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Full Length Research Paper

Effect of processing conditions on cyanide content and colour of cassava flours from West Africa

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The evolution of cyanide content and colour were monitored during the processing of lafu, traditional flour and improved flour from five cassava cultivars from Benin. In addition, the total phenol, polyphe-noloxidase (PPO), peroxydase (POD) and linamarase activities were assessed. The processing of cassava in lafu and improved flour proved superior for producing safe and white non- fermented and fermented cassava flours with total cyanide mean values of 16.6 and 11.4 mg HCN/kg, db and ΔE values of 9.2 and 12.1, respectively. Detoxification appeared to be only linked to processing, in particular to the size reduction level of cassava roots, regardless of the initial cyanide level and the linamarase activity of the fresh roots. Cassava flour yellowness was closely linked to the phenol content (r = 0.95) that decreased after steeping and pressing. The PPO and POD activities did not appear to be linked to flour discoloration.

Key words: Manihot esculenta, cassava processing, cyanide, colour, total phenol, polyphenoloxidase, peroxydase, linamarase.

INTRODUCTION

Cassava (Manioc esculenta Crantz) is one of the most important energy sources for people in tropical regions (Cooke and Cock, 1989). In West Africa, particularly in Benin, cassava is consumed mainly after processing into garri, traditional flour, lafu and improved flour. Lafu, a fermented cassava flour, is processed in Nigeria and South-East Benin by soaking peeled roots in water (sub-merged fermentation) for 3 to 5 days (at ambient tempe-rature, 26 - 28°C), pressing and sun-drying (Padonou et al., 2005). Traditional flour, the most common cassava flour in rural areas, is obtained by sun-drying peeled roots for 3 to 5 days. Improved flour, recently introduced in Benin by the International Institute of Tropical Agriculture (Nweke, 1996), and is processed in one single day by peeling, crushing, pressing and sun-drying. Eventually, the resulting dried products are then milled into flours, which are used to prepare a thick paste named "oka" for lafu, and "gêoun" for the traditional and improved flours.

Colour is by far one of the main quality criteria for con-sumers' acceptance of cassava derived flours (Padonou et al., 2005): consumers prefer white flours to the yel-lowish ones. Unfortunately, the latter is the most fre-quently produced in West African countries, particularly in Benin. The mechanism of cassava flour discoloration is unknown, even if the role of phenolic compounds and of peroxidase (POD) and polyphenoloxidase (PPO) in the post-harvest physiological deterioration of cassava roots is well recognized (Buschmann et al., 2000). In parallel, it has been reported that total phenol, POD and PPO play a large part in the colour change of yam tubers and their derived flours (Akissoé et al., 2003).

Furthermore, cassava roots contain cyanogenic glycol-sides and cassava products that are not adequately pro-cessed have been linked to cyanide poisoning (Essers et al., 1996). Direct sun-drying of roots indeed leads to high cyanide levels in cassava flours (Muzanila et al., 2000) whereas a combination of techniques (such as crushing, fermentation/pressing and cooking) is an efficient way to produce safe cassava products such as garri (Agbor-Egbe and Mbome, 2006).

In Benin, cassava flours (lafu, traditional flour and im-

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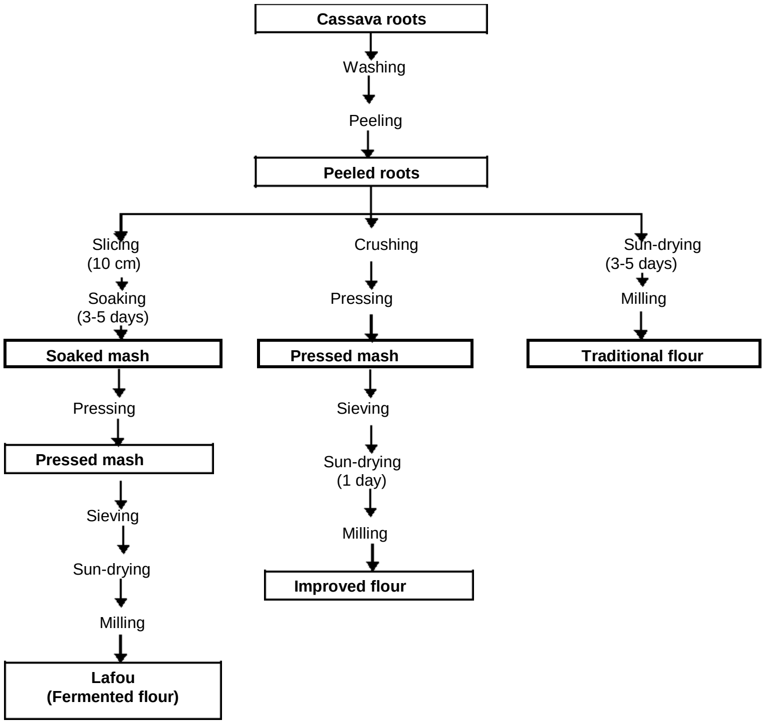

