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Residual cyanogens, chemical composition and aflatoxins in cassava flour from Tanzanian villages

Y.C. Muzanila [b], J.G. Brennan [a], R.D. King [a,*]

[a] *Department of Food Science and Technology, The University of Reading, PO Box 226, Whiteknights, Reading RG6 6AP, UK*
[b] *Sokoine University of Agriculture, Food Science and Technology, PO Box 3006, Morogoro, Tanzania*

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Abstract

Cassava samples processed by wet fermentation, solid-state fermentation and sun-drying were analysed for residual cyanogens and the presence of mycotoxins. Wet fermentation was very effective in reducing cyanogen content in bitter varieties. The total cyanogen content of 5.84 mg HCN kg^{-1} was less than that for samples processed by solid-state fermentation which had a residual cyanogens content of 14.0 mg HCN kg^{-1}. Sun-dried cassava samples (sweet varieties) had 6.8 mg HCN kg^{-1}. The chemical composition of cassava flour, processed by wet fermentation, solid-state fermentation and sun-drying in Tanzanian villages, was also determined. Wet fermentation resulted in lower contents of vitamin C, reducing sugars and protein and also low pH values compared to samples processed by solid-state fermentation and sun-drying. Solid-state fermented samples had higher reducing sugars and protein contents than sun-dried samples. No mycotoxins (aflatoxins) were detected in the cassava samples.

1. Introduction

Cassava is a starchy food low in protein, minerals and vitamins, with the exception of vitamin C (Grace, 1977; Lancaster, Ingram, Lim & Coursey, 1982). The roots are processed by a variety of methods, including, sun-drying and fermentation, into a variety of products depending on local customs and preferences. The main products produced in Tanzania are cassava chips or flour (Berry, 1993). Although some cassava-processing methods result in substantial reduction in total cyanogen content (Gidamis, O'Brien & Poulter, 1993; Padmaja, 1995), they also result in reduction in vitamin and mineral content (Ezeala, 1984; Lancaster, Ingram, Lim & Coursey, 1982). Wet fermentation has been reported to cause losses of protein (Ayankumbi, Keshinro & Egele, 1991; Bokanga, O'Hair, Narayanan & Steinkraus, 1988). Padmaja, Mathew and Moorthy (1994) suggested that this is likely to result partly from the leaching into steeping water. Sun-drying also has been reported to cause a slight decrease in protein content (Fay, 1991) while solid-state fermentation can result in an increase in protein content (Zvauya & Muzondo, 1993).

Processing methods, such as sun-drying and solid-state fermentation, encompass a range of conditions under which mould growth and the production of mycotoxins is likely to occur (Westby, Wareing, Gibbs & Dallin, 1995). Scopoletin, a coumarin compound, which tends to accumulate in the cassava roots, can interfere in aflatoxin analysis. This compound fluoresces blue in UV light and has a similar RF value to aflatoxin B_1 in some thin-layer chromatography (TLC) systems (Wheatley, 1984). This could be responsible for the high aflatoxin values, 500–4600 mg kg^{-1}, that have been reported in cassava flour (Mota & Lourenco, 1974).

In this study we compare the cyanogen content, aflatoxin level and chemical composition of cassava products locally produced in Tanzania and compare these with previous reported laboratory studies using similar processing techniques.

* Corresponding author. Tel.: +44-118-9318711; fax: +44-118-9310080.

E-mail address: r.d.king@reading.ac.uk (R.D. King).

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2. Materials and methods

Samples of cassava chips were collected from three regions in Tanzania: Tanga in the Northeast, and Mwanza and Kagera in the Northwest of the country. In the Tanga region both wet and solid-state fermentation are practised. The solid-state fermented cassava chips are milled into flour without prior removal of the mould. In the Mwanza region, solid-state fermentation is the normal processing method, but the mould is scraped off before milling cassava chips into flour. In Kagera, sun-drying of thin slices of sweet cassava varieties is practised.

Six samples of cassava chips were collected per village from three villages in each region, these were pounded in a pestle and mortar, and sieved to obtain fine flour. The flour was packed into polythene bags and transported to Britain where it was kept at −18°C until required for analysis.

2.1. Cyanide extraction and determination

Cyanogens were determined using the enzymatic colorimetric method of Cooke (1978) with modifications described by O'Brien, Taylor and Poulter (1991). Cassava flour samples (30 g) were homogenised in 160 ml 0.1 M orthophosphoric acid containing 25% ethanol. For free cyanide (HCN), prior to assay, the extract was diluted with phosphate buffer. In the case of non-glycosidic cyanogens (intermediate + free cyanide) the extract was mixed with phosphate buffer and 0.2 M NaOH, then assayed colorimetrically. The assay for total cyanogens was carried out in the same way, with the addition of the enzyme linamarase.

