

Effect of traditional and improved cassava processing on Cassava Derivative Products in Central African Republic

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is a starchy staple food that previous researches have showed to contain cyanogenic compounds, precursors of hydrocyanic acid, undoubtedly toxic for humans. The aim of this study is to compare the effect of traditional and improved cassava processing in Cassava derived products. Data were analysed by unpaired student's t-test at a confidence level of 95% ($P < 0.05$). Carbohydrate, lipids, cyanogenic compounds and Humidity profile proved that there is variability between the measured parameters according to the traditional and improved cassava processing ($P < 0.0001$).

Keywords: Cassava, Derivative Products, Technological Treatment

Introduction

Cultivated cassava is usually *Manihot esculenta* Crantz. Since its domestication thousands of years ago in the Amazon region, cassava is now spread around the world and is widely cultivated for consumption in the tropics and sub-tropic (Siritunga, 2002; Tewe and Lutaladio, 2004; Burns *and al.*, 2010; FAOSTAT, 2012). Cassava is a valuable source of food for developing countries and Central African Republic (CAR) is no an exception (Conaway *and al.*, 2012). Cassava is a nutritionally strategic famine crop could support food security in areas of low rainfall. Mature roots are able to survive for a long time without water and still retain nutritional value. Roots are a valuable source of calories, whereas cassava leaves are a valuable source of protein, minerals, and vitamins (Aboua and Kamenan, 1996; Wobeto *and al.*, 2006; Chijindu and Boateng, 2008; Sahoré Drogba and NemlinGnopo, 2010).

Cassava is grown in CAR as a subsistence crop. Its utilization as food varies from region to region. It is a source of food security, not only because it can be grown on less productive land, but because it is a source of income for urban and rural populations (Kosh-Komba *and al.*, 2016). People in many parts of CAR rely largely on starchy foods for their carbohydrates intake. The population consume it in various forms: tuber without any preparation, chicwangue, porridge soup, roasted, braised (retted or unretted) in the form of cooked pasta prepared from flower made out of pieces of cassava. More than 80% of Central African populations' energetic needs come from cassava. However, the status of cassava as a food security crop to most subsistence farmers is threatened by pests, diseases and weeds and inadequate processing techniques to eliminate the cyanogenic glycoside in cassava (Pohlandt *and al.*, 1983; Ernesto *and al.*, 2002; Diallo *and al.*, 2014).

Usually, cassava is well processed before being consumed. Inadequate processing however may result in appreciable amounts of cyanogenic glycosides remaining and this may pose a public health risk (WHO, 2004; ATSDR, 2006; FSANZ, 2008; Mburu *and al.*, 2012; Abraham *and al.* 2016).

The aim of this study is to assess cyanide content in various processed cassava to determine how different processing techniques can help reduce cyanide content and to provide information to relevant stakeholders on cyanide contents in cassava derived products.

2. Materials and Methods

2.1. Choice of Surveyed Sites

In response to the invitation to participate in the research project, samples were received from Yaloké, Sibut and Pissa (figure 1). The sites were chosen to represent a range of environments and management practices in cassava-based cropping systems in the mid-altitude zone of CAR. The climate in all sites is sub-humid with a bimodal rainfall distribution. This allows for the production of most annual crops during both the long (March–August) and the short rains (September–October). Altitude ranges between 1200 and 1500 masl. Cassava is planted in the first 2 months of the short or long rains and remains in the field for about a year. Agricultural systems are diverse with farmers growing 4–6 main crops on average (Boulvert, 1986).

