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Improved processing methods to reduce the total cyanide content of cassava roots from Burundi

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The identification of highly effective procedures that reduce the cyanogens contained in cassava roots which require no sophisticated equipment, and can readily be adopted by subsistence farmers is of tremendous importance. This study, which used cassava root samples collected in Burundi, included fermentation tests using both selected and native cultures at different temperatures for variable times. Moreover, drying procedures with and without fermentation were carried out. A factorial analysis of variance (ANOVA) showed that the detoxification was mainly affected by fermentation length and by the initial cyanogens content of the roots. When fermentation lasted 48 h and the initial cyanide level was lower than 300 mg/kg dry weight (d.w.), the detoxification was also found to vary based on the microorganism inoculated; *Saccharomyces cerevisiae* demonstrated the greatest effectiveness. In terms of drying conditions, a temperature of 60°C, even for a shorter duration of time (8 h), lowered the initial cyanide level by more than 90%. Finally, when dehydration followed fermentation, the pressed pulp showed a substantial reduction in cyanide content. By means of this last procedure, safe cassava was produced according to FAO/WHO amendments (10 mg HCN equivalent per kilogram flour), if the initial cyanide level of roots did not exceed 200 mg/kg d.w. Actually, the initial maximum total cyanide content was confirmed to be fundamental in order to obtain safe products in relation to processing method adopted.

Key words: Cassava, cyanide, detoxification, drying, fermentation.

INTRODUCTION

The relevance of cassava (*Manihot esculenta* Crantz) in the world is mainly a reflection of the agronomic advantages of the crop; in fact, after rice and maize, it is the third most important food source in the tropics (Cock, 1985) and is widely recognised as a productive crop and a good energy source (Osakwe et al., 2008). Due to its high drought tolerance and low demand for nutrients, it can produce acceptable yields even in unfavourable environmental conditions (Cock, 1982). Due to the perceived agricultural advantages of growing cassava and the increasing population pressures, its usage is being extended to regions in Africa and elsewhere (Cardoso et

al., 2005). Precisely, this perennial root crop is grown and exploited in the tropics, including sub-Saharan Africa, Asia, the Pacific Islands, and Central and South America (Hillocks et al., 2002; Lebot, 2009; Burns et al., 2010; McKey et al., 2010). At present, approximately 800 million people (FAO/IFAD, 2000; Lebot, 2009) rely on cassava as a staple food.

Cassava starchy storage roots are an excellent source of carbohydrates. However, this food source has three major deficiencies: poor shelf-life, low content of protein and free amino acids, and high content of the poisonous cyanogenic glucosides (CNG): linamarin (96%) and

lotaustralin (4%) (Cooke and Coursey, 1981). These cyanogens are distributed widely throughout the plant, with large amounts in the leaves and the root cortex (skin layer) and, generally, smaller amounts in the root parenchyma (interior). The designation of bitter and sweet varieties of cassava depends on the associated levels of toxicity (Sundaresan et al., 1987). Consumption of cassava products with high cyanogens levels may cause acute intoxications (Mlingi et al., 1992), aggravate goiter (Bourdoux et al., 1982) and, in severe circumstances, induce paralytic diseases (Tylleskar et al., 1992). To avoid dietary cyanide exposure, the glycosides and their metabolites, collectively known as cyanogens, must be removed by processing before consumption. The World Health Organization (WHO) has set the safe level of cyanogens in cassava flour at 10 mg/kg d.w. (FAO/WHO, 1991), and the acceptable limit in Indonesia is 40 mg/kg d.w. (Djazuli and Bradbury, 1999).

Effective cassava processing methods disintegrate the root tissue completely, thereby releasing an endogenous enzyme, linamarase; this endogenous β -glucosidase enables the hydrolysis of linamarin into glucose and acetone cyanohydrins (Conn, 1969). These chemical components will decompose above pH 6 into volatile hydrogen cyanide (HCN) that is rapidly lost from the system (Cooke and Maduagwu, 1978).

