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Antinutritional factors changes occurring in trifoliate yam (*Dioscorea dumetorum*) tubers after harvest

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Abstract

Studies were conducted on the changes in antinutritional factors occurring during the storage of *Dioscorea dumetorum* (cv. Yellow) yam tubers. The tubers were harvested and stored under prevailing tropical ambient conditions (19–28 °C, 60–85% RH) for 0, 2, 5, 7, 14, 21, 28, 42 and 56 days. The samples were evaluated for total phenols, tannins, phytic acid, α-amylase inhibitors, trypsin inhibitors and oxalates. Results showed that, during storage, total phenols and tannin contents decreased by approximately 22–28% after 56 days of harvest, due to the hardening phenomenon and sprouting. Phytate and α-amylase inhibitor levels declined, respectively, from 690–416 mg/100 g and 1013–659 AIU/g, while oxalates and trypsin inhibitor contents increased during the first week of storage (days 0–7) and, after this period, they started to decrease progressively. Since sprouting of most tubers was observed after 28 days of storage, the results suggest that post-harvest hardening and sprouting influence antinutritional composition of *D. dumetorum* tubers.

Keywords: *Dioscorea dumetorum*; Antinutritional factors; Storage

1. Introduction

Antinutritional factors, which consist of polyphenols, oligosaccharides (α-galactosides), lectins, proteases and amylase inhibitors, are widely distributed in most plants (Liener & Kakade, 1980; Nakase et al., 1996; Reddy & Pierson, 1994; Roberts & Goldstein, 1984). They have been associated with reduction of food digestibility, decrease in nutrient bioavailability and flatulence production (Brune, Rossander, & Hallberg, 1989; Gillooly et al., 1983; Jood, Mehta, & Singh, 1986).

Among the eight yam species commonly grown and consumed in Cameroon, *Dioscorea dumetorum* is the most important (Agbor-Egbe & Trèche, 1995). It is rich in protein (9.6%) and fairly balanced in essential amino acids (chemical score of 0.94), and the starch is easily digestible. The starch granules are polygonal or spherical (<10 μm) in shape with a type A X-ray diffraction structure, similar to that of cereals (Afoakwa & Sefa-Dedeh, 2001; Mbome Lape & Trèche, 1994).

Earlier *D. dumetorum* storage studies have reported a tuber hardening phenomenon which occurs within a few hours after harvest (Brillouet, Trèche, & Sealy, 1981; Sealy, Renaudin, Gallant, Bouchet, & Brillouet, 1985) and the physicochemical changes have been elucidated (Medoua, Mbome Lape, Agbor-Egbe, & Mbofung, 2005a; Sefa-Dedeh & Afoakwa, 2002). Despite this phenomenon, other studies have shown that hardened yam tubers could adequately provide the dietary requirements for human nutrition (Agbor-Egbe & Trèche, 1995; Trèche & Agbor-Egbe, 1996). However, there is very little information available on the antinutritional factor changes occurring during storage.

The present study was therefore carried out to determine the changes in antinutritional factors occurring during the storage of *D. dumetorum* (cv. Yellow) yam tubers under tropical ambient conditions.

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2. Materials and methods

2.1. Material

Trifoliate yam, *D. dumetorum* (cv. Yellow) tubers were randomly harvested at physiological maturity, 9 month after planting, from at least 10 different randomly selected plants from a farm at Esse in the Centre Province of Cameroon. These were immediately transported to the Centre for Food and Nutrition Research laboratories, in Yaounde, for subsequent storage and chemical analyses. Care was exercised in selecting for storage tubers with little or no skin wounding. Yam tubers were divided into two groups: one group of tubers was individually weighed and left on shelves in a warehouse for 56 days, under prevailing tropical ambient conditions (19–28 °C, 60–85% RH) and the other group was prepared for chemical analyses. Tuber weight losses, decay and sprouting, that occurred during storage, were noted.