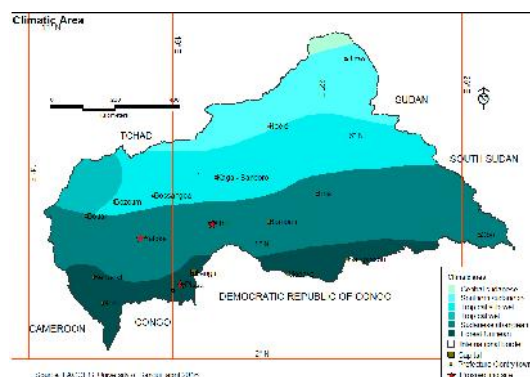


Figure 1. Location of sites for data collection in Central African Republic

2.2 Sample Collection

A questionnaire was prepared and used as a tool for the collection of information in the different sites. The questionnaire focused on the different processing to eliminate the cyanogenic glycoside in cassava. 250- 350 households per site were randomly selected. Structured interviews, in combination with a visit of different processing techniques (traditional and improved processing) to eliminate the cyanogenic glycoside in cassava were documented. Cassava Derivative Products (Locally called cossette of cassava and bread of war) were obtained from farms market vendors from Yaloké, Sibut and Pissa.

2.3 Humidity level

5 g of each sample is putting in an oven at 85 ° C until their mass is stabilized. The mass obtained after this operation, subtracted from the initial mass (5g), give the water content (AOAC 1975) according to the formular $% H = [(M1-M2)/M1] \times 100$. M1 = initial mass (5g), M2 = stabilized mass and % H= Humidity level.

2.4 Glucide dosage

The reducing sugars are assayed according to the **MILLER method (1959)**. At elevated temperature and in basic medium, 3,5-dichlorosilicic acid is reduced to 3-amino-5-nitrosalicylic acid in the presence of reducing sugars. The product of the reaction is yellow-orange, and has a maximum absorption at 575 nm. This method makes it possible to determine concentrations of reducing sugars ranging from 0 to 1 g / l. To 1 ml of the suitably diluted sample was added 1 ml of the Dinitrosilicic acid reagent. The mixture is boiled for 5 minutes and cooled rapidly by immersing it in an ice bath. After the addition of 8 ml of distilled water, the contents of the tubes are vortex-homogenized and the optical density is read at 575 nm. A glucose standard range from 0 to 1 g / l is carried out in parallel for each series of measurements.

2.5 Lipids dosage

100 g of cossette of cassava were extracted with 250 ml of Hexan as solvent during 6 hours on soxhlet (AOAC 1975). The extract was evaporated in the Rotavapor until the total evacuation of the solvent. The mass lipids were determined by making the difference between the full bottle and the empty bottle.

2.6 Cyanogenic glycoside dosage

2.6.1 Extraction of cyanogenic compounds

Samples of 10 g from Cassava Derivative Products (cossette of cassava) were homogenized in 30 ml of acid extraction medium. The amount of sample varied, but the ratio of sample to extraction medium was always approximately 1:3. The homogenized samples were left to stand for 10 min and then centrifuged at 10,000 g for 10 min (Barthet and Bacala, 2010). The supernatant was stored at 4°C until assayed for cyanogenic compounds.

2.6.2 Cyanogenic glycoside determination in cassava derivative products

The cyanide concentration in cossette of cassava was determined using ninhydrin based spectrometer of trace cyanide at 485 nm maximum wavelength (Ikédiobi *and al.*, 1980; Surleva *and al.*, 2013; Lambri *and al.*, 2013). A calibration graph was first constructed using standard solutions of CN⁻ at concentrations of 0.02, 0.04, 0.08, 0.1 and 0.2 µg/mL (which is within the linear range) and was prepared by adding appropriate volumes of cyanide solutions at concentration of 20 µg CN⁻/mL to 1 mL of 2% Na₂CO₃. Ninhydrin solution (0.5 mL) containing 5 mg/mL in 2% NaOH was added to each standard cyanide solution. The mixture was homogenized and incubated for 15 minutes for color development. Similarly, the blank was prepared in the same way as above, except that instead of 1 mL 2% Na₂CO₃ containing CN⁻, 1 mL of 2% Na₂CO₃ without CN⁻ was added. UV-Visible absorption of the reaction product (Cyanide-ninhydrin adduct) of the different concentrations of cyanide was measured using UV/Vis Spectrophotometer at 485 nm. Total cyanide in the samples was determined by adding 0.1 g of the ground sample in a standard volumetric flask (5 mL) and made up to mark with 0.1% NaHCO₃. The samples were sonicated for 20 minutes in a water bath and the mixture centrifuged at 10,000 rpm for 10 minutes. The supernatant was pipetted with automatic pipette, two aliquots (2 mL each) and added to 0.5 mL ninhydrin in NaOH, allowed for fifteen minutes for color development and absorbance measured at 485 nm.