2.2. Chemical composition analysis

Starch content was determined using the acid hydrolysis method according to AOAC method 22 (Association of Official Analytical Chemists [AOAC], 1995), with modifications as described by Rikard and Behn (1987). Vitamin C (ascorbic acid plus dehydroascorbic acid) analysis was done using the AOAC microfluorometric method, AOAC (1990). Reducing sugars were determined by Lane-Eynon's volumetric method (Egan, Kirk & Sawyer, 1988). The moisture content was determined by drying in a vacuum oven (Townson & Mercer Ltd. Croydon) at 70°C and 84.65 kNm^{-2} for 12 h and protein by the micro Kjeldahl method (Egan et al.) and expressed as N% × 6.25. The pH of the samples was determined with a Kent EIL 7045/46 pH meter, immersing the electrode in the homogenised samples.

2.3. Aflatoxins extraction and detection

Aflatoxins were analysed by TLC according to Park, Truckness, Nesheim, Stack and Newell (1994) and AOAC (1990).

3. Results and discussion

Cyanogen levels in cassava flour samples from the villages within each region were not significantly different ($P \geqslant 0.05$). The mean cyanogen levels in cassava flour samples processed by different methods are shown in Table 1. There is no statistical difference ($P \geqslant 0.05$) in the average total cyanogens for cassava samples processed by solid-state fermentation from the Tanga region (13.9 mg HCN kg^{-1}) and Ukerewe in the Mwanza region (14.0 mg HCN kg^{-1}), both are above the 10 mg kg^{-1} recommended safe level. The fermentation is carried out in the same way in the two regions except that, in Ukerewe, the mould is scraped off before milling cassava into flour. Mould growth is said to contribute to cyanogen loss due to enzyme-induced cellular disruption releasing β-glucosidases with linamarase activity (Essers & Nout, 1989; Gidamis et al., 1993). Linamarase-type activity has been observed in a number

Table 1
Cyanogens in cassava flour samples processed by different methods[a,c]

Processing method	Cyanogens content (mg HCN kg^{-1})			
	Total cyanogens	Intermediate cyanide (cyanohydrins)	Free cyanide (HCN)	Cyanogenic glucosides (bound cyanogens)
SSF-Tanga[d]	13.9 (0.51)[b]	0.41 (0.12)	0.96 (0.13)	0.96 (0.13)
SSF-Ukerewe[e]	14.0 (0.77)	0.31 (0.09)	0.88 (0.10)	12.53 (0.49)
Wet fermentation[f]	5.84 (0.51)	4.84 (0.46)	1.08 (0.10)	0.05 (0.17)
Sun-drying[g]	6.8 (0.4)	0.54 (0.43)	0.78 (0.11)	5.15 (0.86)

[a] Mean results from 3 villages within each region (18 samples).
[b] Standard deviation values are in parentheses.
[c] All values are given on a dry weight basis.
[d] SSF-Tanga, solid state fermentation in the Tanga region (Tanga district).
[e] SSF-Ukerewe, solid state fermentation in the Mwanza region (Ukerewe).
[f] Wet-fermentation, wet fermentation in the Tanga region (Muheza district).
[g] Sun-drying, sun-drying in Kagera region.

of yeasts and moulds (Muzondo & Zvauya, 1995). Gidamis et al. (1993) reported 58% reduction in total cyanogens in solid-state fermented roots. The wet-fermented product (Tanga region) had a total cyanogen content of 5.84 mg HCN kg^{-1}, within the recommended maximum levels.

Although the original cyanogen content of cassava samples is unknown, it is reasonable to conclude that there was reduction during fermentation as the bitter cassava varieties typically used have cyanogen contents in excess of 100 mg HCN kg^{-1} (Nartey, 1978). Again, we cannot directly compare the processing methods in this study because they were performed in different places by different people and on different samples. However, the trend observed in this study is similar to that observed by Gidamis et al. (1993) who processed cassava roots and found that wet fermentation causes higher losses in total cyanogens.

The cyanogen content of cassava flour processed by solid-state fermentation was mainly in the form of cyanogenic glucosides while, in wet fermented material, it existed mainly as cyanohydrin. Free cyanide levels in each case were relatively low. The mean total cyanogen levels in cassava flour samples from the Tanga region, which were processed by wet fermentation in the Muheza district, were 5.84 mg HCN kg^{-1}, i.e. within the recommended safe level. Wet fermentation has been reported by others to facilitate the breakdown of cyanogenic glucosides to low total cyanogen levels (Agbor-Egbe, Mbome, Noubi & Treche, 1995; Muzanila, 1993), in one case by up to 99% (Padmaja, 1995).