Figure 1. Processing of cassava into lafu, improved and traditional flours.

improved flour) are processed using various combinations of unit operations such as crushing, pressing and sun-drying. The objective of this study was to investigate the effects of the three processing methods for producing cassava flours on reducing cyanide and on colour change for five Beninese cassava cultivars. The role of some key enzymes in these modifications has also been studied to give tracks for improving the products.

MATERIALS AND METHODS

Plant material

Five cassava cultivars were obtained from International Institute of Tropical Agriculture in Benin (IITA-Benin). Two were sweet (AGRIC and GBEZE) and three were bitter (TMS 30572, TMS 91934 and TMS 920326). They were harvested 12 months after planting. The freshly harvested roots were brought to the laboratory and divided into two parts. The first part was used for direct analysis, and the second part was processed into three different cassava flours as described below. The roots were harvested and processed into flours twice within two weeks.

Cassava flours production

For lafu (fermented flour) production, 10 kg of cassava roots from each cultivar were hand-peeled and cut into 10 cm slices, which were immersed in 15 L of distilled water for 5 days until softening. The soaked root slices were pressed in a jute bag using a manual screw press (IITA, Ibadan, Nigeria). Fibrous material was removed by sieving and the mash was sun-dried at ambient temperature (30 - 35°C) for one day (Figure 1).

For improved flour production, 10 kg of cassava roots from each cultivar were hand-peeled and crushed using a mechanical crusher equipped with a perforated drum (IITA, Ibadan, Nigeria). The resulting mash was pressed in a jute bag using a manual screw press (IITA, Ibadan, Nigeria), sieved and sun-dried for one day.

For traditional flour processing, 10 kg of cassava roots from each cultivar were hand- peeled. The peeled roots were roughly sliced, then sun-dried for 3 - 5 days.

The resulting dried products were milled into fine flours with a laboratory mill (Perten 3100, Sweden), while the freshly peeled roots and intermediate products were freeze-dried and ground into flour with a laboratory mill (Perten 3100, Sweden) . The dried flours and freeze- dried products were packed in plastic bags and stored at 4°C until analysis.

Physico-chemical analysis

The dry matter content was determined after oven-drying at 105°C for 48 h. The pH and titrable acidity were measured following the methods described by Nout et al. (1989): 5 g of cassava product were dispersed in 20 ml of distilled water and homogenized for about 15 s. The pH was measured and titrable acidity was determined by adjusting the pH to 8.0 with 0.1 N NaOH. The total and free cyanides were determined using the methods described by Essers et al. (1996) except that linamarase was replaced by beta-glucosidase from almonds (Sigma # G 0395). The total phenol content, polyphenoloxidase (PPO) and peroxidase (POD) activities were determined following the methods described by Mestres et al. (2004). Total phenol was measured at 760 nm after reaction with Folin reagent. PPO was determined by measuring the oxygen consumption kinetic with a cathechol substrate, and POD was determined by measuring optical density at 460 nm after reaction with Pyrogallol prepared in H_2O_2 solution. The colour (of roots, intermediate products and flours) was measured using a chromameter

Table 1. Dry matter (%), acidity and cyanide content and linamarase activity in fresh roots of different cultivars.

