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Changes in rheological properties and amylase activities of trifoliate yam, *Dioscorea dumetorum*, starch after harvest

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Abstract

Changes in rheological properties and amylase activities occurring in trifoliate yam, *Dioscorea dumetorum*, starch after harvest were investigated. Trifoliate yam tubers were harvested and stored under tropical ambient (28 °C) and cold room conditions (4 °C) for 12, 24 and 36 h. The *D. dumetorum* starches were extracted from the tubers under study and samples were evaluated for changes in their rheological properties (paste characteristics) during storage and to study the action of amylases on *D. dumetorum* starch after harvesting. The post-harvest activities of α and β-amylases were also studied to evaluate their rate of action on *D. dumetorum* starch. Storage caused decreases in the rheological properties (paste characteristics) of the tubers within 36 h of harvest. Similarly, α and β-amylase activities in the tubers more than doubled within 24 h after harvesting. Blanching, however, effectively decreased the action of amylases on *D. dumetorum* starch during storage, with subsequential increase in the paste viscosities of the stored tubers. Low temperature storage of the tubers also slowed down the rates of decrease in both the rheological properties and amylase activities during the 36-h storage period. © 2002 Published by Elsevier Science Ltd.

Keywords: *Dioscorea dumetorum*; Post-harvest changes; Rheological properties; Amylase activities; Starch paste

1. Introduction

Dioscorea dumetorum tubers are characterized by a post-harvest hardening phenomenon that occurs within 24 h after harvest, leading to textural changes which consequently affect the quality and acceptability of the tubers (Afoakwa, 1999). Research conducted on the tubers showed increasing levels of sugars and cell wall polysaccharides constituents and increases in texture during storage of the tubers, with substantial decreases in moisture and starch contents (Afoakwa & Sefa-Dedeh, 2001). This decrease in starch content is suspected to result from the activities of constituent amylases, converting starches into sugars and causing textural changes in the tubers, but this has not been properly justified in the literature.

Starch is composed of semi-crystalline particles comprised of A chains and the exterior B chains of amylopectin (Hizukuli, 1996). The amorphous region consists of free amylose, lipid-complexed amylose (L-AM) and 1,6 branched amylopectin (Morris, 1990). When the starch suspension is heated, the particles absorb water and swell, due to dissolution of amylose molecules and destruction of the crystalline region. According to previous findings (Eliasson, 1986; Fannon & BeMiller, 1992; Morris, 1990), a starch gel is composed of dispersed swollen starch particles embedded in a continuous 3-dimensional network created by dissolved amylose. The concentration, granule size and distribution, extent of swelling, hardness of the particles and state of entanglement, all affect the rheological properties of starch gels.

Many researchers have used rheological methods to study gelatinization in suspensions of a variety of starches (Eliasson, 1986; Svegmark & Hermansson, 1990; Tsai, Li, & Lii, 1997) as well as to determine rheological properties of starch pastes (Evans & Lips, 1992; Reddy, Subramanian, Ali, & Bhattacharya, 1994). It has been shown (Tsai et al., 1997) that, upon heating, the viscosity increases suddenly after a certain temperature is reached and increases to a maximum. Further heating only leads to reduction in viscosity. However, Afoakwa (1999) reported that the effectiveness in reducing the viscosity of yam starches during heating depends largely on the constituent amylases and their potential to

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breakdown polysaccharides in the tubers after harvest. Since changes in rheological properties and amylasic enzyme activities may affect the contribution of starch to texture, which is of considerable importance to the acceptability of *D. dumetorum* tubers, this study was worthy of more indepth justification.

The current study therefore seeks to investigate the changes in rheological properties and amylasic enzyme activities in *D. dumetorum* starch, leading to the hardening phenomenon after harvest.

2. Materials and methods

2.1. Materials

Trifoliate yam, *Dioscorea dumetorum*, tubers were randomly harvested (matured) from a farm at Obomeng in the Eastern Region of Ghana, washed thoroughly with water, kept in ice-packed ice chest containers and transported immediately (within 3 h) to the laboratory for analyses.