Dipping of cassava roots in water during the wet fermentation process makes them soft and causes the cells to rupture, releasing linamarase (Oyewole & Odunfa, 1992). Lactic acid bacteria play an important role in wet fermentation (Oyewole & Odunfa). *Lactobacillus plantarum* was found to be predominant; it produces a linamarase type enzyme with similar physiological properties to the endogenous cassava linamarase (Agbor-Egbe et al., 1995). It has also been reported that cyanogenic glucoside removal can be enhanced by direct leaching into the soaking water (Vasconcelos, Twiddy, Westby & Reilly, 1990).

Cyanogenic glucoside hydrolysis results in the formation of cyanohydrins, which in turn can be hydrolysed by the enzyme hydroxynitrile lyase to yield free cyanide (Cooke, Rikard & Thompson, 1985). This hydrolysis occurs spontaneously at pH 4 and above (Gidamis et al., 1993). Lactic acid bacteria can be expected to cause a pH reduction during fermentation. As the system becomes acidic a greater proportion of cyanohydrins will be retained in the cassava (Agbor-Egbe et al., 1995).

The mean cyanogen levels of cassava flour samples processed by sun-drying in the Kagera region are also shown in Table 1. The total cyanogen content, at 6.8 mg HCN kg^{-1}, is also within the recommended safe level, and mostly in the form of cyanogenic glucosides. The samples collected in this region were from sweet cassava varieties, so the levels of reduction achieved are probably rather less than that achieved by fermentation. Although chipping and slicing of the cassava root will cause considerable cell disruption and the rapid drying of the tissue will restrict mobility of enzymes and prevent further action. Mlingi, Bainsbridge, Poulter and Rosling (1995) reported that sun-drying of whole cassava roots does not cause sufficient cell disruption to achieve an adequate level of hydrolysis. Although sun-drying of bitter cassava varieties reduces the amount of cyanogenic glucosides considerably, about 10% of the initial cyanogenic glucosides remains in the flour (Mlingi et al., 1995). In all cases, the free cyanide produced is always rapidly lost and does not normally contribute to the dietary cyanogens (Mlingi).

Most of the cassava samples processed by wet fermentation had residual total cyanogen levels below 10 mg HCN kg^{-1}. Assuming a normal distribution in the results, the probability of samples containing residual total cyanogen levels greater than the recommended safe level is 6.1%. Likewise, most cassava samples from the Kagera region processed by sun-drying had residual total cyanogen levels below 10 mg HCN kg^{-1}. The probability of these samples containing residual total cyanogen levels greater than 10 mg HCN kg^{-1} is 9%.

4. Chemical composition of cassava flour

Table 2 shows the average chemical composition of cassava flour samples processed by the different methods. In this study, the original chemical composition of cassava before processing is not known but, by comparing the results with the values given in the literature, it is possible to see how these processing methods have affected the chemical composition of cassava samples.

The average starch contents of cassava flour samples from the Tanga and Ukerewe districts were 80.2 g/100 g, and 78 g/100 g, respectively. Comparing these values to the reported starch content of fresh cassava, which ranges from 73.7 to 84.9 g/100 g (Asiedu, 1992), it can seen that solid-state fermentation has little effect on the starch content. Wet fermentation resulted in lower values of starch content (71.5 g/100g), possibly as a result of amylolitic action of the fermenting mixture (Birk, Bravdo & Shoseyov, 1996). Starch levels were significantly higher in the sun-dried material.

The average reducing sugars content of cassava flour samples processed by different methods is also shown in Table 2. Samples processed by solid-state fermentation, in both the Tanga and Mwanza regions, had higher reducing sugars contents, 6.16 and 6.94 g/100 g, respectively than that reported for fresh cassava, 1.9–2.3 g/100 g (Gomez et al., 1984; Muzondo and Zvauya, 1995).

