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Influence of fermentation on some quality characteristics of trifoliate yam (*Dioscorea dumetorum*) hardened tubers

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Abstract

Processing of *Dioscorea dumetorum* tubers into flour could be a means of adding a longer-term value to this tropical plant with a high nutritional potential but which possesses a post-harvest hardening problem, characterised by a hard-to-cook defect. In an attempt to investigate the changes leading to fermentation of hardened tubers, CIE $L^*a^*b^*$ parameters and some physicochemical properties of the yams were monitored. Four fermentation periods (0, 2, 4, 7 and 14 days) under ambient temperature (23 ± 5 °C) in the presence and absence of oxygen were studied. The results showed that fermentation significantly ($p \leqslant 0.05$) increased a^* and b^* parameters, and decreased L^* parameter. However, browning affected more tubers fermented in the presence of oxygen. All the anti-nutrients evaluated (total phenols, phytic acid, oxalates, trypsin inhibitors and α-amylase inhibitors) decreased by significant levels ($p \leqslant 0.05$) within 14 days of fermentation. However, the rates of anti-nutrient losses varied for each constituent and followed first-order reaction kinetics.
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Keywords: *Dioscorea dumetorum*; Storage; Fermentation; Browning; Anti-nutritional factors

1. Introduction

Trifoliate yam, *Dioscorea dumetorum*, is usually used in the form of fresh tubers cooked in boiling water, followed or not by manual pounding into a thick paste, locally called *fufu*. This food preparation is ineffective with hardened tubers, which have lost the ability to soften during cooking. In order to use *D. dumetorum* hardened tuber as food, it is important to find out pre-treatment methods which can reverse tuber hardening, and in this connection, fermentation could be an appropriate solution. Fermentation, a widely used food processing method, has the advantage of reducing anti-nutritional factors and improving digestibility and sensory quality of food (Obiri-Danso, 1994; Reddy & Pierson, 1994). Soaking of vegetables in excess water allows the selection of desirable micro-organisms such as lactic bacteria, yeasts and fungi (Sefa-Dedeh & Cornelius, 2000; Sefa-Dedeh, Sakyi-Dawson, & Sanni, 1999) and the activity of these micro-organisms reduces pH and increases titratable acidity of substrate (Plahar & Leung, 1982).

Mbome Lape (1991) initiated an assay for the processing of hardened yam tubers into low-bulk gruel for infants, using lactic acid fermentation and the elimination of fibrous materials. However, to the best of our knowledge, data on the physicochemical changes occurring during fermentation of *D. dumetorum* tubers are still needed, and fermentation of these yam tubers poses the problem of tuber browning, which is exhibited by a purplish colour.

The present study was therefore carried out in order to standardize the soaking process which has been incriminated in the observed browning of *D. dumetorum* tubers, and to determine the physicochemical changes occurring during fermentation of hardened tubers.

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2. Materials and methods

2.1. Materials

Trifoliate yam, *D. dumetorum* cv., yellow tubers were randomly harvested at maturity from a farm at Esse in the Centre Province of Cameroon. They were immediately transported to the Centre for Food and Nutrition Research laboratories in Yaounde and stored under prevailing tropical ambient conditions (19–28 °C, 60–85% RH) for 14 days. Hardening of the stored tubers was characterised by a rough and fluffy surface of peeled tubers as opposed to the smooth and moist surface of freshly-harvested ones. After the storage period, the samples of hardened tubers were thoroughly washed with water, peeled and chopped into chips of 1-cm thickness.

2.2. Yam tuber fermentation

Hardened tuber chips (100 g) were soaked in 1 l of tap water at ambient temperature (23 ± 5 °C) for 0–7 days. Fermentation was carried out in glass jars and two systems were tested; an open system, with renewal of air, which consisted of jars left open during the soaking period and a closed system, without air renewal, where jars were tightly closed. At the end of the fermentation period, samples were dried at 40 °C in a ventilated oven for 24 h and the dried samples were grounded into flour to pass through a 400-μm sieve for colour measurements.