2.7 Data analysis

Data were analysed by unpaired student's t-test at a confidence level of 95% (P<0.05) to find the significant difference between various parameters. XLSTAT statistical software (version 2008) was used for data analysis. Excel (version 2003) was used for showing the correlation between the traditional cassava processing and improved cassava processing.

3. Results

Cassava derivate products were obtained using traditional and improved processes to closely reflect the way that people consume these products. In this study, the selected products were Cossette of cassava and bread of war.

3.1. Traditional cassava processing to obtain cossette of cassava and bread of war

Traditional processing techniques for cassava are described in Figures 2, 4 and 6. Most of the prospective households do the retting of unpeeled cassava roots in the backwaters with a retting period ranging from 3 to 5 days according to the seasons. The drying time varies from 10 to 20 hours depending on the dry and rainy seasons.

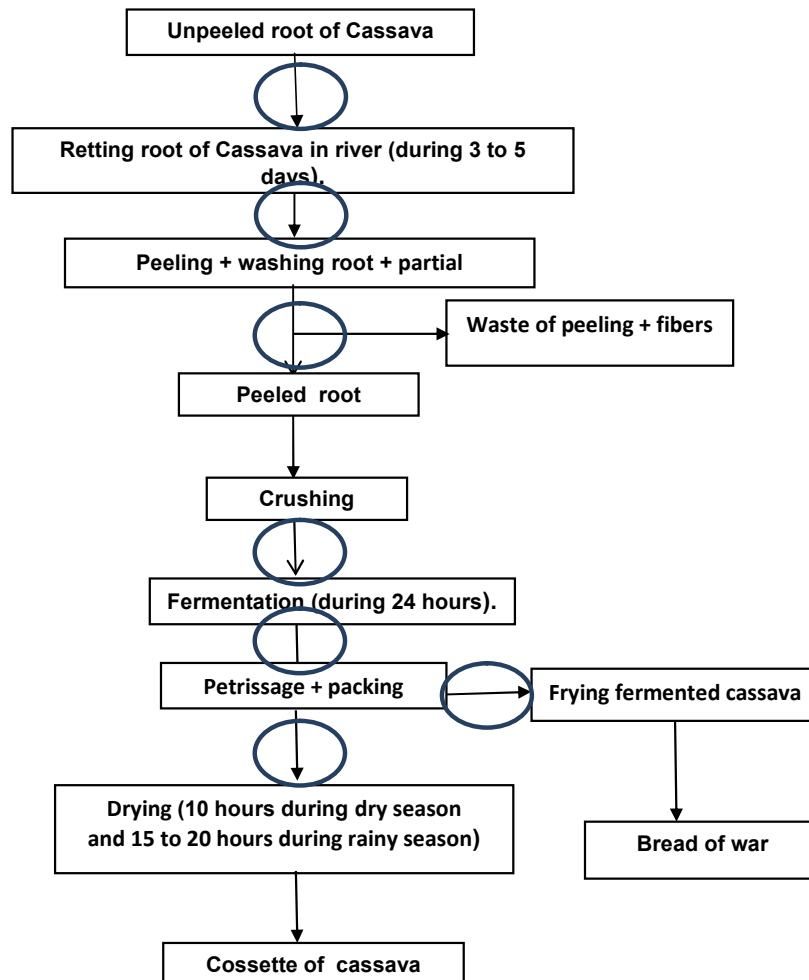


Figure 2. Traditional cassava processing to obtain cossette of cassava and bread of war

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3.2. Improved cassava processing to obtain cossette of cassava and bread of war

Improved cassava processing techniques are described in Figures 3, 5 and 7. The retting process in Plastic Drums ranging in length from 24 to 36 hours is done with peeled roots. Drying time varies from 1 to 2 hours depending on dry and rainy seasons.