2.1.1. Sample preparation for rheological analysis

At the laboratory, the tubers were grouped into three portions. The first group of tubers was blanched in hot water at 100 °C for 15 s, and the second group blanched for 30 s. The third group was left unblanched to serve as a control. The three groups were then stored under tropical ambient temperature conditions (28 °C) for 36 h and samples taken from each group after 0, 12, 24 and 36 h of storage for Brabender Viscoamylograph analyses.

Samples taken after each period of storage were washed, peeled and cut into cubes. Afterwards, 100 ml of CO_2-free distilled water were added to 300 g of freshly cut cubes and blended at high speed for 5 min using a Waring Blender, into a smooth paste. The paste was then subjected to rheological analyses using a Brabender Viscoamylograph. The Brabender Viscoamylograph cycle was monitored and the various viscosity profile indices recorded.

2.1.2. Experimental design

A 3×4 factorial design was used and the principal factors investigated were:

1. *blanching time*: 0, 15 and 30 s; and
2. *storage period*: 0, 12, 24 and 36 h.

2.1.3. Sample preparation for amylases analyses

The tubers for this study were grouped into two, the first group kept under ambient temperature conditions (28 °C) and the second, under cold room temperature conditions (4 °C). The two groups were stored for 36 h and samples taken for analyses after every 0, 12, 24 and 36 h. Samples taken after each defined period of storage were washed, peeled and cut into slices of 0.5 cm thick and 5 cm diameter, deep frozen and freeze-dried. The dried samples were then milled into flour (250 μm) using a hammer mill (Christy and Norris Ltd, England) and packaged in polypropylene bags for amylase enzyme analyses.

2.2. Methods

2.2.1. Rheological properties

The rheological properties of an 8% slurry (dry matter basis), of blended sample were determined by the use of a Brabender Viscoamylograph (Brabender Instrument Inc. Duisburg, West Germany) equipped with a 700 cmg sensitivity cartridge. The weighed sample was then mixed with water and made up to 500 ml in a volumetric flask. The suspension was heated from 25 °C at the rate of 1.5 °C/min to 95 °C, held at this temperature for 30 min, cooled at 1.5 °C/min to 50 °C and held at this temperature for 20 min. The viscosity profile indices recorded include the following; pasting temperature, peak viscosity, viscosity at 95 °C, viscosity after 30 min at 95 °C, viscosity at 50 °C and viscosity after 20 min at 50 °C.

2.2.2. α- and β-amylase activities

The activities of α- and β-amylases of the trifoliate yam samples prepared were determined by the procedure outlined by Marshall and Christian (1978), as modified by Hui (1992). Results of the enzyme activities were expressed as equivalent maltose units liberated per minute.

2.3. Statistical analyses

All the statistical analysis and graphical presentations were done using Statgraphics (Graphics Software System, STCC, Inc. USA) and Lotus Freelance softwares. Comparisons between sample treatments and the indices were done using analysis of variance (ANOVA) with a probability $P \leqslant 0.05$.

3. Results and discussion

3.1. Changes in rheological properties of D. dumetorum starch during storage

The Brabender Viscoamylograms present useful information on the hot and cold paste viscosity characteristics of starch-based foods. The effectiveness in reducing the viscosity of yam starches depends largely on the activities of the constituent amylases and their potential to breakdown polysaccharides in the tubers after harvest. The use of high temperature blanching to effectively regulate the hydration properties of *D. dumetorum* starches after harvest was investigated using the Brabender Viscoamylograph to measure the rheological properties of the tubers.