2.2.1. Colour measurement

Colour was measured using a colorimeter (Lovibond RT Colour Measurement Kit V2.28) with an observation window of 10° and D65 light source. The colorimeter was calibrated with a white standard ($L^* = 93.87$, $a^* = 0.18$, $b^* = 2.71$) and CIE $L^*a^*b^*$ parameters of each sample were determined on the basis of triplicate measurements.

The colour derivative functions of C^* [chroma $= (a^{*2} + b^{*2})^{1/2}$] and H^* [hue angle $= \tan^{-1}(b^*/a^*)$] were also calculated.

2.3. Textural and physicochemical changes occurring during fermentation of hardened tubers

Hardened tuber chips (800 g) were soaked in 4 l of tap water at ambient temperature for 0, 2, 4, 7 and 14 days. Fermentation was carried out in tightly-closed plastic recipients. In order to better define the effect of natural fermentation of *D. dumetorum* hardened tubers, sterile soaking was conducted at the same time using the method of Okafor, Ijioma, and Oyolu (1984). Yam chips were sterilised for 15 min with mercuric chloride (1% in 70% ethanol), then rinsed and soaked in sterile water.

After fermentation for the indicated time, samples were dried at 40 °C in a ventilated oven, packaged in plastic bags and stored at −21 °C. Prior to analysis, the dried samples were ground in a hammer mill (Campsas 82370, Labastide St-Pierre, France), to pass through a 500-μm sieve.

2.3.1. Dry matter estimation

Dry matter (DM) contents of sample were determined using the Association of Official Analytical Chemists approved method 925.10 (AOAC, 1990).

2.3.2. pH and titratable acidity measurement

Dry ground sample (10 g) was mixed with distilled water (100 ml). The mixture was agitated for 15 min and centrifuged at 3000 rpm for 15 min. The pH of the supernatant was measured using a pH meter. Titratable acidity was determined according to AOAC (1990) method; an aliquot of supernatant (10 ml) was titrated against 0.1 N NaOH solution, using 1% phenolphthalein as indicator. Titratable acidity was expressed in mg lactic acid equivalent/g as follows:

$$\text{Titrable acidity} = \frac{V_{\text{NaOH}} \times 90 \times 100}{V_{\text{Tit}} \times S_{\text{w}}},$$

where V_{NaOH} is the volume of NaOH required for titration, V_{Tit} is the volume of extract (10 ml), 90 is the relative molecular mass of lactic acid, S_{w} is the weight of the sample (g) and 100 is total volume of extract.

2.3.3. Softening measurement

Vortex induced cell separation (VICS) was used to evaluate the degree of tuber softening during fermentation, according to the method of Parker and Waldron (1995), with some modifications. For each soaking period, 10 yam slices, randomly obtained, were cut up in cubes of 1 cm width with a rust-proof knife. Ten grams of yam cubes, randomly obtained (P_1) were mixed with 30 ml of distilled water and stirred at 1100 rpm for 30 min with a magnetic agitator. The mixture obtained was then left to settle and the level of soluble solids (P_2) determined after air-drying at 105 °C. VICS, representing the degree of softening, was calculated according to the formula:

$$\text{VICS} = \frac{P_2}{P_1 \times \text{DM}} \times 100,$$

where DM is the dry matter level of soaked yam cubes.

2.3.4. Anti-nutritional factors analysis

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

2.3.4.2. Phenolic compounds. Total phenols were estimated according to the Swain and Hillis (1959) method, using Folin–Ciocalteu reagent and gallic acid as standard.

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

2.3.4.4. Trypsin inhibitors. The extraction of trypsin inhibitors was carried out, as described by Khor, Tan, and Wong (1982), and trypsin inhibitors were determined according to the spectrophotometric method of Kakade, Rackis, MacGhee, and Puski (1974) using benzoyl-DL-arginine *p*-nitroanilide hydrochloride (BAPNA; pH 8.6) as substrate. Trypsin inhibitory units (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):

TIU = (2.632 × dilution factor × 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.

2.3.4.5. 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 α-amylase enzyme inhibitory unit (AIU) was defined as one unit of α-amylase enzyme activity inhibited and expressed as AIU per g of dry matter.