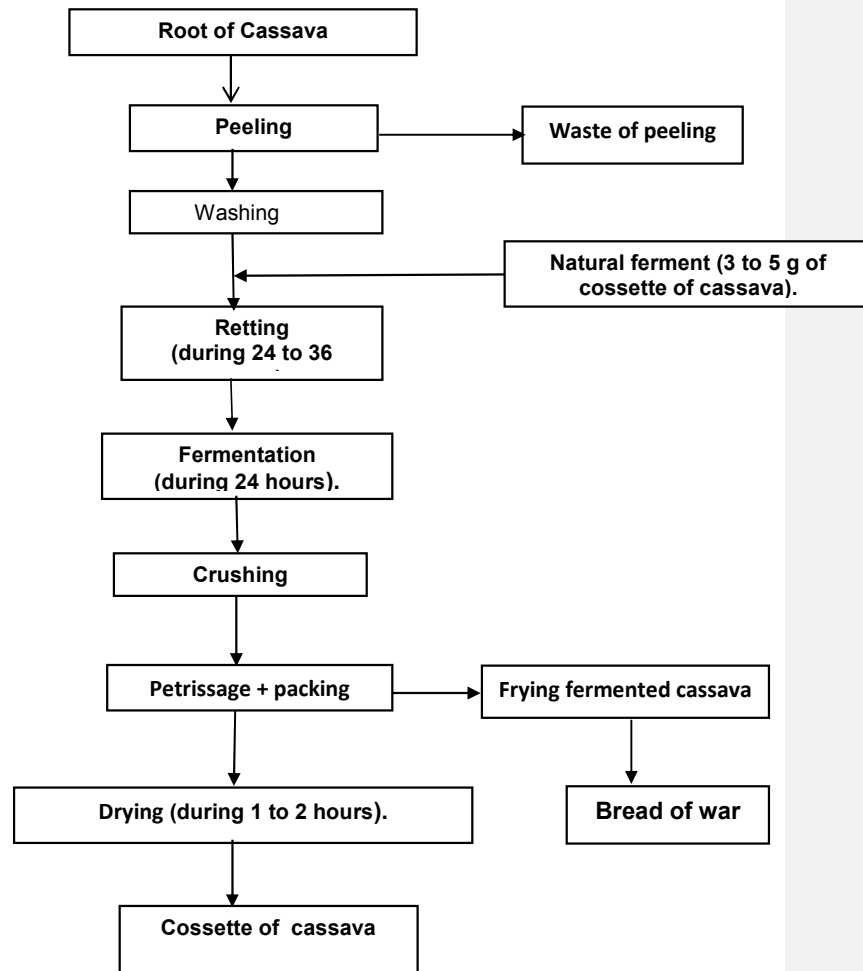


Figure 3. Improved cassava processing to obtain cossette of cassava and bread of war



Figure 4 : Traditional retting



Figure 5 : Improved retting



Figure 6 : Traditional drying



Figure 7 : Improved drying



Figure 8 : Cassava Derivative Products
Locally called Cossette of cassava



Figure 9 : Cassava Derivative Products
Locally called bread of war

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203 **3.3. Result of carbohydrate determination**

204 The Glucide profile of Cassava in traditional and improved processing is depicted in Table 1.

205 The difference observed is significant ($P < 0,0001$). This proves that there is variability between the
206 measured parameters according to the two cassava processing.