Table 2
Chemical composition of cassava flour samples processed by different methods[a,c]

Processing method	Starch (g/100 g)	Reducing sugars (g/100 g)	Protein (g/100 g)	Vitamin C (mg/100 g)	Moisture (g/100 g)	pH
SSF-Tanga[d]	80.2 (0.14)[b]	6.16 (0.45)	2.59 (0.13)	10.51 (0.51)	14.20 (0.34)	5.21 (0.07)
SSF-Ukerewe[e]	78.2 (0.59)	6.94 (0.23)	2.22 (0.02)	8.41 (0.23)	13.59 (0.20)	5.27 (0.05)
Wet fermentation[f]	71.5 (1.86)	3.00 (0.1)	0.74 (0.08)	6.29 (0.7)	13.67 (0.20)	4.29 (0.05)
Sun-drying[g]	84.8 (0.52)	2.37 (0.04)	1.22 (0.08)	25.17 (0.88)	12.66 (0.22)	6.22 (0.06)

[a] Mean results from 3 villages within each region (18 samples).
[b] Standard deviation values are in parentheses.
[c] All values are given on a dry weight basis.
[d] SSF-Tanga, solid state fermentation in Tanga region (Tanga district).
[e] SSF-Ukerewe, solid state fermentation in Mwanza region (Ukerewe).
[f] Wet-fermentation, wet fermentation in Tanga region (Muheza district).
[g] Sun-drying, sun-drying in Kagera region (Bukoba district).

Samples processed by wet fermentation had lower reducing sugars contents. A reduction of reducing sugars by wet fermentation was also reported by Birk et al. (1996). During fermentation, micro-organisms produce polysaccharide- and pectin-degrading enzymes which help to lyse the cell membranes and alter their integrity. These changes are likely to lead to leaching out of the soluble nutrients from cassava (Mathew, Padmaja & Moorthy, 1991).

A low content of reducing sugars is a welcome attribute, as it will reduce the effects of the Maillard reaction when the cassava flour is cooked. Sun-drying did not seem to have a measurable effect on the reducing sugar content of cassava flour samples. This can be compared to a study conducted by Ayankumbi et al. (1991) in which no changes in reducing sugar content of sun-dried cassava flour were observed.

The protein content of cassava flour was generally within the range reported for fresh cassava. The protein content of fresh cassava according to Ayankumbi et al. (1991) and Muzondo and Zvauya (1995) ranges from 1.0 to 3.5 g/100 g dry weight basis. The highest values of protein observed were in samples processed by solid-state fermentation. Soccol, Marin, Raimbault and Lebeault (1994) have observed an increase in protein content which was attributed to an increase in microbial protein.

Considerable variations in the vitamin C content were observed between the different flours. Sun-dried flour had the highest vitamin C content at 25.2 mg/100 g and wet fermentation the lowest at 6.29 mg/100 g.

5. Aflatoxins

Cassava samples processed by solid-state fermentation and sun-drying contained no aflatoxins. Aflatoxin B_1 standards were used since this is the most abundant and likely to be produced in cassava. Studies showed that *Aspergillus* species produce aflatoxin B_1 and G_1 at the same time in cassava. So the absence of aflatoxin B_1 indicates the absence of aflatoxin G_1 too. Westby et al. (1995) found that *A. parasiticus* C7 produces aflatoxins B_1 and G_1 on cassava, while *A. flavus* A41, *A. parasiticus* C7 and *A. parasiticus* MO39 produced aflatoxins B_1 and G_1 in rice.

6. Conclusions

Cassava samples processed by wet fermentation comply with the safe level of not more than 10 mg HCN kg^{-1}. The same applies to samples processed by sun drying. Although samples processed by solid-state fermentation exceeded the safe levels, it is only by about 4%. Again the residual cyanogens are in the form of cyanogenic glucosides (bound cyanogens). Barret, Hill, Alexander and Zitnak (1977) and Mlingi (1995) reported from animal experiments that ingested linamarin can be absorbed unchanged and excreted intact in urine. They further said that ingestion of linamarin can result in cyanide exposure if suitable microbial glucosidases are present in the gut. This mechanism is not confirmed as occurring in humans.

This study indicates that, cassava processed by wet fermentation in Tanzanian villages, is safe. When it is not possible to practise wet fermentation, grating followed by solid state fermentation will reduce cyanogen content to safe levels.

Most of the work on cassava detoxification has been done in the laboratory, but this study has revealed that, under normal conditions, cassava products processed in the villages, especially those processed by wet fermentation, are safe to eat.

Variations in the composition of cassava flour within the regions were remarkably small, considering that cassava samples were from different farms, and may have encountered variation in time of planting, climate and harvesting and post-harvest handling. In general, solid-state fermentation has been found to improve the nutritional value of cassava by increasing its protein content and retaining higher values of vitamin C. Sun-

drying also resulted in high vitamin C retention. The problem is that, these two methods may cause cassava to retain higher residual cyanogens than wet fermentation. Wet fermentation, although it causes a decrease in nutritional value of cassava, has the advantage of reducing cyanogens to low levels. However, in general, processed cassava requires adequate supplementation of protein and vitamins to meet the body requirement.

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