2.2. Sample preparation

Samples of stored tubers were collected at fixed time intervals (days 0, 2, 5, 7, 14, 21, 28, 42 and 56). Collected tubers were thoroughly washed with water, peeled, chopped into chips of 0.5 cm thickness, dried at 40 ± 2 °C in a ventilated oven, packaged in plastic bags and stored at −21 °C. Prior to chemical analysis, the dried samples were ground in a Hammer mill (Campsas 82370, Labastide St-Pierre, France) to pass through a 500 μm sieve.

2.3. Antinutritional factors analysis

2.3.1. Phytate

Phytic acid content of tubers was determined using the chromophore method of Mohammed, Ponnamperuma, and Youssep (1986). About 5 g of sample was treated at ambient temperature with 25 ml of 3% trichloroacetic acid for 45 min under continuous stirring and 8 ml of the mixture obtained were centrifuged at 20,000 rpm for 15 min. Five milliliters of the supernatant obtained were then mixed with 3 ml of $FeCl_3$, $6H_2O$ 1% (prepared in 1 N HCl) and then, boiled, cooled and centrifuged at 20,000 rpm for 10 min. The residue obtained was then incubated at ambient temperature with 1 ml 0.5 N HCl for 2 h; then 7 ml of distilled water and 3 ml of 1.5 N NaOH were added and the mixture was boiled during 15 min. After cooling, the mixture was centrifuged at 20,000 rpm for 10 min. 0.2 ml of the supernatant obtained was mixed with 4.6 ml of distilled water and 2 ml of a chromogenic solution and then heated at 95 °C for 30 min. After cooling, absorbance was read at 830 nm.

2.3.2. Phenolic compounds

Total phenols were determined according to the Swain and Hillis (1959) method, using Folin–Ciocalteu reagent, while condensed tannins were determined by the spectrophotometric method of Bainbridge, Tomlins, Wellings, and Westby (1996), using acidified vanillin and (+) catechin as standard.

2.3.3. Total oxalates

Total oxalates were determined according to the AOAC (1970) titrimetric method, based on the extraction by 6 N HCl and dosage with hot 0.01 M $KMnO_4$ in the presence of sulphuric acid.

2.3.4. Trypsin inhibitors

The extraction of trypsin inhibitors was done as described by Khor, Tan, and Wong (1982), and trypsin inhibitors were determined according to the spectrophotometric method of Kakade, Rackis, McGhee, and Puski (1974) using BAPNA (benzoyl-DL-arginine *p*-nitroanilide hydrochloride), pH 8.6, as substrate. Trypsin inhibitory unit (TIU) were estimated in terms of weight of pure trypsin inhibited per g of dry matter and calculated by using the following formula derived by Smith, Megen, Twaalfhoven, and Hitchcock (1980).

$\text{TIU} = (2.632 \times \text{Dilution factor} \times A_i/S)$ mg of pure trypsin inhibited per g of sample, where A_i is the change in absorbance due to trypsin inhibition per ml of sample extract, and S is the sample weight in grams.

2.3.5. α-Amylase inhibitors

The extraction of α-amylase inhibitors was done as described by Lonstaff and McNab (1991) and α-amylase inhibitors were determined according to the Piergiovanni (1992) spectrophotometric method, using starch and iodine. One unit of enzyme activity was defined as that which liberates, from soluble starch, 1 μmol of reducing groups (calculated as maltose) per min at 30 °C and pH 6.9, under the specified conditions. One unit of α-amylase enzyme activity inhibited was defined as one α-amylase enzyme inhibitory unit (AIU), and expressed as AIU per g of dry matter.

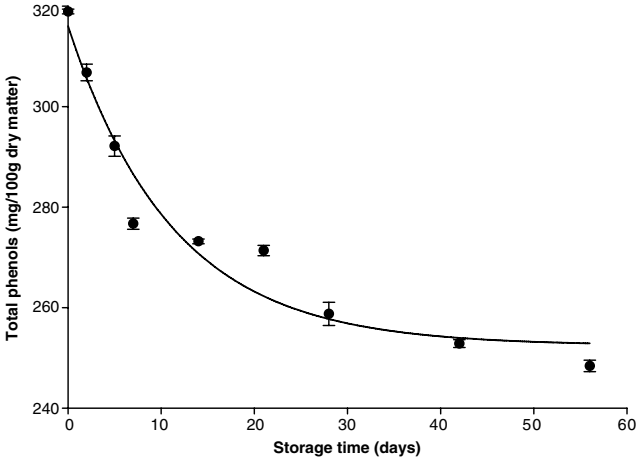
2.4. Statistical analysis

All measurements were carried out in triplicate. Statistical analyses of data were performed using SPSS 10.1 software. Comparisons between dependent variables were determined using analysis of variance (ANOVA), Duncan's multiple range test and correlation analysis. Statistical significance was defined at $P \leqslant 0.05$.