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209 Table 1: Glucide contain in Cossette of Cassava from traditional and improved processing

Difference	91,869
t (observed value)	386,378
t (critical value)	-1,990
Degree of freedom	79
p-value	< 0,0001
alpha	0,05

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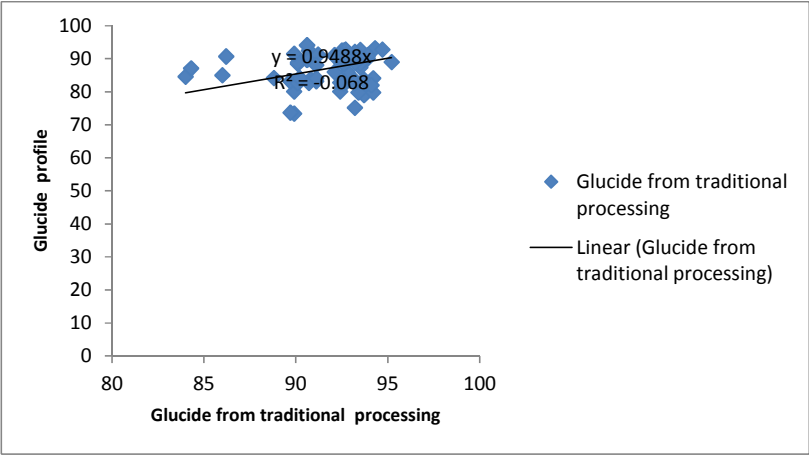
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The correlation test also shows that the two measured parameters through the two techniques are not related (figure 10). Thus, there is a weak negative correlation ($R^2 = -0,068$).



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Figure 10: Glucose contain in Cossette of Cassava from traditional and improved processing

3.4. Result of Lipids determination

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The lipids profile of Cassava in traditional and improved processing is depicted in Table 2. The difference observed is significant ($P < 0,0001$). This proves that there is variability between the measured parameters according to the two cassava processing.

Table 2: Lipid contain in Cossette of Cassava from traditional and improved processing

Difference	0,147
t (observed value)	20,575
t (critical value)	-1,990
Degree of freedom	79
p-value	< 0,0001
alpha	0,05

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The correlation test also shows that the two measured parameters through the two techniques are not related (figure 11). Thus, there is a weak negative correlation ($R^2 = -0,856$).

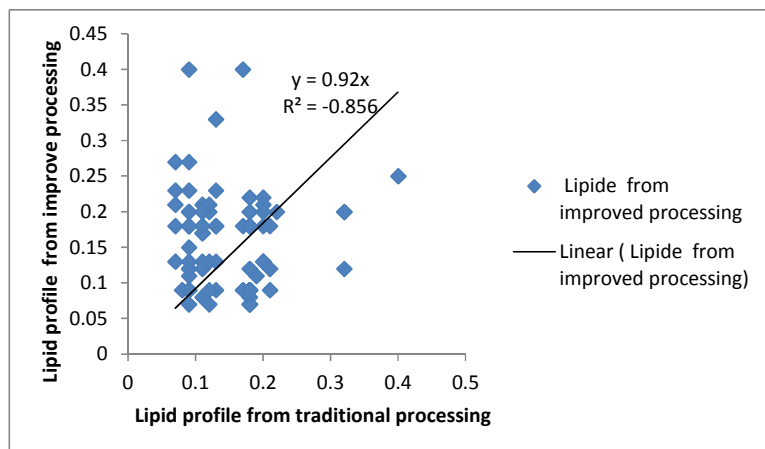


Figure 11: Lipid contain in Cossette of Cassava from traditional and improved processing

3.5. Result of Cyanogenic glycoside

The Cyanogenic glycoside profile of Cassava in traditional and improved processing is depicted in Table 3.

The difference observed is significant ($P < 0,0001$). This proves that there is variability between the measured parameters according to the two cassava processing.

Table 3: Cyanogenic glycoside contain in Cossette of Cassava from traditional and improved processing

Difference	12,184
t (observed value)	102,567
t (critical value)	-1,990
Degree of freedom	79
p-value	< 0,0001
alpha	0,05

The correlation test also shows that the two measured parameters through the two techniques are not related (figure 12). Thus, there is a weak negative correlation ($R^2 = -2,090$).