Many different methods have been developed over hundreds of years (Padmaja, 1995) to improve processing of cassava roots, resulting in less residual cyanide. Some methods remove nearly all residual cyanogens (Nambisan and Sundaresan, 1985; Dufour, 1994), but many methods leave appreciable amounts of cyanogens behind (Mlingi and Bainbridge, 1994; Cardoso et al., 1998). The processing methods generally adopted include a combination of procedures, such as peeling, slicing, fermentation, boiling, drying, pounding or milling and sieving. However, especially for the varieties which are high in CNG, the most popular and efficient processing method for their removal is fermentation (Nambisan, 2011). Actually, cassava is processed by fermentation in most parts of Africa and South America (Nambisan, 2011) where fermentation plays a significant socio-economic role being a highly desirable technique in the rural communities (Chelule et al., 2010). Yeasts have been identified as the predominant microorganism involved in cassava fermentation after lactic acid bacteria (Oyewole and Odunfa, 1990). Akindahunsi et al. (1999) showed that *Saccharomyces cerevisiae* is capable of utilising both the CNG and their metabolites, thus explaining why it is one of the natural floras involved in cassava fermentation during food processing. In addition, Okafor et al. (1998) observed that the combination of the three organisms *Lactobacillus coryneiformis*, *Lactobacillus delbrueckii* and *Saccharomyces* was generally more effective than any of the three organisms acting alone in the first 24 h fermentation. The short-cut method of alternate pounding and drying of cassava roots resulted in a sharp decline in glucoside levels,

but high cyanohydrin levels may remain if the products are not sufficiently dried (Mlingi et al., 1995). This process could be improved by sun-drying the products to obtain flour with low moisture, hence yielding a low cyanohydrin content. However, prolonged sun-drying of cassava roots alone will not reduce CNG in bitter cassava roots to the safe limit established by FAO (FAO/WHO, 1991).

There is a great need for development of improved processing methods for detoxification of cassava, especially for populations living in rural and poor areas, as in Burundi. To standardise the procedures, it is necessary to start with traditional and simple methods that can be modified to obtain safer end products that can be eaten directly or processed into flours.

MATERIALS AND METHODS

Chemicals

The enzymes linamarase (50 EU) and linamarin (BDH, Milano, Italy) were used.

Tubers

Cassava tubers were collected directly from a rural market in Gitega, Burundi. The samples were grouped, according to their toxicity (Bradbury and Holloway, 1988) and the taste properties related to their cyanide parenchyma content (King and Bradbury, 1995), into three groups: bitter white cassava (A, B and C), sweet white cassava (D, E and F), and sweet red cassava (G).

Preparation of cassava grated samples

Cassava tubers were peeled, and any damaged or mouldy parts were removed. Then, the samples were mixed together and the resulting pulp was grated to obtain a uniform mass. This way is useful to homogenize the total amount of pulp in order to overcome the high variability of the tubers, to ensure the homogeneous distribution of the cyanide in the product and to get quantitatively representative samples. The peeling of the tubers followed by grating is a traditional processing method since it allows enhancing the contact between the enzyme and CNG, resulting in higher hydrolysis (Sornyotha et al., 2010; Nebiyu and Getachew, 2011).

Microorganisms and related feedings

The microbial samples were composed of native cultures, called mixed cultures, and of selected cultures of *Lactobacillus plantarum* V22 (Lallemand, Canada), *Oenococcus oeni* (Lallemand, Canada), *S. cerevisiae* (AEB, Brescia, Italy), and *S. cerevisiae* collected from the Department of General Microbiology of the University of Reims, France. The solutions used for the yeast feeding were Yeast Nitrogen Base (OXOID, Hampshire, UK) and a nutrient solution prepared according to the procedure of Oboh and Akindahunsi (2003).

Handling of microorganisms

The native mixed cultures were taken in Burundi at the end of the fermentative process and were developed in malt extract broth (BD, Difco, USA) for three days. The selective *Lactobacillus plantarum*

Table 1. Characterization of cassava root samples^a.