2.3.5. Degradation kinetics calculations

The general reaction rate expression for degradation kinetics of compounds in food (Ramaswami, Van De Voort, & Ghasal, 1989; Van Boekel, 1996) can be written as follows:

$$\frac{-\mathrm{d}[C]}{\mathrm{d}t} = k[C]^m, \qquad (1)$$

where $[C]$ is the quantitative value of the component under consideration, k is the reaction rate constant and m is the order of the reaction. The equation for first-order kinetics after integration of Eq. (1) can be written as follows:

$$\ln\frac{[C]_t}{[C]_0} = kt, \qquad (2)$$

where $[C]_0$ and $[C]_t$ are the concentration of the component under consideration at time zero and time t (day), respectively.

2.4. Statistical analysis

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

3. Results and discussion

3.1. Influence of fermentation system on browning

The colour of fermented hardened yam tubers can be an important quality parameter, which has a direct influence on the acceptability of the developed product. CIE $L^*a^*b^*$ parameters of non-fermented and fermented *D. dumetorum* hardened tubers are presented in Table 1. Fermentation and the system used for soaking significantly affected ($p \leqslant 0.05$) colour $L^*a^*b^*$ parameters of hardened yam tubers. With fermentation, there was a general increase in a^* and b^* parameters (red and yellow, respectively) while L^* parameter (luminance) decreased, indicat-

Table 1
CIE $L^*a^*b^*$ parameters of non-fermented and fermented *D. dumetorum* hardened tubers

	L^*	a^*	b^*	C^*	H^*
NF	99.96 ± 0.01c	−0.55 ± 0.01a	10.27 ± 0.01a	10.28 ± 0.01a	93.05 ± 0.02c
FCS	96.21 ± 0.01b	−0.02 ± 0.01b	12.65 ± 0.01c	12.65 ± 0.01c	90.11 ± 0.01b
FOS	91.51 ± 0.02a	1.95 ± 0.01c	12.08 ± 0.01b	12.23 ± 0.01b	80.84 ± 0.01a

Mean ± SD, $n = 3$.
Means in the same column with different letters are significantly different ($p \leqslant 0.05$), according to Duncan's multiple rage test.
NF: non-fermented.
FCS: fermented in a closed system.
FOS: fermented in an open system.

ing tuber browning during fermentation. Tubers fermented in open jars were significantly more brown ($a^* > 0$) than those fermented in closed jars ($a^* < 0$), suggesting that browning of *D. dumetorum* hardened tubers during fermentation could have been caused by the oxidation of some organic compounds, such as phenolic compounds. Results obtained in this study have shown that fermentation of *D. dumetorum* hardened tubers in a close system without air renewal prevented the browning phenomenon.

3.2. Textural and physicochemical changes occurring during fermentation

3.2.1. Dry matter

Dry matter levels of *D. dumetorum* hardened tubers showed significant variations ($p \leqslant 0.05$) during the fermentation period. The dry matter of tubers decreased from 25.9% to 19.4% during the 14 days of natural fermentation (Fig. 1), which indicated that the tubers underwent a phenomenon of degradation or loss of some constituents during fermentation. Dry matter losses of about 9.6% were observed after 14 days of tubers sterile soaking, suggesting that the decrease noted during natural fermentation was in part due to leaching of some compounds from the tubers.

3.2.2. pH and titrable acidity

Natural and sterile soaking of *D. dumetorum* hardened tubers significantly affected tuber pH. An exponential increase of pH to a stationary state was noted during sterile soaking (Fig. 2). This pH increase could be explained by the loss of some acidic compounds into the soak water. On the contrary, pH decreases were observed during natural soaking, which could be the result of the activities of micro-organisms. During the two first days of natural fermentation, pH decreased from 5.5 to 4.8 and then to 3.9 after 14 days of fermentation. During maize fermentation, Sefa-Dedeh, Kluvitse, and Afoakwa (2001) noted a pH decrease of 6.3–4.0 after 24 h of fermentation. In the same order, O'Brien, Mbome, Taylor, and Poulter (1992) reported a pH decrease of 6.8–4.5 after 48 h of cassava fermentation. The pH decreases obtained in this study were relatively low compared to those observed for other fermentation systems. The relatively low pH decreases could be due to the high protein level of *D. dumetorum* tubers, which acts as a buffer. Similar results were reported by Sefa-Dedeh et al. (2001), who noted that bean protein contributed in lowering pH during the fermentation of bean–maize composite flour.