Cultivars	Dry matter (% wb)	pH	Titrable acidity (mg/g, db)	Total cyanide (mg HCN/kg, db)	Free cyanide (mg HCN/kg, db)	Linamarase (µMol.min^{-1}g^{-1}, db)
AGRIC	28.2 ± 0.7 a	6.7 ± 0.2 c	0.29 ± 0.02 a	98.9 ± 4.7 a	10.8 ± 2.5 a	0.29 ± 0.03 ab
GBEZE	28.5 ± 0.7 a	6.5 ± 0.1 c	0.28 ± 0.03 a	103.2 ± 4.5 a	11.3 ± 2.9 a	0.23 ± 0.04 a
TMS 30572	27.2 ± 0.1 a	6.7 ± 0.1 c	0.23 ± 0.03 a	124.9 ± 1.6 b	13.8 ± 2.2 b	0.22 ± 0.05 a
TMS 91934	27.5 ± 0.1 a	6.7 ± 0.1 c	0.22 ± 0.03 a	140.0 ± 1.8 c	15.8 ± 1.4 b	0.21 ± 0.03 a
TMS 920326	28.2 ± 0.6 a	7.0 ± 0.1 c	0.22 ± 0.01 a	158.6 ± 0.9 d	15.7 ± 2.9 b	0.24 ± 0.05 a
Mean	27.9 ± 0.6	6.7 ± 0.2	0.25 ± 0.04	125.2 ± 22.3	13.5 ± 2.9	0.24 ± 0.04

Average ± standard deviation (n = 2); values in the same column not followed by the same letter are significantly different ($p < 0.05$).

(CR-210, Minolta). The Hunter Lab colour coordinate system, L*(lightness), a*(redness) b*(yellowness) values were recorded and E the total colour difference from the white ceramic standard was calculated according to Nago et al. (1998b): $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$. All the measurements were duplicated.

Statistical analysis

The statistical analyses were performed on duplicate data using Statistica 7.1 (StatSoft, Tulsa, USA) using ANOVA procedure, Newman-Keulss mean comparison tests (with $p < 0.05$), correlation and linear regression.

RESULTS AND DISCUSSION Dry

matter and processing yields

The fresh root dry matter (DM) content range was very narrow (between 27.2 and 28.7%, wb, Table 1), and no significant difference was observed between cultivars.

Irrespective of the cassava cultivars, the mean yield with processed traditional flour was higher (72.9%, db) than with improved flour and lafu (66.4 and 65.8%, db, respectively). The material losses were 2.4% during crushing, 2.1% during soaking and 1.9% during sieving. A similar yield for lafu processing (63 - 67%, db) had been previously reported (Gbaguidi-Darboux et al., 2001).

Irrespective of the cultivar and processing, dry matter content in flour was similar, with a mean value of 89% (wb), an adequate value for storage and transportation.

pH and titrable acidity changes during processing

As regards to pH and titrable acidity, no significant differrences were observed between cultivars (Table 1). The pH significantly decreased and titrable acidity increased after processing: the intensity of acidification was greater for lafu (2.0 mg lactic acid eq/g; pH value of 4.4; Table 2).

The titrable acidity in improved flour was close to 0.4 mg lactic acid eq/g, a value reported in Nigerian flour (Shittu et al., 2007), and the pH of Beninese lafu was similar to 4.5 mg lactic acid eq/g reported on lafu from Nigeria (Oyewole and Afolami, 2001). As for lafu, it may be inferred that the increase in titrable acidity observed in traditional flour may be due to some extent to the fermentation process during a long-term sun-drying; indeed, the water content is sufficient to initiate fermentation during the first days of drying. This phenomenon is therefore much less pronounced for the improved flour, which is dried in one single day.

Cyanide and linamarase activity reduction during processing

The total cyanide content in peeled cassava roots ranged between 98.9 and 158.6 mg HCN/kg, db (Table 1). The lowest total cyanide contents were observed in AGRIC and GBEZE roots (around 100 mg HCN/kg, db), and the highest in the three other cultivars (around 150 mg HCN/kg, db) . These results are in accordance with the classification used by Beninese farmers (Nago and Hounhouigan, 1998a).