3. Results and discussion

3.1. Changes in phenolic compounds during storage

The levels of total phenols and condensed tannins, on a dry weight basis, decreased, respectively, from 319 to 248 mg/100 g and from 22 to 16 mg (+)-catechin equivalents/100 g, respectively (Fig. 1). In general, the phenolic



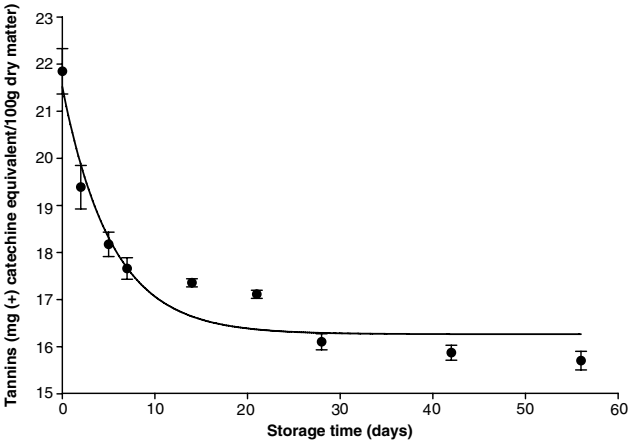


Fig. 1. Changes in phenolic compounds during storage.

compound levels were significantly influenced ($P \leqslant 0.05$) by the storage period; there was about 22–28% reduction in levels of phenolic compounds after 56 days of storage. The decrease in phenolic compounds during tuber storage can be divided into two phases; at the beginning of storage to the 21st day, with 14.9% and 21.7% loss for total phenols and condensed tannins, respectively, and a second phase from the 28th day to 58th day of storage, with a decrease of about 8%.

Since sprouting was observed in most of the tubers after 28 days of storage, it could be suggested that sprouting caused decreases in the levels of phenolic compounds. In this study, analysis of variance showed a significant effect ($P \leqslant 0.05$) of tuber sprouting on phenolic content. The observed decreases in phenolic contents could be attributed to the yam tuber hardening phenomenon. A previous study has shown a strong correlation between the levels of phenolic compounds in *D. dumetorum* tubers and lignification during the tuber hardening process (Medoua et al., 2005a).

3.2. Changes in phytate contents during storage

As seen in Fig. 2, comparatively high phytate contents (690 mg/100 g) were found in the fresh yam tubers. However, this level is relatively low compared to that found in

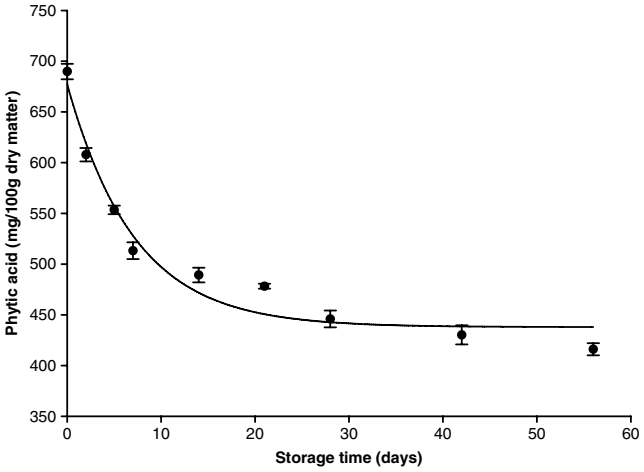


Fig. 2. Changes in phytic acid contents during storage.

cereals (2 g/100 g; Erdman, 1979). Analysis of variance indicated that storage period and sprouting significantly affected the level of phytic acid. A decrease in phytate content was observed concomitantly with increases in storage period. This occurred in two phases: 0 to 21st day and 21st to 56th day. For the first phase, phytate content decreased from 690 to 478 mg/100 g, representing 30.7% loss and, in the second phase, when sprouting occurred, phytate levels decreased from 478 to 416 mg/100 g, representing 13% loss.