Root sample	Humidity (g/100 g)	Ash (g/100g d.w.)	Crude fiber (g/100 g d.w.)	Tannins (mg/100 g d.w.)	Proteins (g/100 g d.w.)	HCN in unpeeled root (mg/kg d.w.)	HCN in peeled roots (mg/kg d.w.)
Bitter white							
A	48.40 ± 0.20 ^d	1.99 ± 0.15 ^{ab}	3.82 ± 0.02 ^d	0.31 ± 0.03 ^b	3.30 ± 0.10 ^b	700 ± 32 ^a	221 ± 26 ^a
B	44.80 ± 0.10 ^e	2.40 ± 0.47 ^a	2.59 ± 0.04 ^f	0.51 ± 0.06 ^a	3.10 ± 0.10 ^{bc}	677 ± 45 ^a	177 ± 10 ^b
C	49.40 ± 0.10 ^c	1.80 ± 0.50 ^b	4.37 ± 0.10 ^c	0.33 ± 0.05 ^b	2.90 ± 0.10 ^{bc}	732 ± 51 ^a	275 ± 24 ^a
Sweet white							
D	45.80 ± 0.20 ^e	0.79 ± 0.12 ^d	3.19 ± 0.01 ^e	0.36 ± 0.05 ^b	2.80 ± 0.20 ^c	563 ± 32 ^b	130 ± 11 ^c
E	54.00 ± 0.10 ^a	1.65 ± 0.21 ^c	5.46 ± 0.10 ^b	0.48 ± 0.02 ^a	3.50 ± 0.10 ^b	285 ± 21 ^c	89 ± 8 ^d
F	49.10 ± 0.10 ^c	1.87 ± 0.33 ^b	3.73 ± 0.02 ^d	0.37 ± 0.02 ^b	4.70 ± 0.10 ^a	500 ± 43 ^b	140 ± 12 ^c
Sweet red							
G	50.80 ± 0.20 ^b	2.12 ± 1.10 ^a	8.39 ± 0.03 ^a	0.43 ± 0.03 ^{ab}	3.20 ± 0.10 ^b	140 ± 15 ^d	63 ± 7 ^e

d.w., Dry weight. ^aValues represent means ± SD (n=3). Within each column different letters indicate significantly different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

V22 cultures were developed for three days in de Man, Rogosa and Sharpe (MRS) broth (OXOID, Hampshire, UK), *O. oeni* cultures were developed in MRS Broth, and *S. cerevisiae* cultures were developed in malt extract broth.

Fermentation tests

Various samples of grated cassava were subjected to fermentation processes using both native and selected microorganisms. These fermentation tests were carried out by varying the following parameters: fermentation time (24, 48 or 72 h), temperature of fermentation (25, 30 or 35°C), addition of nutrient solution for yeast (with or without), and fermentation container (open or closed). For each trial, a fermentation sample that was not inoculated with microorganisms was maintained (control sample). All tests were conducted in triplicate with a constant ratio of 1000 g cassava/1500 ml of water.

Drying tests with or without joining fermentation

The drying tests involved grated cassava, which were put in the oven for 8, 24 or 72 h at varying temperatures of 60 and 105°C. The samples were dried before and after fermentation with the selected microorganisms and the mixed cultures at 35°C for 24, 48 or 72 h with *S. cerevisiae* at 25°C for 24 h.

Chemical and microbiological analysis

Chemical analysis (humidity, ashes, crude fibre) of the cassava root samples was carried out using the standard method (Cunniff, 1995). The protein content was determined using the Kjeldhal method (Cunniff, 1995), and the tannin content was evaluated by means of the Folin-Ciocalteu method (Singleton et al., 1999).

Analysis of toxicity

The cyanogenic glycosides content was determined using the method reported by Bradbury et al. (1999), which requires the use

of a picrate paper kit, and the toxicity of the sample was expressed as mg HCN/kg dry weight (d.w.). The analysis was performed on untreated cassava roots and on samples after fermentation, drying and combined procedures.

Statistical analysis

The test results proceeded from three independent replications, and each analysis was performed in triplicate. Statistics were performed using a factorial ANOVA. Significantly different samples were selected either by Student's t-test at $p \leq 0.05$ or by post-hoc comparison with Tukey's test at $p \leq 0.05$ using SPSS software version 15.0 (SPSS, Chicago, USA).