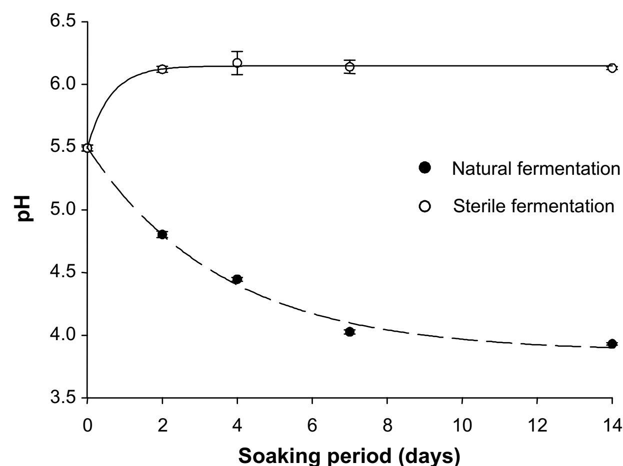

Fig. 2. Variation in pH of *D. dumetorum* hardened tubers during natural and sterile fermentation.

Titrable acidity of *D. dumetorum* hardened tubers increased with soaking period (Fig. 3). Analysis of variance showed a significant effect ($p \leqslant 0.05$) of soaking period on titratable acidity. In addition, it was observed during this study that *D. dumetorum* hardened tubers lost about 46% of titratable acidity after 14 days of sterile soaking, suggesting that some acidic compounds of tubers were lost in the soak water.

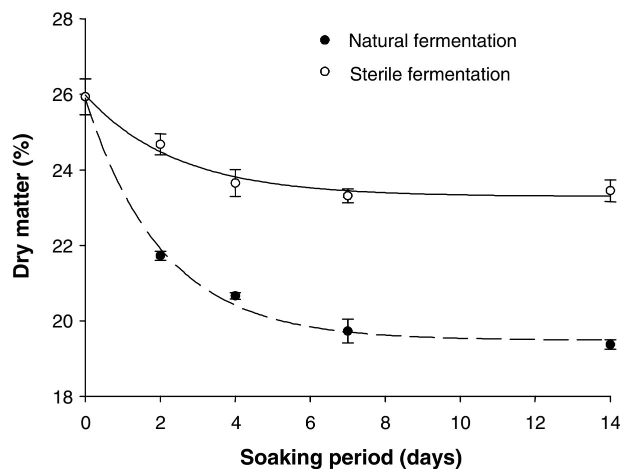

Fig. 1. Variation in dry matter levels of *D. dumetorum* hardened tubers during natural and sterile fermentation.

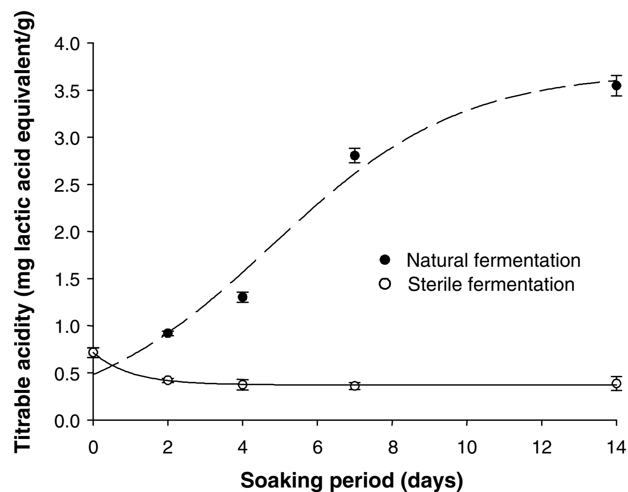

Fig. 3. Variation in titrable acidity level of *D. dumetorum* hardened tubers during natural and sterile fermentation.