Earlier studies have shown that the losses observed in phytate contents could be related to the tuber hardening phenomenon, which has been explained by a pectin–phytate mechanism (Medoua et al., 2005a). Sprouting has been shown to play a significant role in the phytate degradation in vegetables by the activation of phytases (Chavan & Kadam, 1989).

3.3. Changes in total oxalate levels during storage

As shown in Fig. 3, considerably high oxalate levels (502–511 mg/100 g, on a dry weight basis) were found in the fresh tubers as compared to the 780 mg/100 g reported

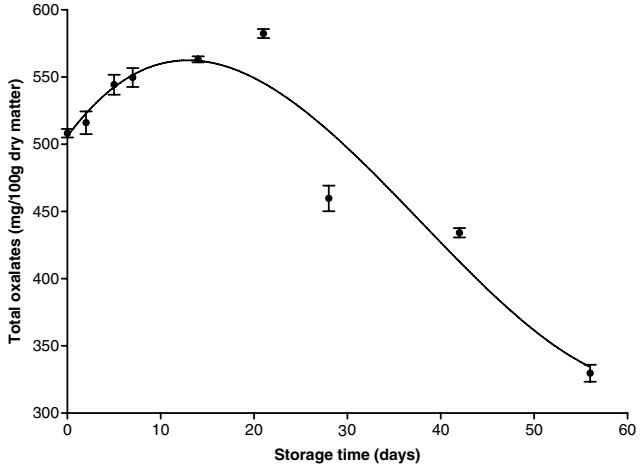


Fig. 3. Changes in total oxalate contents during storage.

in taro (*Colocasia esculenta*) corms (Holloway, Argall, Jealous, Lee, & Bradbury, 1989). However, it should be noted that *D. dumetorum* tubers are generally boiled or transformed into flour in a process that includes a cooking step before consumption (Trèche, Agbor Egbe, Mbome Lape, & Mba Mezoui, 1983), procedures which have been shown to significantly reduce oxalate levels in foods (Ross, Savaga, Martin, & Vanhanen, 1999). Hence, tuber processing would reduce any nutritional problems that the high levels of oxalates could cause.

Results obtained from the analysis of variance showed that total oxalate contents were significantly ($P < 0.05$) affected by the storage period. An increase of about 12.7% in total oxalate levels was observed after 21 days of storage, followed by a rapid decrease of about 43.4% between the 21st and 56th day of storage.

The increase in oxalate levels observed in this study could be related to decreases in moisture content during storage, reported in a previous study (Medoua, Mbome Lape, Agbor-Egbe, & Mbofung, 2005b). Since tuber sprouting was observed after 28 days of storage, in this study, the decreases in oxalate levels between the 21st and 56th of storage could be the result of metabolic activities triggered by the onset of sprouting.

3.4. Effect of storage on trypsin inhibitors

With 631 TIU/g of dry matter, fresh tubers of *D. dumetorum* presented a non-negligible antitryptsic activity. Inhibition of trypsin activity exhibited by *D. dumetorum* tubers could be explained by the presence in these tubers of antinutritional factors, such as condensed tannins, that are known to inhibit the activity of trypsin (Horigome, Kumar, & Okamoto, 1988; Mitjavila, 1986).