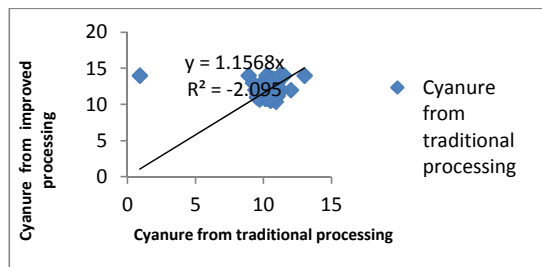


Figure 12: Cyanure contain in Cossette of Cassava from traditional and improved processing

3.4. Humidity level in cossette of cassava and bread of war

The Humidity profile of Cassava derived products is depicted in Table 4.

The difference observed is significant ($P < 0, 0001$). This proves that there is variability between the measured parameters according to the two cassava processing.

Table 4: Humidity level in cossette of cassava and bread of war

Difference	255 49,128
t (observed value)	256 63,956
t (critical value)	-12,580
Degree of freedom	79
p-value	< 0,0001
alpha	0,05

The correlation test also shows that the two measured parameters through the two techniques are not related (figure 13). Thus, there is a weak negative correlation ($R^2 = -0,70$).

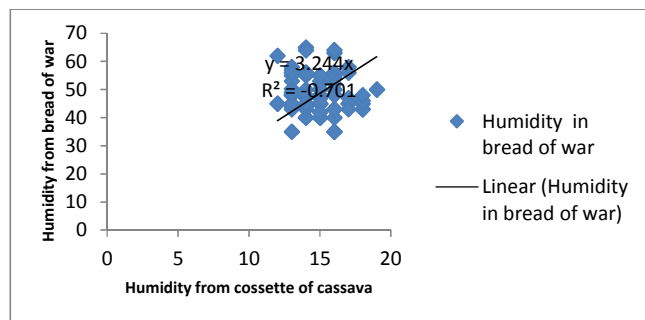


Figure 13: Humidity level in cossette of cassava and bread of war

4. Discussion

In this study, the concentration of cyanogenic compounds is higher in traditional cassava processing. On the other hand, the concentration of cyanogenic compounds in improved cassava processing is significantly lower than the levels in traditional processing ($P < 0.05$) (figure 12). World Health Organization recommends 10 mg/kg body weight as the maximum safe cyanide level (Okafor *and al.*, 2002; WHO, 2004) for fresh cassava intake. The concentration of cyanogenic compounds in cossette of Cassava from improved processing (shucking + fermentation + drying) obtained after retting of cassava root without skin is significantly lower than the levels in traditional processing. Cassava is consumed in various forms. Different traditional processing techniques are relatively effective in removing cyanide from cassava, especially those involving grating and crushing. The efficiency of the technique depends on the duration of the process; however, many small-scale farmers in developing countries do not have the time necessary to adequately process cassava. Even though cassava is an important food, it contains toxic and antinutritional substances that interfere with digestion and uptake of nutrients (Wobeto and others 2007).

The presence of skin in traditional processing could be in the origin of higher of cyanogenic compounds. Cyanide content ranges from 53 to 1300 mg cyanide (HCN) equivalents/kg DW (Siritunga and Sayre 2003; Wobeto and others 2007) in leaves, and from 10 to 500 mg HCN equivalents/kg DW (Arguedas and Cooke 1982; Dufour 1988; Siritunga and Sayre 2003, Cardoso *and al.*, 2005) in root

parenchyma. Some cassava genotypes are considered as highly cyanogenic while others are less cyanogenic. Further, the variation in the content of cyanogenic compounds in the different parts of cassava roots can be explained by differential translocation of cyanogenic glycosides and their metabolizing enzymes in the different cellular compartments (Jorgensen *and al.*, 2011).

