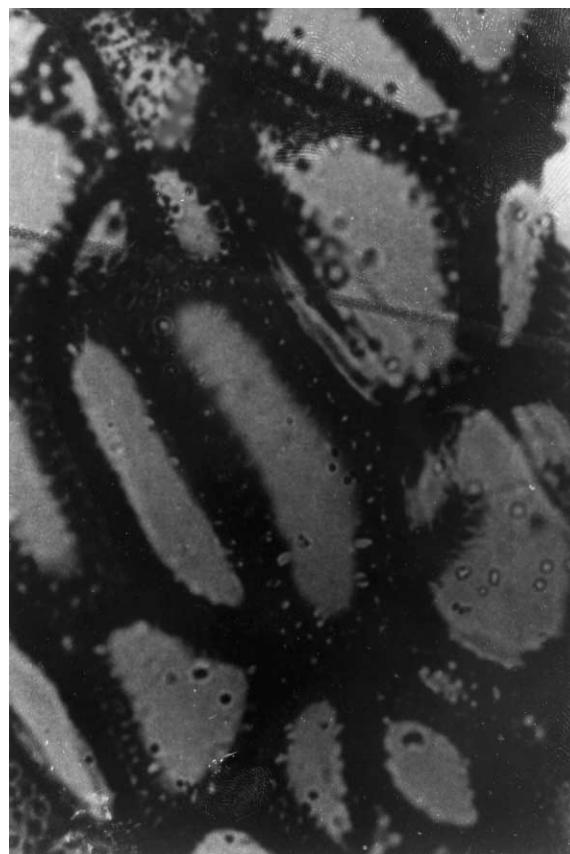


Fig. 6. Micrograph of starch microstructure of *Disocorea dumetorum* tuber stored under low temperature conditions, showing both hardened and unhardened sections ( $\times 150$ ).

microstructure of *D. dumetorum* tubers are directly linked with their textural properties during the post-harvest hardening of the tubers.

#### 4. Conclusions

The levels of hardness and adhesiveness of *D. dumetorum* tubers increase tremendously after harvesting, leading to the hardening of the tubers. However, the treatment of size reduction of *D. dumetorum* tubers after harvesting influences the hardening process as the tubers kept whole during the study showed a comparatively reduced texture. This means that increasing the surface area of the tubers exposed to the external environment, after harvest, enhances the hardening process. Variation in starch microstructure of the *D. dumetorum* tubers occurs after harvesting, which leads to the hardening of the tubers. The walls of the starch granules thicken considerably with storage time, leading to lignification and thickening of the tuber membranes, and thus increasing the hardness of the tuber. This indicates that changes in microstructure of *D. dumetorum* tubers influence the textural properties (hardness and adhesiveness) of the tubers after harvest, leading to the

hardening phenomenon. Low temperature (4 °C) storage of the tubers, however, can effectively be used to minimize the rate of post-harvest hardening of the tubers.

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