A closer look at a graphical representation of the amylograms of the *D. dumetorum* starches revealed that, during the heating cycle from 25 to 95 °C, the viscosity of the paste observed for the freshly harvested tubers showed no significant variation when compared to those of the stored tubers, even though the stored tubers exhibited slight viscosity fluctuations with storage time (Fig. 1). During the cooling cycle, from 95 to 50 °C, the viscosity of the pastes observed for both the freshly harvested tubers and the stored tubers thickened considerably as compared to the viscosity of the pastes observed during the heating cycle. These increases in the viscosities of the paste during the cooling cycle are probably due to the gelatinization and consequent swelling of the starch granules of *D. dumetorum* starches as the elements present in the hot paste (swollen granules, fragments of swollen granules, colloidally and molecularly dispersed starch molecules) begin to associate or retrograde as the temperature of the paste decreases. However, during cooling from 95 °C (HOLD) to 50 °C, the paste viscosities of the stored samples showed consistent decreasing trends with storage time of the tubers, as compared to the paste viscosity of the freshly harvested sample (Fig. 1). These decreasing viscosities, observed in the paste characteristics of the stored tubers during the cooling cycle, are suspected to result from the activities of the constituent amylases in hydrolysing the starch molecules of the tubers during storage.

The blanching treatment given to the tubers prior to storage considerably increased the pasting characteristics of the tubers during storage as compared to the pasting characteristics of the freshly harvested tubers (Figs. 2 and 3). This observation explains that the high temperature treatment given to the tubers prior to

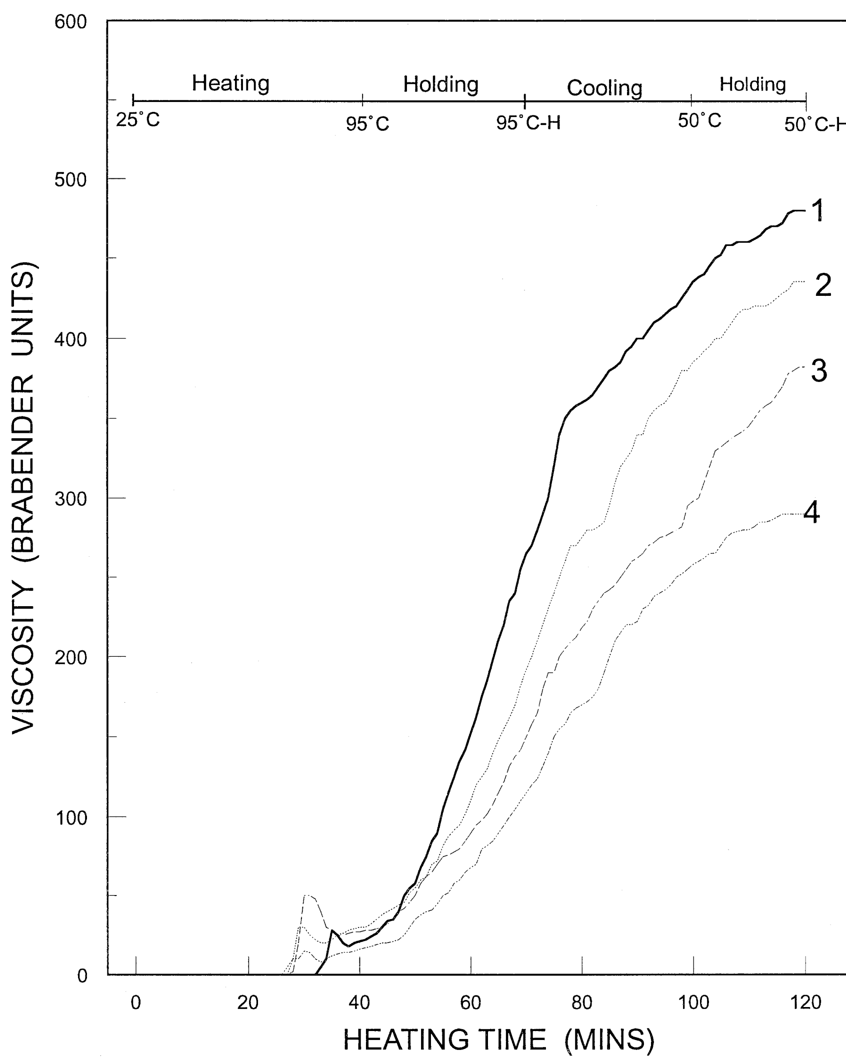

Fig. 1. Effect of storage time on the rheological properties of *Dioscorea dumetorum* starches. 1, freshly harvested tubers; 2, tubers stored for 12 h at ambient conditions; 3, tubers stored for 24 h at ambient conditions; 4, tubers stored for 36 h at ambient conditions.