RESULTS AND DISCUSSION

Characterisation of cassava root samples

The cassava root samples were characterised as reported in Table 1. With the exception of the cyanide content, the data on humidity, ashes, fibres, tannins, and proteins showed no substantial differences among the groups of bitter white, sweet white, and sweet red roots, confirming what was previously reported by Charles et al. (2005) and Sarkiyayi and Agar (2010). The data confirms that peeling represents the first substantial process step to lower the cassava toxicity, as the CNG are distributed in large amounts in the root cortex (skin layer) (Cooke and Coursey, 1981).

Furthermore, grating of the pulp, as the second step of sample preparation, enables linamarin to have contact with its hydrolytic enzyme (linamarase), resulting in hydrolysis and subsequent removal of the breakdown products (Sornyotha et al., 2010). Grating provides a higher surface area for fermentation and allows retting to be completed more quickly than when whole roots are used (Okafor et al., 1984).

Table 2. Significance of factors affecting fermentations when performed at different times.

Time (h)	Microorganism	Temperature (°C)	Initial HCN (mg/kg d.w.)	Cassava root (type)
24	n.s.	n.s.	$p \leq 0.001$	$p \leq 0.05$
48	$p \leq 0.05$	n.s.	n.s.	n.s.
72	n.s.	n.s.	$p \leq 0.001$	n.s.

d.w., Dry weight; n.s., not significant.

Table 3. Effect of the root type used for 24 h-fermentations on the cyanide level detected after fermentation^a.

Cassava root (type)	Initial HCN (mg/kg d.w.)	Final HCN (mg/kg d.w.)	Detoxification (% HCN reduction)
Sweet white	374 ± 23^b	248 ± 37^c	33.8
Bitter white	442 ± 31^a	94 ± 62^d	78.7

d.w., Dry weight. ^aValues represent means \pm SD (n=9). Within data different letters indicate significantly different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

Table 4. Effect of the selected microorganism inoculated for 48 h-fermentations on the cyanide content detected after fermentation^a. The initial cyanide level was 195 ± 12 mg/kg d.w..

Microorganism	Final HCN (mg/kg d.w.)	Detoxification (% HCN reduction)
Control	105 ± 50^a	46.2
<i>Saccharomyces cerevisiae</i>	66 ± 39^c	65.9
<i>Lactobacillus plantarum</i> V22	96 ± 35^{ab}	50.9
<i>Oenococcus oeni</i>	88 ± 32^b	55.1
Mixed cultures	128 ± 56^a	34.2

d.w., Dry weight. ^a Values represent means \pm SD (n=9). Within the column different letters indicate significantly different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

Fermentation tests

The results indicate that fermentation temperature was not significant (Table 2) as no consistent differences between 30 or 35°C fermentation temperatures were shown confirming the results of Bradbury (2006). In addition to 30 and 35°C fermentation temperatures, we also performed fermentation tests at 25°C but no significant HCN reduction was detected from the initial value. The initial HCN content of the root significantly ($p \leq 0.001$) affected the levels of cyanide detected at the end of the fermentation. The statistics divided the roots into two groups depending on the initial CNG content. In first group was samples with a low level of toxicity (HCN < 300 mg/kg d.w.), and the second group was samples with a high level of toxicity (HCN > 300 mg/kg d.w.).

The other key factor was the fermentation length (Table 2) that related to the initial CNG content, or to the root type, or to the microorganism type, significantly ($p \leq 0.001$) affected the level of HCN in the final processed product as already observed by Nambisan (2011).

The statistics highlight that in 24 h fermentations (Table 3), the initial data of toxicity was clustered for the cassava root type, and that the percentage of cyanogen reduction of the bitter cassava roots was more than double that of

the sweet ones. Moreover, taking into consideration all the cassava roots having initial toxicity values greater than 250 mg HCN/kg d.w. a reduction greater than the 80% of the initial cyanide content occurred in 24 h fermentations.