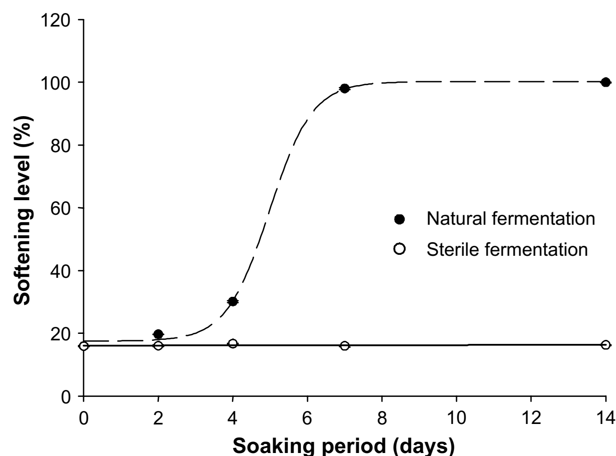

Fig. 4. Variation in softening level of *D. dumetorum* hardened tubers during natural and sterile fermentation.

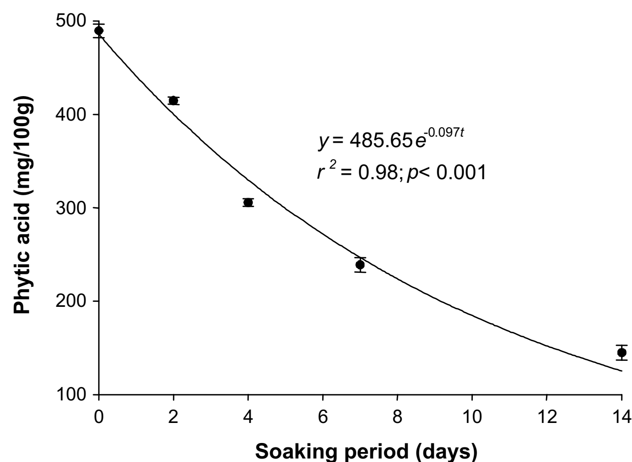

Fig. 5. Variation in phytic acid level during natural fermentation of *D. dumetorum* hardened tubers.

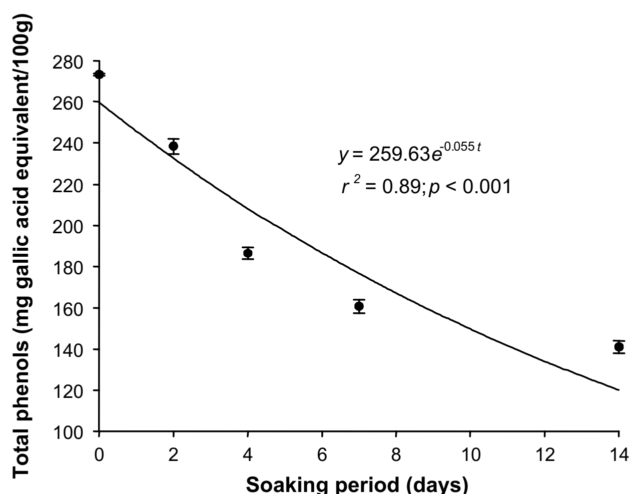

Fig. 6. Variation in total phenols level during natural fermentation of *D. dumetorum* hardened tubers.

3.2.3. Tuber softening

The effect of soaking on hardened tuber softening is presented in Fig. 4. Analysis of variance showed a significant effect of soaking period on the degree of tuber softening during natural soaking and no effect was observed during sterile soaking. During natural fermentation, a slight increase of softening was observed between day 0 and 4, followed by a significant increase from day 7; thereafter completely softened tubers were obtained. The texture of vegetables has been attributed to the cohesion of cell–cell linkage at the middle lamella, through the medium of pectic polymers (Waldron, Smith, Parr, Ng, & Parker, 1997). Results obtained in this study indicated that micro-organisms involved in fermentation had hydrolysed pectic polymers in *D. dumetorum* tubers, allowing them to soften, in spite of the stability conferred to the cell–cell linkage by the hardening phenomenon.