storage deactivates the constituent amylases in the tubers, thereby hindering their ability to hydrolyse the starch molecules during the storage period. However, the paste viscosities observed for the tubers blanched for 30 s increased more than those blanched for 15 s, and stored under ambient conditions. This suggests that the extent of deactivation of the constituent enzymes in the tubers, as rendered by the blanching treatment, is dependent on blanching and storage time.

Statistical analyses indicated that storage and the various blanching treatments significantly affected ($P \leqslant 0.05$) all the measured viscosity indices.

3.2. Changes in amylasic enzymes activities during storage of D. dumetorum *tubers*

3.2.1. Changes in α-amylase

The post-harvest activities of α-amylases were investigated to ascertain their general rate and pattern of activities in *D. dumetorum* starches after harvest. During the storage period, the α-amylase activities in the tubers generally increased with storage time. Mean values observed for the tubers kept under ambient conditions (28 °C) increased rapidly from 2.28 to 6.68 mg/g maltose released per minute (mg/g min) within 36 h after harvest whereas those kept under low temperature conditions (4 °C) increased from 2.28 to 3.25 mg/g min. This indicates that the post-harvest activities of α-amylase in the tuber more than doubled within 24 h after harvest when the tubers were kept under ambient conditions. However, low temperature storage of the tubers considerably minimized the α-amylase activity within the tuber during storage (Fig. 4). The data, however, suggest that storage temperature influenced α-amylase activity in *D. dumetorum* tubers after harvest. Houvet, Diopoh, Ketekou, and Marchis (1982a, 1982b), working on other yam species, found that variations in the total amylasic activity doubled in six *D. esculenta* cultivars by the 55th after harvest.