Analysis of variance (ANOVA) showed that the level of trypsin inhibitors is significantly influenced by tuber storage duration. Trypsin inhibition level increased during the first week of storage, followed by a progressive decrease (Fig. 4). Despite the fact that trypsin inhibition has been attributed, by several authors, to condensed tannins, a

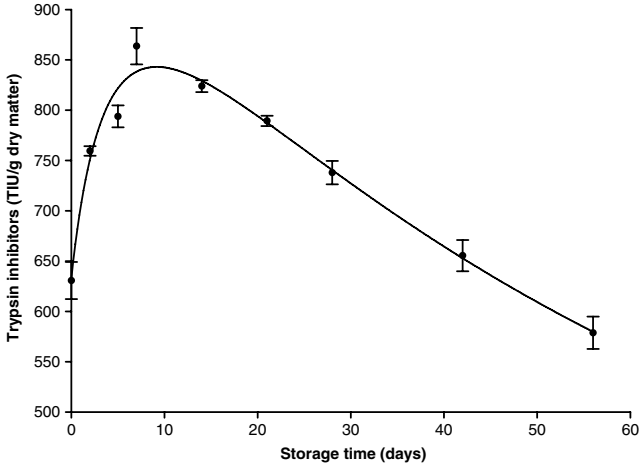


Fig. 4. Changes in trypsin inhibitors levels during storage.

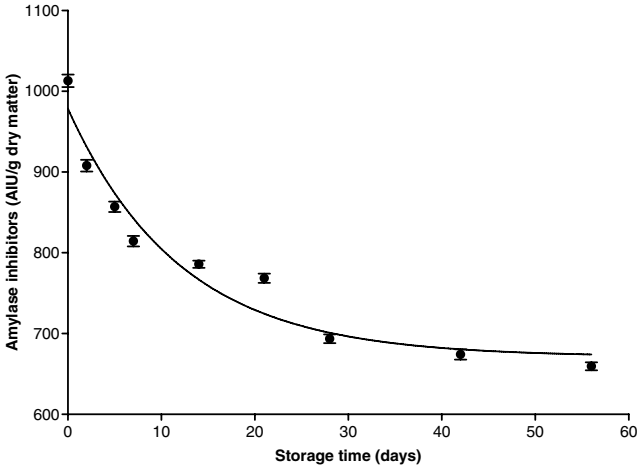


Fig. 5. Changes in α-amylase inhibitors levels during storage.

non-significant correlation ($r = 0.126$; $P \leqslant 0.05$) was found in this study between tannin and trypsin inhibitors level during *D. dumetorum* storage, which suggested that trypsin inhibition found, in this case, is not caused by tannins. However, Mole and Waterman (1985) have showed that tannins could either activate or inhibit trypsin hydrolysis *in vitro*, this activity depending on the ratio tannin/substrate. For low tannin concentrations and excess of substrate, hydrolysis by trypsin is activated.

A significant correlation ($r = 0.84$; $P < 0.05$) was recorded between trypsin inhibitors and oxalate levels during *D. dumetorum* tuber storage, which suggests that oxalates play a significant role in trypsin inhibition, probably by reducing availability of calcium, which is known to be a trypsin effector.

3.5. Effect of storage on α-amylase inhibitors

Fresh tubers of *D. dumetorum* presented non-negligible quantities of α-amylase inhibitors (1013 ± 18 AUI/g of dry matter). Amylase inhibitor levels decreased to a significant level during tuber storage (about 35% of α-amylase inhibitor loss during 56 days of storage) (Fig. 5). The trend observed here is similar to that obtained for phenolic compounds and phytate. Since phenolic compounds and phytic acid are known for their role in inhibition of α-amylase activity (Deshpandes, Salhe, Salunkhe, & Cornforth, 1982; Weselake, Mcgregor, Hill, & Duckworth, 1983), they could be responsible of the α-amylase inhibition exhibited by *D. dumetorum* tubers. Significant correlations have therefore been obtained in this study between α-amylase inhibitors and phenolic compounds ($r = 0.98$; $P < 0.01$) on the one hand and phytic acid ($r = 0.99$; $P < 0.01$) on the other.

4. Conclusion

D. dumetorum tubers undergo antinutritional factor changes as result of post-harvest hardening and sprouting of tubers. Total phenols, tannins, phytic acid and α-amy-

lase inhibitors decreased with tuber storage duration. Total oxalates and trypsin inhibitors rapidly increased during the first week of storage and progressively decreased these after. In general, the results suggested that storage of *D. dumetorum* tubers positively affects its nutritional potential by a significant decrease of its antinutritional factors. This beneficial effect is most accentuated during the second week of storage.

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