3.2.4. Anti-nutritional changes

3.2.4.1. Phytic acid. Analysis of variance showed that phytic acid level in *D. dumetorum* hardened tubers was significantly affected ($p \leqslant 0.05$) by the soaking period. There was an exponential decrease of 489–145 mg/100 g DM during the soaking period (Fig. 5), representing a phytic acid loss of about 70%. Marfo, Simpson, Idowu, and Oke (1990) reported phytic acid decreases of 88%, 98% and 68%, after 96 h natural fermentation of cassava, cocoyam and yam, respectively. These losses were attributed to the phytase, naturally present in tubers or secreted by fermentation micro-organisms.

3.2.4.2. Total phenols. *D. dumetorum* hardened tubers contained 273 mg gallic acid equivalent/100 g DM of total phenols. Analysis of variance showed a significant effect of the soaking period. There was an exponential decrease in total phenols levels with soaking period (Fig. 6). After 14 days of natural fermentation of *D. dumetorum* hardened tubers, about 48% loss of total phenols was noted. A similar effect of fermentation has been reported in an earlier study where 10% loss of phenolic compounds was found after 9 h fermentation of rabadi (Reddy & Pierson, 1994).

3.2.4.3. Total oxalates. Oxalates appear as end-metabolism products in many vegetable tissues. When they are consumed, oxalates can link calcium and other minerals (Noonan & Savage, 1999). *D. dumetorum* hardened tubers contain high levels of oxalates (226 mg/100 DM). There was a significant effect of soaking period on oxalate levels. An exponential decrease of oxalate levels with the soaking period was noted (Fig. 7). About 22% losses of oxalates were noted after 14 days of fermentation. Decreases in oxalate levels observed in this study can be imputed to their solubility in the soak water, since it is known that soaking decreases oxalate levels in food by leaching (Noonan & Savage, 1999; Ross, Savaga, Martin, & Vanhanen, 1999).

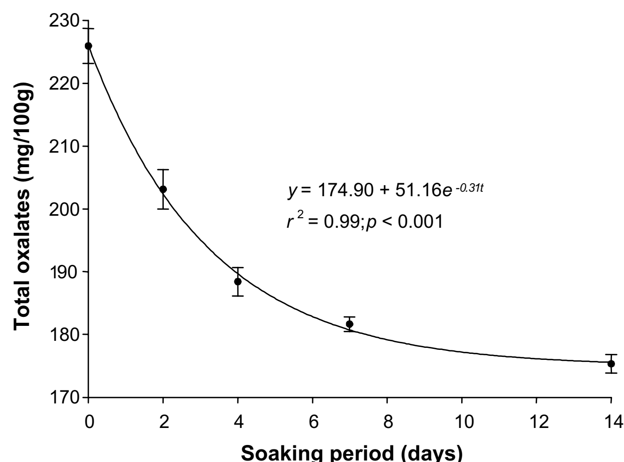

Fig. 7. Variation in total oxalates level during natural fermentation of *D. dumetorum* hardened tubers.

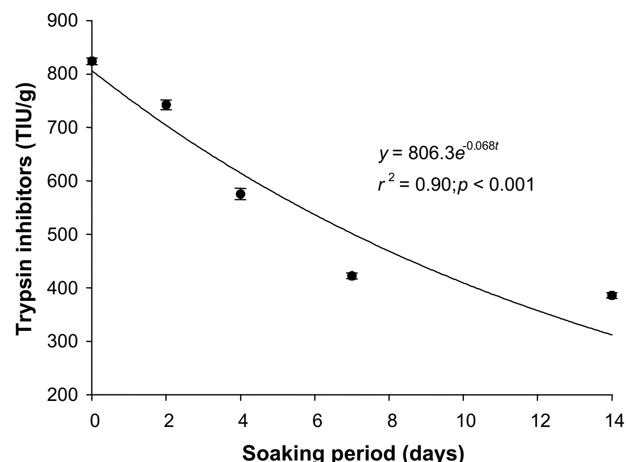

Fig. 9. Variation in trypsin inhibitors level during natural fermentation of *D. dumetorum* hardened tubers.