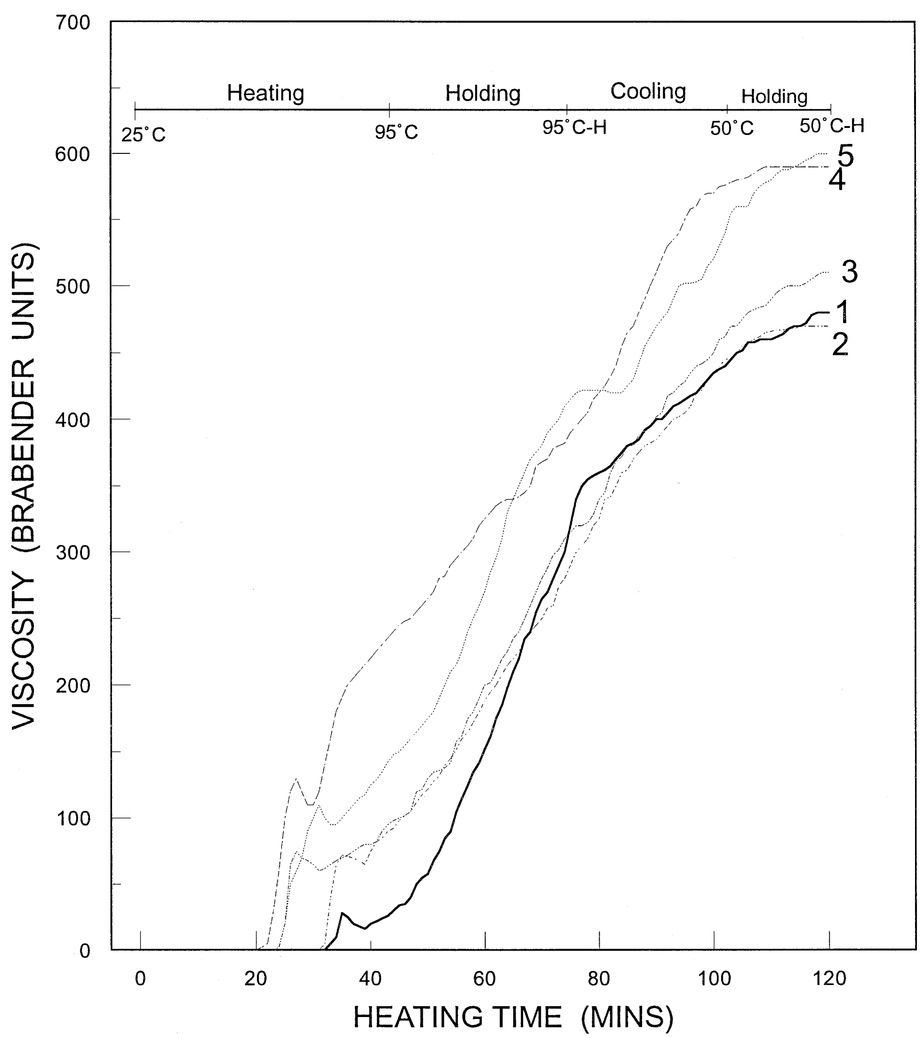

Fig. 2. Effect of storage and blanching (15 s) on the rheological properties of *Dioscorea dumetorum* tubers. 1, freshly harvested tubers; 2, freshly harvested tubers blanched for 15 s; 3, tubers blanched for 15 s and stored for 12 h under ambient conditions; 4, tubers blanched for 15 s and stored for 24 h under ambient conditions; 5, tubers blanched for 15 s and stored for 36 h under ambient conditions.

Contrary to this trend, the α-amylase activities in the tubers blanched prior to storage generally decreased with storage time (Fig. 4). This rapidly decreasing activity is assumed to be partly due to an increase in the thermal agitation of the enzymes and substrate molecules, with a consequent decrease in the affinity of the enzyme for the substrate and partly due to a slow increase in the thermal denaturation of the enzyme.

Statistical analyses conducted on the data showed that the tuber treatment (blanching), storage condition and storage time significantly affected ($P \leqslant 0.05$) the α-amylase activities in the tubers after harvest (Table 1).

3.2.2. Changes in β-amylase

The post-harvest activities of β-amylases in the *D. dumetorum* tubers were generally observed to increase with storage time (Fig. 5). However, the rate of increase was to a greater extent very high in the tubers kept under ambient temperature conditions compared to those kept under low temperature conditions. Similar to the trends observed for α-amylase activities, the β-amylase activities also more than doubled within 24 h after harvesting the tubers and the rate of increase was also reduced with low temperature storage.

Furthermore, the β-amylase activities in the blanched tubers decreased with storage time but to a lesser extent in the tubers blanched for 30 s. This decreasing β-amylase activity in the blanched tubers may be due to the fact that temperature and time of blanching affect

Table 1
ANOVA summary of *F*-values showing the post-harvest activities of &alpha- and β-amylases

Process variables	α-amylase	β-amylase
Tuber treatment	6.076*	5.018*
Storage time	3.611*	1.895
Storage condition	4.951*	3.007*

* Significant *F*-ratios at $P \leqslant 0.05$.