The effect of the initial cyanide content on the final HCN level was no more significant in the case of 48 h fermentation. In this case, an overall reduction was observed from 150 to 250 mg HCN/kg d.w. before fermentation up to 47 to 90 mg HCN/kg d.w. after the process. Indeed, the outputs of the 48 h fermentations (Table 2) emphasize the role of the type of microorganism culture used for fermentation as reported by Sornyotha et al. (2010) who observed a significant effect of most microorganisms in degrading the linamarin after 24 h of treatment. Results in Table 4 highlight the efficiency of the yeast *S. cerevisiae*, followed by *O. oeni* and *L. plantarum* V22, in abating the cyanide levels, while the mixed cultures proved to be less useful for the detoxification. These findings confirm the results of other researches (Tweyongyere and Katongole, 2002), regarding the fermentation of cassava roots soaked in water in which microbial growth was shown to be essential for the efficient elimination of cyanogens (Westby and Choo, 1994). From our investigation (Table 4), *S. cerevisiae* appears to play an important role in root detoxification, as

Table 5. Cyanide loss gained prolonging drying for different times^a.

Time (h)	Initial HCN (mg/kg d.w.)	Final HCN (mg/kg d.w.)	HCN reduction (%)
8	570 ± 32 ^a	31 ± 8 ^d	94.6
24	583 ± 23 ^a	91 ± 26 ^c	84.1
72	142 ± 21 ^b	17 ± 11 ^e	87.8

d.w., Dry weight ^aValues represent means ± SD (n=9). Within the data different letters indicate significantly different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

Table 6. Cyanide loss gained performing drying at different temperatures^a.

Temperature (°C)	Initial HCN (mg/kg d.w.)	Final HCN (mg/kg d.w.)	HCN reduction (%)
60	576 ± 24 ^a	31 ± 8 ^c	94.6
105	162 ± 22 ^b	21 ± 10 ^c	87.1

d.w. = dry weight ^aValues represent means ± SD (n=9). Within the data different letters indicate significantly different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

already reported by Oboh and Akindahunsi (2003).

Conversely to these findings, Okafor et al. (1998) detected that natural mixed cultures were generally more effective in reducing the cyanide content of the mash, and hence of the *gari*, than any of the components acting alone. As the fermentation progressed, a gradual reduction in the number of microorganisms due to the increased acidity of the medium (Oyewole, 2001) may support the fact that within the first 48 h fermentation, *S. cerevisiae* performed detoxification better than the other cultures.

Finally, regarding 72 h fermentations (Table 2), the initial cyanide content returned to being a significant factor affecting the final HCN level. The most significant decreases in the cyanide content were registered in association with the highest initial values (580 mg HCN/kg d.w.), obtaining detoxification rates ranging from 60 to 80%. This finding confirms what was reported by Kobawila et al. (2005) and Agbor-Egbe et al. (1995), who observed that the cyanide content in cassava roots decreases progressively during fermentation. Likewise, the results of Aloys and Zhou (2006) showed 93.8 and 82.4% reductions of the initial cyanide in Ikivunde and Inyange, respectively, at the end of a 120 h fermentation.

In any case, both the addition of feeding and keeping the fermentation container either open or closed did not significantly affect the results.

Drying tests with or without joining fermentation

Because of the hydro solubility of its cyanogenic glycosides and because of its water content (approximately 61% w/w), the dehydration process of cassava roots may result in substantial detoxification. The trials performed to this aim show that drying time, temperature, and initial cyanide content are highly significant ($p \leq 0.001$) factors affecting the final cyanide content of the processed roots.

Table 5 summarises the HCN levels measured after the drying process performed at three different times

and shows that very high reductions (close to 95%) of the initial cyanide contents occurred even for short (8 h) drying periods.

Regarding drying temperature (Table 6), the 60°C processing chosen to simulate the sun-dried conditions that usually take place in the villages of Africa and Latin America, produced better results than heating to 105°C, primarily due to a reduced surface pre-drying phenomenon at lower temperatures. These conditions allow a longer contact period between the glucosidase and the glucoside in the aqueous medium (Tewe, 1984) and therefore tended to result in a greater loss of bound cyanide due to the slower drying rate. On the contrary, the increase in drying temperature has long been observed as being accompanied by an increase in cyanide retention (Cooke and Maduagwu, 1978; Nambisan, 1994) because linamarase activity is inhibited at temperatures above 55°C (Gomez et al., 1984; Nambisan, 2011); therefore, linamarin starts to accumulate in the dried cassava. Our results underline that by standardising processing conditions that simulate the more empirical sun-drying method, more cyanogen degradation is promoted.