Processing significantly ($p < 0.05$) reduced total cyanide content in fresh root. The mean total cyanide contents decreased to 11.4, 16.6 and 36.5 mg HCN/kg, db in lafu, improved flour and traditional flour, respectively, irrespective of the initial fresh root cyanide level (Table 2). The reduction rate was higher in lafu (91%), followed by improved flour (86%) and then traditional flour samples (70%). Cyanide reduction rate for soaked roots was higher than previously reported (70 - 85%) for roots soaked for 3 days (Hahn, 1989), and our flours' total cyanide contents were lower than similar fermented cassava flour (34.2 mg HCN/kg, db) and non-fermented flour samples (64.0 mg HCN/kg, db) from Mozambique (Muzanila et al., 2000). The mean total cyanide level in improved flour (16.6 mg HCN/kg, db) was however similar to that reported in non-fermented flour (17.0 mg HCN/kg, db) processed by oven-drying at 60°C for 20 h (Charles et al., 2005). Despite the drastic reduction, our flours' final total cyanide contents remained higher (1.1 to 3.6 times) than the recommended safe level of 10 mg HCN/kg, db (FAO/WHO, 1991). Sun-drying alone caused a drastic total cyanide reduction (70%); however combining with

Table 2. Dry matter (%), acidity and cyanide content and linamarase activity in different cassava products (mean values for the 5 cultivars)

Product	Process step	Dried matter (% wb)	pH	Titrable acidity (mg/g, db)	Total cyanide (mg HCN/kg, db)	Free cyanide (mg HCN/kg, db)	Linamarase (µMol.min^{-1}g^{-1}, db)
Fresh root	Freeze-drying peeled root	27.9 ± 0.6 a	6.7 ± 0.2 c	0.25 ± 0.04 a	125.2 ± 22.3 f	13.5 ± 2.9 b	0.24 ± 0.04 c
Traditional flour	Sun drying	89.4 ± 0.3 d	6.1 ± 0.2 b	1.4 ± 0.3 b	36.5 ± 4.6 b	6.5 ± 1.3 a	0.19 ± 0.05 b
Improved flour	Pressed mash	47.7 ± 0.7 c	6.7 ± 0.2 c	0.37 ± 0.06 a	75.5 ± 12.6 e	13.6 ± 3.5 b	0.09 ± 0.01 a
	Sun drying	89.2 ± 0.4 d	6.2 ± 0.3 b	0.60 ± 0.01 a	16.6 ± 3.3 b	6.2 ± 1.0 a	0.07 ± 0.02 a
Lafu	Soaked mash	34.6 ± 0.6 b	5.1 ± 0.6 ab	2.4 ± 0.2 c	51.9 ± 8.0 d	17.5 ± 2.4 cd	0.08 ± 0.01 a
	Pressed mash	50.9 ± 0.7 c	4.5 ± 0.5 a	2.5 ± 0.3 c	30.1 ± 5.2 c	10.2 ± 1.3 b	0.05 ± 0.01 a
	Sun drying	89.0 ± 0.1 d	4.4 ± 0.5 a	2.0 ± 0.4 c	11.4 ± 1.4 a	5.3 ± 0.6 a	0.04 ± 0.01 a

Average ± standard deviation (n = 10); values in the same column not followed by the same letter are significantly different ($p<0.05$).

other unit operations such as - crushing and pressing (improved flour processing), or soaking and pressing (lafu processing) - appeared to be more efficient. Similarly, combining soaking and pressing was more efficient than pressing alone. The differences between total cyanide contents of the flours appeared only due to bound cyanide. Cyanide reduction is thus limited by the hydrolysis of linamarine, due to the action of the linamarase, but not by the free cyanide elimination.