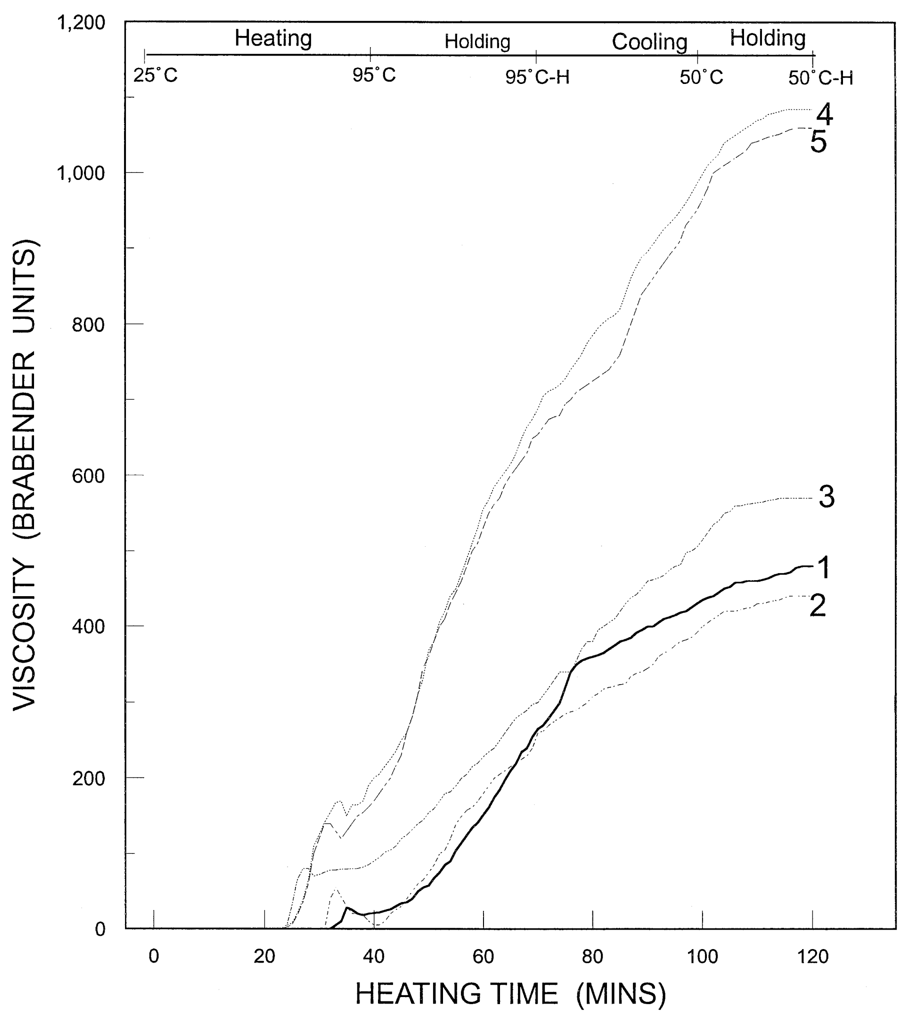

Fig. 3. Effect of storage and blanching (30 s) on the rheological properties of *Dioscorea dumetorum* tubers. 1, freshly harvested tubers; 2, freshly harvested tubers blanched for 30 s; 3, tubers blanched for 30 s and stored for 12 h under ambient conditions; 4, tubers blanched for 30 s and stored for 24 h under ambient conditions; 5, tubers blanched for 30 s and stored for 36 h under ambient conditions.