Finally, it is of interest to observe that independently of the initial toxicity of the cassava root, the drying process lowered the HCN level by more than 90% (Table 7). In the drying experiments, the glucoside degradation was highest at the beginning of the process, decreasing slightly over the following hours. The primary initial decrease coincided with the moisture loss: the glucoside degradation stopped at a moisture level between 33 and 13%, according to Essers et al. (1996), while Mlingi et al. (1995) expected to achieve values of 12 or 13%. During the drying process, water evaporates at the root surface. To restore the water balance in the tissue, water must pass through the cell walls. Therefore, the integrity of the membrane system ultimately determines whether the glycosides remain within the vacuoles or are transported along through the linamarase-containing cell wall. This

Table 7. Cyanide loss gained by means of drying for cassava roots clustered on the base of initial cyanide content ^a.

Initial HCN (mg/kg d.w.)	Final HCN (mg/kg d.w.)	HCN reduction (%)
97 ± 8 ^b	8 ± 1 ^d	91.5
586 ± 32 ^a	49 ± 14 ^c	91.4

d.w., Dry weight ^aValues represent means ± SD (n=9). Within the data different letters indicate significantly different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

Table 8. Cyanide loss gained with drying joint with fermentation^a. The cyanide content of the root samples before fermentation was 226 ± 29 mg/kg d.w..

Parameter	Final HCN (mg/kg d.w.)	HCN reduction (%)
Post fermentation	120 ± 68 ^a	46.9
Post drying	22 ± 19 ^b	82.1

d.w. = dry weight ^aValues represent means ± SD (n=9). Within the column different letters indicate significantly different values according to post-hoc comparison (Tukey's test) at $p \leq 0.05$.

forced transport of liquid might be a factor in enabling contact between the glycosides and the linamarase (Essers et al., 1996). The process of grating/pounding followed by simulated sun-drying is the most effective method because it puts linamarase directly into contact with linamarin and then reduces cyanohydrin and free cyanide to low levels. However, drying alone is not an efficient means of detoxification, especially for cassava varieties with high initial cyanogen glucoside content, as also observed by Mlingi and Bainbridge (1994).

Table 8 displays the percentage of toxicity reduction that occurred during the fermentation process followed by drying. While the treatment of fermentation enabled a toxicity decrease of almost 47%, the subsequent drying process further reduced the toxicity by more than 80%. Overall, processing cassava roots with a combination of fermentative treatment and a single stage of drying permitted the breakdown of the cyanogens content up to 90%. This simple procedure produces safely dried roots according to the FAO/WHO amendments, as the initial cyanide level does not exceed 200 mg/kg d.w.

Conclusion

Although processed foods are generally safe, lack of standardization in the methods used, the environment and the hygiene of the people that prepare them, will determine the quality of the product (Achi, 2005). So there is need to educate the citizens on the need of consuming treated and safe foods.

In view of the importance of cassava as a major source of food to the local people in developing countries, fear of HCN toxicity still exists by these people. Hence, searching for and application of different post harvest practices that can significantly reduce HCN will have great role in promoting the wider production and consumption

of cassava (Nebiyu and Getachew, 2011). A reduction in the cyanogenic potential of cassava can occur during every unit operation in the processing of the roots, resulting in near detoxification of the product. While all methods abate the cyanide levels, the effectiveness of these methods depends on the processing steps and the sequence utilised and is often time-dependent.

This study confirms the results of Chelule et al. (2010), outlining that fermentation of foods, as a hurdle technology, is profitable in terms of food quality, preservation and decontamination of toxins, often found in crops. In detail, the results of this survey show that peeling and grating cassava before a 48 h fermentation process by means of selected cultures of *S. cerevisiae*, followed by oven-drying for 8 h at 60°C resulted in end products with low cyanogen levels. Moreover, based on the results presented here, it is strongly advisable that before processing the initial CNG content of the cassava roots should be carefully monitored in order to ensure safe cassava end-products.

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