Cyanide is the most toxic factor restricting the consumption of cassava roots and leaves. Cassava contains cyanogenic glucosides that are toxic for humans and can lead to serious health disorders. Cassava contains cyanogenic glycosides, mostly linamarin (2- β -D-glucopyranosyloxy-2-methylpropanenitrile) and lutostralin (2- β -D-glucopyranosyloxy-2-methylbutyronitrile) (ATSDR, 2010; Jorgensen *and al.*, 2011). These glycosides are supposed not to be toxic, but their decomposition into hydrogen cyanide (HCN) by intestinal flora enzymes would take place if humans ingested them (Sahoré and NemlinGnopo, 2010). The produced HCN is toxic for humans and it was reported to be responsible for certain pathological disorders such as Konzo diseases, thyroid goiter and tropical ataxic neuropathy (Ernesto *and al.*, 2002). The (per)acute oral toxicity of cyanide ions and HCN in mammals is well known. The small molecule is readily absorbed and distributed via systemic circulation. Above a certain tissue level, cyanide inhibits the cytochrome c oxidase (complex IV), the terminal enzyme of the mitochondrial electron transport chain by competitively binding to the oxygen-reducing cofactor of the protein (Piero and al., 2015). This crucial effect causes decreased utilization of oxygen and increased anaerobic metabolism, leading to excess of lactic acid and metabolic acidosis, and finally to cell death through energy deprivation. Due to its high dependence on oxidative metabolism, the central nervous system is particularly vulnerable to cyanide intoxication. With high oral doses, symptoms occur within a few minutes and may include nausea, vomiting, giddiness, headache, palpitations, hyperpnoea then dyspnoea, bradycardia, unconsciousness, and violent convulsions, followed by death (JECFA 2012; WHO 2004). In terms of the minimal lethal dose of cyanide in humans, the number of about 0.5 mg/kg body weight is commonly cited. It dates back to a paper by Gettler and Baine (1938) who applied a formula for back-calculation of the dose derived from experimental data in dogs to tissue levels found in humans after lethal intoxications. Often, a range of 0.5–3.5 mg/kg body weight is cited in the literature for the acute lethal dose of cyanide in humans which first was published by Halstrøm and Møller (1945). However, in some cases, much higher doses have been survived (e.g. 2.5 g potassium cyanide equivalent to 1000 mg cyanide: Tassan et al. 1990). Methods which use grating and crushing are very effective in removing cyanide because of the intimate contact in the finely-divided wet parenchyma between linamarin and the hydrolyzing enzyme linamarase, which promotes rapid breakdown of linamarin to hydrogen cyanide gas that escapes into the air (Cardoso *and al.*, 2005). This in combination with wetting, fermentation and drying can reduce cyanide contents up to 99%.

The Glucide profile of Cassava in traditional and improved processing is depicted in Table 1. The difference observed is significant ($P < 0, 0001$). This proves that there is variability between the measured parameters according to the two cassava processing. The carbohydrate content varies between 90% and 95% in the sample cossettes. Studies by other authors have revealed the content in some cassava varieties of 94% (Sahoré Drogba and NemlinGnopo, 2010). Cassava roots are rich in energy and contain mainly starch and soluble carbohydrates (Abraham and al., 2016).

The Humidity profile of Cassava derived products is depicted in Table 4. The difference observed is significant ($P < 0, 0001$). This proves that there is variability between the measured parameters according to the two cassava processing. The Humidity profile of Cassava is the main origin of post-harvest deterioration and it is the most important cause of loss in cassava production and this is mainly as a result of microbial invasion of the tuber (Okigbo, 1980). Post-harvest deterioration can render cassava unpalatable and un-marketable (Onabolu et al. 2002).

5. Conclusion

The concentration of cyanogenic compounds in cossette of Cassava from improved processing (shucking + fermentation + drying) obtained after retting of cassava root without skin is significantly lower than the levels in traditional processing.

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