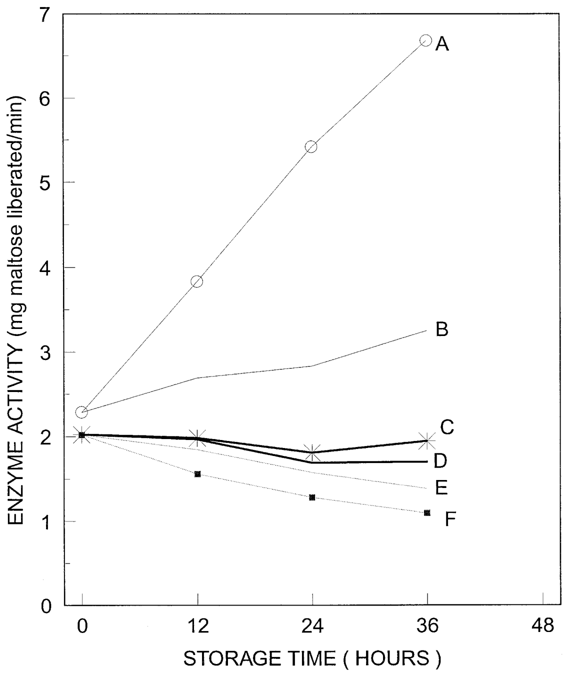

Fig. 4. Changes in α-amylase activities in *Dioscorea dumetorum* tubers during storage. A, unblanched samples kept under tropical ambient conditions (28 °C); B, unblanched samples kept under cold room conditions (4 °C); C, samples blanched for 15 s kept under tropical ambient conditions (28 °C); D, samples blanched for 15 s kept under cold room conditions (4 °C); E, samples blanched for 30 s kept under cold room conditions (4 °C); F, samples blanched for 30 s kept under tropical ambient conditions (28 °C).

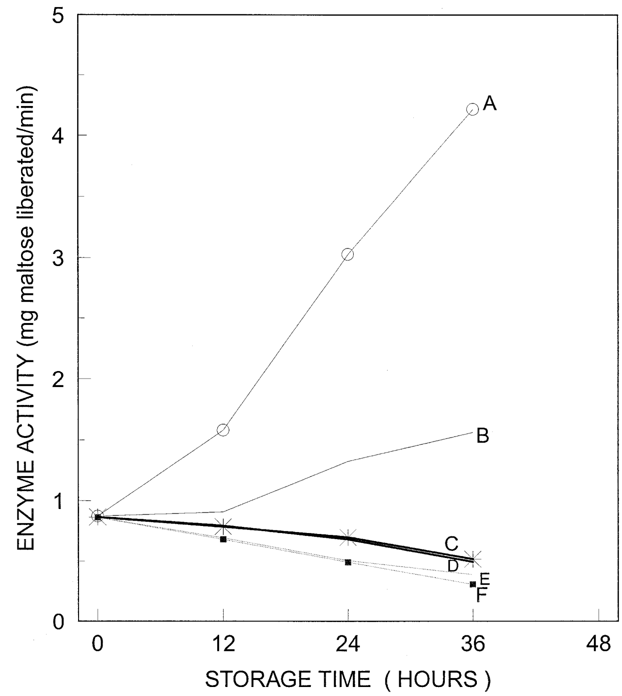

Fig. 5. Changes in β-amylase activities in *Dioscorea dumetorum* tubers during storage. A, unblanched samples kept under tropical ambient conditions (28 °C); B, unblanched samples kept under cold room conditions (4 °C); C, samples blanched for 15 s kept under tropical ambient conditions (28 °C); D, samples blanched for 15 s kept under cold room conditions (4 °C); E, samples blanched for 30 s kept under cold room conditions (4 °C); F, samples blanched for 30 s kept under tropical ambient conditions (28 °C).

β-amylase activity in *D. dumetorum* tubers. Also, at sufficiently high temperatures, the decrease in activity is probably due to the thermal denaturation of the enzymes.

Analysis of variance showed that, with the exception of storage time, the tuber treatment (blanching) and storage condition had significant effects ($P \leqslant 0.05$) on the β-amylase activities in *D. dumetorum* starches during storage (Table 1).

4. Conclusion

Changes in rheological properties and amylasic enzyme activities occur in the starches of *D. dumetorum* tubers after harvesting. The paste characteristics of the tubers decrease with storage time, signifying the action of amylases on the constituent starch. However, blanching of tubers before storage increases the various pasting characteristics of the tubers due to amylase denaturation. Blanching *D. dumetorum* tubers in boiling water (100 °C) for 15–30 s can therefore effectively decrease the action of amylases on the *D. dumetorum* starches after harvest. The post-harvest activities of both α- and β-amylases increase very rapidly within 36 h after harvest. These rapid changes in amylase enzyme activities result in the hydrolysis of starches into sugars in *D. dumetorum* tubers after harvest, thereby reducing the rheological properties (starch pasting viscosities) of the stored tubers and leading to the hardening of the tubers. The changes, as observed, are therefore suspected to be one of the factors responsible for the hardening phenomenon in *D. dumetorum* tubers after harvest.

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