The linamarase activity in peeled root varied between 0.22 and 0.29 µMol.min^{-1}g^{-1}; AGRIC presented the highest level and TMS 91934 the lowest (Table 1). No relationship was observed between linamarase activity and total residual cyanide content in the products. The linamarase activity decreased after processing, but the drop was low for traditional flour. Sun-drying at ambient temperature (30°C) therefore kept a large part of the linamarase active. Correa et al. (2002) also found high linamarase activity in sun-dried cassava leaves. A drastic decrease in linamarase activity was however observed in improved flour, and in lafu; Linamarase activity was more than halved after crushing/pressing for improved flour, and after soaking for lafu. It is therefore questionable whether this decrease in linamarase activity is detrimental to the detoxification process. The time required for the total hydrolysis of fresh root cyanides can be calculated: it is 16 min for original linamarase activity in fresh roots, and 2 h for residual activity in lafu. Linamarase activity is therefore theoretically not limiting. The hydrolysis of linamarine is however limited due to a lack of contact between the enzyme (present in the cell wall) and the substrate (in the vacuoles). Fragmentation is therefore necessary to initiate hydro-lysis (Agbor -Egbe and Mbome, 2006). Soaking (that causes cell well degradation) or root size reduction by crushing increases the surface area of contact between cyanogen glycosides and linamarase (Muzanila et al., 2000), and the cyanide reduction level has been found to be strongly correlated to the degree of root size reduction (Agbor- Egbe and Mbome, 2006). This explains the fact that traditional flours retain the highest cyanide level (in bound form) whereas they present the highest linamarase residual activity.

Effect of processing on colour and phenolic compounds of cassava flour

∆E values in fresh roots, the total colour difference from standard white tile, ranged from 16.3 to 17.1 (Table 3) and were close to the values reported by Padonou et al. (2005) for other Beninese cultivars (range of 17.0 - 24.8). After processing, lafu and improved flours became whiter with a significant decrease in ∆E and b* and an increase in luminosity (Table 4). Conversely, an increase in ∆E value was observed for traditional flour. Consumers generally look for a white colour in cassava flours (Nweke, 1996; Padonou et al., 2005). Hence they will prefer improved cassava flours to traditional flours in terms of this attribute. Total phenol content and PPO activity in fresh root were similar for all cultivars, with mean values of 1.5 µMg^{-1} and 15.4 µM O$_2$ g^{-1} min^{-1}, respectively. How-ever, POD activity varied depending on the cultivar; GBEZE and AGRIC showed the lowest POD activity in fresh roots, while TMS 30572 and TMS 91934 had the highest (Table 3). Total phenol content in traditional flour (1.7 µMg^{-1}) was close to that of fresh roots (1.5 µMg^{-1}). It decreased drastically in lafu and improved flour (Table 4). This drop can be partly explained by partial phenol leaching into the waste water after soaking and/or pressing. Similarly, phenol leaching was observed during yam chip bleaching (Akissoé et al., 2003). Total phenol content in the cassava flours was significantly and positively correlated with yellowness (r = 0.95; Figure 2).

PPO activity dramatically decreased after pro-

Table 3. Colour, phenolic compounds content and PPO and POD activities in fresh roots.

Cultivars	Luminosity (L*)	b*	ΔE	Total phenol (μMg^{-1}, db)	PPO (μM O$_2$ g^{-1} min^{-1}, db)	POD (mDO s^{-1}g^{-1}, db)
AGRIC	86.1 ± 1.5 a	13.5 ± 1.4 b	16.8 ± 1.3 a	1.5 ± 0.05 a	16.1 ± 0.8 ab	9.6 ± 2.0 a
GBEZE	86.3 ± 1.1 a	14.2 ± 1.4 b	17.0 ± 1.3 a	1.5 ± 0.1 a	14.3 ± 1.6 a	7.8 ± 1.2 a
TMS 30572	84.8 ± 1.3 a	12.4 ± 1.4 ab	17.1 ± 1.3 a	1.5 ± 0.03 a	15.6 ± 0.5 ab	17.7 ± 0.8 c
TMS 91934	85.2 ± 1.3 a	12.0 ± 1.5 ab	16.3 ± 1.4 a	1.7 ± 0.1 ab	14.6 ± 0.7 a	19.7 ± 1.4 c
TMS 920326	85.1 ± 1.2 a	13.3 ± 1.4 b	17.2 ±1.5 a	1.4 ± 0.04 a	16.3 ± 0.9 ab	11.7 ± 1.2 b
Mean	85.5 ± 1.2	13.1 ± 1.3	16.9 ± 1.1	1.5 ± 0.1	15.4 ± 1.1	13.3 ± 4.9

Average ± standard deviation (n = 2); values in the same column not followed by the same letter are significantly different ($p < 0.05$).