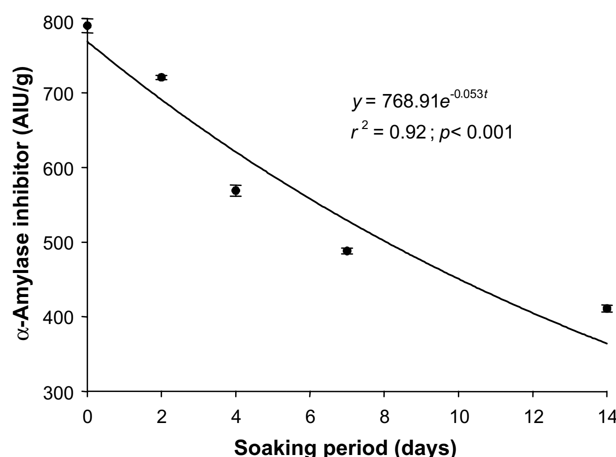

Fig. 8. Variation in amylase inhibitors level during natural fermentation of *D. dumetorum* hardened tubers.

3.2.4.4. α-Amylase inhibitors. α-Amylase inhibitor levels decreased from 790 to 411 AIU/g DM during the 14 days of soaking (Fig. 8). This means that about 48% of α-amylase inhibitors were lost after 14 day of natural fermentation, suggesting that the α-amylase inhibitors had been degraded. Since phenolic compounds and phytate are known for their roles in α-amylase inhibition (Deshpendes, Salhe, Salunkhe, & Cornforth, 1982; Westlake, McGregor, Hill, & Duckworth, 1983), they could be responsible for the amylase inhibition exhibited by *D. dumetorum* hardened tubers. Significant correlations were obtained in this study between α-amylase inhibitors and total phenols ($r = 0.987$; $p < 0.001$) and phytic acid ($r = 0.991$; $p < 0.001$).

3.2.4.5. Trypsin inhibitors. *D. dumetorum* hardened tubers possessed an antitrypsic activity of 824 TIU/g DM. During natural fermentation of *D. dumetorum* hardened tubers, an exponential decrease of trypsin inhibitors was noted (Fig. 9). After 14 days of soaking, there was about 53.7% trypsin inhibitors loss ($p < 0.05$). Reddy and Pierson (1994) reported a trypsin inhibitor loss of 47% during fermentation of vegetables. Decreases in trypsin inhibitors reported during food fermentation have been attributed to hydrolysis by micro-organisms involved in the fermentation.

3.2.4.6. Kinetic studies. Data obtained for phytic acid, total phenols, trypsin inhibitors and α-amylase inhibitors were analysed using Eq. (2), in order to determine the overall order and rate constant of the degradation reactions. The results obtained for the parameters studied showed that, the correlation coefficients were greater than 0.9 (Table 2), indicating that the degradation reactions of anti-nutritional factors in *D. dumetorum* hardened tubers during natural fermentation followed first-order reaction kinetics. The value of $t_{1/2}$, the time required for a compound to degrade to 50% of its original value was also calculated from the rate constant as $0.693/k$, and the results showed that degradation of phytate is more rapid than that of trypsin inhibitors, which is more rapid than that of total phenols and amylase inhibitors.

Table 2
Kinetic parameters for the degradation of some anti-nutritional factors, during natural fermentation of *D. dumetorum* hardened tubers

Kinetic parameters	Anti-nutritional factors							
	Phytic acid		Total phenols		α-Amylase inhibitors		Trypsin inhibitors	
	Constant	r^2	Constant	r^2	Constant	r^2	Constant	r^2
Rate constant, k (day^{-1})	0.097	0.98	0.055	0.89	0.053	0.92	0.068	0.90
$t_{1/2}$ (day)	7	0.98	12.6	0.89	13	0.92	10	0.90

4. Conclusion

Soaking of *D. dumetorum* hardened tubers in a closed system without air renewal prevented tuber browning, suggesting that browning of tubers during fermentation could have been caused by the oxidation of some organic compounds, such as phenolic compounds. In spite of the stability conferred to the cell–cell linkage by the hardening phenomenon, natural fermentation to completely softened *D. dumetorum* hardened tubers. Natural fermentation decreased anti-nutritional factor levels of the tubers, as a result of micro-organism activities. Except for oxalate levels, the decreases in anti-nutritional factors followed first-order reaction kinetics.

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