Table 4. Colour, phenolic compounds content and PPO and POD activities in different cassava products (mean values for the 5 cultivars).

Product	Process step	Luminosity (L*)	b*	ΔE	Total phenol (μMg^{-1}, db)	PPO (μM O$_2$ g^{-1} min^{-1}, db)	POD (mDO s^{-1}g^{-1}, db)
Fresh root	Freeze-drying peeled root	85.5 ± 1.2 b	13.1 ± 1.3 d	16.9 ± 1.1 bc	1.5 ± 0.1 c	15.4 ± 1.1 d	13.3 ± 4.9 b
Traditional flour	Sun drying	78.7 ± 1.4 a	11.2 ± 1.7 c	18.7 ± 1.9 c	1.7 ± 0.2 c	1.8 ± 0.3 a	0 a
Improved flour	Pressed mash	89.6 ± 1.3 b	9.8 ± 1.0 bc	16.0 ±1.2 bc	1.1 ± 0.1 b	15.5 ± 1.2 d	3.4 ± 1.4 a
	Sun drying	93.8 ± 1.1 c	5.1 ± 0.7 b	12.1 ±1.3 b	0.4 ± 0.1 b	3.8 ± 0.5 b	0 a
Lafu	Soaked mash	84.9 ± 1.3 b	11.2 ± 1.1 c	20.8 ±1.1 d	1.0 ± 0.1 b	14.8 ± 1.0 d	0 a
	Pressed mash	90.5 ± 1.1 b	9.1 ± 0.7 bc	15.5 ± 0.6 bc	0.6 ± 0.1 b	10.9 ± 1.3 c	0 a
	Sun drying	95.3 ± 1.1 c	2.4 ± 0.3 a	9.2 ± 0.6 a	0.1 ± 0.05 a	4.1 ± 0.6 b	0 a

Average ± standard deviation (n = 10); values.

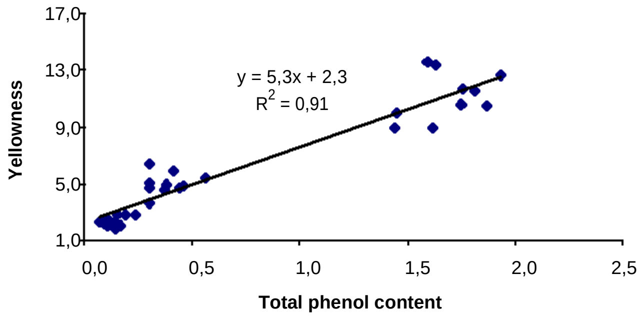

Figure 2. Relationship between total phenol content and yellowness of cassava flours.

processing (Table 4). Traditional flours presented the lowest residual PPO activity, but were also the darkest. As for yam (Akissoé et al., 2003), PPO was not the pri-mary agent responsible for cassava flour staining. The relatively low inhibition of PPO observed for improved flour and lafu could be due to the shortness of their drying (one day versus 3 - 5 days for traditional flour), which should be not sufficient to cause the long-term PPO de-gradation suggested by Akissoé et al. (2003). POD was completely inactive in all the dried flour samples. Indeed POD is highly sensitive to heat and is completely inactive after 20 h at 65°C (Akissoé et al., 2003) for yam. Unlike yam, no significant phenol content increase could be observed after drying. The role of PPO and/or POD in the discoloration phenomenon could therefore not be demonstrated. If they play a role in the discoloration phenomenon of cassava flour, it is minor compared with that of pressing and/or steeping, which induces flour bleaching.

Conclusion

The processing of cassava roots in improved flour and lafu proved superior for producing safe and white non-fermented and fermented cassava flours, respectively. Safe products were produced regardless of the initial cyanide level and the linamarase activity of the fresh roots.
Cassava flour colour was closely linked to the phenol content; steeping and pressing promotes phenol leaching and cassava flour bleaching. The PPO and POD activeties did not appear to be linked to flour discoloration.

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